DOCTOR OF PHILOSOPHY

The role of plant-fungus and plant-insect interactions in the dynamics of secondary and mature tropical rainforests

Weissflog, Anita

Award date:
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A thesis submitted to Bangor University by

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In candidature of the degree of

Doctor of Philosophy

Supervised by

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Prof. Bettina M.J. Engelbrecht

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Declaration

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

June 2nd, 2022

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Abstract

Tropical tree species richness and community composition varies along natural and anthropogenic gradients. Microbial pathogens and mutualists, insect herbivores, and predators of insect herbivores may contribute to such variation. Plant-microbial and plant-insect interactions affect the seedling performance and local abundance of tropical tree species. Evidence from temperate systems suggests that variation in the amount of insect herbivory can limit the distribution of plant species. Further, plant-soil feedbacks (PSF), the reciprocal effects between a plant and the soil microbial community it grows in, have been shown to drive the successional trajectories of grassland ecosystems. However, we know little about the role of plant-microbial and plant-insect interactions in determining the dynamics of secondary and mature tropical rainforests. This thesis aims at contributing knowledge to fill this research gap in four data chapters.

Two large greenhouse experiments and two manipulative field studies were performed to address the role of biotic interactions on tropical plant communities in Panama from different perspectives. The greenhouse experiments described in Chapter 2 and 3 tested the variation in PSF with successional stage (i.e. in soils from forests differing in the duration of their recovery since agricultural abandonment), experimental light level, tree species' association with specific stages of succession, and phylogenetic distance between soil conditioning and succeeding tree species. Chapter 4 assessed variation in seedling vigour of the widespread tropical tree species *Lacistema aggregatum* across four forest sites along a rainfall gradient within its distributional range and correlated these changes with measurements of pest effects (fungal pathogens and insect herbivores) on plant performance. Chapter 5 explored whether predation pressure on insect herbivores varies with time of the day and host plant pubescence and assessed the effect of model prey shape on estimates of predation in two lowland rainforests.

The results of this thesis are threefold. First, the greenhouse experiments showed that species-specific PSF acted in soils from all successional stages and affected most tree species tested. The occurrence of negative and positive net PSF suggests roles of both microbial pathogens and mutualists in structuring rainforest communities. PSF may contribute to

driving the rate and direction of successional tree species turnover by overall more positive PSF occurring at successional stages in which a species is naturally abundant, a lower susceptibility of tree species that are abundant in late successional stages, and a phylogenetic signal in heterospecific PSF. Second, pest effects varied across the rainfall gradient and peaked where plant vigour was highest, providing evidence that pest effects may contribute to determine tree species' local abundance but no support for pests limiting the distribution of this tree species. Third, this thesis shows that model prey shape may not be as important in prey recognition as assumed in previous studies indicating the need for caution when interpreting model-prey-derived estimates of predation.

The findings of this thesis indicate widespread effects of biotic interactions on plant community dynamics in secondary and mature rainforests. This contributes to closing the knowledge gap on the role of plant-microbial and plant-insect interactions in driving patterns of plant diversity and community structure in tropical rainforests. Its findings contribute to advancing fundamental science on the drivers of tropical biodiversity. Further, they provide information that enhances our ability to predict the consequences of projected changes in climate on tree species distributions and to develop effective restoration strategies to promote the recovery of tropical rainforests after severe disturbance.

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Chapter 1: Introduction

1.1 BACKGROUND

1.1.1 Why are there so many tree species in tropical rainforests?

Tropical rainforests host the highest diversity of vascular plants (Kreft & Jetz, 2007) of any biome and are home to at least 40,000-53,000 tree species (Slik et al., 2015). The question on why trees developed so many different mechanisms that enable them to live and reproduce, which combine to shape biodiversity as a whole, has sparked the interest of generations of scientist as expressed in a large number of ecological and evolutionary theories (Brown, 2014; Givnish, 1999; Leigh et al., 2004; Macarthur, 1965). While several mechanisms may act synergistically, high rates of co-evolution and biotic interactions have been suggested to play a key role (Brown, 2014; Kreft & Jetz, 2007).

1.1.2 Plant-microbial and plant-insect interactions

The interactions between tropical rainforest plants, microbes, and insects are intense. Fungal pathogens (Augspurger, 1984; Chanthorn et al., 2013; Spear et al., 2015) and insect herbivores (Eichhorn et al., 2010; Leigh et al., 2004) are main agents of seedling mortality with implications for local tree species abundance and richness (Bagchi et al., 2014; Connell, 1971; Janzen, 1970; Norghauer & Newbery, 2013).

Soil microbial communities, and fungal pathogens and mutualists in particular, may affect the presence and performance of current and future host plants via biotic plant-soil feedbacks (PSF) (Bennett & Klironomos, 2019). Both positive and negative PSF have been shown to affect tropical trees (Eck et al., 2019; Mangan, Herre, et al., 2010). Their density-dependence (Bagchi et al., 2014) and the species-specificity of pathogens (Eck et al., 2019; Spear & Broders, 2021) may affect plant species abundance and community structure (Bever et al., 2015). As well as fungi and bacteria, other soil biota such as nematodes (Wilschut et al., 2019; Wilschut & Geisen, 2021) and protists (Chandarana & Amaresan, 2022) in addition to

soil chemical and physical properties (reviewed in Bennett & Klironomos, 2019) can drive and modulate PSF in complex and likely interactive ways (Fig. 1.1) which remain largely unexplored in natural rainforest systems.

Insects are the most important herbivores of tropical rainforests (Coley & Barone, 1996) and can directly affect the plant community composition of rainforests (Szefer et al., 2020). Further, they are thought to have driven the evolution of a broad array of plant defences and by mediating the coexistence of plants with different defensive strategies, they may further contribute to promote tropical biodiversity (Coley & Kursar, 2014).

Intraspecific variation in these species' interactions could limit the distribution of a tree species or decrease its abundance at a locality. Interspecific variation may determine the relative success of a tree species establishment in a site when competing with other species. However, their role in driving patterns of tropical tree diversity is largely unknown.

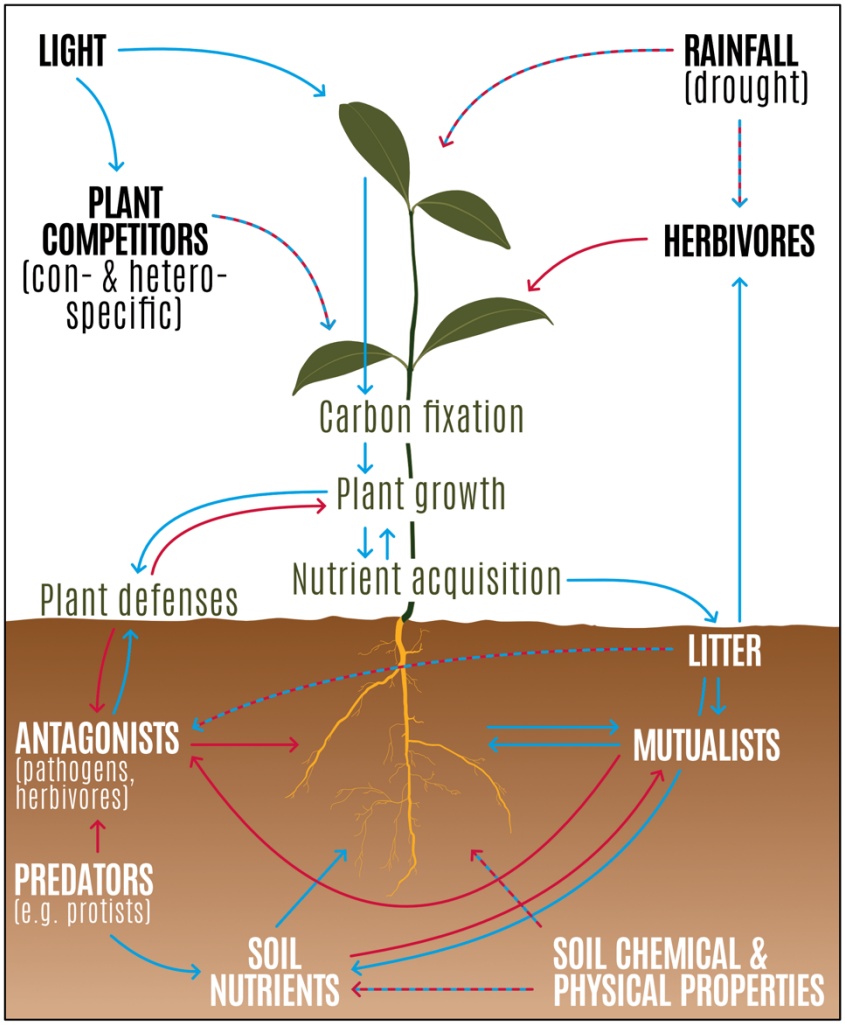


Figure 1.1. Schematic overview of plant-soil feedbacks. Abiotic (e.g. light, rainfall, soil chemical properties) and biotic (e.g. plant competitors, antagonists, mutualists) factors (capital letter case) affect plant performance and plant processes (normal letter case). Blue arrows show positive effects and red arrows show negative effects. Dotted arrows indicate that a factor can have positive or negative effects. Strongly modified from Bennett & Klironomos (2019).

1.1.3 Gradients – playgrounds for science

Tree species richness is not uniformly high across rainforests, but varies along environmental gradients, such as the pronounced increases with rainfall and aseasonality (Gentry, 1988; Givnish, 1999; ter Steege et al., 2003) and with recovery time since disturbance (Guariguata & Ostertag, 2001; Poorter et al., 2021; van Breugel et al., 2013). Such variation along natural and anthropogenically-caused gradients offer fantastic opportunities to study the mechanisms that shape and maintain tropical tree diversity and community structure.

1.1.4 Natural rainfall gradients

Spatial and seasonal variation in rainfall has strong effects on seedling mortality (Fortunel et al., 2016; Solé et al., 2019) and species-specific drought sensitivity of seedlings may affect the regional distributions of tree species along rainfall gradients (Brenes-Arguedas et al., 2009; Engelbrecht et al., 2007; Gaviria et al., 2017). Further, the damage caused by fungal pathogens and insect herbivores (hereafter "pests") is expected to increase towards wetter and less seasonal forests (Coley & Barone, 1996; Givnish, 1999), because of reduced resource limitation (Coley & Barone, 1996; Leigh et al., 2004) and improved dispersal and virulence of pathogens (Romero et al., 2022). However, the reasons why individual tree species differ from plant community trends in their patterns of pest damage along rainfall gradients (Spear et al., 2015; Weissflog et al., 2018) is unclear.

Variation in plant vigour along environmental gradients could affect the amount of pest damage as well as the effects of a given amount of damage on plant performance. The plant vigour hypothesis predicts that more vigorous plants experience higher pest damage (Price, 1991). Therefore, stronger growth of tropical saplings in nutrient-rich, wet sites than in resource-limited sites can result in greater pest damage (Boege & Dirzo, 2004). Resource limitation may also affect a plant's capacity to compensate for tissue loss (Wise & Abrahamson, 2008). Under low resource availability, meadow plants were shown to have lower tolerance and suffer greatest effects of herbivore damage (Cronin et al., 2010). In contrast, overcompensation for pest damage (Garcia & Eubanks, 2019; Wise & Abrahamson,

2008) has been suggested to explain positive effects of herbivory on subtropical tree growth (Schuldt et al., 2017).

Variation in the effects of insect herbivory has been shown to restrict the geographic range of a temperate plant species (Benning et al., 2019). However, we know little about variation in plant-pest interactions on seedling performance along rainfall gradients within the distributional range of tropical tree species.

1.1.5 Secondary succession of tropical rainforests

Secondary succession of tropical rainforests, i.e. the natural regrowth of forest after severe disturbance, where the soil remains intact (Chazdon et al., 2016), is characterized by constant competition amongst trees in a changing environment, which strongly influences the turnover of tree species (Poorter et al., 2021; van Breugel et al., 2013). While tree species richness generally recovers fast and typically returns to old-growth forest levels within only 30 to 60 years (Poorter et al., 2021), the recovery of species composition is less predictable and can take centuries (Guariguata & Ostertag, 2001; Poorter et al., 2021). Along with locality-specific land use history and stochastic dispersal (van Breugel et al., 2013), PSF may contribute to this pattern.

Species-specific PSF have been shown to affect the probability of tree seedlings establishing at a site (Sarmiento et al., 2017; Segnitz et al., 2020), to exclude tree species from habitats by enhancing niche differences (McCarthy-Neumann & Kobe, 2008, 2019; Spear et al., 2015), and to affect local tree abundance through reducing seedling survival at high conspecific densities (Bagchi et al., 2014; Krishnadas et al., 2018; Mangan, Schnitzer, et al., 2010). Plant-soil feedbacks can thereby mediate plant competition and contribute to the maintenance of plant diversity in intact rainforests (Bagchi et al., 2014; Eck et al., 2019; Krishnadas et al., 2018). Plant-soil feedbacks have also been shown to drive the successional plant species turnover in grassland systems (Bennett et al., 2017; Bennett & Klironomos, 2019; van der Putten et al., 2013). It thus seems likely that PSF may affect the successional trajectories of tropical rainforests, but empirical evidence is lacking.

1.1.6 Plant-soil feedbacks and the direction of secondary succession

The direction of successional tree species turnover could be determined by phylogenetic signals in PSF. The evolutionary conservatism of plant defence traits against pathogens (Gilbert & Webb, 2007; Gougherty & Davies, 2021) and growth benefits from mutualists (Reinhardt et al. 2012) may explain a greater likelihood of sharing a pathogen among closely related tropical tree species (Gilbert & Webb, 2007) and more positive microbial-mediated legacy effects between closely-related predecessor-successor pairs in grassland species (Anacker et al. 2014).

Studies suggest that pathogens could be more strongly associated with their hosts than mutualists (Eck et al., 2019; Schroeder et al., 2019), driven by the evolutionary arms race between hosts and pathogens and their co-diversification (Gilbert & Webb, 2007; Gougherty & Davies, 2021). Indeed, tropical seedlings grew worse in soils conditioned by adult trees of the same species than other species (Mangan, Schnitzer, et al., 2010).

Whether such phylogenetic signals in the effects of soil microbes extend beyond the species-level in tropical trees is unknown. Phylogenetic PSF could affect the performance of the species that follows during succession more negatively if it is closely related to the predecessor species and could thereby explain the successional transition from phylogenetic clustering to dispersion (Purschke et al., 2013).

1.1.7 Plant-soil feedbacks and the speed of secondary succession

The rate of successional tree species turnover could be affected by variation in PSF with tree life-history strategy and forest successional stage. The growth-defence trade-off predicts that the fast-growing, less well defended species that are typically abundant at the early stages of succession ("early-successional species") are more susceptible to pathogens than the slower-growing, better-defended species that are associated with late stages of succession ("late-successional species"). A high local abundance of such less well defended species during the early stages of succession (Guariguata & Ostertag, 2001) may favour the accumulation of species-specific soil microbes and a fast turnover of tree species (Gougherty & Davies, 2021).

Assuming a phylogenetic signal in PSF, these microbes may also drive the phylogenetic diversification of plant communities. The developing more-diverse host communities may in turn support a greater microbial diversity and dilute the average pathogen load per host (Gougherty & Davies, 2021). Overall more negative PSF in early-successional species, compared with overall more positive PSF in late-successional species, have been suggested to drive plant species turnover during secondary succession in temperate grasslands (Kardol et al., 2006; van de Voorde et al., 2011). A similar effect in tropical rainforests could drive an overall decrease in the strength of negative PSF and thus a decrease in the rate of tree species turnover with successional stage.

In addition to such general trends in PSF, individual species may have strongest PSF where they are most abundant, and thus at "home" because of a build-up of host-plant-specific microbes. The dominant tree species has been shown to be the main determinant of successional fungal community (Zhang et al., 2017) and negative PSF effects on seedling survival have been found to disappear at low host abundance (Y. Liu et al., 2015). Variation in soil-microbial-mediated PSF with host species association was shown in grassland mesocosms, where late-successional species had the strongest positive PSF in late-successional soil and weakest positive PSF in early-successional soil (Kardol et al., 2006) . Therefore, more positive PSF at a specific "home" stage could stall succession and promote plant species dominance, while more negative PSF could facilitate plant species turnover.

Lastly, the successional decrease in light availability below the canopy (Guariguata & Ostertag, 2001) may explain the simultaneous decrease in the density of mutualistic fungi (Zangaro et al., 2012) and could affect the overall strength of PSF during succession (reviewed in: De Long et al., 2019). Light availability can modulate PSF (Janos, 1980; Mangan, Herre, et al., 2010; McCarthy-Neumann & Kobe, 2019). High light levels may favour mutualists through increased sporulation (Mangan, Herre, et al., 2010) and carbon transfer from host plants (Kiers et al., 2011). This may drive greater pathogen-mediated seedling mortality in shaded understory than in light-gaps (Augspurger, 1984). Considering the effect of light when assessing successional variation in PSF is important as it could counteract some of the effects of plant community composition on soil microbiota detailed above.

The composition of soil microbial communities has been shown to change with forest succession (Chen et al., 2020; Yu et al., 2021; Zhang et al., 2017). Whether this translates into variation in the magnitude of PSF with successional stage is however unknown. Inter- and intraspecific variation in PSF with successional stage and light may affect the establishment success of specific tree species at different successional stages and drive tree species turnover.

1.1.8 Can it get more complex, please? Yes!

Insect herbivores have been suggested to have driven the evolution of a large range of plant defences and mediate the coexistence of plants with different defensive strategies (Coley & Kursar, 2014). Predation is the greatest source of larval mortality for many insects (Cornell & Hawkins, 1995), and it can effectively limit insect herbivory (Harrison & Banks-Leite, 2020; Kalka et al., 2008; Styrsky et al., 2006) and thereby have cascading effects on plant performance (Styrsky et al., 2006), community composition (Harrison & Banks-Leite, 2020), and diversity (Chesson & Kuang, 2008). Assessing temporal and spatial variation in plant-herbivore-predator interactions is thus crucial to advance our understanding of the mechanisms affecting the structure and stability of tropical rainforest communities.

Predation may affect where and when insect herbivory is greatest. Predation may vary with many variables including daytime (Ferrante et al., 2017; Seifert et al., 2016) (Ferrante et al., 2017) and the morphology of the plant on which the insect larva is feeding.

One example of a morphological trait potentially affecting predation of herbivorous insects is trichomes, plant hairs that are thought to present a structural defence against herbivores (Gorb & Gorb, 2019; Hanley et al., 2007; Levin, 1973), which may nonetheless provide herbivores with enemy-free space. Stem trichomes may increase friction of movement along the stem (Vermeij, 2015), increasing the energy expenditure needed to climb up stems. However, as well as deterring herbivores this may also deter their predators such as opportunistically foraging ants. Foliar trichomes may impede predation efficiency of bat predators of herbivorous insects. Rough surfaces can scatter echolocation calls (Clare &

Holderied, 2015) and reduce the detectability of prey via the acoustic specular effect (Geipel et al., 2019). In conclusion, trichomes, a plant defence against herbivores, may have a conflicting effect of compromising the ability of two main predator groups to detect and access their insect prey.

1.1.9 Are common methods good methods?

The complexity of processes in hyper-diverse rainforest ecosystems, makes ecologists commonly rely on proxies. One such example is artificial, plasticine caterpillars in which predators leave distinctive attack marks without removing the objects. These are commonly used to estimate the predation risk of insect herbivores (Howe et al., 2009; Lövei & Ferrante, 2017). Their lack of olfactory and chemical cues that predators may use for prey identification is accepted as an explanation for the fact that this kind of model prey underestimates real predation pressure (Lövei & Ferrante, 2017). However, whether attacks on model prey – that are expected to be recognized as prey purely based on mimicking its shape and size – represent predation events at all, is unclear. The repeated appearance of marks left by non-predators on plasticine objects (Pfennig et al., 2007) and the fact that the majority of attacks on plasticine prey in tropical forests are caused by chemically-oriented arthropod predators (X. Liu et al., 2020; Seifert et al., 2016) raises the question of whether plasticine caterpillars record predation specifically rather than a broad range of animal responses to a novel object.

1.1.10 Knowledge gap

Secondary forests are increasingly prominent features of tropical landscapes (FAO, 2020) and the dynamics of their plant communities have been the focus of much research in recent years (e.g. Poorter et al., 2021; Rozendaal et al., 2019). At the same time, interest in the role of “hidden” soil organisms in determining the stability and diversity of plant communities has emerged as a research field (De Long et al., 2019; van der Putten et al., 2013). Studies connecting these two topics by empirically investigating the role of PSF during the secondary succession of tropical rainforests are, however, lacking.

Evidence from temperate systems suggest that plant-microbial and plant-insect interactions have the potential to affect tropical tree species distributions along environmental gradients and drive the successional trajectories of rainforests. Further, pest effects on plant performance are likely to vary across rainfall gradients because variation in rainfall can influence pest damage, plant vigour, and plant tolerance. Empirical evidence on the variation of plant-microbial and plant-insect interactions along natural and anthropogenic gradients in tropical rainforests is scarce.

1.2 THESIS OBJECTIVES

The overall aim of this thesis is to assess the role of plant-microbial and plant-insect interactions on the tree community and species dynamics along a temporal gradient in the recovery of tropical rainforests following anthropogenetic disturbance and over a natural rainfall gradient.

More specifically, the objectives are to:

1) Explore the potential of PSF to differentially affect tree species performance at different successional stages and reveal how such effects may alter the rate and direction of tree species turnover during secondary succession of tropical rainforests (Chapter 2 + 3).

2) Determine the variation in effects of microbes and insect herbivores on seedling performance along a rainfall gradient within the distribution of a tree species (Chapter 4).

3) Test the diurnal and spatial variation in predation pressure on insect herbivores, and thus provide evidence for the contribution of tri-trophic interactions in shaping plant diversity (Chapter 5).

4) Assess the validity of a common ecological method and thereby promote the reliability of scientific results (Chapter 5).

To narrow the knowledge gap on the role of plant-microbial and plant-insect interactions on tropical plant diversity and address the specific research objectives stated above, I combined large-scale greenhouse experiments with manipulative field studies along an anthropogenically-caused regeneration gradient and a natural gradient in rainfall in the tropical rainforests of Panama.

1.3 RELEVANCE

The results of this thesis will advance our understanding of the fundamental ecological processes that structure hyper-diverse communities. This focus is very timely, and the results may serve two applied purposes.

First, understanding variation in species interactions and their effects on plant performance along current gradients in rainfall will promote our ability to predict how projected changes in climate will affect these interactions and determine future distribution of species. The predicted increase in the intensity and frequency of droughts (Pokhrel et al., 2021) may disrupt plant-pest interactions by shifting species distributions or increasing pest damage in some habitats but not others (Gely et al., 2020; Hamann et al., 2021). Understanding patterns of plant-pest interactions along abiotic gradients within the current range of tree species is crucial to determine the relative contribution of variation in pest damage and plant vigour on the strength of pest effects under different environmental conditions. This will allow us to better predict the role of pests in determining future tree species distributions under changed rainfall regimes.

Second, by providing empirical data, this thesis – in combination with further studies – may help to inform forest restoration efforts. More than 50% of the world's tropical forests are already lost (Malhi et al. 2014, Lewis et al. 2015) while tropical deforestation rates continue to exceed five million hectares per year (Barlow et al., 2018). Together with climate change (Malhi et al., 2014), this may have pushed the Amazon rainforest close to a dieback (Boulton et al., 2022). Given the enormous amount of ecosystem services that rainforests provide to humanity, including climate regulation and the provision of freshwater, timber, and food to a

large proportion of the global population, their continued degradation will have catastrophic consequences for our life on this planet (Foley et al., 2007). Secondary rainforests present a glimmer of hope. They are highly productive, and hold enormous, low-cost potential for carbon sequestration that may mitigate climate change with benefits for biodiversity (Chazdon et al., 2016; Guariguata & Ostertag, 2001; Poorter et al., 2021). However, their often slow and unpredictable recovery of species composition limits their potential to restore all of the original forests' ecosystem services (Guariguata & Ostertag, 2001; van Breugel et al., 2013). Our ignorance of the drivers of secondary forest succession hampers our ability to develop effective restoration strategies that promote the recovery of the ecosystem functionality that underpins the delivery of these services.

1.4 THESIS STRUCTURE

This general introduction is followed by four data chapters. In the following paragraphs, I introduce each chapter and provide an overview of its specific content and methods. The chapters have been developed to address the role of biotic interactions on tropical tree communities from different perspectives and thereby contribute to the overall aim of the thesis. All the research has been performed in the tropical lowland rainforests of Panama, including use of the facilities of the Smithsonian Tropical Research Institute in Gamboa.

Chapter 2 "Home sweet home: Plant-soil-feedbacks vary with tree species' association with forest successional stages" is the first of two chapters addressing the impact of microbial-mediated plant-soil feedbacks (PSF) on the rate and direction of tree species turnover during secondary succession of neotropical rainforests.

In this fully factorial, greenhouse experiment, I assessed variation in the magnitude and direction of PSF effects on seedling performance with forest successional stage, i.e. duration of forest recovery since agricultural abandonment, and light level for seven tree species that differ in their abundance across the stages of succession. I compared emergence, survival

and biomass of seedlings that were grown in sterilized soils against that of seedlings grown in soils containing the living microbial communities of four successional forest stages (0, 15, 25, and 115 years of recovery) under two light levels. This experiment specifically aimed at determining variation in PSF during secondary succession and assessing the potential for biotic PSF to contribute to the association of tree species with specific successional stages.

Chapter 3 "Negative plant-soil feedbacks decrease with plant phylogenetic distance and are stronger in late- than early-successional soil" used the soils conditioned in the first greenhouse experiment (Chapter 2) to determine the existence and strength of phylogenetic signals in microbial-mediated PSF and their variation with successional stage. A plant may affect the establishment of the plant following it in succession, i.e. its successor, through legacies mediated by soil microbial communities. Phylogenetic signals in these PSF may favour distantly-related successors over conspecifics and may drive successional diversification.

In a reciprocal greenhouse experiment, I grew seedlings of three tree species in soils originally collected from an early- and a late-successional forest stage (15 and 115 years) that had been conditioned by their conspecifics and two to four heterospecific species. I assessed PSF effects on the survival, growth, and biomass of successor species that varied in their phylogenetic distance to the conditioning species. I compared conspecific against heterospecific PSF and the magnitude of PSF against phylogenetic distance between species pairs. Further, I tested whether the strength of PSF and the phylogenetic signal varies with the successional stage that soils had been collected from.

Chapter 4 "Effects of insect herbivores and fungi on seedling performance of the tropical tree *Lacistema aggregatum* vary along a natural rainfall gradient" describes the findings of a field experiment in four forest sites across the steep natural rainfall gradient across the isthmus of Panama. Rainfall and dry season length may strongly affect plant vigour. How this may determine variation in pest effects on plant performance within the distributional range of a

tree species is unknown. The goal of this experiment was to advance our understanding of variation in pest effects within a tree species' current distributional range. Such knowledge may promote our ability to predict species' responses under projected changes in rainfall regimes.

I planted 400 seedlings of the widespread tropical tree species *Lacistema aggregatum* in four forest sites across a steep natural rainfall gradient and measured variation in the effect of partial and full pest exclusion on seedling growth and biomass. In each forest site, four plots each containing 25 planted seedlings were subject to one of four treatments respectively: fungicide, insecticide, combination of fungicide plus insecticide, and water-sprayed control. The combination and thus full pest exclusion treatment allowed me to assess seedling performance when pests are absent and thus the effect of variation in rainfall on seedling vigour. I tested how pest effects varied with seedling vigour across the rainfall gradient.

Chapter 5 "Do prey shape, daytime, and plant trichomes affect the predation rate on plasticine prey in tropical rainforests?" had the dual goals of assessing diurnal and spatial variation in predation pressure on insect herbivores, and testing the effect of model prey shape on predation rates to assess the validity of this commonly used method.

Predation is the main cause of mortality for many insects. It may strongly affect the times and locations of greatest insect herbivory with ultimate consequences for plant communities. However, we know little about variation in predation pressure on insect herbivores in tropical rainforests and many of the existing estimates are derived from model prey.

I used artificial model prey to test the potential of plant hairs, considered to be a structural defence against insect herbivores, to secondarily provide insect herbivores with enemy-free space by reducing prey detectability and predation efficiency of ants and bats. I compared attacks on models of caterpillars placed on glabrous versus pubescent plants in the forest understory. By checking for attack marks at dusk and dawn, I assessed variation between day- and night-time predation. Further, I tested the assumption that predators recognize model prey by its shape, and thus the validity of inferring predation pressure from attack rates on

artificial caterpillars, by comparing attacks on caterpillar-shaped plasticine models against those on similar-sized plasticine models shaped like the “superhero” The Incredible Hulk.

The data chapters are followed by ***Chapter 6***, which synthesizes the research findings of the data chapters in a general discussion and conclusion. This chapter aims to provide a wider picture of the impacts of plant-microbial and plant-insect interactions on plant dynamics of tropical forests along anthropogenic (chapter 1 and 2) and natural (chapter 3) environmental gradients. It ends with a short section identifying directions for future research and providing some advice for reforestation efforts.

1.4.1 Structure of data chapters

All data chapters are written in the format of scientific research articles. Thus, each data chapter is a self-contained entity, consisting of an abstract, an introduction including a brief literature review, a description of research methods and results, as well as a discussion and conclusion.

Chapter 5 has been submitted for publication. For this chapter, I provide details on co-authors and author contributions. Further, I presented preliminary results of Chapter 2 at two international conferences in 2019, the IAVS 62nd Annual Symposium and the ATBC 56th Annual Meeting, where I won the New Phytologist Prize for Best Poster.

Chapter 2: Home sweet home: Plant-soil-feedbacks vary with tree species' association with forest successional stages

2.1 ABSTRACT

Secondary forests, i.e. forests that naturally regrow on intact soils after severe disturbance, represent ~30% of all neotropical rainforests. While they offer many ecosystem services that a large part of the global population directly or indirectly depends on, we still lack of an understanding of the mechanisms that drive their recovery. Biotic plant-soil feedbacks (PSF), by which soil microbial communities and plants affect each other's presence and performance, have been shown to affect seedling survival and abundance of tropical tree species and to drive secondary succession in grasslands. The role of PSF in determining the rate and direction of successional tree species turnover in tropical rainforest, however, is unknown.

In a greenhouse study in Panama, we assessed variation in PSF with the successional stage of soil (extracted from forests recovering for 0, 15, 25, and 115 years) and light level (5% vs 40% ambient light) on seven tree species that vary in their abundance during succession. We assessed PSF as the difference in seedling performance (emergence, survival, and biomass) in live (including soil microbes) and sterilized soil. For each tree species, we compared PSF in soils extracted from forests of "home" successional stage(s) that the species is naturally abundant at versus soils from "other" successional stages it is not abundant at.

We found species-specific positive and negative PSF. Six of our seven species were affected by PSF in at least one parameter of seedling performance. Further, we found PSF at all four stages of succession. PSF on emergence and survival time were significantly more positive in home than in other soil for three species and when averaged across species.

Our results indicate that many tropical tree species are affected by species-specific PSF that reflect the relative effects of soil pathogens and mutualists. Complex interactions of PSF, species identity and successional stage with light suggest that the magnitude and direction of

PSF is strongly context-dependent. Lastly, most positive PSF at home soil may promote seedling establishment and allow species to persist in specific successional stages. Our data thus provide evidence that microbial-mediated PSF affect seedling establishment during the regeneration of tropical rainforests with potential effects on their successional trajectories.

2.2 INTRODUCTION

Secondary forests are increasingly prominent features of tropical landscapes (FAO, 2020). They are highly productive and provide many ecosystem services (e.g. carbon sequestration, timber provision; Chazdon et al., 2016; Guariguata & Ostertag, 2001). Yet, the often slow and unpredictable recovery of species composition limits their potential to restore all of the original forests' services (Guariguata & Ostertag, 2001; Poorter et al., 2021; Rozendaal et al., 2019; van Breugel et al., 2013). Improved understanding of the mechanisms that drive tropical forest dynamics after severe disturbance is needed to promote the recovery of ecosystem functionality (Chaparro et al., 2012) and will advance our understanding of the fundamental ecological processes that structure hyper-diverse communities.

Plant-soil feedbacks (PSF), by which soil-borne microbes and their plant hosts reciprocally affect each other's presence and performance (Bennett & Klironomos, 2019), could affect the rate and direction of species turnover during secondary succession. Soil microbial communities have been shown to strongly affect tree seedling performance (Augspurger, 1984; Packer & Clay, 2004). Via density-dependent effects (Bagchi et al., 2014) and differences in plant species susceptibility to pathogens (Eck et al., 2019; Spear & Broders, 2021), soil microbial communities may further affect plant species' local abundance and plant community structure (Bever et al., 2015; Lekberg et al., 2018).

Direct PSF, i.e. effects from a predecessor to a successor plant mediated by soil microbiota, have been shown to affect tropical tree seedlings in greenhouse experiments (Eck et al., 2019; Mangan, Herre, et al., 2010). This provides important insight into the differential effects of conspecific and heterospecific PSF and allows for an assessment of the role of PSF effects in local abundance dynamics. In a natural setting however, a seed will be exposed to a mixture of predecessor-specific microbial communities depending on the plant community composition and its distance to individual trees. Thus seedling establishment of a species may be largely affected by net community-level PSF, effects of the pathogenic and mutualist microbial community that is conditioned by the entire plant community (van der Putten et al., 2013). Empirical research on the role of such community-level PSF during secondary succession of tropical rainforests however is completely lacking.

Secondary succession of tropical rainforests is characterized by a deterministic turnover of tree species (Poorter et al., 2021; van Breugel et al., 2013) that peak in abundance at different stages of succession. Such an association of tree species to particular stages of succession where they reach high abundance and thus are at “home”, may affect the magnitude of PSF effects on seedling establishment.

Soil microbial communities of intact rainforests have been shown to be affected by tree species community composition (Barberán et al., 2015; Schroeder et al., 2019) and the identity of the most abundant tree species was the main determinant of fungal community composition in subtropical secondary forests (Zhang et al. 2017). Species-specific soil microbes may thus accumulate at successional stages at which their host species is most abundant. Pathogens have been suggested to be more host species-specific than mutualists (Bennett & Klironomos, 2019). Indeed, studies reported higher specialization of fungal pathogens than mycorrhizas in meadows (Klironomos 2002) and subtropical forests (Chen et al. 2019). Further, tree community composition of a mature rainforest was reflected more strongly in the soil fungal community composition of putative pathogens than mutualists (Schroeder et al., 2019). In combination, a host species-specific accumulation of soil microbes and a higher specificity in pathogens than mutualists could result in strongest negative PSF for tree species at their home successional stage.

PSF may vary with tree life-history strategy. The growth-defence trade-off (Wright et al., 2010) predicts that fast-growing, less-defended species that are abundant at the early stages of succession (hereafter “early-successional species”) should be more susceptible to PSF than slow-growing, better-defended species that are abundant at late stages of succession (hereafter “late-successional species”). Strong negative PSF effects in early successional species and positive PSF effects in late-successional species (Cortois et al., 2016; Kardol et al., 2006) have been suggested to drive the successional plant species turnover in grasslands (Kardol et al., 2006; van de Voorde et al., 2011; van der Putten et al., 2013). Whether similar patterns apply to tropical rainforests and whether early-successional tree species are more negatively affected by PSF than late-successional tree species is however unclear.

The overall strength of PSF may decline with succession: The increasing proportion of well-defended late-successional tree species (Guariguata & Ostertag, 2001) and the accumulation of a larger variety of microbiota (Zhang et al. 2017) may reduce the impact of individual microbial species on a host. Further, the direction of PSF effects may change. Grassland studies reported a successional shift in PSF that were dominated by pathogens at early stages of succession and dominated by mutualists at late stages of succession (Kardol et al., 2006; van de Voorde et al., 2011; van der Putten et al., 2013). This notion is further supported by a meta-analysis that found mycorrhizal abundance to increase during the first 50 years of succession across 85 temperate chronosequences (X. Zhou et al., 2017). While soil microbial community composition has been shown to change during secondary succession in tropical forests as well (Silva et al., 2016), mycorrhizal abundance decreased during the secondary succession of tropical rainforests in Brazil (Zangaro et al., 2012). Thus, the overall strength and direction of PSF may vary in soils of forests at different successional stages, i.e. in their recovery time since agricultural abandonment, yet the direction of this effect remains to be investigated.

Lastly, the drastic changes in abiotic condition during secondary forest succession may affect PSF (reviewed in: De Long et al., 2019). The successional decrease in light level below the canopy (Guariguata & Ostertag, 2001) may in particular affect PSF. Previous studies reported mixed results and found plant-soil feedbacks to be stronger (Janos, 1980; Mangan, Herre, et al., 2010; McCarthy-Neumann & Kobe, 2019) or weaker (Packer & Clay, 2004) at lower light levels. Low light conditions have been suggested to favour pathogen growth through increased air humidity (reviewed in: Bennett & Klironomos, 2019). Indeed, the proportion of seeds infected with fungal pathogens was higher under low than high light (Pringle et al., 2007). Pathogen-mediated seedling mortality has been shown to be greater in the shaded understory compared with light gaps (Augspurger, 1984) and increased pest-driven mortality in the shade for 53 woody rainforest species (Kobe & Vriesendorp, 2011). In contrast, microbial mutualist may benefit from high light levels, directly by inducing increased sporulation (Mangan, Herre, et al., 2010) or indirectly from effects of high light levels on plant physiology (increased carbon transfer to roots, altered root exudates, etc.) that enhance root colonization with mycorrhizas (as shown in the laboratory: Kiers et al., 2011). Plant-soil feedbacks may thus be less negative at high light levels.

Plant-soil feedbacks may play a larger role in determining the speed and direction of rainforest recovery than previously realized. Microbial communities may change during secondary succession of tropical rainforests mirroring changes in plant communities (and their functional traits) and changes in abiotic conditions. This could affect the strength of PSF for individual tree species and thus their establishment success at specific successional stages. However, no previous study has addressed the impact of PSF variation on the rate and direction of successional tree species turnover.

We performed a greenhouse experiment to assess variation in the strength and direction of PSF with successional stage (soils collected from below secondary rainforests of different successional ages) and light level for seven tree species that vary from early- to late-successional species in Panamanian rainforests. We aimed at assessing the potential of soil microbial communities to drive tree species turnover during secondary succession of Neotropical rainforests.

We asked whether PSF vary with soil successional stage, i.e. in soils collected from secondary forests that differ in their time of recovery since agricultural abandonment. More specifically, we hypothesize that 1) tree species experience strongest negative PSF when growing in soils from their "home" successional stages, i.e. in soils from secondary forests of the successional age that the species is naturally abundant at. We further hypothesize that 2) PSF vary with plant species identity and are stronger for species that are abundant at early stages of succession. Lastly, we hypothesize that 3) PSF will be less negative under high light levels.

2.3 METHODS

We conducted a greenhouse experiment at the Smithsonian Tropical Research Institute in Gamboa (9°07'N, 79°42'W), Republic of Panama, using seeds and soils from forests in and adjacent to the Soberanía National Reserve. In a fully factorial design, we quantified the effects of presence of microbial communities in soils (live vs sterilized) collected from forests of four successional stages (0, 15, 25, and 115 yrs) under two experimental light levels (40% vs 5%) on the establishment success (emergence) and performance (survival, biomass) of

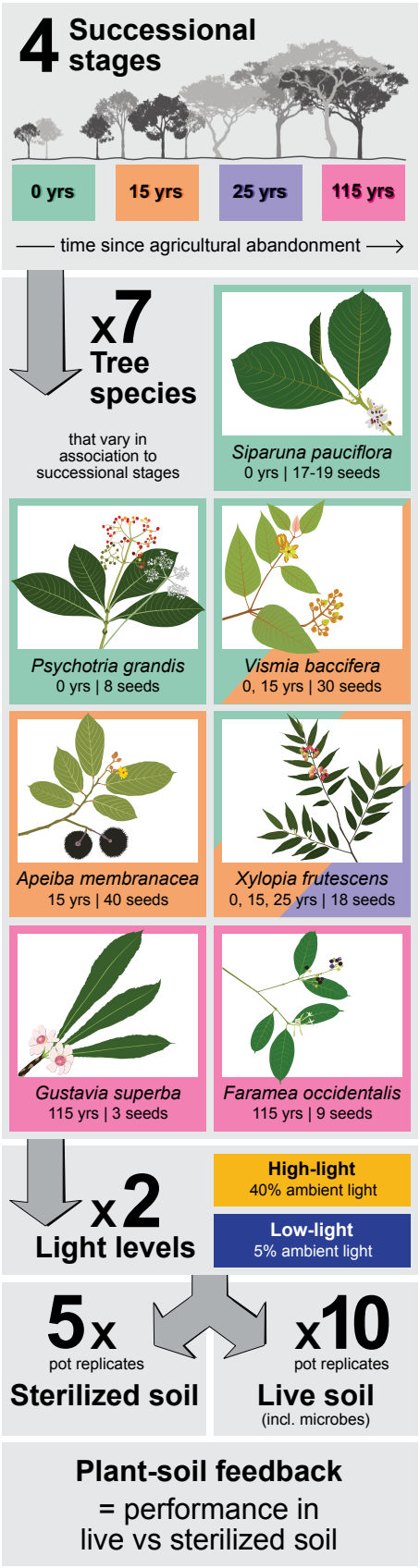
seven tree species. These tree species vary in their abundance in forests of the four successional stages and thus their association to these successional stages (home vs other; Fig. 2.1). We measured the magnitude and direction of plant-soil feedbacks (PSF) as the ratio of performance in non-sterilized, live soil (containing its live microbial community) to the performance in sterilized soil. Interspecific variation in the timing of seed production and the rapid decline in viability of seeds of many tropical tree species (Garwood, 1983; Román et al., 2012) necessitated a staggered start to the experiment, with three species being sown in November 2018, two species in January 2019 and two species in June 2019. All plants were harvested in August 2020. The duration of the experiment was 631, 552 and 416 days respectively (Table S2.1).

We additionally assessed PSF effects on the biomass allocation of seedlings by analysing root and leaf biomass fractions. As these analyses did not reveal any systematic PSF effects on biomass allocation, we focused our manuscript on seedling emergence, survival, and biomass as three main parameters of seedling establishment success. The analyses of root and leaf biomass fractions are presented in the supplement (Table S2.2, Fig. S2.2 – S2.5).

2.3.1 Soil collection

Tree species turnover is highest within the early decades of secondary succession (Rozendaal et al., 2019), but it can take more than a century for forest composition to recover (Derroire et al., 2016; Guariguata & Ostertag, 2001; Rozendaal et al., 2019). To capture the impact of PSF on secondary succession, we thus included three young successional stages (0-, 15-, and 25-yrs) and one old (~115-yrs) successional stage (Fig. S2.6). Soils of the three younger stages were collected in the Agua Salud landscape, a matrix of agricultural fields and secondary lowland rainforests of different successional stages (9°13’N, 79°47’W; 330 masl; 2,700 mm yr-1 rainfall; van Breugel et al., 2013). All sites have a similar land-use history (cattle pasture and low-intensity crop agriculture; Craven et al., 2015). Soils are nutrient poor Oxisols and vary little in texture (silty clays to clays) and nutrient levels (described in: van Breugel et al., 2013). Soils of the oldest successional stage (115 yrs) were collected in proximity to five

ForestGeo plots in a continuous semi-deciduous forest (9°9'N 79°44'W; 2,300 mm yr^{-1}; Condit, 1998; Fig. S2.6).



Our soil sampling protocol aimed at encompassing for small-scale variation in soil microbial communities (Schroeder et al., 2019) and achieve our goal to assess effects of community-conditioned soils. For each successional stage, we thus collected soils in two locations (50 m apart, avoiding canopy gaps) in each of five replicate sites. These sites were separated by ≥ 1 km with forests of different successional stage between them, except for the old successional stage for which soils were collected in a continuous forest. We removed surface litter and extracted the top layer of soils (0-10 cm). Soils were sieved (4 mm) to remove large roots and stones, pooled across sites for each successional stage and mixed thoroughly. Soils were stored in a ventilated and dark room before being used within 22 days after collection.

We pooled soil samples per successional stage to achieve our research objective to identify differences in PSF effects amongst our four successional stages. We aimed at assessing whether soil microbial communities that are "typical" for a specific successional stage affect

Figure 2.1. Overview of full-factorial experimental design. Soils were collected in secondary forests of four successional stages (0, 15, 25, and 115 years of recovery). In soils of each successional stage, seeds of seven tree species were sown. Tree species differ in their abundance during succession and are associated to the successional stage(s) at which they are among the 20 most abundant species. These "home" stage(s) are indicated by the colour(s) of species boxes and are printed below the species name, next to the number of seeds sown per pot. Seedlings were grown under two light levels. Per successional stage x species x light combination, we had five pot replicates filled with sterilized field soil and ten pot replicates with live (including microbes) field soil. We assessed plant-soil feedbacks as the difference in seedling performance in live versus sterilized soil.

the strength and direction of PSF on plant performance and interact with plant species identity and light level and thus sought to reduce bias from variation among sampling sites of the same successional stage. We were not primarily interested in determining the level of variation in PSF effects among sites of the same successional stage. For our objectives, not pooling would have presented two major problems. Firstly, sites of different successional stages vary strongly in their tree density (van Breugel et al., 2006) and thus the distance to adult trees at which soil samples can be collected in the field. Therefore, without pooling soil samples, we potentially would have tested effects of microbial communities that have been conditioned by one specific adult tree, and thus testing for conspecific vs heterospecific PSF instead of differences among successional stages. The strength of this "species-specific" bias would have been confounded with successional stage because of the difference in stem densities but also because of among-site differences in the similarities of forest composition with successional time (Poorter et al., 2021; van Breugel et al., 2013). Secondly, incorporating variation in soil amongst sites within each successional stage, and replicating sufficiently to overcome the potential "species-specific" bias, into the design would have made the experiment unfeasibly large and complex. It would still have been essential to retain high numbers of replicate pots per species X treatment combination in order to get sufficiently accurate measures of emergence and survival because of the high intraspecific variation.

2.3.2 Tree species

We aimed to include species that are associated with one or more of the four successional stages in our study, and thus represent typical species of various stages of succession in Panamanian rainforests. We selected seven tree species based on their abundance in forests of each of the four successional stages (Fig. 2.1). As measure of relative abundance of each species at a successional stage, we calculated the importance value (IV) based on tree census data from forest sites across Panama (Condit, 1998; Condit et al., 2019; M. van Breugel *unpublished data)*. In this case, the IV is the sum of relative basal area (i.e. basal area of all trees >10 mm DBH of a species/ basal area of all trees per site) and relative density (number of trees of a species/ number of all trees per site) divided by two (Schroeder et al., 2019). Data on the frequency of species occurrence across forest sites of each of our forest

successional stages on a larger landscape-scale were not available and thus could not be included. Based on the IV, we pre-selected the 20 most abundant species per successional stage. Final species selection was dependent on the availability of field-collected seeds and aimed at including species that are associated to different successional stages (Table S2.1). Associations with arbuscular mycorrhizas are a common feature of Neotropical tree species (Mangan, Herre, et al., 2010) and are documented for all seven of our focal species at the species (*Faramea occidentalis*: Husband et al., 2002; *Apeiba membranacea*: Lovelock et al., 2003; *Gustavia superba*: Pizano et al., 2017) or genus level (*Psychotria, Siparuna, Vismia*: Kottke & Haug, 2004; *Xylopia*: Soudzilovskaia et al., 2022). While additional associations with ectomycorrhizal fungi are possible (Corrales et al., 2018), we found no published records of such associations for any of our focal species.

Association translates the four successional stages into a binary variable by pooling all successional stages that a species is naturally abundant at (among the 20 most abundant species in that successional stage) into "home" and pooling all other successional stages into "other". While species may be present at other stages of succession as well, presence is often stochastic depending on the abundance of seed dispersing trees in the vicinity. The stages at which abundance is particularly high however is relevant for a build-up of species-specific microbiota, and thus the focus of this experiment. As some of our species are abundant at several successional stages, the association analysis enables us to investigate whether the soil microbial community of the successional stage a species is abundant at is more important than a specific successional stage.

2.3.3 Seed collection and preparation

External colonization of seeds by beneficial and pathogenic soil microbes can affect seed viability (Sarmiento et al., 2017) and germination. Impacts on seeds may therefore be an important component of PSF during secondary succession and so were included in our study. To avoid pre-experimental infection with soil fungi, we collected seeds directly from trees or obtained seeds from intact fruits found on the forest floor. Undamaged seeds were cleaned and dried. For each species we collected seeds from a minimum of six mother trees (Table

S2.1). All seed collection sites were > 4 km away from all the soil extraction sites to minimize bias from adaptation to local microbial communities or plant genotype-specific pathogens (Eck et al., 2019). All seeds of a species were pooled and stored in paper bags at 23°C.

2.3.4 Greenhouse set-up

We germinated and grew seedlings of seven tree species in soils that contained living microbial communities or sterilized soils (Inoculum: live vs sterile) and had been collected from forests of four successional stages (Successional stage: 0, 15, 25, 115 yrs) under two light levels (Light: 5% and 40%; Fig. 2.1 & S2.1). We compared seedling emergence, survival, and biomass in 10 pot replicates of live vs 5 pot replicates of sterilized soil per species-treatment combination. The experiment thus comprised 840 pots (7 species x 4 successional stages x 2 light levels = 56 species-treatment combinations in 56 x 10 live + 56 x 5 sterilized pot replicates) and 15,211 tree seeds (Table 2.1, Fig. 2.1).

The greenhouse is part of a large experimental area, has a concrete floor, and is located at ~70 m to the edge of a mature rainforest. It has a plastic roof to shelter experimental pots from rain and the roof and sides are covered in shade-cloth allowing for airflow. The experiment was set up on metal-grid 30 tables (to promote drainage and minimize risk of microbial contamination between pots, height 90 cm). Tables were systematically assigned to either the high-light level or the low-light level. Overall light level at bench level in the greenhouse was at 40% of ambient light and constituted the high-light treatment. To simulate conditions in the understory of dense secondary forests, we created a low-light environment by shading individual tables with standardized shade cloth ensuring a consistent shading to 5% of ambient light. Each table was divided into two separate sections, one of pots with sterilized soil and one of pots with live microbial communities. These two zones were separated by a 100 cm tall clear plastic wall that prevented any transfer of liquid water or fungicide spray between them. Within each of these zones, pot position was randomized with the restriction of allowing a maximum of two pots per species-treatment combination per table (Fig. S2.1).

Seeds were sown within 30 days of collection, except for *A. membranacea* for which seeds were stored for ≤ 103 days. Seeds of *A. membranacea* remain viable for up to 6 months after collection (Román et al., 2012). All seeds were soaked in water for 24h and surface-sterilized (70% ethanol, 10% bleach, 70% ethanol, distilled water: Fitzpatrick et al., 2017) directly before sowing. The number of seeds per pot varied among species depending on seed size (for seed number per pot for each species see Fig. 2.1). We chose large 5-l pots (ø 21 cm) to reduce the risk of root limitation and provide a large surface for seedling emergence. Each experimental pot had five drainage holes (0.7 cm diameter, covered with mesh) and was placed on a 1 cm high plastic ring at a distance of > 5 cm in any direction to other pots.

All pots were filled to 90% with a steam-sterilized (2 x 2.5 h at ≥ 75 °C) soil and sand mix (1:1). The soil used to create this sterilized, generic growing medium had been collected from various forest sites (ranging from 60 to 90 years recovery since agricultural abandonment) within the Barro Colorado Nature Monument, had been collected more than two years prior to our experiment and had been stored in a well-ventilated shed with walls on three sides and a roof. As a surface layer, we added 400 ml inoculum, i.e. 10%, of the soil collected from the successional forests that either contained a live microbial community ('live') or was autoclaved ('sterilized', 2 x 57 min at 121 °C; Sterilmatic, Market Forge Industries Inc., Everett, USA). Using a small proportion of soil inoculum reduces potential effects of increased nutrient availability through microbial lysis in sterilized soils (S. Mangan, *pers. comm.*) and potential effects of successional changes in soil pH and nutrients on microbial communities (Yu et al., 2021), while providing an effective fungal inoculum (Mangan, Herre, et al., 2010). Seeds were placed directly onto the inoculum soil layer and then the entire surface was sprinkled with a ~1 mm thin layer of sterilized sand to reduce mould development. Any developing mould patches were carefully removed with sterile small wooden spatulas with minimal removal of the underlaying soil. Any emerging seedlings from species other than our focal species were removed from the experimental pots as soon as detected but never later than two days after emergence (equalling the interval between survival censuses).

All pots were given equal amounts of water every other day. We avoided wetting of leaves. Pots leaked different quantities of water depending on plant density and size. To minimize bias from different levels of nutrient leaching, we applied the slow-release fertilizer Osmocote Plus (N15–P9–K12; 18.55g per pot; Scotts Co. LLC, Marysville, USA).

Herbivorous insects forced us to apply the broad-spectrum insecticide Pyrethrin (Pyrethrin 1%, Naphtha 0.78%; Bonide LLC, Oriskany, USA) to all pots twice (19.10.2019 and 20.01.2020). Further, we removed dead leaves from all pots directly after abscission to reduce the risk of insect herbivore infestations. We thinned seedling densities to a maximum of three plants per pot to limit competition seven months after seed sowing (Table S2.1).

To continuously suppress fungal growth in the sterilized soils, we applied the broadband systemic fungicide Amistar XTRA® (200g/ l Azoxystrobin, 80g/ l Cyproconazole; Syngenta Ltd., Basel, Switzerland) to seedling shoots and soil with a hand mister and according to the manufacturer's guidelines at a rate of 575 ml/ ha at intervals of six weeks. Amistar XTRA® is active against Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes and thus all the most common pathogens of seedlings in our forests (Spear & Broders, 2021). The preceding version of Amistar XTRA® has previously been used in studies of tropical forests before (Bagchi et al., 2014) and no phytotoxic effects are known.

2.3.5 Measurements

We recorded seedling emergence and survival every other day. All plants alive at the end of the experiment were harvested, washed, and divided into leaf, stem, and root material (including the vast majority of fine roots). Abscised leaves were not included in the final biomass measurements. Samples were dried for > 72 hours at 60 °C in drying ovens and subsequently weighed with a fine balance (d: 0.0001 g; TORBAL AGN 200, Scientific Industries Inc., Bohemia, USA) to determine biomass. We measured plant height (stem base to apex) at the end of the experiment in July and August 2020.

2.3.6 Statistical Analyses

We analysed the effects of presence of microbiota in the soil (Inoculum: sterilized vs live soils), soil successional stage (Successional stage: 0, 15, 25, 115 yrs) and light level (Light: 40% vs 5%) on seedling emergence, survival, and biomass of our seven tree species. For each parameter, we performed two separate analyses using A) soil successional stage ("Successional stage"), and B) species' associations with each successional stage ("Association: home vs other"). All statistical analyses were conducted in the same way for successional stage and for species' association. In the following, "successional stage/ association" will refer to conducting the same but separate analyses for both factors. All statistical analyses were performed in R (R Core Team, 2021).

Species were sown at different times due to variation in their time of seed production and thus had different experimental durations (Table S2.1). We cut off emergence and survival data after the maximum study length for the species that were sown last minus one day, i.e. after 415 days to give all species equal time to emerge and die. Five *Siparuna pauciflora* seedlings emerged after this threshold and were excluded from the emergence analysis. For the biomass analyses we included plant age (number of days between emergence and death) in the models to encompass for the different experimental durations.

Emergence. – We analysed the odds to emerge (emerged seedlings by seeds that did not emerge) using binomial logistic regression. We constructed separate models for each of our species for both, the successional stage and association analyses.

In one of our species x treatment combinations (*G. superba*, 40% light, sterilized, 15-yrs) no seedling emerged. To avoid inflated and unreliable estimates due to complete separation we fitted a binomal Bayesian generalized linear model with logit link distribution and the default prior scale of 2.5 for logistic regression and used the number of predictors in the model as a prior for the degrees of freedom (function bayesglm in R package arm). The effect of association was analysed with binomial generalized linear models (glm in stats) with logit link.

We performed model selection based on the Bayesian information criterion (BIC; Burnham & Anderson, 2004) and compared the fit of a random intercept model against the fit of models including all meaningful combinations of single and interactive effects of our treatment factors, i.e. species, successional stage/ association, light, and inoculum. The final model included all treatment factors, all two-way interactions, and the three-way interaction between inoculum x successional stage/ association x light. Including the position of a plant in the greenhouse as a random effect contributed less than 0.3% of the explained variance so it was omitted. We found no collinearity among our factors (vif in performance) and tested for overdispersion by dividing the residual deviance through the degrees of freedom; no corrections were necessary.

We performed type III Anovas (Anova in car) to evaluate the overall impact of our main effects and interaction terms. We calculated PSF as the logarithmic odds ratio log(OR) of estimated marginal mean emergence between live and sterilized soils for each species x treatment combination (emmeans) (Bennett et al., 2017). A log(OR) > 0 indicates a positive PSF, i.e. higher emergence in live than in sterilized soil. We tested whether PSF differed among successional stages and association with Tukey-adjusted pairwise contrasts within each species at high light, low light, and averaged across light levels (contrasts in emmeans).

Additionally, to provide a more intuitive measure, we present simple odds ratios (OR) of emergence by dividing the odds to emerge in live soil against the odds to emerge in sterilized soil. An OR > 1 indicated higher emergence in live than sterilized soil.

Survival. – We calculated the risk ratio (RR) of survival as the proportion of seedlings surviving in live vs sterilized soil for 415 days. A RR = 1 indicates equal survival in live and sterilized soil and a RR > 1 indicates a positive PSF and higher survival in live than sterilized soil. This basic analysis allowed for the inclusion of species-treatment combinations with 100% survival, in contrast to the statistical models described below.

We additionally analysed the effect of time on treatment effects in all species-treatment combinations where survival was not 100%. We analysed the probability of survival over 415 days using mixed effects Cox proportional hazards regression (coxme). Plants that were removed during thinning were not included in the analysis as they did not die of a natural cause. Cox models cannot compute survival probabilities if a species-treatment combination is without a single death event. As we aimed to compare survival between live and sterilized soil, we excluded species-treatment combinations that had no deaths as well as their corresponding live or sterilized treatment. We could therefore not analyse survival of *G. superba* and survival of *F. occidentalis* under 40% light and in 0-yr old soils under 5% light.

We included the position of a plant in the greenhouse as a random effect and included inoculum, successional stage/ association, and light, and the interactions between inoculum x successional stage/ association and inoculum x light as fixed effects. We tested the proportional hazard assumption (cox.zph in survival) for each covariate and model. As the effects of inoculum and light varied over time, thereby violating the proportional hazard assumption, we split our data into time intervals of 100 days (timesplitter in Greg) and included an interaction term of inoculum x time (all models except *F. occidentalis*) and light x time where necessary (all models except *F. occidentalis* and *S. pauciflora*).

We performed type III Anovas to obtain estimates of the effect of each variable. We assessed PSF as the hazard ratio (HR) of death between sterilized and live soils for each successional stage/ association at 40% and 5% light, overall PSF averaged across light levels and successional stages, and the overall HR of growing at 40% vs 5% light using estimated marginal mean survival times (emmeans at the response scale). A HR = 1 indicates no difference in the hazard of death between the compared groups. A HR > 1 indicates a reduced hazard of death per unit time in live soil compared to sterilized soil and thus a positive PSF (and for light it represents better survival at 5% than at 40% light). A HR < 1 indicates a negative PSF (or better survival at 40% light). We calculated pairwise contrasts of HR to identify differences of PSF between successional stages and association using a Bonferroni correction for multiple comparisons. Hazard ratios were translated into percentage change over time as %=(HR-1)*100.

Biomass. **–** We analysed biomass using generalized linear models (glm in stats) and linear mixed effects models (lmer in lme4) separately for each species. We added the number of days between emergence and harvest for each plant as a fixed effect to the models to control for the effect of plant age on biomass. Only plants ≥ 105 days old since emergence were included (Table S2.3). The position of a plant in the greenhouse and the number of plants per pot were considered as random effects and included in a model if they explained more than 5% of the variance.

For *A. membranacea*, *G. superba*, and *F. occidentalis,* we present models including light as a factor, as well as separate models for plants growing under 40% and 5% light to facilitate comparisons among species. In four of our species, all plants in the 5% light treatment died before the harvest (Table S2.3) precluding the analysis of the effect of light level on biomass of these species.

To test our hypotheses, we *a priori* defined inoculum, successional stage/ association, and (where possible) light and the interaction between inoculum x successional stage/ association as fixed effects. Among all plausible models, we selected the best fitting one using BIC. We log-transformed biomass data and tested for the normality of residuals, non-multicollinearity of predictors using a variance inflation factor of <5, and homoscedasticity (package DHARMa).

For all successional stage and association models, we performed type III Anovas and calculated PSF as the log(OR) of estimated marginal mean biomass in live vs sterilized soils at each treatment level. We compared PSF between levels of successional stage and association to identify differences in the strength of PSF as described above.

2.4 RESULTS

From the 15,214 seeds sown 5,969 seedlings and thus 39.2% emerged within 415 days after sowing (Table 2.1). We harvested 789 plants at the end of the experiment, of which 746 were older than 105 days and included in the analysis of biomass and survival. A summary table of all PSF and the results of pairwise comparisons between PSF is provided in the Supplement (Table S2.4).

2.4.1 Emergence

Emergence was overall lower in live than sterilized soils for four of the seven tree species and when averaged across species and light levels (OR = 0.94, in Table 2.1). This negative PSF on emergence was significant for two species (*A. membranacea*, *X. frutescens*), while *V. baccifera* had a significant positive PSF on emergence (significant effect of inoculum in Table 2.2A, Fig. 2.2). When looking at the four successional stages separately, we found significant PSF in at least one successional stage for five of seven species and significant pairwise differences between PSF among successional stages in four species (Fig. 2.2).

Table 2.1. Overview of seedling performance of seven tropical tree species. In a greenhouse experiment, we germinated and grew seedlings of seven tropical tree species. Tree species vary in their abundance (basal area and stem density) across four successional forest stages (0, 15, 25, and 115 years of recovery since agricultural abandonment). We classified all those successional stages at which a species is among the 20 most abundant species as "home" and grouped the remaining successional stages into "other". In a factorial design, seedlings were grown under two light levels ("40% light", "5% light") in soils collected from below forests of four successional stages that either had been sterilized ("sterile") or contained a living microbial community ("live"). We present summary data for each species (across all successional stages) as well as separately for home and other stages. We provide data on **i) Emergence** as the number of plants emerged against the number of seeds sown and percentage of emergence in brackets. We show the odds ratio (OR) which divides the odds to emerge in live soil by the odds to emerge in sterilized soil. A OR > 1 indicates a higher emergence in live than sterilized soil and thus a positive plant-soil feedback (PSF). Further, we show **ii) Survival** as the number of plants that survived the first 415 days of our experiment against the sum of plants that died and survived in the same time span, and again, in brackets, the percentage survival. For survival, we provide risk ratios (RR) as the percentage survival in live soil against percentage survival in sterilized soil. A RR > 1 signifies higher survival in live than sterilized soil (positive PSF). **iii) Biomass** is the across-plant mean of dry weight (roots, stems, and leaves) and the number of plants used for this measurement in brackets.
The table is printed on the following page.

	i) EMERGENCE								ii) SURVIVAL								iii) BIOMASS			
	40% light			5% light			Across light		40% light			5% light			Across light		40% light		5% light	
	sterile	live	OR	sterile	live	OR	total	OR	sterile	live	RR	sterile	live	RR	total	RR	sterile	live	sterile	live
Siparuna pauciflora	78/ 370 (21.1%)	157/ 740 (21.2%)	1.01	71/ 370 (19.2%)	151/ 740 (20.4%)	1.08	457/2220 (20.6%)	1.04	40/ 62 (64.5%)	81/ 122 (66.4%)	1.03	17/ 59 (28.8%)	89/ 117 (76.1%)	2.64	227/ 360 (63.1%)	1.51	1.0 (16)	3.0 (5)	– (0)	0.2 (5)
Home	21/ 95 (22.1%)	34/ 190 (17.9%)	0.77	16/ 95 (16.8%)	29/ 190 (15.3%)	0.89	100/ 570 (17.5%)	0.82	10/ 17 (58.8%)	18/ 28 (64.3%)	1.09	3/ 15 (20.0%)	19/ 25 (76.0%)	3.80	50/ 85 (58.8%)	1.72				
Other	57/ 275 (20.7%)	123/ 550 (22.4%)	1.10	55/ 275 (20.0%)	122/ 550 (22.2%)	1.14	357/1650 (21.6%)	1.12	30/ 45 (66.7%)	63/ 94 (67.0%)	1.01	14/ 44 (31.8%)	70/ 92 (76.1%)	2.39	177/ 275 64.4%	1.45				
Psychotria grandis	115/ 160 (71.9%)	183/ 320 (57.2%)	0.52	114 /160 (71.3%)	189/ 320 (59.1%)	0.58	601/ 960 (62.6%)	0.55	49/ 85 (57.6%)	115/ 135 (85.2%)	1.48	0/ 101 (0.0%)	98/ 138 (71.0%)	–	262/ 459 (57.1%)	2.96	3.9 (43)	6.5 (66)	– (0)	0.2 (23)
Home	29/ 40 (72.5%)	46/ 80 (57.5%)	0.51	26/ 40 (65.0%)	56/ 80 (70.0%)	1.26	157/ 240 (65.4%)	0.80	9/ 22 (40.9%)	30/ 33 (90.9%)	2.22	0/ 23 (0.0%)	24/ 37 (64.9%)	–	63/ 115 (54.8%)	3.86				
Other	86/ 120 (71.7%)	137/ 240 (57.1%)	0.53	88/ 120 (73.3%)	133/ 240 (55.4%)	0.45	444/ 720 (61.7%)	0.49	40/ 63 (63.5%)	85/ 102 (83.3%)	1.31	0/ 78 (0.0%)	74/ 101 (73.3%)	–	199/ 344 (57.8%)	2.76				
Vismia baccifera	207/ 600 (34.5%)	519/1200 (43.3%)	1.45	156/ 600 (26.0%)	449/ 1200 (37.4%)	1.7	1331/3600 (37.0%)	1.56	4/ 191 (2.1%)	32/ 428 (7.5%)	3.57	0/ 155 (0.0%)	0/ 447 (0.0%)	1.00	36/ 1221 (2.9%)	3.16	1.0 (2)	10.7 (11)	– (0)	– (0)
Home	102/ 300 (34.0%)	273/ 600 (45.5%)	1.62	103/ 300 (34.3%)	207/ 600 (34.5%)	3.15	670/1800 (37.2%)	2.16	2/ 92 (2.2%)	13/ 215 (6.0%)	2.78	0/ 53 (0.0%)	0/ 242 (0.0%)	1.00	15/ 602 (2.5%)	2.06				
Other	105/ 300 (35.0%)	246/ 600 (41.0%)	1.29	53/ 300 (17.7%)	242/ 600 (40.3%)	1.01	661/1800 (36.7%)	1.14	2/ 99 (2.0%)	19/ 213 (8.9%)	4.42	0/ 102 (0.0%)	0/ 205 (0.0%)	1.00	21/ 619 (3.4%)	4.57				
Apeiba membranacea	413/ 840 (49.2%)	724/1600 (45.4%)	0.85	427/ 800 (53.4%)	890/1600 (55.6%)	1.09	2454/4840 (50.7%)	0.97	56/ 130 (43.1%)	90/ 370 (24.3%)	0.56	26/ 270 (9.6%)	38/ 571 (6.7%)	0.69	210/ 1341 (15.7%)	0.66	5.8 (54)	8.6 (88)	0.01 (8)	0.1 (20)
Home	105/ 240 (43.8%)	189/ 400 (47.3%)	1.15	111/ 200 (55.5%)	222/ 400 (55.5%)	1.00	627/1240 (50.6%)	1.10	12/ 35 (34.3%)	25/ 97 (25.8%)	0.75	4/ 75 (5.3%)	12/ 133 (9.0%)	1.69	53/ 340 (15.6%)	1.11				
Other	308/ 600 (51.3%)	535/1200 (44.6%)	0.76	316/ 600 (52.7%)	668/1200 (55.7%)	1.13	1827/3600 (50.8%)	0.93	44/ 95 (46.3%)	65/ 273 (23.8%)	0.51	22/ 195 (11.3%)	26/ 438 (5.9%)	0.53	157/ 1001 (15.7%)	0.56				
Xylopia frutescens	83/ 360 (23.1%)	38/ 720 (5.3%)	0.19	75/ 360 (20.8%)	42/ 720 (5.8%)	0.24	238/2160 (11.0%)	0.21	47/ 65 (72.3%)	23/ 38 (60.5%)	0.84	0/ 74 (0.0%)	1/ 42 (2.4%)	–	71/ 219 (32.4%)	0.89	2.3 (13)	9.7 (16)	– (0)	– (0)
Home	62/ 270 (23.0%)	34/ 540 (6.3%)	0.23	55/ 270 (20.4%)	38/ 540 (7.0%)	0.30	189/1620 (11.7%)	0.26	38/ 47 (80.9%)	21/ 34 (61.8%)	0.76	0/ 54 (0.0%)	1/ 38 (2.6%)	–	60/ 173 (34.7%)	0.81				
Other	21/ 90 (23.3%)	4/ 180 (2.2%)	0.07	20/ 90 (22.2%)	4/ 180 (2.2%)	0.08	49/ 540 (9.1%)	0.08	9/ 18 (50.0%)	2/ 4 (50.0%)	1.00	0/ 20 (0.0%)	0/ 4 (0.0%)	1.00	11/ 46 (23.9%)	1.06				
Gustavia superba	17/ 60 (28.3%)	36/ 120 (30.0%)	1.08	19/ 60 (31.7%)	40/ 120 (33.3%)	1.08	112/ 363 (30.9%)	1.06	17/ 17 (100%)	36/ 36 (100%)	1.00	19/ 19 (100%)	38/ 40 (95.0%)	0.95	110/ 112 (98.2%)	0.97	10.6 (17)	15.1 (36)	3.9 (19)	4.3 (37)
Home	7/ 15 (46.7%)	12/ 33 (36.4%)	0.65	6/ 15 (40.0%)	9/ 30 (30.0%)	0.64	34/ 93 (36.6%)	0.65	7/ 7 (100.0%)	12/ 12 (100.0%)	1.00	6/ 6 (100.0%)	9/ 9 (100.0%)	1.00	34/ 34 (100.0%)	1.00				
Other	10/ 45 (22.2%)	24/ 90 (26.7%)	1.27	13/ 45 (28.9%)	31/ 90 (34.4%)	1.29	78/ 270 (28.9%)	1.28	10/ 10 (100.0%)	24/ 24 (100.0%)	1.00	13/ 13 (100.0%)	29/ 31 (93.5%)	0.94	76/ 78 (97.4%)	0.96				
Faramea occidentalis	138/ 180 (76.7%)	249/ 360 (69.2%)	0.68	135/ 180 (75.0%)	254/ 351 (72.4%)	0.87	776/1071 (72.5%)	0.77	58/ 2 (96.7%)	117/ 6 (95.1%)	0.98	60/ 63 (95.2%)	112/ 119 (94.1%)	0.99	347/ 365 (95.1%)	0.99	1.9 (51)	3.8 (100)	0.3 (46)	0.4 (70)
Home	27/ 45 (60.0%)	54/ 90 (60.0%)	1.00	26/ 45 (57.8%)	78/ 90 (86.7%)	4.75	185/ 270 (68.5%)	1.92	13/ 15 (86.7%)	29/ 29 (100.0%)	1.15	15/ 16 (93.8%)	30/ 31 (96.8%)	1.03	87/ 91 (95.6%)	1.09				
Other	111/ 135 (82.2%)	195/ 270 (72.2%)	0.56	109/ 135 (80.7%)	176/ 261 (67.4%)	0.49	591/ 801 (73.8%)	0.53	45/ 45 (100.0%)	88/ 94 (93.6%)	0.94	45/ 47 (95.7%)	82/ 88 (93.2%)	0.97	260/ 274 (94.9%)	0.95				
Across species	1051/2570 (40.9%)	1906/5063 (37.7%)	0.87	997/2530 (39.4%)	2015/5051 (39.9%)	1.02	5969/15214 (39.2%)	0.94	271/ 610 (44.4%)	494/1252 (39.5%)	0.89	122/ 741 (16.5%)	376/1474 (25.5%)	1.55	1263/4077 (31.0%)	1.10				
Home	353/1005 (35.1%)	642/1933 (33.2%)	0.92	293/ 965 (30.4%)	674/1930 (34.9%)	1.23	1962/5833 (33.6%)	1.05	91/ 235 (38.7%)	148/ 448 (33.0%)	0.85	28/ 242 (11.6%)	95/ 515 (18.4%)	1.59	362/1440 (25.1%)	1.07				
Other	698/1565 (44.6%)	1264/3130 (40.4%)	0.84	654/1565 (41.8%)	1341/3121 (42.9%)	0.92	4007/9381 (42.7%)	0.88	180/ 375 (48.0%)	346/ 804 (43.0%)	0.90	94/ 499 (18.8%)	281/ 959 (29.3%)	1.55	901/2637 (34.2%)	1.13				

Table 2.2. Summary statistics on seedling emergence of seven tree species. Seeds of seven tree species were germinated in a greenhouse experiment in soils including a living microbial community or sterilized soils ("Inoculum") collected from below forests of four successional stages ("Successional Stage") under two light levels ("Light"). We show Chi-square and p-values for the analyses of variance (type III) on **A) Successional stage** Bayesian generalized linear models. In separate analyses, we translated the four successional stages into the binary variable Association that compares the effect of successional stages that a respective species is naturally abundant at (home) versus successional stages that a species not abundant at (other). We show the results of these **B) Association** models (glm). Degrees of freedom (df) were identical for all seven species and are presented in the rightmost column. Significant values ($p < 0.05$) are printed in bold.

	Siparuna pauciflora		*Psychotria grandis*		*Vismia baccifera*		*Apeiba membranacea*		*Xylopia frutescens*		*Gustavia superba*		*Faramea occidentalis*		All
	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	df
A) Successional stage															
Inoculum	0.68	0.409	2.46	0.117	**14.56**	**<0.001**	**7.05**	**0.008**	**18.18**	**<0.001**	2.39	1.000	1.85	0.174	1
Successional stage	0.88	0.831	2.34	0.505	**10.18**	**0.017**	6.18	0.103	1.97	0.579	**9.72**	**0.021**	**11.98**	**0.007**	3
Light	0.81	0.367	0.36	0.548	**20.44**	**<0.001**	0.46	0.495	1.01	0.315	2.20	1.000	3.11	0.078	1
Inoculum x Successional stage	3.57	0.312	0.65	0.884	**11.66**	**0.009**	6.43	0.093	4.18	0.243	6.95	0.074	1.22	0.747	3
Inoculum x Light	0.07	0.786	2.17	0.141	**8.93**	**0.003**	**4.85**	**0.028**	1.50	0.221	2.44	1.000	1.12	0.291	1
Successional stage x Light	1.38	0.710	0.63	0.889	**15.33**	**0.002**	5.06	0.168	0.97	0.809	5.71	0.127	6.06	0.109	3
Inoculum x Successional stage x Light	2.17	0.539	2.29	0.514	**13.26**	**0.004**	4.41	0.221	0.90	0.825	5.54	0.136	7.74	0.052	3
B) Association															
Inoculum	0.29	0.590	**7.39**	**0.007**	3.05	0.081	**7.31**	**0.007**	**30.43**	**<0.001**	0.32	0.572	**5.06**	**0.024**	1
Association	0.08	0.777	0.01	0.919	0.07	0.797	**3.95**	**0.047**	0.01	0.942	3.13	0.077	**8.65**	**0.003**	1
Light	0.04	0.832	0.08	0.772	0.03	0.864	0.21	0.644	0.03	0.859	0.53	0.468	0.10	0.754	1
Inoculum x Association	1.00	0.318	<0 .01	0.960	1.20	0.273	**4.60**	**0.032**	**3.80**	**0.050**	0.76	0.382	1.59	0.207	1
Inoculum x Light	0.02	0.894	0.19	0.659	1.40	0.236	**7.63**	**0.006**	0.01	0.937	<0 .01	0.978	0.12	0.724	1
Association x Light	0.47	0.491	0.59	0.441	**10.80**	**0.001**	3.48	0.062	0.05	0.828	0.50	0.480	<0 .01	0.990	1
Inoculum x Association x Light	0.05	0.831	2.38	0.123	**8.82**	**0.003**	3.67	0.055	0.06	0.808	<0 .01	0.977	**6.26**	**0.012**	1

In four species, differences in PSF among successional stages were more pronounced under 5% light, while for *A. membranacea* PSF varied among successional stages only under 40% light (Fig. 2.2). These effects of light on the pairwise differences between PSF in different successional stages did, however, not translate into overall significant two- or three-way interactions between inoculum, successional stage, and light except for *V. baccifera* (Table 2.2A).

Plant-soil feedbacks on emergence were positive in home soil, i.e. soil collected from successional stages that a species is naturally abundant at, but negative in other soil when averaged across species (OR_{home} = 1.05, OR_{other} = 0.88, Table 2.1). Similarly, five of our seven tree species had more positive PSF in home than other soil (Table 2.1). These differences in PSF between home and other soil were significant for four species, of which two had a significant interaction inoculum x association (*A. membranacea, X. frutescens*), and two had a significant interaction of inoculum x association x light (*V. baccifera, F. occidentalis*; Table 2.2B). For the two latter species, *F. occidentalis* and *V. baccifera*, the strength of the difference of PSF between home vs other soil was stronger under 5% than under 40% light (Table 2.1, Fig. 2.2). In contrast, the difference between positive PSF in home and negative PSF in other soil in *A. membranacea* was significant only at 40% light (Fig. 2.2). *X. frutescens* had less negative PSF in home than in other soils and this effect was consistent across light levels (Table 2.1, Fig. 2.2).

Table 2.3. Summary statistics on seedling survival in six tropical tree species. We assessed the probability to survive during the first 415 days after sowing in six tree species that were grown in live or sterilized soil ("inoculum") collected from forests of four successional stages ("Successional stage") under two light levels ("Light"). We show results of type-III Anovas of mixed effects Cox proportional hazard models for separate analyses on **A) Successional stages** and **B) Association**. Association is a binary variable, that pools successional stages into home stages, at which a species is naturally abundant at and other stages, at which the species is naturally not abundant at. For the interactive terms "inoculum x time" and "light x time" +/- symbols are derived of model coefficients and represent (+) an increase of the effect of the variable with time or (-) a decrease in effect over time. Statistically significant values ($p < 0.05$) are printed in bold.

	Siparuna pauciflora		*Psychotria grandis*		*Vismia baccifera*		*Apeiba membranacea*		*Xylopia frutescens*		*Faramea occidentalis*		All
	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	df
A) Successional stage													
Inoculum	**4.01**	**0.045**	**6.82**	**0.009**	**6.65**	**0.010**	**17.04**	**<0.001**	0.38	0.536	0.17	0.684	1
Successional stage	6.96	0.073	0.75	0.861	4.40	0.221	4.90	0.179	**8.21**	**0.042**	<0.01	1.000	3
Light	**19.52**	**<0.001**	<0.01	0.960	0.31	0.576	**9.69**	**0.002**	**74.68**	**<0.001**			1
Inoculum x Successional stage	1.91	0.592	3.77	0.287	2.89	0.408	5.25	0.155	1.02	0.796	0.01	0.997	3
Inoculum x Light	**21.02**	**<0.001**	0.19	0.664	**6.82**	**0.009**	**11.84**	**0.001**	**9.81**	**0.002**			1
Inoculum x Time	**-22.20**	**<0.001**	**-49.52**	**<0.001**	**-155.41**	**<0.001**	**-3.94**	**0.047**					
Light x Time			**+55.15**	**<0.001**	**+69.43**	**<0.001**	**+147.92**	**<0.001**	**+7.35**	**0.007**			
B) Association													
Inoculum	**8.61**	**0.003**	**11.46**	**<0.001**	**25.92**	**<0.001**	**22.21**	**<0.001**	0.57	0.449	0.36	0.547	1
Association	2.49	0.114	0.10	0.757	1.09	0.296	3.21	0.073	**6.08**	**0.014**	0.10	0.747	1
Light	**19.52**	**<0.001**	<0.01	0.953	0.35	0.553	**9.61**	**0.002**	**72.40**	**<0.001**			1
Inoculum x Association	0.59	0.443	<0.01	0.955	2.35	0.125	**4.43**	**0.035**	<0.01	0.986	0.51	0.474	1
Inoculum x Light	**20.62**	**<0.001**	0.23	0.629	**5.55**	**0.018**	**11.97**	**0.001**	**9.21**	**<0.001**			1
Inoculum x Time	**-19.95**	**<0.001**	**-49.74**	**<0.001**	**-166.66**	**<0.001**	-3.62	0.057	-0.39	0.530			
Light x Time			**+55.13**	**<0.001**	**+63.83**	**<0.001**	**+147.43**	**<0.001**	**+5.24**	**0.022**			

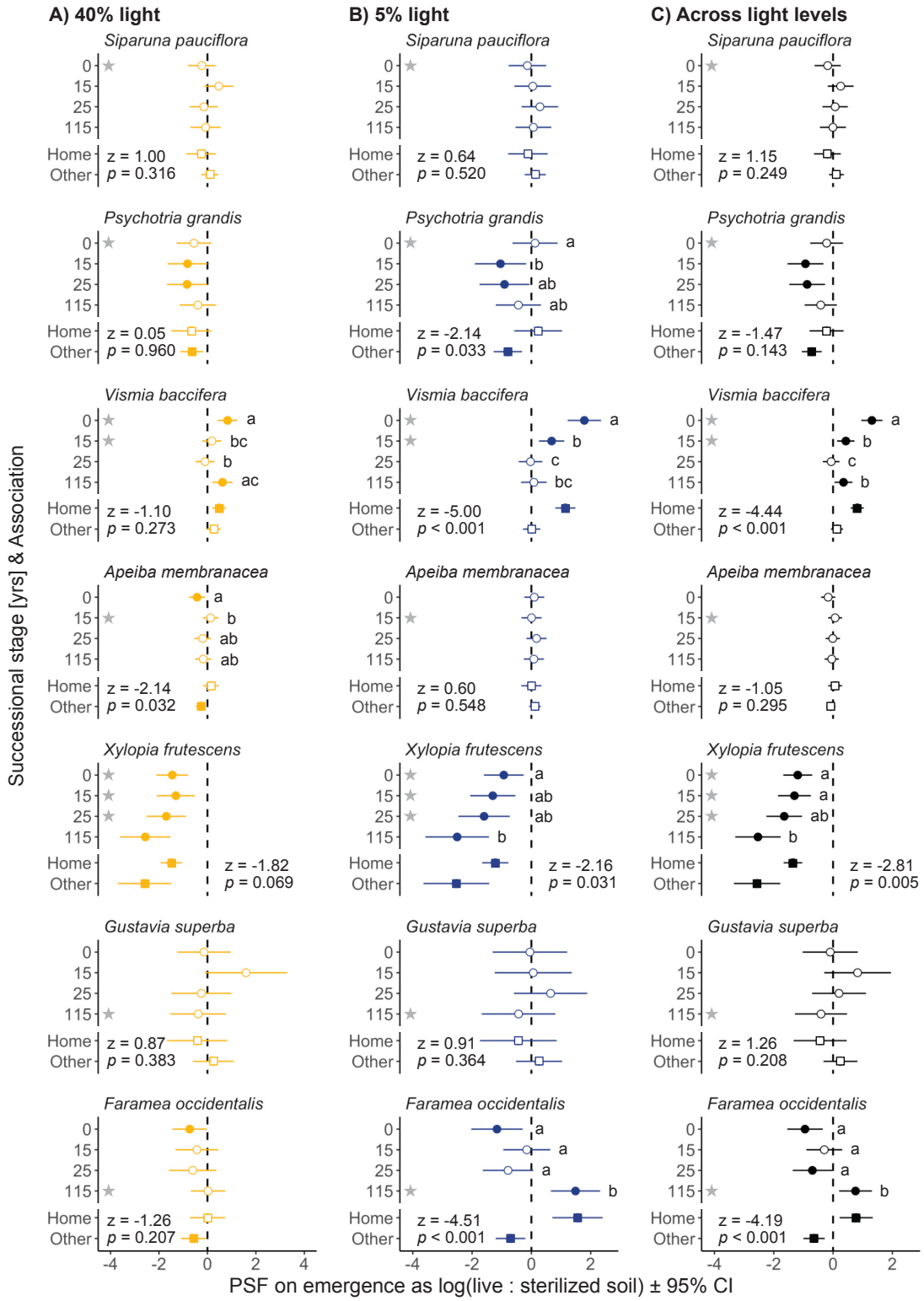


Figure 2.2. Plant-soil feedbacks (PSF) on seedling emergence in seven tree species. We germinated seeds in soils that contained a living microbial community ("live") or had been "sterilized". Soils were collected from forests of four successional stages (0, 15, 25, and 115 years of recovery). Further, we pooled successional stages according to a species' association to them: "Home" pools all stages that a species is abundant at, and "other" pools all stages that a species is not abundant at. Grey stars indicate the home successional stage(s) for each species. We show PSF in our four successional stages (circles) and for home vs other (squares) at **A) 40% light** (yellow), **B) 5% light** (blue), and **C) averaged across light** levels (black). Positive PSF > 0 mean more plants emerged in live than in sterilized soil. Filled symbols indicate significant PSF ($p < 0.05$). Letters (successional stages) and z-ratios (home vs other) highlight significant differences between PSF within a species x light combination.

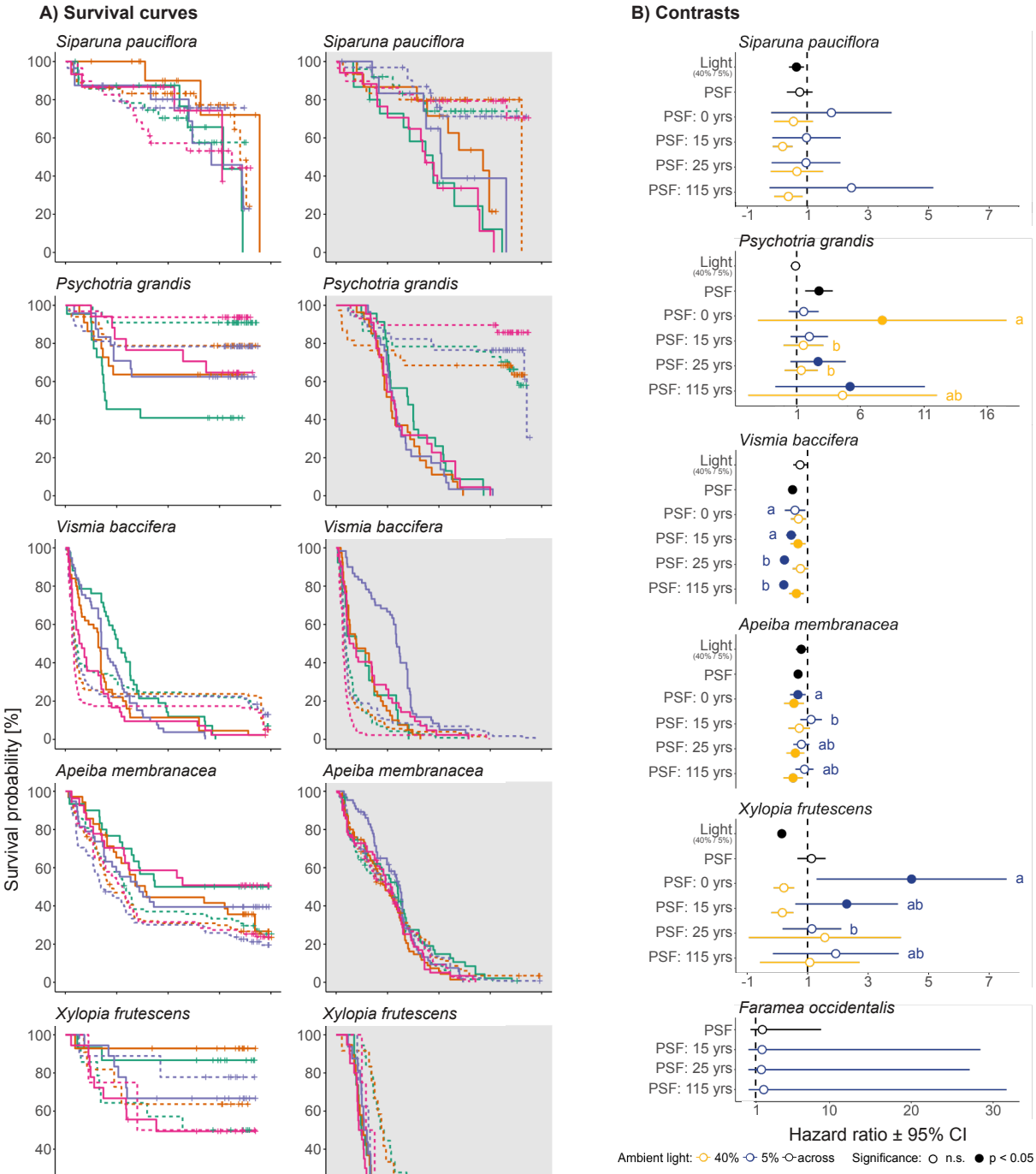


Figure 2.3. Seedling survival in seven tree species. **A) Kaplan Meier curves** of survival probabilities over 415 days under 40% (white background) and 5% light (grey background) in sterilized and live soils from four successional forest stages. **B) Hazard ratios** (HR) as pairwise contrasts between the hazard of death when growing under 40% vs 5% light ("Light") and in sterilized vs live soil ("PSF"). We show average PSF across successional stages and for each stage separately. Filled circles indicate HR that differ significantly from 1. HR > 1 indicate positive PSF with higher survival in live than sterilized soil. Letters indicate significant ($p < 0.05$) differences in PSF among stages.

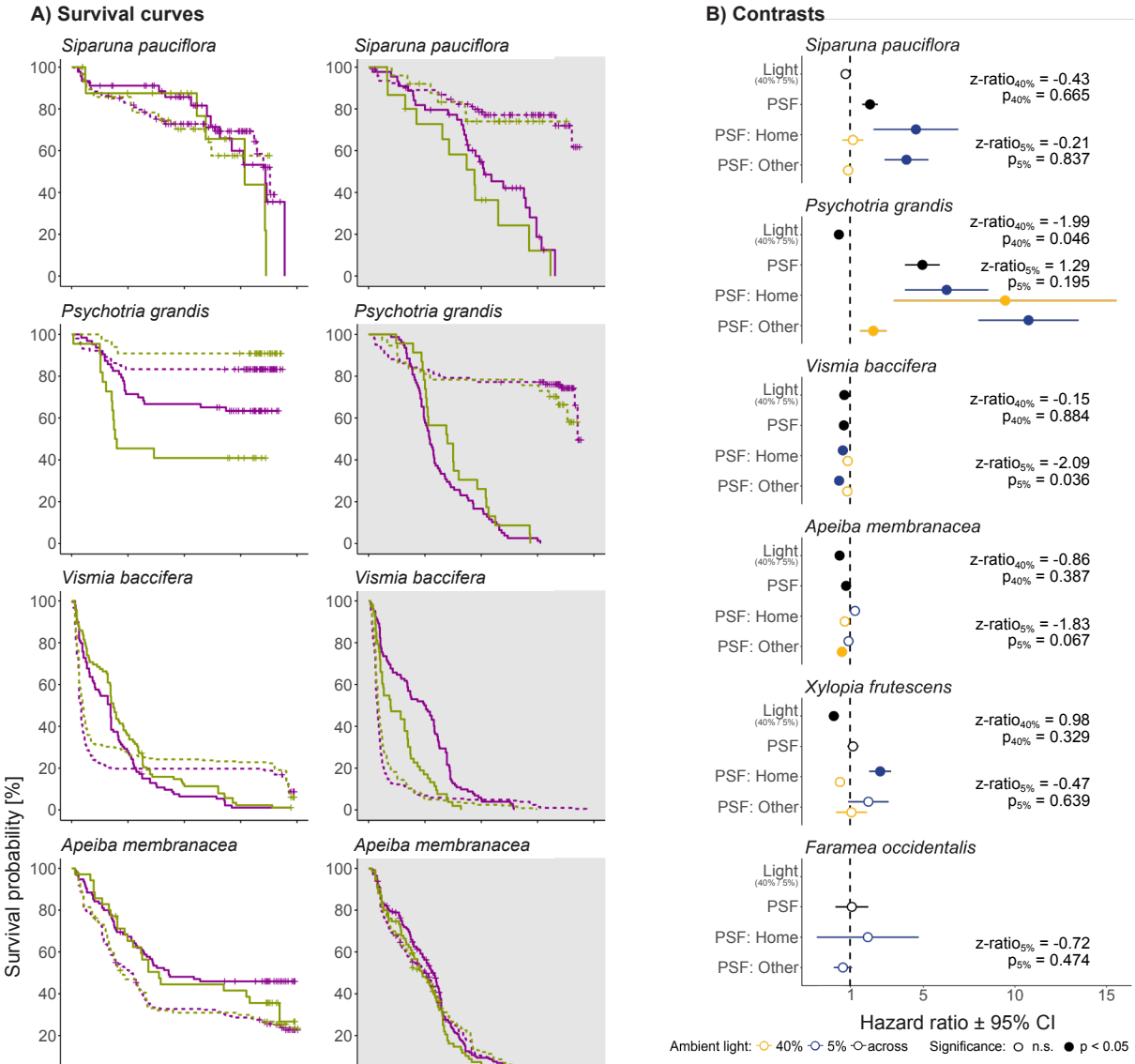


Figure 2.4. Seedling survival at "home" for seven tree species. A) Kaplan Meier curves of survival probabilities over 415 days when grown under 40% light (white background) and 5% light (grey background) in sterilized and live soils (= incl. soil microbiota). Soils were collected from forests of successional stages in which a species is abundant at ("home") and successional stages, where it is not abundant at ("other") collected from four successional forest stages. **B) Hazard ratios (HR)** are pairwise contrasts between the hazard of death when growing under 40% vs 5% light ("Light") and in sterilized vs live soil ("PSF"). We show PSF averaged across stages and for home and other stages separately. Filled circles indicate HR that are significantly different from 1. HR > 1 indicate positive PSF and thus higher survival in live than sterilized soil. We compared PSF between home and other stages, with positive z-ratios representing more positive PSF in home than in other stages.

2.4.2 Survival

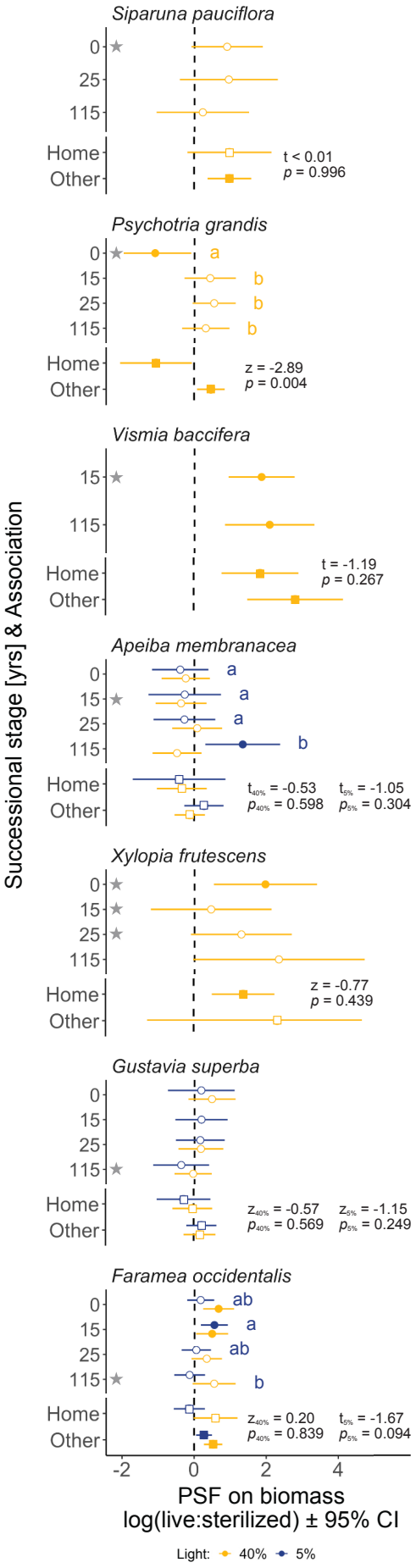
Survival differed largely among tree species and was highest for the two late successional species *G. superba* (98.2%) and *F. occidentalis* (95.1%). Survival in the five species associated to our three younger successional stages was on average only 34.2% (range 2.9 - 63.1%; Table 2.1).

We found significant PSF on survival in four of six analysed species (inoculum in Table 2.3; Fig. 2.3). While the direction of PSF varied among tree species (Fig. 2.3), PSF averaged across species and treatments were positive with seedlings surviving 28% longer (mean HR =1.28; Table S2.4) and having a 1.10-times higher chance to survive in live than in sterilized soil for the first 415 days (mean RR =1.10; Table 2.1). The effect of PSF significantly decreased with time for all four species for which this interactive effect could be analysed (Table 2.3).

We found no significant interaction of inoculum and successional stage, and the interaction of inoculum and association was significant only for *A. membranacea* (Table 2.3). However, four of the six species showed significant differences between pairs of successional stages (Fig. 2.3). The overall chance to survive the first 415 days was on average 1.07x higher in live than sterilized soil for home soil and 1.13x higher in live than sterilized soil for other soil (RR in Table 2.1). Hazard ratios were generally more positive in home (HR = 1.83; $HR_{40\%}$ = 2.05; $HR_{5\%}$ = 1.62) than in other soil (HR= 1.11; $HR_{40\%}$ = 0.93; $HR_{5\%}$ = 1.29; Table S2.4) and seedlings thus survived 83% or 11% longer in live than sterilized soil from home and other stages, respectively.

The difference in PSF on survival in home vs other soil varied with light level. Averaged across species, the relative risk to survive was higher in home than in other soil under 5% light (RR_{home}= 1.59, RR_{other} = 1.55) but lower in home than in other soil under 40% light (RR_{home}= 0.85, RR_{other} = 0.90). PSF were significantly more positive in home than other soil under 40% light in *P. grandis* and under 5% light in *V. baccifera* (Fig. 2.4).

For four species, PSF varied significantly with light level (significant interaction inoculum x light in Table 2.3, Fig. 2.3). Across successional stages, PSF were significantly more positive under 5% than under 40% light for *S. pauciflora, A. membranacea*, and *X. frutescens*, but



more negative under 5% than under 40% light for *V. baccifera* (Fig. 2.3). The effect of light on survival increased significantly over time in four species but did not vary with time in two species (Table 2.3).

2.4.3 Biomass

We found significant PSF on biomass in five of our seven species (inoculum in Table 2.4). All species had an overall higher biomass (on average 3.7 times higher) in live than sterilized soil under both light levels (Table 2.1). PSF were significant in at least one successional stage for five species: for four of seven species under 40% light and for two of three species under 5% light (Fig. 2.5). We found however no consistent pattern of an interaction between PSF with light, successional stage, or when comparing home to other soil across species (Table 2.4). PSF differed with association only for *P. grandis,* which had more negative PSF in home than in other soil (Fig. 2.5). Light level had a strong and significant effect on the biomass of all three analysed species, that had a ~3.5-, 10-, and 75-times higher biomass under 40% than 5% light (Table 2.1 & 2.4).

Figure 2.5. Plant-soil feedbacks (PSF) on seedling biomass in seven tree species. PSF are the logarithmic odds ratio ± 95% confidence intervals between estimated marginal mean biomass of seedlings growing in live vs sterilized soils from forests of four successional stages (0, 15, 25, and 115 years of recovery). We show PSF for the four successional stages (circles) and for home vs other soil (squares). "Home" pools all successional stages that a respective tree species is abundant at (indicated by stars), and "Other" pools all stages at which it is not abundant. Filled circles and squares indicate statistically significant PSF ($p < 0.05$). Letters (successional stages), and z-ratios and p-values (home vs other), show results of pairwise contrasts between PSF.

Table 2.4. Summary statistics on seedling biomass in seven tropical tree species. Seedlings of seven tree species were grown under high light ("40% light") or low light level ("5% light") in live vs sterilized soils ("Inoculum") collected from forests of four successional stages ("Successional stage"). We included "Plant age" to encompass for variation in the days between emergence and harvest per plant. In separate analyses, we translated the four successional stages into the binary variable "Association" that compares the effect of successional stages that a respective species is naturally abundant at (home) versus successional stages that a species not abundant at (other). We show results of analyses of variance (type-III) on linear mixed effects models on **A) Successional Stage** and **B) Association** for seedlings of the seven species growing under 40% light and 5% light (upper table) and for three species across light levels (lower table). Significant values ($p < 0.05$) are printed in bold.

	Siparuna pauciflora		*Psychotria grandis*		*Vismia baccifera*		*Apeiba membranacea*				*Xylopia frutescens*		*Gustavia superba*				*Faramea occidentalis*			
	40% light		40% light		40% light		40% light		5% light		40% light		40% light		5% light		40% light		5% light	
	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
A) Successional stage																				
Inoculum	**4.32**	**0.038**	**4.56**	**0.033**	**25.52**	**<0.001**	0.51	0.475	0.98	0.320	**7.27**	**0.007**	2.12	0.146	0.16	0.689	**9.37**	**0.002**	0.80	0.370
Successional stage	**10.73**	**0.013**	7.23	0.065	**12.02**	**0.007**	1.21	0.750	1.73	0.630	2.02	0.569	**6.24**	**0.044**	0.99	0.803	2.65	0.449	2.41	0.493
Inoculum x Successional stage	1.41	0.493	**8.46**	**0.037**	0.17	0.676	1.52	0.677	**7.92**	**0.048**	2.48	0.478	1.52	0.467	1.42	0.701	1.18	0.758	6.29	0.098
Plant Age	0.65	0.419	**12.79**	**<0.001**	0.68	0.411	**95.81**	**<0.001**	**137.49**	**<0.001**	0.11	0.742	**48.23**	**<0.001**	**38.55**	**<0.001**	**20.73**	**<0.001**	**4.35**	**0.037**
B) Association																				
Inoculum	**12.73**	**<0.001**	**5.76**	**0.016**	**39.15**	**<0.001**	0.33	0.566	1.21	0.271	3.89	0.048	0.54	0.461	1.04	0.307	**16.72**	**<0.001**	**6.03**	**0.014**
Association	0.14	0.710	**6.91**	**0.009**	0.65	0.421	0.56	0.453	0.51	0.477	0.01	0.913	**6.02**	**0.014**	0.43	0.514	0.05	0.818	0.73	0.393
Inoculum x Association	<0.01	0.996	**8.33**	**0.004**	2.32	0.128	0.28	0.595	1.26	0.262	0.60	0.439	0.33	0.569	1.33	0.249	0.04	0.839	2.80	0.094
Plant Age	0.39	0.530	**12.33**	**<0.001**	0.09	0.759	**46.32**	**<0.001**	**104.15**	**<0.001**	0.16	0.688	**60.11**	**<0.001**	**45.64**	**<0.001**	**12.85**	**<0.001**	**4.26**	**0.039**

	Apeiba membranacea		*Gustavia superba*		*Faramea occidentalis*	
	χ^2	*p*	χ^2	*p*	χ^2	*p*
A) Successional stage						
Inoculum	0.01	0.933	2.36	0.125	**15.61**	**<0.001**
Successional stage	2.76	0.430	2.32	0.508	3.32	0.345
Light	**14.85**	**<0.001**	**11.62**	**0.001**	**198.87**	**<0.001**
Inoculum x Successional stage	2.69	0.442	3.07	0.382	4.07	0.254
Inoculum x Light	0.10	0.756	0.92	0.337	**5.35**	**0.021**
Plant Age	**113.87**	**<0.001**	**91.28**	**<0.001**	**21.28**	**<0.001**
B) Association						
Inoculum	1.07	0.301	1.25	0.264	**26.81**	**<0.001**
Association	2.12	0.146	2.13	0.145	0.16	0.685
Light	**24.29**	**<0.001**	**20.83**	**<0.001**	**199.26**	**<0.001**
Inoculum x Association	2.53	0.111	1.20	0.273	0.58	0.445
Inoculum x Light	0.06	0.812	0.10	0.756	**5.57**	**0.018**
lant Age	**81.66**	**<0.001**	**92.88**	**<0.001**	**17.44**	**<0.001**

2.5 DISCUSSION

Plant-soil feedbacks (PSF) significantly affected six of our seven tree species in at least one parameter of seedling performance (Table 2.5). We found positive and negative net PSF, indicating that both microbial mutualists and pathogens may affect seedling performance in a species-specific manner. Strength and direction of PSF were affected by tree species identity, species association with forest successional stages, and light level.

PSF effects on emergence were overall negative, while PSF effects on survival and biomass were positive for most of our species. PSF effects on emergence and survival time were more positive in soils collected from forest successional stages at which a species is abundant and thus at "home" at than in soils from other successional stages. Three tree species had significantly higher performance if microbiota were present in their home soils, but not in other soils, indicating that PSF may promote tree species in soils from forest successional stages at which a species is abundant at.

Light affected the strength and, in some cases, the direction of PSF in a species-specific manner. The overall positive effect of light level on survival increased with time since emergence, while that of PSF decreased with time, which may indicate a shift in survival-determining processes during the initial year of a seedling's life.

Overall, our data suggest that PSF specific to some species and to successional stages exist and that PSF may promote the emergence and survival of seedlings in their home successional stages. If our findings translate into general patterns, PSF may contribute to determine the establishment success of tropical tree species during secondary succession.

Species	Emergence	Survival	Biomass	Total
Siparuna pauciflora			X (+)	1
Psychotria grandis		X (+)	X (+/ -)	2
Vismia baccifera	X (+)	X (+)	X (+)	3
Apeiba membranacea	X (-)	X (+)	X (+/ -)	3
Xylopia frutescens	X (-)	X (+)	X (+)	3
Gustavia superba				0
Faramea occidentalis	X (-/+)		X (+)	2

Table 2.5. Overview of PSF on three tree seedling performance parameters. Statistically significant positive (+) or negative (-) PSF and the total number of parameters for which we detected a significant PSF in at least one of four successional stages for seven tree species.

2.5.1 Community-level PSF in all four successional stages

Community-level PSF affected seedling performance in soils from all four successional stages tested. We found no general trend in strength or direction of PSF with successional stage and thus the time that forests had been recovering since agricultural abandonment. Direct PSF, the soil-mediated effect of a predecessor plant on its successor plant, have been shown to affect tree recruitment in intact tropical rainforests (McCarthy-Neumann & Kobe, 2019; Spear et al., 2015; Xi et al., 2020). Here we show that community-level PSF, i.e. effects from the microbial community conditioned by the entire plant community (van der Putten et al., 2013), can act at all stages of secondary succession. This may suggest that the microbes responsible for PSF are generally abundant in soils throughout succession and can rapidly be activated by arriving plant species.

2.5.2 More positive PSF in home soil

PSF on emergence and survival time were more positive in home soil i.e. soil collected from forest successional stages that a species is naturally abundant at (Fig. 2.2 & 2.4). This finding contrasts our hypothesis that expected more negative PSF in home soil. Our measurement are net effects that reflect the balance of negative effects of pathogenic and positive effects of mutualistic soil microbiota. Our finding of net positive PSF in home soils could thus emerge through the absence of species-specific pathogens – e.g. if pathogens accumulate with a lag phase – or an increased importance of species-specific mutualists. Pathogens are often assumed to have a higher host-specificity than mutualists (Bennett & Klironomos, 2019; Klironomos, 2002) with negative effects of fungal pathogens on seedlings in Panamanian rainforests being plant species- (Spear & Broders, 2021) or even genotype-specific (Eck et al., 2019). Yet, the composition of mycorrhizal fungi can be affected by plant species identity (Mangan, Herre, et al., 2010) and effective specialization was reported for the majority of fungal taxa resulting in tree-species specific negative or positive effects (Gallery et al., 2010; Sarmiento et al., 2017). Given that associations with arbuscular mycorrhiza are a common feature of Neotropical tree species (Mangan, Herre, et al., 2010), species-specific positive

effects of mycorrhiza could widely affect plant successional dynamics. The species-specific positive and negative PSF that we found add evidence that microbial mutualists may be more specialized than historically assumed and that both, pathogenic and mutualistic soil microbes may contribute to species-specific PSF.

Positive PSF are expected to promote dominance and arrest secondary succession (Rozendaal et al., 2019; van der Putten et al., 2013). Yet, positive PSF could drive successional turnover if tree species with low competitive ability experience positive feedback only at a specific successional stage, but not at others. In laboratory studies, mycorrhizal fungi increased nutrient transfer to those plant roots that provided most carbohydrates (Kiers et al., 2011). Such context-dependency of PSF, with tree species accumulating most mutualists and profiting from most positive PSF where they grow best, could enhance effects of environmental filtering of tree species during secondary succession. In conclusion, we provide evidence of a newly discovered mechanism (in addition to direct effects of plant competition) that may cause tree species to be abundant in different stages of succession: The probability of establishment at home stages is increased by positive effects of the soil microbial community on emergence. This PSF could contribute to allow species to persist for more than one generation at these successional stages, counteracting the competitive advantage of species associated with later successional stages.

We found species-specific PSF effects on seedling emergence, the first stage of plant recruitment. PSF studies typically focus on seedlings due to practicality. Fungal-mediated, negative PSF effects on emergence of seeds resting in intact rainforests (Gallery et al., 2010; Sarmiento et al., 2017) and in successional soils in our study suggest that current perspectives of plant community dynamics may miss to include a differential mechanism affecting plant communities prior to the seedling stage. This is especially important, as the more positive PSF on emergence in home soils, i.e. successional stages a species is abundant at, that we found could enhance the effects of dispersal limitation, a main determinant of secondary succession in tropical forests (Guariguata & Ostertag, 2001; Poorter et al., 2021). In the successional forest metacommunity surrounding our soil collection sites, the local abundance and frequency of species was found to be determined by the speed and uniformity with which seeds arrive at sites while the recruitment of most tree species was

not *per se* restricted to a specific successional stage (van Breugel et al., 2013). In our study, the overall likelihood of emergence was 5% higher in live than in sterilized home soils, but 12% lower in live than in sterilized other soils. While the overall chance to survive did not differ between home and other soil, microbiota increased the time a seedling survived almost 8x as much in home soil than in other soil (Table S2.4). The greater positive PSF effect on emergence and survival in home soil we found may be important in species' establishment success given the high overall mortality rates (70-90%) of seedlings in Panamanian rainforests (Augspurger, 1984). Similar effects of some species experiencing stronger positive PSF in the successional stage that they are naturally abundant at have been reported in grassland systems (Kardol et al., 2006). Here, for the first time, we show such effects in tropical forest succession. We suggest that our finding of a 17% net increase in likelihood to emerge in soil from a forest of a stage associated with the species can assist species in colonizing a site and may thereby magnify dispersal limitation and contribute to successional trajectories.

2.5.3 PSF varied with tree species identity and between early- and late-successional species

Species differed strongly in the strength and direction of PSF effects on different parameters of seedling performance. One of our two late-successional species (i.e. species abundant at late stages of succession) did not show any significant PSF and survival of both of these species was unaffected by PSF (Table 2.5), supporting our hypothesis. In combination with their very low mortality rates – 1.8% of seedlings died in *G. superba* and 4.9% in *F. occidentalis* – this suggests that survival was largely independent of microbial presence, successional stage and association, and light level in the two late-successional species tested here. In contrast, mortality rates for the five species associated to forest successional stages of 0-25 years was more than an order of magnitude larger and on average 65.8% (range 36.9 – 97.1%; Table 2.1).

Seedlings of shade-tolerant tropical tree species have been shown to be less susceptible than shade-intolerant species to fungal pathogens (Spear & Broders, 2021) and to experience less negative PSF effects (McCarthy-Neumann & Kobe, 2008). The higher susceptibility to pathogens and negative conspecific PSF in early-successional species in contrast, may drive

successional turnover of species in grasslands (van de Voorde et al., 2011). Here, we assessed the actual association of species with different successional stages of forest by their relative abundance, rather than assuming it based on functional traits, which were shown to be poor predictors of microbial community composition in a Panamanian forest (Barberán et al., 2015). Our results support the theory of a growth-defence trade off (Wright et al., 2010), that predicts that a high investment into fast growth and low investment into defence associated with species abundant at early stages of succession makes them more susceptible to natural enemies than late successional species. The complete lack of any, including positive, PSF on one of our two species associated with the late stage of succession could indicate that some late-successional tree species are less influenced by the composition of fungal communities, including mutualists.

2.5.4 PSF strength (and direction) varied with light level

The effect of light on PSF was species-specific, with some species having more negative and some species having more positive PSF at low light (e.g. on emergence, Fig. 2.2), and differed among successional stages (e.g. on biomass, Fig. 2.3). Further, the effect of light interacted with successional stages in a complex way, resulting in more positive PSF on emergence in home vs other soil being greater under 5% light for two species and greater under 40% for one species and did not differ between light level for the rest of the species (Table 2.1, Fig. 2.2). Home PSF on survival were more positive under low light, but more negative at high light (Table 2.1). Overall, our results do thus does not support our hypothesis of generally more negative PSF at low light levels.

Previous studies suggested negative PSF to have a stronger effect on forest communities under low light levels (Bennett & Klironomos, 2019) and reported greater pathogen-mediated seedling mortality in the shade (Augspurger, 1984; Kobe & Vriesendorp, 2011). Further, overall soil microbial abundance (Xi et al., 2020), the sporulation and host-specific relative abundance of mycorrhizal taxa (Mangan, Herre, et al., 2010), and carbon transfer from plants to mutualistic fungi (Kiers et al., 2011) has been shown to be higher at high than low light levels. Our results however suggest that the effect of light on plant-microbial

interactions and PSF effects on seedling performance is highly context-dependent and interacts with species identity and successional stage in a complex way.

To complicate matters further, we found an increase in the importance of light level and a decrease in the importance of PSF on survival with seedling age (Table 2.3). Young seedlings of tropical forest trees have repeatedly been shown to be highly susceptible to PSF (Mangan, Herre, et al., 2010; Sarmiento et al., 2017) and younger *Eucalyptus* (Simamora et al., 2017) and tomato (Thomas & Upreti, 2014) seedlings were more likely to die from fungal pathogen infection than older seedlings, potentially because of a slower lignification of infected tissues (McClure & Robbins, 1942). Light limitation (and potentially also the dependence on mutualists) may in contrast become increasingly important for photosynthesis in larger seedlings. Our data suggest a shift in the relative importance of factors determining survival over the first 415 days of a seedling's life and call for longer studies when assessing the effects of PSF on seedling establishment.

In summary, we suggest that the interactive and species-specific effects of PSF and light level could enhance tree species niches and accelerate environmental filtering of tree community composition during secondary succession. Early-successional species may emerge in high numbers and grow well in post-agricultural sites due to optimal abiotic conditions. High light level stimulates the sporulation of AMF (Mangan, Herre, et al., 2010) that receive high carbohydrate supplies and return stronger positive PSF (Kiers et al., 2011) that strengthen the fast establishment of these early colonizing species. Similar to invasive species (van der Putten et al., 2013), these colonizing species may additionally profit from the absence of pathogens that have not yet accumulated. While pathogens start accumulating, abiotic conditions get less favourable for light-demanding species, potentially reducing the positive effect of PSF and ultimately decreasing a colonizing trees' competitive ability. At the same time, positive PSF may start to support new tree species that have better growth under reduced light. Positive, PSF at the home successional stage that a tree species is most abundant at can thereby accelerate tree species turnover at this stage of succession. If our result of higher independence from PSF in species associated with later stages of succession is a general pattern, this would indicate a decrease in the relative importance of PSF in structuring forest communities with succession. We argue that the interaction between light,

tree species' association with a successional stage, and PSF interact in affecting succession via differential recruitment among trees of different species. By increasing species niche differentiation, PSF may thus contribute to tree species turnover during the recovery of tropical rainforests.

2.5.5 Limitations

While unavoidable for short-term studies of the recovery of hyper-diverse rainforests, we acknowledge that certain limitations need to be considered when interpreting our results. Most importantly, our study is based on a chronosequence of secondary succession, which substitutes space for time and cannot fully control for variation among sites (Powers & Marín-Spiotta, 2017) and the strong impact of local factors on successional trajectories (e.g. Poorter et al., 2021) limits the validity of generalizing findings from one site. In addition, the interspecific variation in PSF reported in our and earlier studies (McCarthy-Neumann & Kobe, 2008) means that results will be highly dependent on species selection. These limitations are, however, unavoidable for short-term studies of the recovery of hyperdiverse rainforests. Lastly, many other factors (e.g. nutrients, moisture, herbivory, competition), can modulate PSF (De Long et al., 2019) and could reduce their overall impact on seedling performance under natural conditions (Forero et al., 2019). We minimized artefacts due to factors such as pot size limitation, short experimental duration, or nutrient pulses in sterilized soils. Given the enormous knowledge gap on the mechanisms that drive successional plant community dynamics in rainforests, controlled greenhouse conditions however are ideal to assess the role of individual factors in complex ecosystems.

2.6 CONCLUSION

We found PSF to be affected by complex interactions between species identity, successional stage that soils are extracted from, and light level in our experiment. Together with our small number of species this complicates drawing general conclusions from our results.

Nonetheless, we present evidence that microbial-mediated PSF can affect the recruitment of tree species across the successional stages of a Panamanian rainforest community. Variation of PSF with successional stage was species-specific and overall PSF effects on emergence were more positive in soil collected from the successional stage of forest at which the species is naturally abundant. Thus, our data indicate that plant species alter microbial communities and mutualist microbes have a higher plant-species specificity than expected. Our data may further indicate that PSF may enhance tree species niche differentiation by 1) affecting species differently according to their association to successional stages (and thus shade tolerance), and 2) species-specific variation in PSF strength with light. Differences in soil seed banks and dispersal limitation have been suggested to account for large dissimilarities in the community composition of secondary forests of similar age and land use history and to contribute to explain the slow and stochastic recovery of species composition (Guariguata & Ostertag, 2001). Our results indicate that soil microbes could strengthen such effects through PSF on emergence and survival.

Chapter 3: Negative plant-soil feedbacks decrease with plant phylogenetic distance and are stronger in late- than early-successional soil

3.1 ABSTRACT

Plant-soil feedbacks (PSF), the reciprocal interaction between plants and soil microbes, affect tropical tree performance, local abundance, and diversity in intact rainforests. Phylogenetic signals in PSF, whereby a plant affects closely related successor species more negatively than unrelated successor species, could drive the directional species turnover and diversification of plant communities during secondary succession of rainforests. Variation in the magnitude of PSF with successional stage could explain decreasing turnover rates.

In a greenhouse experiment in Panama, we compared conspecific vs heterospecific PSF and assessed variation in PSF with phylogenetic distance between predecessor-successor pairs and with the successional stage of soil. We planted tree seedlings of three successor species into early-successional (15 years of recovery) and late-successional (115 years) forest soils that had been conditioned by conspecific and four heterospecific predecessors. We measured growth and biomass of seedlings after seven months.

We found an increase in positive PSF on the performance of heterospecific successor seedlings with increasing phylogenetic distance from the predecessor. Conspecific PSF varied, however, ranging from more positive, more negative, or equal to heterospecific PSF. PSF were generally more negative in late- than early-successional soils. Our results suggest complex interactions between specialist pathogen and mutualist microbes in their effect on the identity of successor tree species through net PSF. If the phylogenetic signal in heterospecific PSF that we found is common, PSF could contribute to the phylogenetic diversification and directional turnover of tree species during the secondary succession of tropical rainforests.

3.2 INTRODUCTION

Crop rotation is one of the oldest agricultural principles and has been practiced by farmers around the globe for millennia (Bullock, 1992; van der Putten et al., 2013). In the 20th century, researchers revealed that accumulations of host-specific pathogenic soil microbes contribute to the increased yields when alternating unrelated crop species cultivated in the same soil (Bullock, 1992). In plant-soil feedbacks (PSF), plants attract or deter soil microbes through root exudates, while the developing fungal and bacterial communities in turn affect the performance of current or future plants in a positive or negative way (Bennett & Klironomos, 2019). Whether such PSF may also explain plant species turnover during the natural recovery of ecosystems has received much attention lately (Bennett et al., 2017; Bennett & Klironomos, 2019; van der Putten et al., 2013), yet research in tropical rainforests is lacking.

Host-specific PSF can affect the chances of tree seedlings to establish at a site (Sarmiento et al., 2017; Segnitz et al., 2020), exclude tree species from habitats by enhancing niche differences (McCarthy-Neumann & Kobe, 2008, 2019; Spear et al., 2015), and affect tree abundance through reducing seedling survival at high conspecific densities (Bagchi et al., 2014; Krishnadas et al., 2018; Mangan, Schnitzer, et al., 2010). PSF can thus mediate plant competition and contribute to the maintenance of plant diversity in grasslands (van de Voorde et al., 2011; van der Putten et al., 2013) and intact rainforests (Bagchi et al., 2014; Eck et al., 2019; Krishnadas et al., 2018). It thus seems likely that PSF affect secondary succession of tropical rainforests, which is characterized by intensive competition during plant establishment, with a change in outcomes with the change in environmental conditions, contributing to a turnover of tree species and increasing tree diversity (Poorter et al., 2021; van Breugel et al., 2013).

A tree may affect the establishment of the tree following it in succession, i.e. its successor, through legacy effects mediated by soil microbial communities. Phylogenetic signals, i.e. an impact of tree species phylogeny, on the host range and effect of these microbes (Mangan, Schnitzer, et al., 2010; Schroeder et al., 2019) could affect the identity of successors and thereby determine the direction of successional tree species turnover. Closely related plant

species are likely to be more susceptible to the same pathogen because of an evolutionary arms race between hosts and pathogens driving their co-diversification and conservatism of plant defence traits (Gilbert & Webb, 2007; Gougherty & Davies, 2021). Indeed, closely related crop species were found to have a higher likelihood of sharing pathogens (Benítez-Malvido et al., 2021). In trees, the probability of developing foliar disease (Gilbert & Webb, 2007) and severe damage (Gilbert et al., 2015) was reported to decline with phylogenetic distance to the most affected host. Similarly, phylogenetically conserved growth benefits of arbuscular mycorrhizal fungi (AMF) (Reinhardt et al. 2012) resulted in more positive PSF between closely related grassland species (Anacker et al. 2014). Studies on direct PSF between predecessor-successor pairs of plant species are however rare and reported inconsistent results. A study on six tropical tree species found conspecific PSF to be overall more negative than heterospecific PSF (Mangan, Schnitzer, et al., 2010). Other studies however reported large interspecific variation in conspecific PSF that ranged from being more positive to more negative than heterospecific PSF in grasslands (Miller et al., 2019) or found no effect of phylogenetic distance between soil conditioning species and heterospecific successor species on PSF (Kaplan et al., 2020). Understanding host-specificity in microbial pathogens and mutualists is crucial, as their combined effects, i.e. net PSF, will determine the degree to which a tree affects the performance of subsequently growing conspecifics relative to other species. A stronger host-specificity in pathogens than mutualists (Bennett & Klironomos, 2019; Eck et al., 2019; Schroeder et al., 2019) could result in more negative PSF between closely related plants. Such phylogenetic signals in PSF could explain the decrease in phylogenetic relatedness of plant communities during rainforest succession (Letcher et al. 2012). However, we know little about phylogenetic signals in PSF effects and their extension beyond conspecifics in tropical forests.

The strength of PSF between predecessor-successor pairs may vary with successional stage. A high local abundance of less well defended tree species during early stages of succession (reviewed in: Guariguata & Ostertag, 2001) could facilitate the accumulation of species-specific pathogens (Gougherty & Davies, 2021), which may suppress the establishment of closely related successors and promote phylogenetically distinct successor trees. The developing phylogenetically diverse host communities might in turn support a greater pest diversity and dilute the average pathogen load per host through physical interception of

spores or vectors by non-host species and larger distances between hosts (summarized in: Gougherty & Davies, 2021). In contrast, the successional decrease in light availability below the canopy (Guariguata & Ostertag, 2001) has been suggested to drive a decrease in AMF density and infection in tropical forest succession (Zangaro et al., 2012; but see: Z. Zhou et al., 2017) and drive a successional shift towards a greater importance of pathogens relative to AMF in structuring plant communities (Bennett & Klironomos, 2019). More diverse microbial communities in late successional sites (Chen et al., 2020; Zhang et al., 2017) may then facilitate the activation and accumulation of host-specific microbes. Studies have shown changes in fungal and bacterial community composition (Yu et al., 2021; Z. Zhou et al., 2017) and an overall increases in microbial diversity (Chen et al., 2020; Zhang et al., 2017) with forest succession. Further, the dominant tree species was the main determinant of successional fungal community composition (Zhang et al., 2017) and negative PSF on seedling survival disappeared at low host abundance suggesting that a certain host abundance is needed to maintain effective PSF (Y. Liu et al., 2015). Whether these changes in microbial community composition translate into differences in overall strength of PSF with successional stage, however, is unknown yet could affect the stability of successional plant communities and the rate of successional tree species turnover.

We conducted a greenhouse experiment to determine the existence and strength of plant phylogenetic signals in microbial-mediated PSF and variation with soil successional stage to advance our understanding of the contribution of PSF to secondary succession of tropical rainforests. We assessed PSF effects on seedling performance of successor tree species with varying phylogenetic distance from predecessor species in soils collected from early- and late-successional forests. We hypothesized that 1) conspecific PSF are more negative than heterospecific PSF and that 2) the magnitude of negative PSF decreases with increasing phylogenetic distance between predecessor and successor species. We further tested for 3) effects of the soil successional stage on PSF.

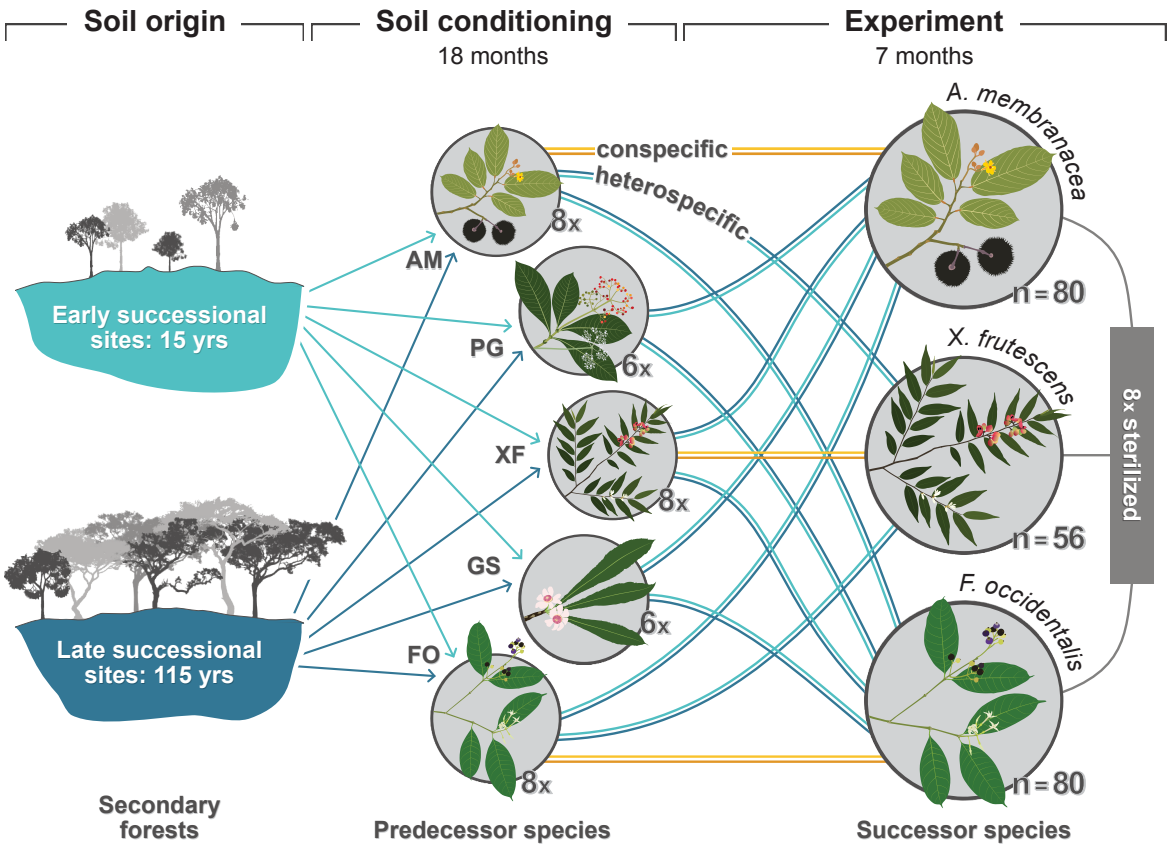


Figure 3.1. Experimental design. Forest soils collected from early- and late-successional stages were conditioned by seedlings of five predecessor tree species (AM = Apeiba membranacea, PG = Psychotria grandis, XF = Xylopia frutescens, GS = Gustavia superba, FO = Faramea occidentalis). Conditioned soils were then used to inoculate three successor species (AM, XF, and FO). Successor seedlings were grown in early- (yellow), and late-successional (orange) soils conditioned by conspecifics and early- (turquoise) and late-successional (dark blue) soils conditioned by two (XF) or four (AM, FO) heterospecific predecessors as shown by lines. Additionally, successor seedlings were grown in sterilized soils. Replicate number per successional stage X predecessor X successor combination (x) and the total number of seedlings per successor species (n) are given.

3.3 METHODS

We analysed microbial-mediated PSF on the performance (biomass and height growth) of seedlings of three tropical tree species in a greenhouse experiment at the Smithsonian Tropical Research Institute in Gamboa, Panama (9°07'11.0"N 79°42'06.2"W). In a fully factorial design, we transplanted seedlings of the three successor species into soils that were originally extracted from early- or late-successional forest sites and had been conditioned by seedlings of the same species (conspecific predecessors) or conditioned by seedlings of two to four other species (heterospecific predecessors; Fig. 3.1). Additionally, we planted successor seedlings into a sterilized soil mix (microwave radiation) that pooled soils

conditioned by all predecessor species for each of the two successional stages. We assessed PSF as the difference in performance of seedlings growing in sterilized (i.e. conditioned soils without microbiota) vs live soils (i.e. conditioned soils including microbiota) for seven months (mean duration = 208 days, range = 203-215 days). We analysed differences in PSF between conspecific and heterospecific predecessors and variation in PSF with the phylogenetic distance between predecessor and successor species in early- and late-successional soils. In total, the experiment comprised 216 successor seedlings, with sample sizes of six or eight per predecessor-successor-successional stage combination, due to limitation in the number of seedlings (Fig. 3.1).

3.3.1 Soil collection & conditioning

To assess variation in the effects of microbial communities during secondary succession, we measured PSF in soils collected from forests at two successional stages: early-successional (15 years of recovery) and late-successional (115 years). For each successional stage, we collected soils at two locations (at a distance of 50 m, avoiding canopy gaps, and at the largest possible distance from mature trees) in each of five replicate sites. Sites were separated by ≥ 1km to reduce bias through local variation in soil microbial community composition among secondary forest sites (Chen et al., 2020). Early-successional soils were collected within the Agua Salud landscape, a matrix of agricultural areas and secondary lowland rainforest sites of different successional stages (9°13'N 79°47'W ; 330 masl; 2,700 mm yr-1 rainfall; van Breugel et al., 2013). The five early-successional sites were surrounded by forests of other successional stages and had been used as cattle pastures or for low-intensity crop agriculture before abandonment (Craven et al., 2015). Late-successional soils were collected in proximity to ForestGeo plots in a continuous semi-deciduous lowland rainforest (9°9'N 79°44'W; 2,300 mm yr-1 rainfall; Condit, 1998). We removed surface litter and extracted the top layer of soils (0-10 cm). Soils were sieved (4 mm) to remove large roots, stones, and debris, pooled and mixed for each successional stage, and stored in the dark at 23° C for not more than 22 days. Soils were then conditioned by one of five predecessor species (*Apeiba membranacea* (Tiliaceae), *Psychotria grandis* (Rubiaceae), *Xylopia frutescens* (Annonaceae), *Gustavia superba* (Lecythidiaceae), *Faramea occidentalis*

(Rubiaceae)) growing in pots in a greenhouse at 40% ambient light for 1.5 years (Fig. 3.1). At the beginning of the conditioning phase, we set up ten replicate pots per predecessor species X successional stage combination and pooled soils from all pots that contained living plants at the end of the conditioning phase (number of pots from which conditioned soil was pooled per early-/ late-successional stage were *A. membranacea:* 10/ 10; *P. grandis*: 9/ 10; *X. frutescens*: 4/ 2; *G. superba* = 8/ 8; *F. occidentalis*: 10/ 10). Soils conditioned by on average 6 seedlings per predecessor species (median = 24, range = 2-30 seedlings) were pooled and used as inoculum in this experiment. For further details on the conditioning phase see Chapter 2 of this thesis.

3.3.2 Successor species

We selected three tree species that are widespread and common in Panamanian secondary forests as successor species for our experiment (Fig. 3.1). *Apeiba membranacea* Spruce ex Benth. is a tall forest tree abundant in secondary lowland forests and clearings in mature forests. *Xylopia frutescens* Aubl. is a tree abundant in disturbed and secondary forests that is also found on roadsides and in farmland. *Faramea occidentalis* (L.) A.Rich. is a treelet abundant in the understory of late-successional and mature forests. All species have animal-dispersed seeds (Condit et al., 2010) and form associations with AMF (*F. occidentalis:* Husband et al., 2002; *A. membranacea*: Lovelock et al., 2003; *Xylopia*: Soudzilovskaia et al., 2022). While associations with ectomycorrhizal fungi are possible (Corrales et al., 2018), no published records exist for any of our three successor species. We sampled seeds directly from ≥ 4 mother trees per species or as undamaged fruits from the forest floor. All seed collection sites were > 4 km away from our soil collection sites to minimize bias from adaptation to local microbial communities or plant genotype-specific pathogens (Eck et al., 2019). We cleaned seeds from pulp, dismissed all seeds with visible damage, and stored the remaining seeds in paper bags at 23°C for a maximum of 50 days. Seeds were soaked in tap water for 36 h and surface sterilized (70% ethanol, 10% bleach, 70% ethanol, distilled water) before sowing in February (*F. occidentalis, X. frutescens*) and March 2020 (*A. membranacea*). Seeds were germinated and grown in trays filled with sterilized 3:1 garden soil to sand mix and covered with a ~1 mm thin layer of sterilized sand to counteract mould development.

3.3.3 Seedling transplantation

Seedlings were transplanted into experimental pots during August 20-26, 2020. We filled pots (8.5 x 8.5 x 18.5 cm; filled to 1 cm below the rim, i.e. approx. 1.25 l volume) to 90% with a 1:1 mix of sterilized early- and late-successional forest soils. We used microwave radiation to sterilize soils. Following conservative reports on sterilization effectiveness (Ferriss, 1984; Speir et al., 1986), we exposed soils with 20% water content and spread to 2 cm thickness to 900 W for 180 sec in open containers, mixed the soil and repeated the treatment. Microwave radiation is rarely used in ecological research due to comparably high costs, yet has been shown to effectively reduce soil microbial biomass and respiration (Speir et al., 1986; Trevors, 1996) and to be equally effective in reducing fungi as autoclaving (Ferriss, 1984).

An inoculum of 125ml (10% of pot volume) of sterilized conditioned soil (sterilized inoculum) or conditioned soil (live inoculum) was placed into a planting hole. Similarly small amounts of inoculum have been shown to allow for an effective transmission of microbes (Mangan, Herre, et al., 2010) and to reduce potential bias through increased nutrient availability through microbial lysis in sterilized soils or edaphic variation between early- and late-successional soils. Successional variation in soil pH and nutrients can affect soil microbial communities (Chen et al., 2020; Yu et al., 2021) but effects in neotropical forests were weak (Barberán et al., 2015; Peay et al., 2013; Sarmiento et al., 2017) and soil properties varied little among our soil collection sites (van Breugel et al., 2013).

Before transplanting the successor seedlings, their roots were washed in tap water. A single seedling was placed in each planting hole on top of the inoculum and covered with sterilized soil. To keep microbial communities as intact as possible, removal of predecessor plants from conditioned soils and transplantation of successor seedlings was completed within two hours per predecessor-successor combination.

3.3.4 Greenhouse set-up

This experiment was set up in a greenhouse that is located in an experimental area with a concrete floor at a distance of ~70 m to the edge of a mature rainforest. The greenhouse has

a plastic roof that sheltered pots from rain and roof and sides of the greenhouse are covered in shade cloth allowing for airflow and a transmission of 40% daylight. Pots were placed at a height of 90 cm on 20 tables with a metal grid surface allowing drainage of excess water and avoiding microbial transmission through standing water. Each table was split by a 100 cm tall transparent plastic wall separating pots with live soil from pots with sterilized soil. Pot position was randomized with the restriction of allowing only one seedling per predecessor-successor combination per table. Each pot had five drainage holes (0.5 cm diameter, covered with mesh) and was placed on a 1 cm high plastic ring at a distance of > 25 cm in any direction to other pots. Plants were watered daily.

3.3.5 Measurements

We harvested all plants during March 16-23, 2021, to assess biomass. We carefully uprooted plants to extract the vast majority of fine roots and washed the roots to remove soil. We dried plants in a dry oven at 60°C for ≥ 72 hours and measured plant biomass as the dry weight of roots, stems, and leaves with a precision scale (d: 0.0001 g; TORBAL AGN 200, Scientific Industries Inc., Bohemia, USA). Abscised leaves were not included in the biomass measurements.

To check for the robustness of biomass as our performance measure, we additionally measured height growth. We assessed height growth as the difference between each seedling's aboveground height at transplantation and after the ~7 months of the experiment, in the week preceding the harvest.

3.3.6 Statistical analyses

We analysed biomass at harvest with linear mixed effects models (function lmer in package lme4). For each successor species, we built three models that included the main effects and interaction of (i) inoculum (live vs sterilized soil), soil successional stage (early- vs late-successional soil), and a binary comparison between conspecific and heterospecific

predecessors (con/ hetero); (ii) inoculum, soil successional stage, and predecessor identity (five predecessor species); (iii) inoculum, soil successional stage, and phylogenetic distance between predecessor and successor species. All statistical analyses were performed in R (R Core Team, 2021).

To analyse phylogenetic distance, we grouped predecessor species into phylogenetic categories based on cladograms. We constructed phylogenetic trees using the online tools phyloT v2 and iTOL v6 (Letunic & Bork, 2021) which are based on the taxonomy of the National Center for Biotechnology Information. We pruned all intervening taxa and joined species sharing the youngest common ancestor into a phylogenetic category. For *F. occidentalis* the five predecessor species classified into five distinct categories of phylogenetic difference, for *A. membranacea* into three categories, and for *X. frutescens* there were three predecessor species classified into two categories (see Fig. 3.2). Note that for *F. occidentalis*, each predecessor formed a separate phylogenetic category and phylogenetic analysis equalled the predecessor analysis. For *X. frutescens* the two heterospecific predecessors merged into one phylogenetic category and the phylogenetic analysis thus equalled the comparison of conspecific versus heterospecific PSF.

A priori, we defined models to include inoculum and conspecific vs heterospecific (or predecessor identity or phylogenetic distance) as fixed effects. We added soil successional stage and its three-way interaction terms, as their inclusion increased the coefficient of determination (R^2) by on average 0.11 and allowed for pairwise comparisons of PSF within soil successional stages. The position of a plant within the greenhouse was included as a random effect as it explained more than 5% of the total variance in each model. Lastly, we included plant height at the beginning of the experiment as a fixed effect to control for pre-experimental differences among seedlings. We tested whether model assumptions were met with diagnostic plots and no corrections were necessary.

We performed type III Anovas (function Anova in R package car) and calculated the explained variance, i.e. conditional R^2, for each model (rsq in rsq). As a measure of PSF, we calculated logarithmic odds ratios between the estimated marginal mean biomass in live (i.e. containing microbes) versus sterilized soil (emmeans in emmeans). A positive PSF indicates a higher biomass in live than sterilized soil.

We then calculated pairwise contrasts to compare conspecific PSF with (i) overall heterospecific PSF, (ii) PSF of each predecessor species, and (iii) PSF of each phylogenetic category. We calculated these contrasts for our two soil successional stages separately. Contrasts were Bonferroni-adjusted for multiple comparisons. For a more intuitive understanding, we reversed the sign of t-ratios. A positive contrast can now be interpreted as a more positive PSF in soils conditioned by the (i) heterospecific predecessors overall, (ii) a specific heterospecific predecessor species, or (iii) the phylogenetic category of predecessors as compared to the PSF in soils conditioned by conspecific predecessors.

We further contrasted PSF between early- and late-successional soils for all predecessor-successor combinations. A positive contrast here means a more positive PSF on seedling performance in early- than late-successional soils.

We analysed height growth in the same way as biomass. To meet normality assumptions of linear regression, we log-transformed height growth data and original plant height was not included.

Eight of 232 plants died during the experiment (six *A. membranacea*, two *X. frutescens*) and were excluded from the analysis. The low number of deaths precluded survival analysis but provided high replication for the analysis of PSF effects on height growth and biomass.

3.4 RESULTS

Results of the biomass and height growth analysis were – as expected – similar. We thus focused our manuscript on biomass and added graphs and tables for height growth to the appendix (Fig. S3.1 & S3.2, Table S3.1 - S3.4).

We found widespread and significant PSF (inoculum: live vs sterilized) on seedling performance in 18 of 26 predecessor-successor-successional stage combinations (for numerical presentation of all PSF see Table S3.1). Biomass was greater in live (including microbes) than sterilized soil in five of these cases, i.e. positive PSF, and lower in live than

sterilized soil in 13 cases, i.e. negative PSF (Fig. 3.2). Our models explained 45-86% of the total variance in seedling performance (Table 3.1, S3.2 & S3.5).

Plant-soil feedbacks varied between conspecific- and heterospecific predecessors (con/ hetero), with predecessor identity (predecessor: five species), with phylogenetic distance between heterospecific predecessor and successor (phylogeny: 1-5 categories), and soil successional stage (stage: early- vs late-successional soil) as described in the following paragraphs.

3.4.1 Conspecific vs heterospecific PSF

Conspecific PSF differed significantly from overall heterospecific PSF (i.e. all heterospecific predecessors pooled) for *A. membranacea* ($X^2 = 6.64$; $p = 0.010$) and *F. occidentalis* ($X^2 = 15.77$; $p < 0.001$) but not for *X. frutescens* ($X^2 = 0.86$; $p = 0.355$) as indicated by the interaction term inoculum X con/ hetero (Table S3.5). These differences between conspecific and heterospecific PSF interacted with soil successional stage. In *A. membranacea*, the overall difference was driven by significantly more positive conspecific than heterospecific PSF in late-successional soil, while con- and hetero-specific PSF did not differ in early-successional soils. For *F. occidentalis*, conspecific PSF was significantly more negative in late-successional soil, but significantly more positive in early-successional soils as compared to heterospecific PSF (Fig. 3.2, Table S3.3).

3.4.2 Predecessor identity

The identity of the soil conditioning, i.e. predecessor, species significantly affected PSF in all three successor species (significant interaction inoculum X predecessor) and interacted with soil successional stage in two species (significant interaction inoculum X predecessor X stage for *A. membranacea* and *F. occidentalis*; Table 3.1, Fig. 3.2).

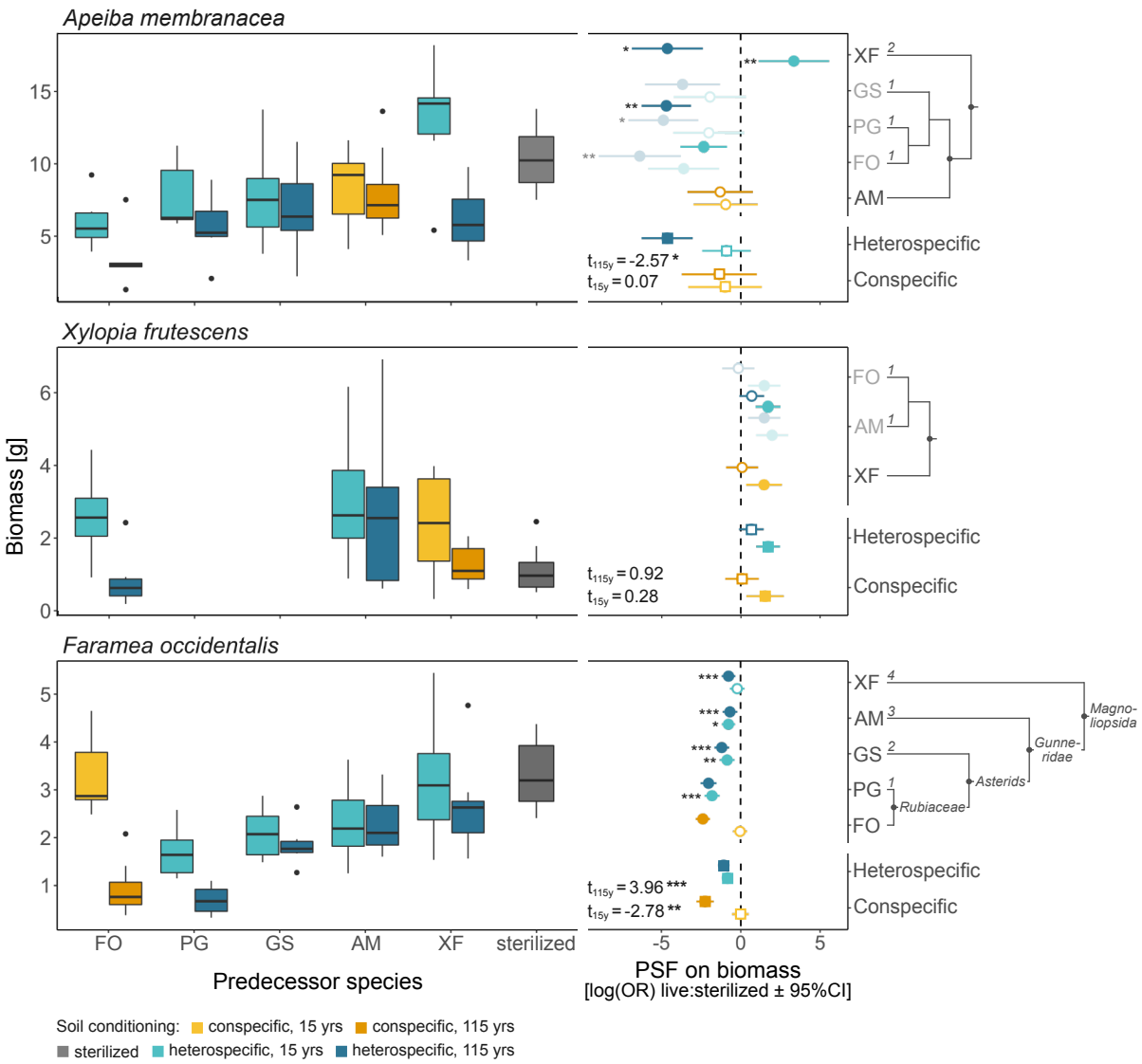


Figure 3.2. Effects of predecessor species and soil successional stage on biomass of three successor species. The left side of the panels show seedling biomass of successor species grown in early- (15 years of recovery) or late- (115 years) successional soils that were sterilized or conditioned by conspecifics and heterospecific species. The centre of the panels shows plant-soil feedbacks (PSF) as the logarithmic odds ratio of the biomass of seedlings growing in live vs sterilized soil ± 95% confidence intervals. The vertical dashed line denotes an effect size of zero at which the conditioning species had no impact on biomass relative to sterilized soil. Positive PSF represent a higher biomass in conditioned than sterilized soils. PSF is averaged over all heterospecific predecessors and conspecific predecessor (squares) and separately for each predecessor species (circles). Cladograms on the right side of the panels illustrate the phylogenetic relatedness between successor and predecessor species. We grouped heterospecific predecessors into phylogenetic categories (1-4), based on their last common ancestor with the respective successor species. Where several predecessor species were pooled into a phylogenetic category, the category's PSF is plotted on top of the PSF of each individual predecessor species (faded). Filled symbols represent significant PSF ($p < 0.05$). Stars indicate significant differences between PSF of conspecifics and a heterospecific or a phylogenetic category ($p < 0.001$*** < 0.01** < 0.05*). Results of pairwise contrasts between conspecific and overall heterospecific PSF are shown for early- and late-successional soils. A positive t-ratio indicates a more positive hetero- than conspecific PSF.

FO = *Faramea occidentalis*, PG = *Psychotria grandis*, GS = *Gustavia superba*, AM = *Apeiba membranacea*, XF = *Xylopia frutescens*.

Table 3.1. Summary statistics of effects on seedling biomass of three tree species. We assessed effects of inoculum (soils including microbiota vs sterilized soil), successional stage of the soil (Stage: 15 years vs 115 years of succession), and identity of soil conditioning species (Predecessor: conspecific and 2-4 heterospecific tree species) on seedling biomass of three successor species after 7 months. Results of analysis of variance (type III) and overall explained variance (R^2) of linear mixed effects regressions. Significant p-values (< 0.05) are highlighted in bold. For factors for which degrees of freedom (df) differed among successor species, one value for each species is provided.

	Successor species						
	Apeiba membranacea		*Xylopia frutescens*		*Faramea occidentalis*		
	X^2	*p*	X^2	*p*	X^2	*P*	*df*
Inoculum	1.54	0.215	8.51	**0.004**	8.17	**0.004**	1
Predecessor	21.93	**<0.001**	12.38	**0.002**	80.78	**<0.001**	4; 2; 4
Stage	0.12	0.733	0.95	0.330	0.17	0.678	1
Inoculum x Predecessor	12.78	**0.012**	6.40	**0.041**	46.36	**<0.001**	4; 2; 4
Inoculum x Stage	0.06	0.802	0.50	0.480	0.09	0.759	1
Predecessor x Stage	30.84	**<0.001**	2.71	0.258	70.34	**<0.001**	4; 2; 4
Inoculum x Predecessor x Stage	16.92	**0.002**	1.43	0.489	38.95	**<0.001**	4; 2; 4
Conditional R^2	0.67		0.50		0.86		

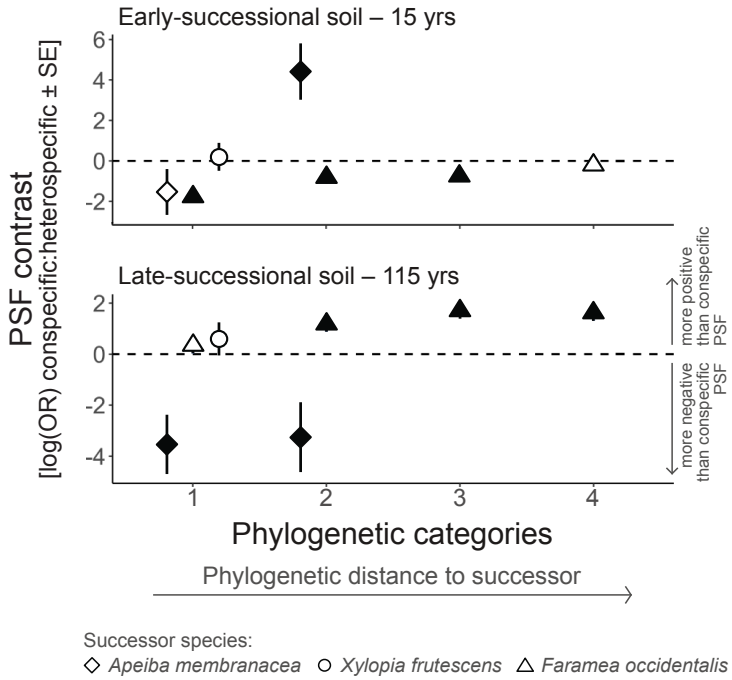


Figure 3.3. Phylogenetic signal in plant-soil feedback (PSF) on seedling biomass. We plotted the contrast between heterospecific and conspecific PSF against the phylogenetic distance of the heterospecific predecessor-successor pair as the logarithmic odds ratio ± one standard error. Positive values, above the dashed line, indicate a better performance in heterospecific than conspecific conditioned soils and negative values indicate the opposite. Error bars represent one standard error. Phylogenetic distance between predecessor and successor species was measured as a categorical variable based on cladograms (see Fig. 2). Filled symbols represent significant PSF ($p < 0.05$).

3.4.3 Phylogenetic distance

Plant-soil feedbacks differed with phylogenetic relatedness of successor and predecessor species for two of the three species (significant interaction inoculum X phylogeny; Table S3.5). Differences interacted with soil successional stage (significant interaction inoculum X phylogeny X stage; Table S3.5). When contrasted against conspecific PSF, heterospecific PSF transitioned from negative or neutral to positive with increased phylogenetic distance between successor and predecessor species (Fig. 3.3, Table S3.3).

Table 3.2. Contrasts between plant-soil feedbacks (PSF) on seedling biomass in late- vs early-successional soils. Seedlings of three successor species were grown in early- (15 years of forest recovery) and late- (115 years) successional soil conditioned by one of five predecessor species. We calculated PSF as the logarithmic odds ratio of biomass in each conditioned soil versus sterilized soil and pooled for all heterospecific conditioned soils versus sterilized soil ("Heterospecific"). We then calculated pairwise contrasts between late- and early-successional soils for each predecessor-successor pair and for the pooled heterospecific PSF. Negative t-ratios indicate more negative PSF in late- than early-successional soils. Significant values ($p < 0.05$) are printed in bold.

	Successor species					
	Apeiba membranacea		*Xylopia frutescens*		*Faramea occidentalis*	
Predecessor species	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*
Apeiba membranacea	-0.25	0.803	-0.70	0.485	0.31	0.760
Faramea occidentalis	-1.83	0.070	-2.32	**0.023**	-7.62	**<0.001**
Gustavia superba	-1.10	0.273	–	–	-1.04	0.299
Psychotria grandis	-1.94	0.055	–	–	-0.65	0.517
Xylopia frutescens	-5.55	**<0.001**	-1.88	0.064	-1.74	0.084
Heterospecific	-4.40	**<0.001**	-2.04	**0.045**	-1.30	0.198

For *F. occidentalis*, for which p heterospecific predecessors were grouped into four phylogenetic categories, the increase towards more positive PSF with increased phylogenetic distance was gradual. For *A. membranacea* (two phylogenetic categories of heterospecific predecessors) PSF were also more positive in soils conditioned by more distantly related predecessors, and for *X. frutescens* (with both heterospecific predecessors being in the same phylogenetic category) heterospecific PSF tended to be more positive than conspecific PSF (Fig. 3.3).

3.4.4 Soil successional stage

Seedlings of all three successor species experienced more negative PSF in late- than early-successional soils (Fig. 3.2). This pattern was consistent for 12 of the 13 predecessor-successor combinations (Table 3.2) and significant for *A. membranacea* and *F. occidentalis*, but not *X. frutescens* (three-way interaction terms including stage; Table 3.1 & S3.5). Averaged across all predecessor species, PSF reduced seedling biomass more than two-times as strongly in late- than in early-successional soils in *F. occidentalis* ($W_{sterilized}$ = 3.3 g, W_{15yrs} = 2.6 g, W_{115yrs} = 1.7 g) and in *A. membranacea* (10.4 g; 8.1 g; 5.5 g), and PSF were three-times less positive in *X. frutescens* (1.1 g; 2.5 g; 1.6 g) in late- than in early-successional soils.

3.5 DISCUSSION

Predecessors widely affected the seedling performance of their successors through soil microbial legacies. Conspecific PSF varied greatly from positive to negative, indicating that for some tree species PSF may promote conspecific over heterospecific successors. However, we found strong evidence for a phylogenetic signal in heterospecific PSF that may favour the establishment of unrelated successors. PSF were expressed in soil from both early and late stages of succession yet were consistently more negative in late- than early-successional soil. Overall, our results suggest that microbial-mediated PSF may affect the identity of successors and could thereby contribute to determine successional trajectories of tropical rainforests.

3.5.1 Conspecific versus heterospecific PSF

Effects of predecessors on conspecific successors varied greatly being either more positive, more negative, or similar to the PSF of the average heterospecific predecessor (Fig 2). This result contradicts our first hypothesis and the findings of an earlier study that reported conspecific PSF to be more negative than heterospecific PSF (Mangan, Schnitzer, et al., 2010). Instead, our finding suggests contributions of both species-specific pathogens and species-specific mutualists with considerable variation in their net impact. Host-specificity is often reported to be stronger in pathogens than mutualists (Bennett & Klironomos, 2019; Eck et al., 2019). Yet, host-specific communities of AMF (Mangan, Herre, et al., 2010) and the positive conspecific PSF in our conditioning phase (Chapter 2 of this thesis) and earlier studies (Anacker et al., 2014; Mangan, Herre, et al., 2010; Miller et al., 2019) suggest specialization in at least some mutualists. Metagenomic analyses are needed to conclusively clarify the contribution of different microbial groups. Nonetheless, the net PSF measured here have ecological relevance as the combined effects of mutualists and enemies will have a major impact on plant performance (Segnitz et al., 2020) and could have broad implications for community composition (Anacker et al., 2014; Bennett et al., 2017) during succession. A dominance of positive PSF favouring conspecific over heterospecific successors can erode plant diversity (Bennett et al., 2017; van der Putten et al., 2013) and may stall succession. Negative conspecific PSF, however, represent one end of the spectrum of the plant

phylogenetic signal in PSF that we found (described below) and could contribute to explain the observed transition from phylogenetic clustering to evenness in plant communities with secondary succession of rainforests (Letcher et al. 2012).

3.5.2 Phylogenetic signal in heterospecific PSF

Heterospecific PSF transitioned from negative to positive with increasing phylogenetic distance between predecessor and successor species (Fig. 3.3 & S3.2). This finding supports our second hypothesis and could be driven by a stronger phylogenetic signal in the association of trees with pathogens than with mutualists. In support of this idea, Schroeder et al. (2019) reported stronger effects of tree host phylogeny on the community composition of putative soil fungal pathogens than mutualists in a Mexican rainforest. The negative effects of pathogens could therefore overwhelm the positive effects of more generalist mutualists in soils conditioned by closely related predecessors. While our experimental design did not enable us to directly assess the relative abundance of host-specific versus generalist pathogenic or mutualistic soil microbial species, we have shown that plant phylogeny does influence net PSF effects beyond the species level in rainforest trees. Combined with reports of a phylogenetic signal in the susceptibility to foliar pathogen damage(Gilbert et al., 2015; Gilbert & Webb, 2007) our results suggests that phylogenetic relatedness can be useful to predict effects of predecessors on successor species during secondary succession and thus to guide species selection for tree plantations or assisted natural regeneration for forest restoration.

Our results are based on only three successor species, and it is important to note that other studies have shown large species-specific variation in PSF (e.g. Miller et al., 2019; Spear & Broders, 2021) and plant-fungal interactions in the greenhouse may vary from those realized in the forest (in: Gilbert & Webb, 2007). Thus, further studies, including a large number of predecessor-successor combinations, are needed to assess the generality of phylogenetic signals in heterospecific PSF. If microbial legacies of trees commonly affect closely-related successors more negatively, they could provide more distantly related successors with a

competitive advantage and contribute to the directional turnover of tree species during secondary succession of rainforests.

3.5.3 Stronger negative PSF in late-successional soil

PSF varied with the successional stage that soils were collected from and were consistently more negative in late-successional than early-successional soils (Table 3.2). The diversity of microbial communities has been shown to increase during succession in temperate (Chen et al., 2020) and subtropical forests (Zhang et al., 2017). A more diverse microbial community in late-successional soils could have enabled a rapid increase in the abundance of host-specific microbes in the presence of the predecessor species, resulting in the greater PSF found in late-successional soils in our experiment. Future studies will reveal whether and how changes in microbial community composition and their potential interaction with abiotic conditions (e.g. McCarthy-Neumann & Kobe, 2019) can explain the more negative PSF in late-successional soils reported here.

While they varied in intensity, PSF were present in both early- and late-successional soils. This suggests that microbial communities are sufficiently diverse at both stages of succession to cause this effect through rapid formation of host-specific accumulations that exert legacy effects after the death of the predecessor plant. The ability of soil microbes to remain as inactive resting structures or to infect suboptimal hosts in the absence of their preferred host (Katan, 2017) may result in a high resilience of microbial communities that could explain our results. Plant phylogenetic signals in the PSF produced by such resilient microbial communities could reinforce the stochastic effect of plant dispersal, which has been suggested to a primary driver of the unpredictability in the recovery of plant community composition through succession (e.g. Poorter et al., 2021; van Breugel et al., 2013). Comprehensive *in situ* studies are needed to reveal whether resilient microbial communities and fast activation of host-specific microbes could affect the phylogenetic composition of recovering vegetation through PSF.

3.5.4 Limitations

Our experimental design makes it likely that the PSF effects measured have been driven by soil microbial organisms. However, we cannot determine the causal agents of these PSF effects (microfauna, e.g. fungi, bacteria, protists, nematodes, mites) with certainty without analysing soil microbial community composition (e.g. through genomic sequencing of soil samples collected before and after the experiment). We reduced the potential for differences in soil chemical or physical properties to affect our results by adding only a small inoculum to our experimental pots, which were filled to 90% with a standardized growing medium.

Secondly, our small set of predecessor and successor species and the high interspecific variation found in PSF effects suggesting that the results depended on species selection, limit the generalizability of our results. This is further complicated by the dependency of studies like ours on obtaining a large number of available seeds, and thus their focus on abundant tree species, while most tree species in tropical rainforests are rare (Slik et al., 2015).

Thirdly, greenhouse-derived estimates of PSF may differ from those in the field (Forero et al., 2019) because of unmeasured environmental variation (e.g. in soil nutrients, plant competition, herbivory), which may affect both microbial communities and plants and thus PSF (De Long et al., 2019). As an example, leaf litter may strongly affect soil microbial communities, yet, we had to remove abscised leaves from our experimental pots to avoid herbivore infestations. Further, our study measured PSF of plants growing individually in pots. Plant-soil feedback effects may however disappear or intensify when plants are in competition with neighbours in the field. Whether the occurrence of more positive PSF with increasing phylogenetic distance between plant species can provide a large enough advantage to influence the relative abundance of species in the field remains to be tested (e.g. by putting species' fecundity, germination success, and growth and survival rate of young plants as terms in a model together with the strength of PSF).

Lastly, a previous study suggested that fine-scale phylogenetic information (i.e. considering phylogenetic distance as continuous variable rather than phylogenetic categories) may provide a better insight into phylogenetic signals in plant-pathogen interactions (Gilbert & Webb, 2007). Given our small set of species and the uncertainty of molecular clocks methods

(e.g. due to non-constant mutation rates: Rodríguez-Trelles et al., 2001), we decided to use phylogenetic categories as a simple, yet reliable and intuitive measure.

These limitations highlight that results from greenhouse studies like ours need to be carefully interpreted and should not be broadly generalized. Nonetheless, controlled, multi-factorial greenhouse studies that simplify some aspects of species interactions are crucially needed first steps into gaining an understanding of the impact of individual variables and the interactions between them in driving tropical rainforest recovery.

3.6 CONCLUSION

We found clear evidence of a phylogenetic component of plant-soil feedbacks (PSF) in tropical rainforest trees. This phylogenetic signal was varied as exemplified by the large variation in the difference of PSF between conspecific and heterospecific predecessor seedlings, ranging from strongly positive to negative. Our results suggest that both specialist mutualist and pathogenic microbes have a potentially large impact on secondary forest dynamics. Negative effects of microbial legacies of a tree seedling decreased with the phylogenetic distance between predecessor and successor species. If this phylogenetic signal in PSF is widespread, it may favour the establishment of unrelated successor tree species over close relatives. PSF could thereby contribute to the directional turnover of tree species during rainforest secondary succession. Phylogenetic effects rather than negative conspecific PSF *per se* indicate that microbiota may promote tree diversity at high taxonomic levels. Understanding the role of phylogenetic PSF in structuring plant communities can inform species selection in forest restoration and may allow us to enhance the potential of secondary rainforests to conserve biodiversity.

Chapter 4: Effects of insect herbivores and fungi on seedling performance of the tropical tree *Lacistema aggregatum* vary along a natural rainfall gradient

4.1 ABSTRACT

Temporal and spatial variation in rainfall affects seedling damage and mortality due to insect herbivores and pathogens ("pests" hereafter) along rainfall gradients. Whether the effects of pests on plant performance vary along abiotic gradients within the range of tree species however remains unresolved. Amongst other mechanisms, variation in plant vigour with abiotic conditions could affect the susceptibility of plants to pest effects via altering resistance to pests and tolerance of pest damage.

In a field experiment, we tested for variation in the effects of insect herbivores and fungal pathogens on seedling performance along a rainfall gradient within the distributional range of the tree species *Lacistema aggregatum*. We planted young seedlings into four forest sites along the isthmus of Panama. At each site, we established four plots each containing 25 seedlings that were subject to one of four treatments: fungicide, insecticide, combination (fungicide + insecticide), or control (water-sprayed). We measured seedling survival, height growth, and – for a subset of seedlings – biomass after 16 months.

Survival increased with rainfall and was not affected by pesticide application. In contrast, growth and biomass were highest in intermediately wet sites when pests were excluded, but lowest when pests were present. This resulted in a significant interaction between site and treatment with the strongest positive effects of pesticide application in these intermediately wet sites. This result suggests that more vigorous seedlings experience strongest pest effects.

Understanding the variation in plant-pest interactions with environmental context will improve our ability to predict how projected changes in rainfall patterns will affect plant distributions and communities.

4.2 INTRODUCTION

Spatial and seasonal variation in rainfall has strong, species-specific effects on seedling mortality in tropical rainforests (Fortunel et al., 2016; Solé et al., 2019) and contributes to shape the regional distribution of tree species (Brenes-Arguedas et al., 2009; Engelbrecht et al., 2007; Gaviria et al., 2017). Rainfall also affects the amount of damage caused by insect herbivores and fungal pathogens (Coley & Barone, 1996; Givnish, 1999; Romero et al., 2022) that are major agents of tropical seedling mortality (Augspurger, 1984; Bagchi et al., 2014; Spear et al., 2015). Plant-insect and plant-fungi interactions may thus vary with abiotic conditions within the distributional range of a plant species. This may drive local adaptation to pests in some habitats (Muehleisen et al., 2020) and has been shown to restrict the geographic range of a temperate plant species (Benning et al., 2019). Yet, we know little about variation in the effects of insect herbivore and fungal pathogen ("pests" hereafter) damage on tree performance along tropical rainfall gradients within the distributional range of tree species. A better knowledge of the variation in plant-pest interactions along current abiotic gradients will enhance our ability to predict how projected climatic changes (Pokhrel et al., 2021) will affect plant distributions and communities.

Pest pressure in tropical forests is intense. Insect herbivores (Eichhorn et al., 2010; Leigh et al., 2004) and fungal pathogens (Augspurger, 1984; Chanthorn et al., 2013; Spear et al., 2015) cause high seedling mortality with implications for local tree species abundance and richness (Bagchi et al., 2014; Janzen, 1970; Norghauer & Newbery, 2013). Pest damage is expected to increase towards wetter and less seasonal forests (Givnish, 1999). Insects may profit from reduced desiccation risk and the absence of seasonal reductions in plant productivity (Coley & Barone, 1996; Leigh et al., 2004). Higher humidity should favour fungal pathogens (Romero et al., 2022) by promoting spore dispersal and fungal infection by preventing drought-induced stomatal closure in leaves (Garrett et al., 2006). Indeed, the frequency of watering increased fungal-induced seedling mortality of a tropical tree species in a greenhouse experiment (Swinfield et al., 2012) and pathogen damage and insect herbivory of tree seedlings were higher in a wet than a dry forest (Brenes-Arguedas et al., 2009). Large interspecific variation in herbivory (Muehleisen et al., 2020) and pathogen damage (Spear &

Broders, 2021) may however explain why other studies reported on average lower (Coley & Barone, 1996; Muehleisen et al., 2020; Weissflog et al., 2018) or similar levels of pest damage in wet and dry rainforests (Baltzer & Davies, 2012; Spear et al., 2015). Individual tree species can however differ in their patterns of pest damage along rainfall gradients from plant community trends (Spear et al., 2015; Weissflog et al., 2018), the reasons of which have rarely been studied.

Plant vigour may vary along environmental gradients and could affect the attraction of and resistance to pests (reflected in pest damage), as well as plant tolerance of damage (reflected in pest effects on plant performance). The plant vigour hypothesis predicts higher pest damage on more vigorous plants (Price, 1991). In support of this idea, beetle borer damage increased with the nutritional quality of branches in a tropical tree (Uribe-Mú & Quesada, 2006) and a meta-analysis reported insect herbivore abundance to be higher on more vigorous plants (Cornelissen et al., 2008). The few studies that directly linked plant vigour, pest pressure and variation in abiotic resources reported mixed results. Studies from temperate systems reported insect herbivore abundance (Trotter et al., 2007) and damage (Louda & Collinge, 1992) to be higher for water-stressed plants. In contrast, the stronger growth of tropical saplings in gaps than in the understory (Norghauer & Newbery, 2013) or in nutrient-rich, wet sites than in resource-limited sites (Boege & Dirzo, 2004) correlated with higher pest damage.

Plant vigour may also affect a plant's tolerance of a given amount of pest damage. The common practice to infer pest effects from measuring leaf area loss underestimates damage, e.g. by excluding damage from sap-sucking insects. More importantly, pest damage can be a poor predictor of pest effects on plant performance (Cronin et al., 2010) that are the sum of pest damage and plant response. Effects of herbivore exclosure on survival were found to be stronger in the light-limited understory than in gaps for two of three species (Norghauer & Newbery, 2013), suggesting that resource limitation can intensify pest effects by reducing a plant's ability to compensate for tissue loss (Wise & Abrahamson, 2008). In meadow plants, similar amounts of meristem damage caused a 10-fold higher reduction in seed production in nutrient stressed than in fertilized plants (Wise & Abrahamson, 2008). Also better access to limiting resources increased plants' tolerance of herbivory with effects of herbivory being

greatest under low resource availability (Cronin et al., 2010). Such effects could be counteracted by plants overcompensating for pest damage (Garcia & Eubanks, 2019; Wise & Abrahamson, 2008). Overcompensation has been shown to explain positive effects of herbivory on tree growth in a subtropical forest (Schuldt et al., 2017) and of pathogen infection on seed production in meadow plants (Bradley et al., 2008). Thus, variation in plant vigour with abiotic conditions could have a complex and species-specific impact on pest effects on plant performance.

In conclusion, pest effects on plant performance are likely to vary along rainfall gradients because variation in rainfall can influence pest damage, plant vigour, and plant tolerance. The predicted increase in the intensity and frequency of droughts (Pokhrel et al., 2021) has been suggested to disrupt plant-pest interactions by shifting species distributions or increasing pest damage in some habitats but not others (Gely et al., 2020; Hamann et al., 2021). Understanding patterns of plant-pest interactions along abiotic gradients within the current range of tree species is thus crucial to determine the relative contribution of variation in pest damage and plant vigour on the strength of pest effects under different environmental conditions. This will allow us to better understand the role of pests in determining tree species distributions under climate change.

To assess variation in the relative and combined effects of insect herbivores and fungal pathogens on seedling performance, we planted seedlings of a tropical tree species *Lacistema aggregatum* in four forest sites along a steep rainfall gradient in Central Panama. The combined treatment of protection from herbivores and pathogens allowed us to assess seedling performance when both pest types are absent and thus the effect of variation in rainfall on the vigour of *L. aggregatum.* We hypothesized that 1) seedling vigour varies along the rainfall gradient, and that 2) both insect herbivores and fungal pathogens reduce seedling performance. We asked whether pest effects on seedling performance vary with plant vigour along the rainfall gradient and whether such patterns are identical for insect herbivores and fungal pathogens.

4.3 METHODS

We planted 400 shade-house grown seedlings of the widespread forest tree *Lacistema aggregatum* in four forest sites along a steep rainfall and seasonality gradient along the isthmus of Panama (Fig. 4.1), ranging from an average ~1,700 mm/ yr and ~147-day duration dry season at the Pacific to ~3,200 mm/ yr and ~117-day duration dry season at the Atlantic coast (Condit et al., 2013; Engelbrecht et al., 2007).

In each site, we tested the effects of pests on plant performance by comparing survival, height growth, and biomass after 16 months of seedlings growing under natural conditions ("control") to that of seedlings treated with insecticide, fungicide, and a combination of both pesticides. We then compared pest effects among sites to assess the interaction between effects of pests and variation in rainfall on plant performance.

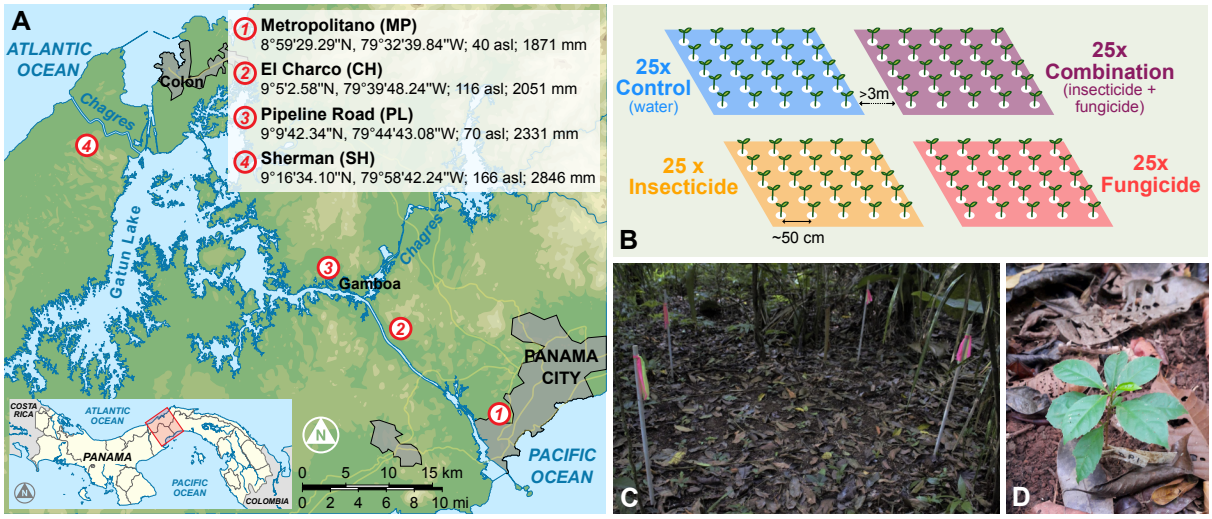


Figure 4.1. Overview of study sites and field set-up. (A) Maps show the location of four forest sites along the steep rainfall gradient across the isthmus of Panama from the drier Pacific to the wetter Atlantic (Caribbean) coast. Field site coordinates, their elevation above sea level (asl) in metres and average annual rainfall (Condit et al., 2013) are presented. **(B)** In each forest site, we established four 2x2 m plots with 25 seedlings each that were subject to one of four treatments: water-sprayed control, insecticide application, fungicide application, and a combination treatment of insecticide plus fungicide application. Plants were spaced at a 50 cm distance between them in all directions and plots were separated by more than 3 m. **(C)** All plots were located in the shaded forest understory. **(D)** *Lacistema aggregatum* seedling.

Maps are strongly modified creative commons: Country: Alexrk, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=6909551; Canal zone: Kaidor, CC BY-SA 4.0 https://upload.wikimedia.org/wikipedia/commons/a/a2/Panama_Canal_Map-ru.svg?.

4.3.1 Focal species

Lacistema aggregatum (P.J. Bergius) Rusby (Lacistematacea) is a 6-20 m tall widespread tree species that is common in all forest zones of Panama including all our study sites. In its seedlings, leaves are alternate, simple, slightly toothed, and oblong-elliptic (10-16 cm long, 4.5-7 cm wide, Fig. 4.1). Fruit production of this monoecious tree typically peaks between April and May. The black, 7 mm long seeds are predominately bird-dispersed and surrounded by a white, fleshy aril in red capsules (Condit et al., 2010; Croat, 1978).

4.3.2 Seed collection and processing

We collected 1,440 seeds directly from the branches of 12 parent trees, avoiding soil contact and pre-experimental pathogen infection, in May 2019. These 12 trees grew in various locations inside lowland rainforests of the Panama Canal Area (seven trees between Metropolitano and El Charco and five trees between El Charco and Pipeline Road). Seed collection sites did not correspond to the four experimental sites and were > 3 km away from any of our experimental sites to minimize bias from potential local adaptations of plants and pests (Eck et al., 2019; Muehleisen et al., 2020). We pooled seeds from all collection sites and mixed them thoroughly.

Undamaged seeds were cleaned from pulp, surface sterilized (70 % ethanol, 10 % bleach, 70 % ethanol, distilled water) and stored in the dark at 23 °C. Seeds were soaked in tap water for 24 h prior to sowing them into seedling trays filled with a steam-sterilized 1:1 sand to garden soil mix on June 21, 2019. 418 seedlings and thus 29% of the seeds germinated. Among those we selected the tallest seedlings for this experiment. With respect to seed origin, seedlings were randomly allocated to the four experimental sites and the treatments within each site. Seedlings were planted into the field sites during December 2-5, 2019. We recorded above-ground seedling height one week after planting to allow for settlement in the soil and to control for bias of initial size effects on survival or biomass (García-Guzmán & Benítez-Malvido, 2003).

4.3.3 Experimental design

We assessed seedling performance in four forest sites spanning almost the entire rainfall gradient crossing the Panamanian isthmus. Sites were directly adjacent to long-term monitoring plots which provided data on average annual rainfall and dry season length (Anderson-Teixeira et al., 2015; Condit et al., 2013). While soil nutrient availability varies in the region (Condit et al., 2013), soil water availability and seasonal drought severity were the main drivers of variation in seedling performance along the isthmus (Engelbrecht et al., 2007; Gaviria et al., 2017).

In each forest site, we established four 2x2 m plots with a minimum separation of 3 m (Fig. 4.1). We chose shaded understory locations with minimal prior vegetation and cut all above-ground plant material in the plots. We initially cleared some of the leaf litter to prepare the site for planting but did not remove litter afterwards to minimize effects on pest damage (García-Guzmán & Benítez-Malvido, 2003).Twenty five seedlings were planted into each plot in five rows with ~50 cm distance between plants. In total, the experiment comprised 400 seedlings (4 sites x 4 plots x 25 seedlings). To reduce bias through potential accumulation of species-specific soil fungal communities or a spillover of insects, each plot was located at least 5 m away from any adult *L. aggregatum* trees. Seedlings were watered immediately after planting (8 l tap water per plot) and one week later to promote seedling establishment.

Plots were randomly assigned to one of four treatments: water-sprayed control, insecticide, fungicide, and combination (fungicide plus insecticide). We used the broad spectrum, insecticide Engeo® (141 g/ l thiamethoxam, 106 g/ l lambda-cyhalothrin; Syngenta Ltd., Basel, Switzerland), which has contact and systemic activity against a wide range of insect pests. The fungicide was the broad-spectrum Amistar XTRA® (200 g/ l Azoxystrobin, 80 g/ l Cyproconazole; Syngenta Ltd., Basel, Switzerland), which has systemic activity against Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes, and thus all the most common fungal and pseudo-fungal pathogens of seedlings in Panamanian forests (Spear & Broders, 2021). Seedling shoots and the soil surface were sprayed with pesticides directly after planting and at bi-weekly intervals hence forward. Insecticide and fungicide were applied according to manufacturer's guidelines at rates of 150 ml/ ha and 575 ml/ ha, respectively. Panamanian COVID-19 regulations restricted the access to our field sites and

pesticide applications were suspended from March 2020 onwards. In total, we applied pesticide nine times.

4.3.4 Measurements

To determine survival, we recorded the number of seedlings that survived and the number of seedlings that were found dead at the end of the experiment. Some of our seedlings could not be found at the end of the experiment (NAs in Table S4.1) and were missing presumed dead. We observed no evidence for damage by vertebrates of branch fall that could have explained the missing of these seedlings.

We measured growth as the difference in above-ground seedling height (stem base to apex) between the start and the end of the experiment of all surviving seedlings. We harvested a randomly selected subset of five seedlings per treatment X site combination during March 25-28, 2021. We carefully uprooted seedlings, extracted as many of fine roots as possible and cleaned them with water. We dried plants at 60 °C for > 72 h and measured biomass with a precision scale (d: 0.0001 g; TORBAL AGN 200, Scientific Industries Inc., Bohemia, USA) as the sum of the dry weight of roots, stems, and leaves.

4.3.5 Statistical analyses

We analysed seedling survival, height growth, and biomass. A treefall made the insecticide plot in Metropolitano inaccessible, and we could not obtain data for this site X treatment combination. The lack of replicate blocks within sites and the fact that the missing data were non-random prevented the application of imputation methods. We thus performed two separate analyses (1) considering all plants in the insecticide treatment at Metropolitano as dead ("incl. Metropolitano") and (2) excluding Metropolitano entirely from the analyses ("excl. Metropolitano") to evaluate the effect of missing measurements of the insecticide plot. Pairwise contrasts are based on the models including Metropolitano. All statistical analyses were performed in R (R Core Team, 2021).

Survival. – We analysed survival using binomial regression models (function glm in R package stats) that included treatment (4 levels: control, insecticide, fungicide, combination) and site (4 levels: Metropolitano, El Charco, Pipeline Road, Sherman) and their interaction as fixed effects. We performed chi-square tests (type III Anova in car) and calculated the total proportion of variance explained by the model as conditional R^2 (rsq in rsq).

To assess the effect of pesticide application, we calculate risk ratios (riskratio in fmsb) between the probability of survival in each of the pesticide treatments versus the control treatment. To test whether the effect of pesticides varied along the rainfall gradient, we then contrasted risk ratios between sites (contrasts in emmeans). Risk ratios can be interpreted as the multiplicator by which the probability of survival is increased or decreased in a pesticide treatment relative to the control, with a risk ratio >1 representing higher survival in the pesticide treatment.

We added the missing presumably dead seedlings (NAs in Table S4.1) to the dead count in the survival analysis presented in the main text. A survival analysis that excluded all missing seedlings did not differ in results except for a weakened R^2 (Table S4.2). For this latter analysis we used survival data from a previous census (March 10, 2020) for the insecticide treatment in Metropolitano.

Height growth and biomass. – We used logistic regression models (glm in stats) with gaussian error distribution to assess the effects of treatment, site and their interaction on height growth and biomass. We log-transformed growth and biomass data to achieve normality of residuals. We tested for the inclusion of plant height at the beginning of the experiment as a random effect but decided against it as it had no effect on the direction or significance of results and explained less than 5% of the total variance in all models. We used diagnostic plots to check that model assumptions were met (simulateResiduals in DHARMa), and no further corrections were necessary. We performed chi-square tests (type III Anova in car) and calculated the conditional R^2 (rsq in rsq).

We performed Tukey-adjusted pairwise post-hoc tests to compare treatments and sites (emmeans in emmeans). First, we tested for variation in growth and biomass with treatment

within each site by calculating pairwise contrasts among all treatments. Second, we calculated the effect of pesticide treatments per site as the logarithmic odds ratios (log(OR)) between each pesticide treatment and the control treatment. A negative log(OR) represents a lower performance in the pesticide treatment than in the control. Lastly, we calculated t-ratios of pesticide effects between sites to assess whether the effect of a pesticide varies among sites.

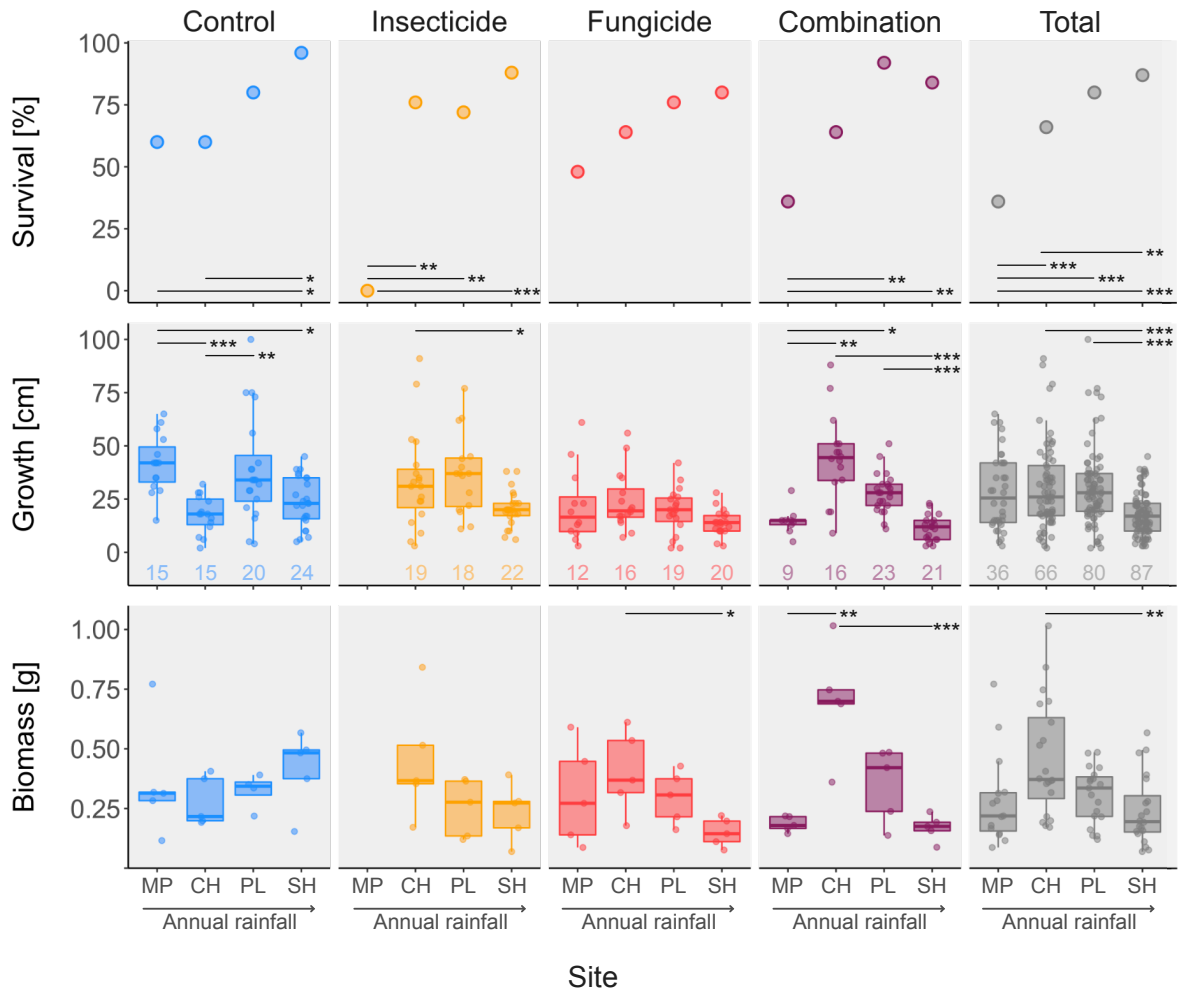


Figure 4.2. Seedling performance varies with pesticide treatment in four forests along a rainfall gradient. Percentage survival, aboveground height growth ("Growth", stem base to apex), and biomass (dry weight of roots, stems, and leaves) of seedlings of the tree species *Lacistema aggregatum* planted to four tropical forest sites (MP = Metropolitano, CH =El Charco, PL = Pipeline Road, SH =Sherman). Per site, 25 seedlings were exposed to each of four treatments: a water-sprayed control (i.e. natural growing conditions at the site; blue), insecticide application (yellow), fungicide application (red), or combination of insecticide plus fungicide application (purple). Additionally, we show seedling performance averaged across treatments ("Total", grey). Sites are sorted from the driest and most seasonal to the wettest and least seasonal (left to right) based on average annual rainfall. Dots in the height growth and biomass plots represent individual plants. For height growth, the number of seedlings measured per treatment X site combination are printed below each box. For biomass, sample size was always n = 5. Stars indicate significant pairwise differences between sites within each treatment ($p < 0.001^{***} < 0.01^{**} < 0.05^{*}$).

4.4 RESULTS

4.4.1 Survival

At the end of the experiment 90 of the 400 planted seedlings were recorded as dead and an additional 41 seedlings could not be found giving an overall mortality rate of 22.5 % or (including the missing seedlings) 32.8% over 16 months (Table S4.1). Survival varied greatly and significantly amongst the sites, increasing over the rainfall gradient from the driest to the wettest site (Fig. 4.2, Table 4.1). However, treatment and the interactive effect of treatment X site had a significant effect on survival only when Metropolitano was included in the analysis, suggesting that the absence of seedlings in the insecticide treatment (through treefall) has biased the results of this analysis. The overall variance explained by the models was low, with R^2 = 21% including and R^2 = 6% excluding Metropolitano (Table 4.1).

Within each treatment and averaged across treatments, percentage survival increased with rainfall (Fig. 4.2). Survival was significantly higher in the wettest (Sherman) than in the driest site (Metropolitano) in all but the fungicide treatment (Fig. 4.2; Table S4.3). In the combined treatment, the probability of survival was 2.4 times higher in the wettest than in the driest site (Table S4.3).

We found no significant effects of pesticide treatments on survival, except for a negative effect of the insecticide treatment in Metropolitano, which was most likely caused by the inflated death count due to the treefall on this plot (Fig. 4.3 & S4.1; Table S4.4 & S4.5).

4.4.2 Height growth and biomass

Results for height growth and biomass showed very similar trends, though the statistical test results were heavily influenced by the much larger number of replicate plants (269) for height growth than for biomass (75). Height growth was significantly affected by treatment, site, and their interaction. Results were similar in both analyses but less pronounced when excluding Metropolitano (Table 4.1). Biomass was significantly affected by treatment X site interaction in both analyses and by treatment when excluding Metropolitano (Table 4.1).

Table 4.1. Summary statistics on seedling A) survival, B) height growth, and C) biomass. Seedlings of Lacistema aggregatum were grown in four tropical forest sites ("Site") spanning a steep rainfall gradient across the Isthmus of Panama. In each site, 25 seedlings each were exposed to four different treatments ("Treatment"): water-sprayed control, insecticide application, fungicide application, and a combination of insecticide plus fungicide application. In one of our forest sites, Metropolitano, all seedlings of the insecticide treatment were buried by a treefall causing survival to be zero (as missing seedlings were counted as dead). To gauge the potential bias introduced by this non-pest related absence of data, we present results of an analysis including Metropolitano and a separate analysis that excluded Metropolitano entirely. Chi-square values, p-values and the degrees of freedom (df) of a type-III Anova and the variance explained by each model (conditional R^2) are shown. Statistically significant values ($p < 0.05$) are highlighted in bold.

	Incl. Metropolitano		Excl. Metropolitano		
A) SURVIVAL	χ^2	*p*	χ^2	*p*	df
Treatment	24.34	**< 0.001**	2.13	0.547	3
Site	11.76	**0.008**	11.40	**0.003**	3
Treatment X Site	26.12	**0.002**	9.29	0.158	9
Conditional R^2	0.21		0.06		
B) HEIGHT GROWTH	χ^2	*p*	χ^2	*p*	df
Treatment	20.18	**< 0.001**	18.40	**< 0.001**	3
Site	21.02	**< 0.001**	10.95	**0.004**	2
Treatment X Site	49.59	**< 0.001**	37.49	**< 0.001**	6
Conditional R^2	0.33		0.28		
C) BIOMASS	χ^2	*p*	χ^2	*p*	df
Treatment	6.69	0.082	9.60	**0.022**	3
Site	1.35	0.716	1.45	0.485	2
Treatment X Site	21.19	**0.007**	19.98	**0.003**	6
Conditional R^2	0.45		0.48		

Under the natural conditions of the control treatment mean height growth varied significantly amongst sites without any trend with rainfall being highest in Metropolitano, followed by Pipeline Road, then Sherman and lowest in El Charco (Fig. 4.2) Mean biomass did not differ significantly among sites in the control treatment but tended to be lowest in El Charco, too. In contrast, in the insecticide, fungicide, and combination treatments, height growth and biomass were highest in the two sites with intermediate rainfall, with El Charco generally being the highest (especially in the combination treatment). This pattern was statistically significant in the insecticide treatment for height growth, the fungicide treatment in biomass, and in the combination treatment, i.e. with full pest exclusion, for both height growth and biomass (Fig. 4.2; Table S4.3).

Fungicide application reduced height growth significantly compared with the control in three sites (Metropolitano, Pipeline Road, and Sherman) and averaged across sites (Fig. 4.3 & S4.1). Insecticide had no significant effect. However, the combination treatment significantly reduced height growth in Metropolitano and Sherman, but enhanced height growth in El

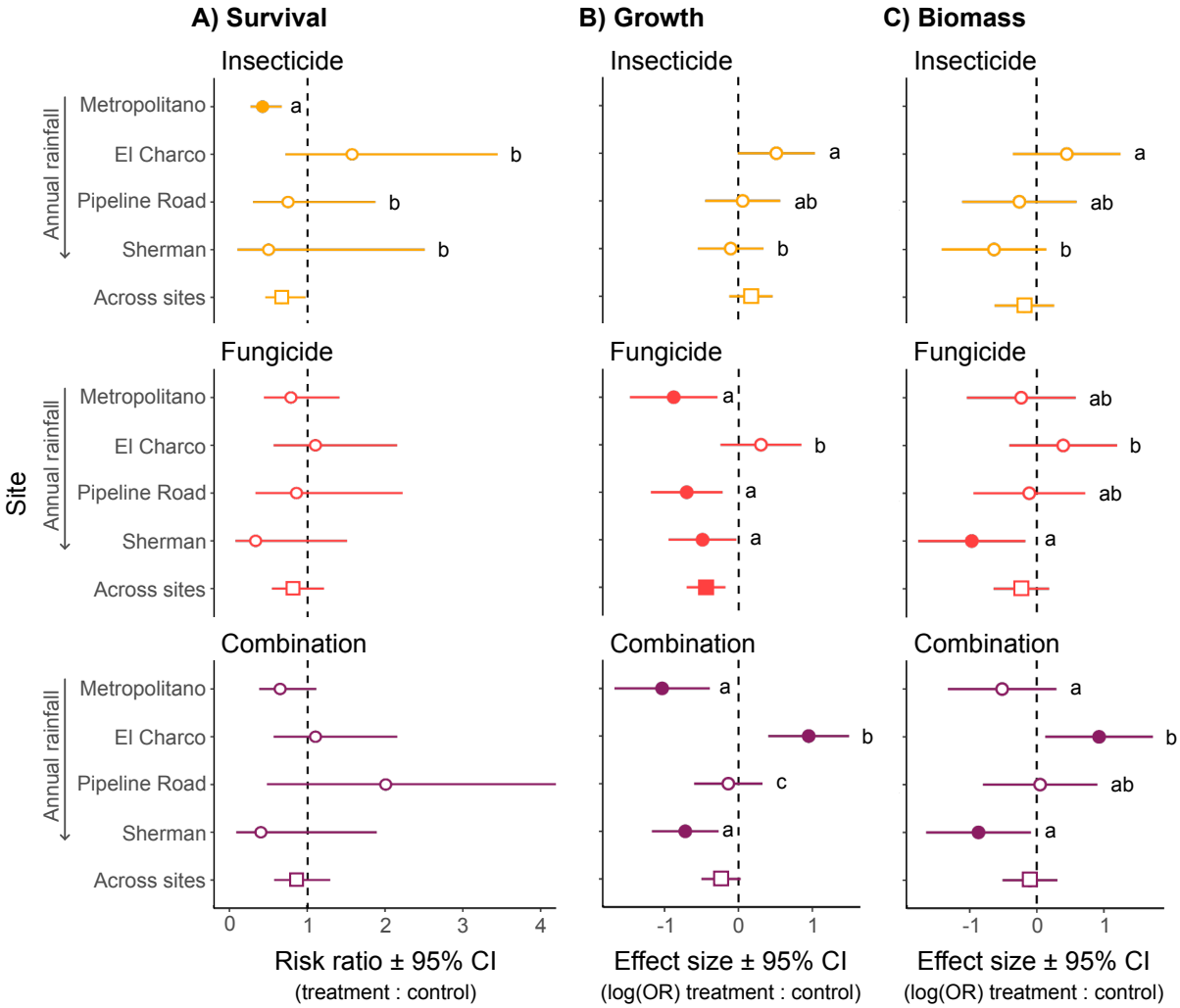


Figure 4.3. Effects of pesticides vary among forest sites along a rainfall gradient. We planted seedlings of the tree species *Lacistema aggregatum* to four tropical forest sites and measured **A**) survival, **B)** height growth ("Growth", stem base to apex), and **C)** biomass (sum of dry weight of roots, stems, and leaves) after 16 months. In each site, we exposed seedlings to four treatments: water-sprayed control, insecticide application, fungicide application, and a combination of insecticide plus fungicide application. We show effects of pesticide application as pairwise contrasts between each pesticide treatment and the control treatment in each site (circles) and averaged across sites (squares). Filled symbols indicate a significant effect of pesticide application ($p < 0.05$), with symbols to the right of the dashed line equaling positive effects on seedling performance of pesticide application relative to control. Letters represent statistically significant differences ($p < 0.05$) in effects among experimental forest sites.

Charco. Pesticide effects were mirrored in the biomass analyses (with less replicates), but fewer were significant than for height growth. While fungicide and combination treatments tended to reduce height growth in comparison with the control in three of the sites, they had positive effects on height growth and biomass in El Charco, significantly and more than doubled for the combination treatment (Fig. 4.3; Table S4.1, S4.4 & S4.5).

4.5 DISCUSSION

Seedling performance of *L. aggregatum* varied both among our four forest sites along the rainfall gradient and with pesticide treatment. Survival was greater with increased annual rainfall and lower seasonality. Chemical exclusion of insect herbivores and fungal pathogens ("pests" hereafter) did not affect survival but had site-specific effects on height growth and biomass. Height growth and biomass were highest in the two intermediately wet sites in all the pesticide treatments, yet were lowest in one of these sites in the natural control conditions, i.e. when pests were present. These results suggest that more vigorous seedlings of *L. aggregatum* in one of the intermediate rainfall sites experience stronger negative pest effects. In contrast, in the other three sites pesticide treatments had neutral or negative effects, which could be due to the seedlings overcompensating for pest damage or negative effects of pesticides on mutualisms. Lastly, the similar and additive effects of fungicide and insecticide treatments suggest that both insect herbivores and fungal pathogens contribute to variation in seedling performance of *L. aggregatum* along the rainfall gradient.

4.5.1 Seedling performance varied along the rainfall gradient

Seedling survival increased with rainfall, whereas biomass and height growth were highest in the two intermediately wet sites when pests were excluded (combination treatment in Fig. 4.2). Our results add to existing evidence that spatio-temporal variation in rainfall has strong effects on tropical seedling mortality (Fortunel et al., 2016; Muehleisen et al., 2020) and can drive large intraspecific variation in survival, with seedlings being 2.4 times as likely to survive in our wettest than our driest site (Table S4.3). Direct and indirect effects of moisture may limit seedling height growth and biomass at both extremes of our rainfall gradient. Restricted access to soil moisture and limited photosynthesis through drought-induced stomatal closure might reduce seedling performance in the driest site, whereas increased nutrient leaching or soil anoxic conditions due to temporal waterlogging might reduce seedling performance in the wettest site (Santiago & Mulkey, 2005). It is important to consider that other unmeasured environmental variables may vary amongst the four sites, e.g. soil phosphorus levels, which can have a strong effect on seedling performance (Condit et al., 2013; Gaviria et

al., 2017). Therefore, the design of our experiment does not allow a direct test of the effect of rainfall on seedling performance or its modification by the applied treatments. In conclusion, our results show the magnitude of variation in seedling performance amongst sites that are distributed along an environmental gradient within the distributional range of a tropical tree species.

4.5.2 Pest effects on plant performance

Pest exclusion significantly affected seedling height growth and biomass but not survival. Our results contrast with those of an earlier study that found pest effects on survival but not growth (Eichhorn et al., 2010). However, regional variation in seedling survival of tropical tree species was found to be independent of insect herbivory and pathogen damage (García-Guzmán & Benítez-Malvido, 2003) and local intraspecific variation in herbivory did not affect seedling survival (Fortunel et al., 2016; Solé et al., 2019). The widespread distribution of *L. aggregatum* (Condit et al., 2010) and thus its ability to establish in a wide range of different habitats might explain the absence of pest effects on its seedling survival rate.

Contrary to our expectation, effects of pest exclusion on height growth and biomass were not always positive but resulted in lower or similar plant performance relative to controls in some sites, with the strongest negative effects being those of the fungicide and the combination treatment on height growth (Fig. 4.3 & S4.1). Negative and neutral effects of pesticides could be driven by three mechanisms: phytotoxicity of pesticides, the elimination of fungal mutualists alongside pathogens, or overcompensation for damage.

First, no phytotoxic effects were reported by earlier studies using the same insecticide and the precursor of our fungicide in the field (Bagchi et al., 2014; Solé et al., 2019). Together with the positive effects of pesticides on performance in one of our sites, phytotoxicity of the pesticides seems an unlikely cause of our results.

Second, it is important to remember that our measurements are net effects reflecting the sum of pest damage, plant response, and mutualisms. Like most neotropical tree species (Mangan, Herre, et al., 2010), *L. aggregatum* is likely to be associated with mycorrhizal fungi.

Mycorrhizas can increase seedling growth (Mangan, Herre, et al., 2010) and their elimination through fungicide application could thus have caused the negative effects on plant performance compared with untreated controls. For this to cause the inconsistent direction of effects among sites (Fig. 4.3 & S4.1) it would require a context-dependent strength of mutualism, which has been shown in the laboratory (Kiers et al., 2011), or variation in the mutualist-pathogen ratio across sites, which is quite plausible but for which we found no published evidence nor did we address this experimentally ourselves in this study.

Lastly, (over-)compensation for pest damage resulting in better performance in damaged than undamaged seedlings (Garcia & Eubanks, 2019; Wise & Abrahamson, 2008) could have caused negative effects of pest exclusion. Overcompensation for insect herbivory has been shown to be prevalent among plant families and growth forms (Garcia & Eubanks, 2019). Variation in the ability of plants to overcompensate with environmental context has been suggested to explain positive effects of herbivory on tree growth and variation in the net effects of pest damage on trees across forest sites (Schuldt et al., 2017).

We did not measure pest damage and thus cannot finally conclude on the cause of negative effects of pesticides on seedling performance. Nonetheless, our results clearly show that pest effects on seedling performance can be both positive or negative. While the negative effects of fungicide were generally stronger than those of insecticide, the direction of effects was identical, suggesting that both insect herbivores and fungal pathogens contribute to variation in seedling performance in an additive manner.

4.5.3 Pest effects varied along the rainfall gradient

When both insect and fungal pests were excluded (combination treatment), seedling height growth and biomass were highest at El Charco, where they were lowest under the natural control conditions (Fig. 4.2). The exclusion of insect herbivores and fungal pathogens increased seedling height growth and biomass by 150% at this site (Fig. 4.3, Table S4.1). Seedlings thus experienced strongest negative pest effects and benefited most from pest exclusion where they were most vigorous (amongst the four tested sites).

Plant tolerance of pest damage may change with plant vigour and could have driven this pattern. The majority of studies, however, report increasing tolerance with plant vigour, whereas limited resources intensify pest effects by reducing a plant's ability to compensate for tissue loss in meadow plants (Cronin et al., 2010; Wise & Abrahamson, 2008) and tropical saplings (Norghauer & Newbery, 2013). In only 2 of 18 studies was tolerance higher in low resource environments (Wise & Abrahamson, 2008). Reduced tolerance is thus a possible, yet an unlikely explanation of our result.

More vigorous plants are expected to attract more pest damage (Price, 1991). Earlier studies provided support for the plant vigour hypothesis and found insect herbivores to cause higher damage on tropical saplings that have higher nutritional quality or growth (Boege & Dirzo, 2004; Norghauer & Newbery, 2013; Uribe-Mú & Quesada, 2006) and insect abundances to be higher on more vigorous plants (Cornelissen et al., 2008). A lower allocation to defences at high resource availability could further strengthen the attraction of pests to more vigorous plants (Boege & Dirzo, 2004; but see: Coley & Barone, 1996). While we did not measure pest damage in this experiment, we had previously found insect herbivory on naturally growing *L. aggregatum* saplings to be highest in El Charco among six sites along the rainfall gradient (including all sites of the present study) (Weissflog et al., 2018). In combination, our results provide support for the plant vigour hypothesis and suggest that in *L. aggregatum* vigorously growing seedlings attract more pest damage. They also provide new evidence that this effect may exist for fungal pathogens as well as for insect herbivores, and particularly for their combination.

Seasonal and temporal variation in rainfall has been suggested to affect pest abundance and damage (Garrett et al., 2006; Givnish, 1999; Romero et al., 2022) and may drive increases or decreases of pest damage with rainfall at the community level (Brenes-Arguedas et al., 2009; Muehleisen et al., 2020; Weissflog et al., 2018). Here we show that variation in plant vigour along a rainfall gradient may be equally or more important in determining pest pressure experienced by individual species.

4.5.4 Limitations

This study needs to be considered as exploratory. We tested the effects of pesticide treatments over a range of environments across the distributional range of a tree species in Panama and explored the amount of variation in these treatment effects across this range. We acknowledge that there are various environmental factors in addition to rainfall that vary between the study sites.

Our sampling strategy limits the impact of our results. Treatments were applied in blocks with seedlings being spatially grouped, rendering individual seedlings non-independent and increasing the risk of losing entire treatment X site combinations (as happened for the insecticide treatment in Metropolitano). Within the constraints of the treatment method, future studies should preferably use a smaller number of seedlings per plot (i.e. several multi-seedling plots per treatment X site combination, allowing for binomial analyses) or set out seedlings individually so that each can be treated as an independent replicate of the treatment, thus increasing the power of the statistical test of treatment effects.

The small number of forest sites, the lack of data on among-site variation in other environmental variables, and the absence of direct manipulations of rainfall mean that our results are not necessarily explained by variation in rainfall. Variation in plant performance may result from changes in other environmental factors (e.g. the interaction of soil phosphorus and drought severity: Condit et al., 2013; Gaviria et al., 2017) across the isthmus of Panama. While previous studies found rainfall to be a main determinant of seedling survival and performance along this gradient and often compare only one “dry” against one “wet” site (e.g. Baltzer & Davies, 2012; Engelbrecht et al., 2007) and show that herbivory varies with tree species X site combination (Muehleisen et al., 2020) we cannot ultimately determine the driver of variation in plant performance among our forest sites within the species’ range.

4.6 CONCLUSION

We show strong intraspecific variation in seedling performance of *L. aggregatum* along a rainfall gradient. Pest pressure had site-specific effects on seedling height growth and biomass but not survival. Insect herbivores and fungi had the strongest negative effects on *L. aggregatum* performance at the site with highest seedling vigour, suggesting a higher attraction of insect herbivores or fungal pathogens. Similar and additive effects of insects and fungi on height growth and biomass suggest that both types of pests have important effects on seedling performance.

The unavoidable early termination of pesticide applications weakened the power of this experiment to test treatment effects. The presence of performance differences more than a year after the last pesticide application however adds to existing evidence (Norghauer & Newbery, 2013) that pest damage during the initial stage of seedling establishment can have persistent effects on plant performance at later life stages with potential implications for species distribution and plant community composition.

Variation in annual rainfall and its seasonality has been shown to affect seedling performance and pest damage at the community level (e.g. Baltzer & Davies, 2012; Brenes-Arguedas et al., 2009). We show that environmentally-driven variation in plant vigour may have an important effect on pest pressure along a rainfall gradient. Understanding the relative contribution of pest damage and plant vigour to plant performance in different environmental contexts will enhance our ability to predict how projected changes in rainfall patterns (Pokhrel et al., 2021) will affect species distributions and ultimately plant community composition.

4.7 ACKNOWLEDGEMENT

Lydia J. Martin helped to plant seedlings and to apply pesticides in the first four months of the experiment during which she measured seedling performance and pest damage for her masters thesis. The data presented here are entirely different to those collected by Lydia. It will be interesting to combine our data on pest effects with Lydia's pest damage measurements. This will allow us to assess the relative contribution of pest damage, plant vigour and overcompensation to variation in pest effects along the rainfall gradient.

Chapter 5: Do prey shape, daytime, and plant trichomes affect the predation rate on plasticine prey in tropical rainforests?

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Author contributions: AW, LM and IG developed the study idea and design. AW and IG conducted the field experiments. AW led the writing of the manuscript and conducted the statistical analyses. All authors contributed to the manuscript and gave final approval to the publication.

5.1 ABSTRACT

Predation can effectively limit insect herbivores with cascading effects on plant community composition and diversity of tropical rainforests. Assessing variation in predation is therefore important to understand the mechanisms structuring complex rainforest ecosystems. Variation in predation with daytime may provide herbivores with temporal enemy-free space. Trichomes (plant hairs) may provide spatial enemy-free space by increasing climbing resistance for walking arthropod predators and by scattering bat echolocation calls. Artificial model prey is commonly used to measure predation pressure on insect herbivores. Whether model prey shape is sufficient to deceive predators and whether attacks represent actual predation however remain unresolved.

We used artificial, plasticine prey to assess temporal and spatial variation in predation in two Panamanian rainforests and tested whether model prey shape is as important for prey recognition by predators as often assumed. We assessed the effect of prey shape and size, daytime, and trichomes on predation by comparing attacks on caterpillar- and humanoid-shaped figurines.

We find higher nocturnal than diurnal predation in one but not the other forest, suggesting that herbivores may benefit from enemy-free space during the day in some forests. We find no evidence for an effect of trichomes on predation in the two plant species tested. Equal attack numbers on caterpillar- and humanoid-shaped objects challenge the idea that the visual resemblance of model prey alone is sufficient to deceive predators. We conclude that attacks on model prey represent a variety of responses to novel objects (e.g. exploration, aggression, possibly predation), and urge caution when interpreting their results.

5.2 INTRODUCTION

Insect herbivores cause tropical trees to lose around a quarter of their foliage (Arnold & Asquith, 2002) and can directly affect the plant community composition of rainforests (Szefer et al., 2020). Insect herbivores may further contribute to the high diversity of tropical rainforests by mediating the coexistence of plants with different defensive strategies (Coley & Kursar, 2014). Predation can effectively limit herbivory (Harrison & Banks-Leite, 2020; Kalka et al., 2008; Styrsky et al., 2006) with cascading effects on plant performance (Styrsky et al., 2006), community composition (Harrison & Banks-Leite, 2020), and diversity (Chesson & Kuang, 2008). Assessing temporal and spatial variation in plant-herbivore-predator interactions is crucial to advance our understanding of the mechanisms affecting the structure and stability of tropical rainforest communities.

Predation can affect the chemical traits, physiology, and behaviour of insect herbivores (Greeney et al., 2012) and drives their behavioural search for enemy-free space, i.e. times or localities at which predation risk is reduced. Direct evidence of diurnal variation in predation within forests is scarce and the few existing studies report inconsistent results of either higher (Seifert et al., 2016) or lower (Ferrante et al., 2017) daytime versus nighttime predation. Nocturnal feeding by undefended caterpillars of some temperate and subtropical Lepidoptera species has been suggested to be behaviour that avoids predominately diurnal predators (Berger & Gotthard, 2008; Heinrich, 1979). In tropical forests, however, caterpillar activity seems not to vary between day and night (Novotny et al., 1999) and predator exclosures had a stronger positive effect on insect herbivore abundance at night than during the day (Kalka et al., 2008). We thus suggest that higher nocturnal predation in tropical forests may provide herbivores with temporal enemy-free space during the daytime.

Spatial enemy-free space could be provided by trichomes (plant hairs). Non-glandular trichomes are a common feature of tropical rainforest plants (Ichie et al., 2016) and present a structural defence against insect herbivores by reducing the insects' mobility and feeding efficiency (Gorb & Gorb, 2019; Hanley et al., 2007; Levin, 1973). It thus seems surprising that butterflies often prefer pubescent plants for oviposition on which their offspring suffer from reduced growth rates and survival (Jones & Agrawal, 2019; Kariyat et al., 2017). Such

oviposition preference in swallowtails has been explained by a reduced mobility of arthropod predators, which captured fewer caterpillars on pubescent plants (Fordyce & Agrawal, 2001). Predation is the greatest source of larval mortality for many insect herbivores (Cornell & Hawkins, 1995) and may drive the selection of pubescent food plants because the positive effect of reduced predation outweighs associated costs in larval growth.

While trichomes should not affect predation by birds or flying arthropods, they may compromise the ability of two main predator groups to detect and access their insect prey. Walking arthropods, and ants in particular, are important and abundant predators of insect herbivores in tropical rainforests (Leponce et al., 2021; Novotny et al., 1999). Ants have been shown to be impaired by trichomes (Davidson et al., 1989) and to prefer climbing glabrous stems over stems covered with stiff trichomes while foraging (Gorb & Gorb, 2019) possibly due to the increased friction imposed by downward pointing stem trichomes (Vermeij, 2015). Stem trichomes could provide herbivores with enemy-free space because walking arthropod predators avoid the higher energy expenditure of climbing pubescent stems during opportunistic foraging if other substrates are available.

Predation efficiency of bats, another major group of predators of insect herbivores in neotropical rainforests (Kalka et al., 2008; Kalka & Kalko, 2006), could be affected by foliar trichomes. The acoustic specular effect by which smooth surfaces, e.g. glabrous leaves, reflect echolocation calls like a mirror makes it easy for bats to locate prey resting on a smooth surface (Geipel et al., 2019). Rough surfaces however can scatter echolocation calls (Clare & Holderied, 2015) and reduce the detectability of prey. Surface protrusions of just 30-μm can alter reflected echoes sufficiently to impact bat behaviour (Habersetzer & Vogler, 1983). We thus hypothesize that several millimetre-long foliar trichomes can scatter bat echoes and reduce the predation risk of insects. In conclusion, the primary plant defences stem and foliar trichomes may reduce predation pressure and provide herbivores with enemy-free space on pubescent plants (Fig. 5.1).

Measuring predation of insect herbivores in natural settings is complicated as predation often happens quickly with predators leaving no traces of their prey. Artificial, plasticine caterpillars in which predators leave distinctive attack marks without removing the objects (Howe et al., 2009; Lövei & Ferrante, 2017) are thus commonly used to estimate predation

risk of insect herbivores. Such model prey offers many advantages including its cheap and simple production, wide applicability (Howe et al., 2009), and comparability of results across studies and treatments. However, model prey lacks olfactory and chemical characteristics that predators use as cues for prey identification (summarized in Howe et al., 2009; Lövei & Ferrante, 2017). This is generally accepted as an explanation for the typically lower estimates of predation derived by using model prey compared to those derived using real prey (Lövei & Ferrante, 2017). However, we know little about the effect of model prey shape on predation estimates. It is widely assumed that attacks on model prey represent predation events because predators perceive objects mimicking prey colour and shape as real prey. This assumption may hold true for birds and some arthropod predators, e.g. wasps and mantids (Greeney et al., 2012), that mainly use visual cues and start attacks from a distance. Most arthropods – that typically cause most attacks on plasticine prey in tropical forests (X. Liu et al., 2020; Seifert et al., 2016) – however, get much closer to their prey before attacking it and other cues may be of greater importance. Together with the repeated appearance of marks left by non-predators on plasticine objects (Pfennig et al., 2007) this fuels a debate on whether plasticine caterpillars record predation specifically, or otherwise a range of animal responses to a novel object, possibly including predation.

Here, we use plasticine prey to quantify variation in predation pressure on insect herbivores in tropical rainforests. We hypothesize that *(a) predation will be higher during the night than during the day*. Stem trichomes may increase climbing resistance for walking arthropod predators and foliar trichomes may disturb the acoustic specular effect for bats. We thus hypothesize that *(b) predation will be higher on glabrous than on pubescent plants*. Lastly, we test the widespread assumption that prey shape is a decisive factor for prey recognition by comparing attacks on caterpillar- versus humanoid-shaped objects resembling The Incredible Hulk superhero (hereafter “hulks”). We hypothesize that *(c) predation will be higher on caterpillars than on hulks* because predators recognize model prey by its shape.

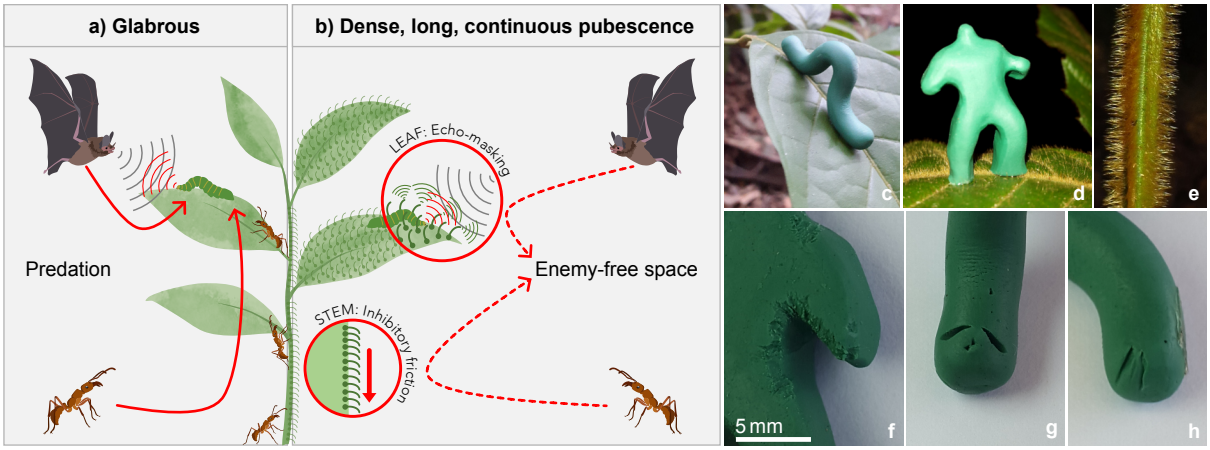


Figure 5.1. Illustration of hypothesis 2. (a) Successful echolocation of a caterpillar by bats and ascent of walking arthropod predators on glabrous plants. (**b)** Foliar trichomes reduce the acoustic specular effect and mask prey echoes, and stem trichomes inhibit arthropod ascent, thereby creating enemy-free space for herbivores on pubescent plants. (**c)** Artificial caterpillar on a glabrous *Cupania cinerea* leaf, and (**d)** hulk on a pubescent *C. rufescens* leaf. (**e)** Stem pubescence of *C. rufescens.* Attack marks by (**f-g)** chewing arthropods, and (**h)** a bird (scale bar applies to f-h only).

5.3 METHODS

We conducted two experiments in the understory of two lowland rainforest sites in Panama to assess temporal and spatial variation in predation and test the importance of model prey shape and size for predator recognition. Both experiments were performed in closed-canopy sites with flat terrain and at the end of the dry season to minimize the impacts of frequent rainfall which can dislodge model prey (Howe et al., 2009).

5.3.1 Island experiment

We compared diurnal and nocturnal attacks on plasticine caterpillars and hulks placed on glabrous and pubescent plants in the understory of Barro Colorado Island (BCI) in Panama [9°09’37’’N, 79°50’45’’W; 2600 mm yr^{-1} rainfall, 3 month dry season (Leigh, 2019)] in February 2020. To standardize host plant height and distance among hosts, we cut similar-sized branches and arranged them in a 10 x 10 matrix with a distance of 1 m between individual branches and alternating between a pubescent and a glabrous species. Branches were cut from young trees with a maximum height of 3 m that grew individually (not

clustered) within a 1 km radius of the experimental site. We cut between one and three 40 to 80 cm long branches per plant, that had a minimum of two undamaged leaves, a branch diameter of 0.8 to 1.2 cm, and vertical growth with leaf angles similar to those of small saplings. Directly after they were cut, branches were placed into sealed water containers that were buried in the soil to keep the branches hydrated for the duration of the experiment and mimic a naturally growing plant. We selected sites with minimal understory vegetation and our experimental branches did not touch any plants that were naturally growing in our sites. Small flags marked the experimental area and facilitated retrieval in the understory, while avoiding effects on predator movement and bias through direct tagging of plants.

The congeneric tree species *Cupania rufescens* Triana & Planch. and *C. cinerea* Poepp. (Sapindaceae) were selected as focal species as both coexist in the BCI forest and their structural similarity precludes any potential effects of leaf shape and general plant architecture on predation. *Cupania rufescens* is a medium-sized tree growing in tropical rainforests from Mexico to Brazil. Young plants have dense pubescence on stems, branches, and both surfaces of the compound leaves (Fig. 5.1) with obovate-oblong leaflets with serrated margins [7-22 cm long x 3.5-10 cm wide; (Woodson et al., 1976)]. *Cupania cinerea* occurs in lowland tropical rainforests from Costa Rica to Bolivia as a medium-sized tree, has compound leaves, obovate leaflets and also a serrated margin (6-17 x 3.5-7cm), but all plant parts are glabrous (Fig. 5.1) except for soft trichomes covering the lower leaf surface (Woodson et al. 1976) that should not affect predation by bats or arthropods. None of the species is myrmecophytic. Many lepidopteran caterpillars feed on the upper leaf surfaces of *C. rufescens* and *C. cinerea*, with six species known to feed on both (Janzen & Hallwachs 2019). Six out of seventeen and four out of twelve recorded herbivore species associated with *C. rufescens* and *C. cinerea* respectively, are inconspicuously brown or green, without obvious defences, and 28–46 mm long (Janzen & Hallwachs 2019). We used plasticine caterpillars to visually mimic this subset of the tree species' known natural herbivores.

Predation pressure was measured using model prey. Caterpillars (50 x 4 mm) and hulks (with a height of 30 mm that equalled the maximum height of the bent caterpillars) were moulded from green, odourless (to humans), non-toxic Newplast (Newclay Products Ltd., Newton Abbot, UK). Caterpillars were shaped and bent to mimic the posture of common geometrid

caterpillars. Hulks resembled small (but fearsome) humanoid figurines, modelled on the superhero of Marvel comics (Marvel Worldwide Inc.), and were shaped using custom made plastic moulds. We used hulks as a control to the more naturally shaped caterpillar models to test whether objects resembling natural prey are more recognized as such by potential predators. All objects were modelled and handled using surgical gloves to avoid leaving unwanted cues (e.g. scent) to predators. One hundred hulks and one hundred caterpillars were placed on 50 plants per host species (i.e. one object of each type per plant). For each plant, we selected two undamaged leaves that grew in opposite directions and at a similar height, 30 to 80 cm above the ground. We attached the objects close to the midrib on the upper leaf surface with a small amount of fast-setting glue (Loctite Superglue, Henkel AG, Düsseldorf, Germany).

For four consecutive days (total of 96 h), plasticine objects were inspected at 12-hour intervals at dusk and dawn (18.15 to 19.15 and 06.15 to 07.15 h) to differentiate diurnal from nocturnal predation. We removed attacked objects from the sites without replacement and identified predator attack marks using reference pictures (e.g. Low et al., 2014).

5.3.2 Mainland experiment

To assess the impact of model size as an additional effect and test for a possibly confounding effect of host plant transplantation in the island experiment, we conducted a follow-up experiment using uncut saplings growing in a close-by mainland forest site in Gamboa, Parque Nacional Soberanía (9°9’42’’N, 79°44’43’’W) in early-March 2021. Following the same procedure as for the island experiment described above, we glued 27 caterpillars, 27 hulks, and 27 small caterpillars (30 x 2 mm) close to the midrib on the upper side of leaves of 27 tree saplings (one object of each type per sapling). In this follow-up experiment, however, we did not select specific plant species, but chose saplings of various species that were naturally growing in the field site. All plants were of similar size (40-100 cm tall), with simple, elliptic to ovate, smooth-edged leaves, and without any foliar or stem pubescence.

5.3.3 Statistical analysis

All statistical analyses were performed in R (R Core Team, 2021).

Predation risk. – We analysed predation risk as the total percentage of attacked objects at the end of each experiment using pairwise two-sided Fisher's exact tests and calculated the estimated odds ratio (OR) as a measure of effect size (R package rstatix). Given our relatively small sample sizes and attack numbers, many values in the contingency table of expected results for Chi-square tests were ≤ 5, in which case the Fisher's test is the preferred analytical method as it does not rely on an approximation but is exact (McDonald, 2009). We performed pairwise tests with Holm-adjusted p-values to correct for multiple comparisons to identify variation in predation risk with daytime and pubescence for each possible object-experiment combination and for the predation risk averaged across objects per experiment. To test for interaction effects, we calculated pairwise tests for all combinations of pubescence, daytime, and object in the island experiment and all combinations of object and daytime in the mainland experiment. Lastly, we compared overall, diurnal, and nocturnal predation risk across objects between the island and mainland experiment. Missing objects were excluded from the analysis.

To facilitate comparison with other studies we additionally provide mean daily predation risk, which is the predation risk divided by the number of exposure days (e.g. Molleman et al., 2016; Seifert et al., 2016).

Predation rate. – To incorporate the temporal component of predation, we calculated predation rates (PR) as the ratio between the number of attacked objects and the time at risk, i.e. the number of hours that each object was exposed, totalled over all objects per object type. For each object we added the hours of exposure until one of three possible events: attack, loss, or the end of the experiment. An object that remained without attack until the end of the experiment added 0 to the nominator and the full experimental time of 96 hours to the denominator. An attacked object added 1, and a lost object added 0 to the

Table 5.1. Predation risk of three model prey objects in two Panamanian rainforests. Risk is the total percentage of attacked objects at the end of the island and mainland experiment. Mean predation risk is predation risk averaged per 24 hours. Predation rate (PR) ± standard error (SE) is the number of attacked objects divided by the time at risk, i.e. the sum of hours each individual object was exposed to predation totalled over all objects of each type. PR can be interpreted as number of attacked objects per 1,000 objects exposed for 1 hour.

	Day			Night			Total		
	Risk (%)	Mean risk (% $24h^{-1}$)	PR ± SE	Risk (%)	Mean risk (% $24h^{-1}$)	PR ± SE	Risk (%)	Mean risk (% $24h^{-1}$)	PR ± SE
Island experiment									
Caterpillar									
Glabrous	4.00	2.00	0.85 ± 0.75	4.00	2.00	0.86 ± 0.77	8.00	2.00	0.87 ± 0.51
Pubescent	8.00	4.00	1.71 ± 0.10	6.00	3.00	1.29 ± 0.90	14.00	3.50	1.55 ± 0.66
Total	6.00	3.00	1.28 ± 0.59	5.00	2.50	1.08 ± 0.55	11.00	2.75	1.21 ± 0.40
Hulk									
Glabrous	10.00	5.00	2.21 ± 1.13	4.00	2.00	0.85 ± 0.76	14.00	3.50	1.60 ± 0.68
Pubescent	0.00	0.00	0.00 ± 0.00	8.00	4.00	1.73 ± 1.01	8.00	2.00	0.86 ± 0.50
Total	5.00	2.50	1.07 ± 0.55	6.00	3.00	1.29 ± 0.60	11.00	2.75	1.22 ± 0.40
Combined									
Total	5.50	2.75	1.17 ± 0.39	5.50	2.75	1.18 ± 0.39	11.00	2.75	
Mainland experiment									
Small caterpillar									
Total	4.17	2.08	0.90 ± 1.27	20.83	10.42	4.99 ± 2.56	25	6.25	3.09 ± 1.43
Caterpillar									
Total	3.70	1.85	0.80 ± 1.13	11.11	5.56	2.42 ± 1.67	14.81	3.70	1.66 ± 0.97
Hulk									
Total	3.85	1.92	0.83 ± 1.17	7.69	3.85	1.67 ± 1.48	11.54	2.88	1.29 ± 0.90
Combined									
Total	3.90	1.95	0.84 ± 0.58	12.99	6.50	2.90 ± 1.00	16.88	4.22	

nominator. As we cannot know when exactly an attack or loss occurred within a 12-hour census interval, we assume that the event happened on average halfway through the respective census period (Cummings, 2019) and thus added 6 hours to the time at risk. Hence, each attacked or lost object contributed the hours of pre-event censuses plus the 6 hours of the census period during which the event occurred to the denominator. Predation rates can thus be understood as the predicted number of attacked objects in 1,000 object-hours of exposure (Cummings, 2019).

We calculated predation rates for the full experimental period (96 hours) and separately for diurnal and nocturnal time periods (48 hours each) for each object and each object-pubescence combination (function epi.conf in R package epiR). To test for statistic differences, we conducted pairwise comparisons of estimated point differences at a confidence level of 0.95 (ratedifferences in fmsb).

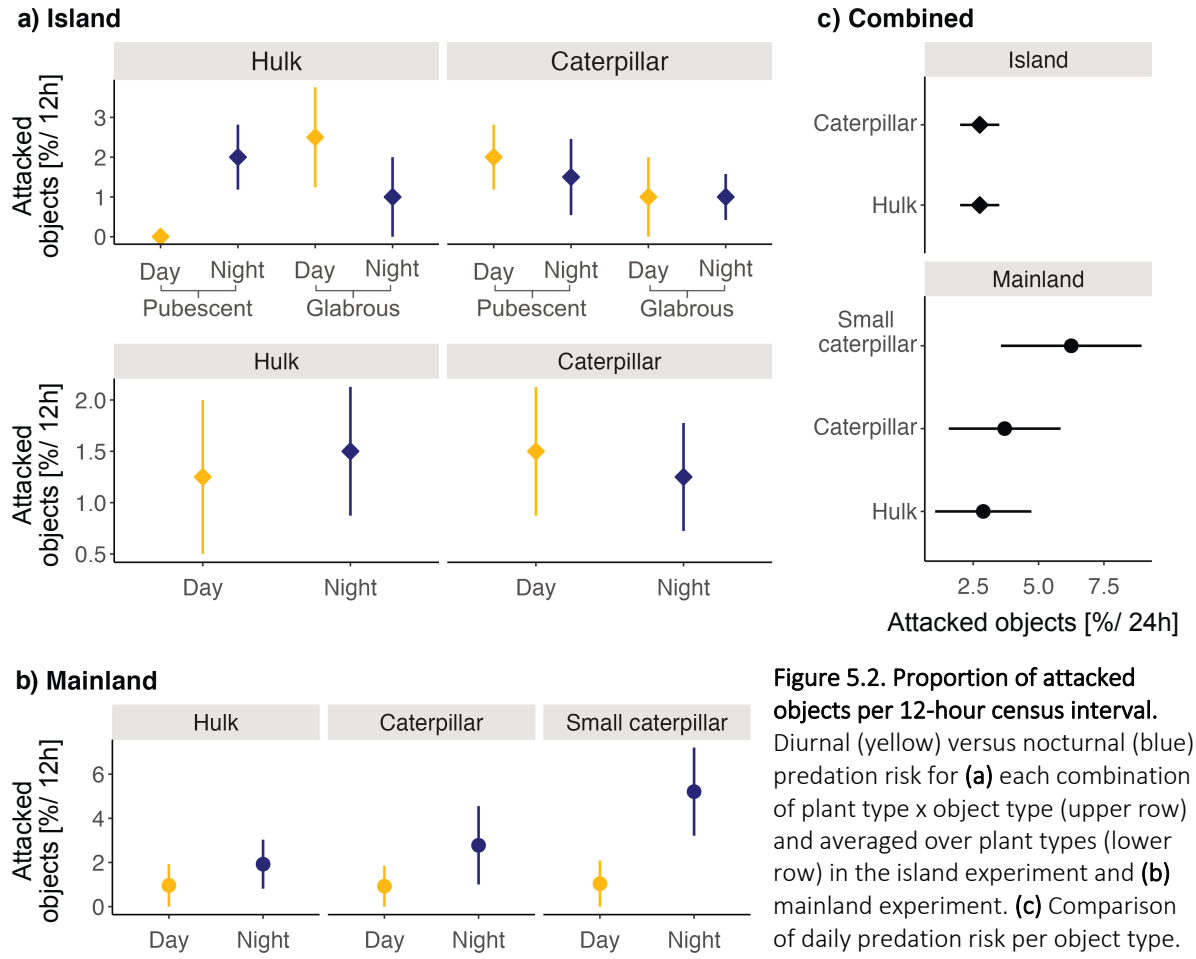


Figure 5.2. Proportion of attacked objects per 12-hour census interval. Diurnal (yellow) versus nocturnal (blue) predation risk for **(a)** each combination of plant type x object type (upper row) and averaged over plant types (lower row) in the island experiment and **(b)** mainland experiment. **(c)** Comparison of daily predation risk per object type.

5.4 RESULTS

A total of 11.0% and 16.9% of objects were attacked in the island and mainland experiment, resulting in mean daily predation risks of 2.8% and 4.2%, respectively (Table 5.1). In both experiments, predation was almost exclusively due to arthropods (Fig. S5.1). In the island experiment, one out of 22 attacks was due to a bird (Fig. 5.1) and on the mainland, one out of 13 attacks was caused by a small mammal (Fig. S5.1). We lost one hulk during the island experiment, and one hulk and three small caterpillars during the mainland experiment, equalling 0.5% and 4.9 % of exposed objects.

5.4.1 Variation with daytime

In the island experiment, predation risk across objects was 5.5% both during the day and during the night (Table 5.1). Even within objects, diurnal and nocturnal predation risk was almost identical being 1% higher at daytime for caterpillars (OR = 1.2, p = 1) and 1% higher at nighttime for hulks (OR = 0.8, p = 1; Fig. 5.2a & Table S5.1). Likewise, predation rates (± *SE*) did not differ between day (1.2 ± 0.4) and night (1.2 ± 0.4) across objects (p = 0.99) but was slightly higher at night for hulks on pubescent plants (p = 0.046; Table S5.2).

In the mainland experiment, the predation rate was significantly higher at night than during the day (p = 0.047; Fig. S5.2, Table 5.1 & S5.2). A non-significant, yet consistent trend of a higher nocturnal predation rate was evident for all three object types but was strongest for small caterpillars that were five times more likely to be attacked during the night than during the day (PR_{day} = 0.9 ± 1.3, PR_{night} = 5.0 ± 2.6, p = 0.09; Table 5.1). Similarly, nocturnal predation risk was three times higher for caterpillars, and doubled for hulks (Table 5.1, Fig. 5.2 & 5.3).

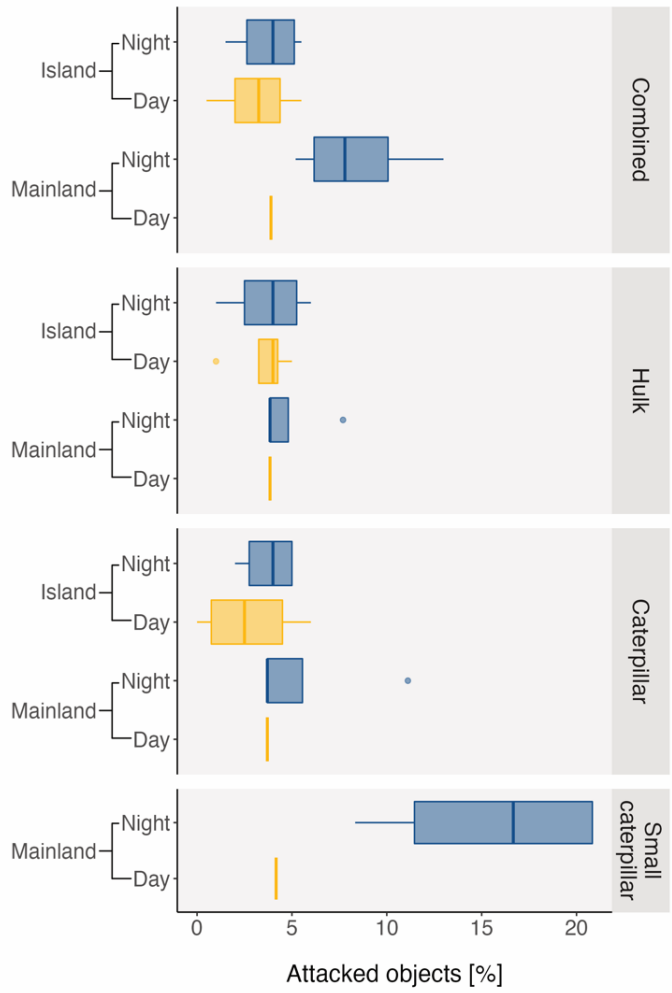


Figure 5.3. Diurnal variation in predation. Daytime (yellow) and nighttime (blue) predation expressed as cumulative percentage of attacked model prey objects over time for small caterpillars, caterpillars, and hulks in the mainland and island experiment in two Panamanian rainforests.

5.4.2 Variation with host plant surface

Neither predation rate (Table S5.2, Fig. 5.2a), nor predation risk differed between glabrous and pubescent host plants when averaged across objects and in the separate analyses for hulks and caterpillars, with a total of 11 objects attacked on each plant type (OR = 1, p = 1; Table S5.1).

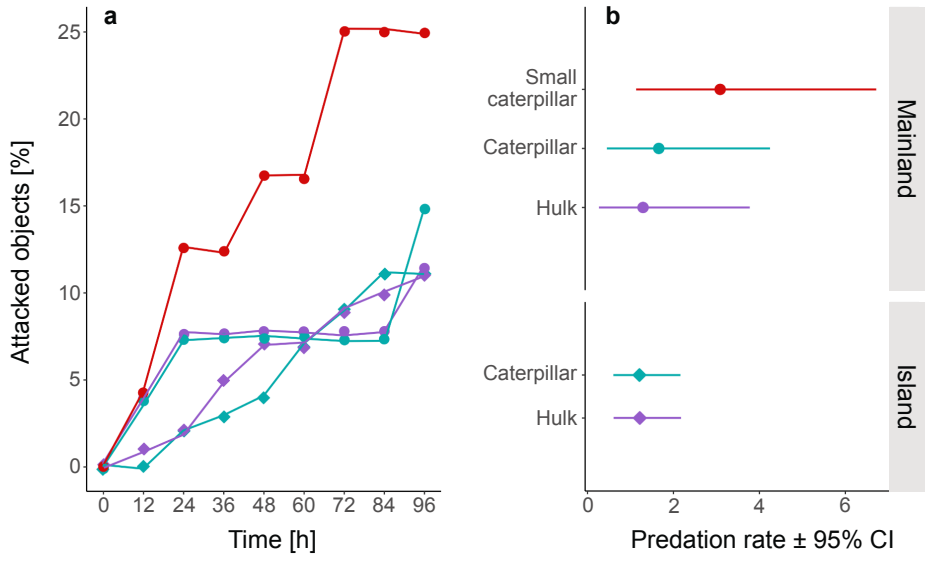


Figure 5.4. Predation risk over time and predation rate on three model prey objects. (a) Cumulative percentage of attacked objects over time and (**b**) mean predation rate with 95% confidence intervals for small caterpillars (red), caterpillars (turquoise), and hulks (purple) in the island (diamond) and mainland (circle) experiments.

5.4.3 Variation with prey object

In the island experiment, hulks and caterpillars experienced an equal predation risk of 11% (OR = 1, p = 1; Table S5.1) and equal predation rates of 1.2 ± 0.4. In the mainland experiment, we found a strong, albeit statistically not significant (all $p > 0.2$, Table S5.1 & S5.2), trend towards higher predation risk and predation rate in small caterpillars (risk = 25%; PR = 3.1 ± 1.4) as compared to caterpillars ($risk_{mainland}$ = 14.8%, $risk_{island}$ = 11%; $PR_{mainland}$ = 1.7 ± 1, PR_{island} = 1.2 ± 0.4) and hulks ($risk_{mainland}$ = 11.5%, $risk_{island}$ = 11%; $PR_{mainland}$ = 1.3 ± 1, PR_{island} = 1.2 ± 0.4; Table 5.1, Fig. 5.2 & 5.4). Predation risk and rate did not differ between the experiments for caterpillars, hulks or across the two object types (Table S5.1 & S5.2).

5.5 DISCUSSION

We used artificial prey to evaluate spatial and temporal variation in predation pressure on insect herbivores in two tropical rainforest sites and test the importance of model prey shape for predator recognition. Predation was higher at night than during the day in one of our sites, but did not vary with daytime in the other site. Whether insect herbivores benefit from temporal enemy-free space may thus be locality-specific. Predation did not differ between objects placed on pubescent or glabrous *Cupania* plants, suggesting that in this plant genus

trichomes may not provide enemy-free space to insect herbivores. Object shape did not affect the number of attacks, suggesting that prey shape may not be as important for predator recognition as previously assumed. Our results thus highlight the need for caution when interpreting model-prey-derived estimates of predation.

Mean daily predation risk was 2.8% in the island experiment and 4.2% in the mainland experiment (Table 5.1). While these levels are low, they are comparable to the 2.4% of artificial caterpillars attacked per day in other lowland rainforests (Tvardikova & Novotny, 2012). Model-prey-derived estimates of predation typically underestimate actual predation pressure (Lövei & Ferrante, 2017) and a daily mortality rate of 5% has been projected to result in a mortality risk of 66% over a caterpillar's life span (Tvardikova & Novotny, 2012). Lastly, parasitoid attacks are not captured well by this method, yet further increase the mortality risk of real caterpillars. Thus, our results support previous evidence that predation strongly affects the abundance of herbivorous caterpillars in tropical rainforests.

5.5.1 Variation of predation with daytime

Nocturnal predation was significantly higher than diurnal predation in our mainland forest experiment, where objects were 3.5 times more likely to be attacked at night than during the day. However, this effect was not seen in the island experiment, where diurnal and nocturnal predation were notably similar (Fig. 5.3, Table 5.1). The result of our mainland experiment is in accordance with a higher nocturnal predation on stick insect nymphs in the BCI forest (Berger & Wirth, 2004) but contrasts with studies in other tropical rainforests that found that attacks peaked during the day (Novotny et al., 1999; Seifert et al., 2016).

In our study, 83% of attacks were caused by chewing arthropods and we suspect that beetles, orthopterans, and ants were main predators (Fig. S5.1). Carabid beetles (Ferrante et al., 2017) and orthopterans (Novotny et al., 1999) have been identified as important nocturnal predators of insects in forests. Ants also are dominant predators in tropical rainforests (Leponce et al., 2021), where they typically cause the overwhelming majority of attacks on artificial caterpillars (e.g. Molleman et al., 2016; Seifert et al., 2016). While ant attacks were reported overall not to vary between night and day (Novotny et al., 1999; Seifert et al., 2016),

shifts in ant community composition were shown to explain a marked increase in attacks within the first hours of the night in a tropical rainforest in China (X. Liu et al., 2020). We suggest that spatial variation in predator community composition and temporal niche partitioning might explain contradicting results between our experiments and among studies on diurnal variation in predation. A few particularly active or aggressive species that peak in their activity at a specific day- or nighttime might be sufficient to skew trends in either direction. Ultimately, our results do not support the assumption that nocturnal feeding by caterpillars is an effective strategy to escape predation (Berger & Gotthard, 2008) and in our mainland forest site the opposite may be the case with herbivores potentially benefiting from reduced predation pressure during the day.

5.5.2 Plant trichomes of *Cupania rufescens* do not provide enemy-free space

For our two host plant species we found no effect of pubescence on predation. An equal 11 out of the 100 objects placed on glabrous and pubescent plants respectively were attacked (Table 5.1, Fig. 5.2a), hence providing no support for the idea that trichomes of *Cupania* species provide insect herbivores with enemy-free space.

Due to the complete lack of detected attacks by bats, we cannot draw conclusions on the effect of trichomes on bat predation. The gleaning bat *Micronycteris microtis* – a common insectivore on BCI (I. Geipel, personal communication) – has been shown to use echolocation to discriminate between the surface structure of prey and attacked model prey made of crumbled aluminium similarly as real prey, while neglecting dummies made from paper and smooth aluminium (Geipel et al., 2013). Thus, some bats may use the combination of object shape and surface structure to differentiate between motionless insect prey and the environment. Given the complexity of the forest understory and the similarity of prey and background (e.g. imagine stick insects versus sticks) these skills can be expected to be extremely precise. It remains to be tested whether the difference in reflective properties between plasticine and chitin could explain the lack of bat attacks on plasticine model prey in our study. We recommend using a different material or real sentinel prey in future studies to address the question of whether trichomes may reduce the acoustic specular effect.

Arthropods readily attacked our objects despite the lack of prey-specific behavioural or chemical cues (Fig. 5.1f-g). Coming from the forest floor, walking arthropods can neither visually detect model prey, suggesting that foraging may be largely opportunistic. Earlier studies have shown that the identity of glabrous plants does not affect attacks on artificial (Molleman et al., 2016) nor live prey in tropical rainforests (Novotny et al., 1999). Trichomes were reported to be overall harmful for predatory insects across 47 studies (Riddick & Simmons, 2014) and to present a physical barrier to ants (Gorb & Gorb, 2019). However, here we show that walking arthropod predators are not deterred by dense, downward pointing stem trichomes of *C. rufescens* (Fig. 5.1e) and attack model prey on both glabrous and pubescent plants. In conclusion, we did not find evidence that *C. rufescens* foliar or stem trichomes can provide enemy-free space.

5.5.3 Not object shape, but size matters

Small caterpillars received twice as many attacks as caterpillars and hulks (Fig. 5.4a). Our finding agrees with a reported negative correlation between model prey length and number of arthropod attacks across 45 studies (Lövei & Ferrante, 2017). Smaller predators may prefer smaller prey due to a reduced risk of injury as compared to attacking large prey (but see: Molleman et al., 2016), while vertebrate predators may prefer bigger prey (Remmel & Tammaru, 2009).

Object shape did not affect our predation estimates and the humanoid-shaped hulks were attacked as frequently as large caterpillars (Fig. 5.3). Predators are argued to confuse plasticine caterpillars with palatable and undefended prey due to their resemblance in colour and shape (Howe et al., 2009; Lövei & Ferrante, 2017), hence this method has been used widely to test for predation of herbivores, as we did here. While predators were shown to not be attracted by the plasticine material itself (Tvardikova & Novotny, 2012), bite marks by non-predatory insects and rodents on plasticine snakes (Bateman et al., 2017; Pfennig et al., 2007), and video recordings of tree crickets and moths feeding on artificial caterpillars (Molleman et al., 2016) show that a variety of species can cause “attack marks” on model prey with only some of them being predators of true caterpillars. Some of the attacks on our

hulks may present opportunistic predation triggered by object size and unsuspicious coloring. Given their humanoid shape, we however suggest that many attacks result from defensive, exploratory, or aggressive behaviour and do not reflect predation attempts.

Artificial prey simulates visual prey cues but lacks any motion, chemical, and tactile cues. It is thus surprising that arthropods that rely on chemical and tactile cues accounted for 21 of 22 attacks in our island and 12 of 13 attacks in our mainland experiment. They were also responsible for more than 90% of attacks on artificial caterpillars in other rainforests (X. Liu et al., 2020; Seifert et al., 2016). Birds, which are mainly visually oriented, however caused only one out of 35 attacks in our study and are typically responsible for less than 5% of attacks on model prey in the understory of tropical lowland forests (e.g. X. Liu et al., 2020; Seifert et al., 2016). Bird attacks on model prey might increase in relative importance with disturbance (Posa et al., 2007), habitat openness (Williams-Guillen et al., 2008), and elevation (Tvardikova & Novotny, 2012). Nonetheless, the coarse identification of taxa causing attack marks in studies like ours, the inability to distinguish the context of attacks, and a lack of knowledge of how predators perceive and process prey-derived information, raise the question of whether attacks on artificial prey actually present predation or whether they include other behaviours too. We show that model shape is less important than it theoretically should be and thus call for caution when interpreting attack marks as caterpillar specific predation.

5.5.4 Limitations

Studies using model prey in the field typically do not continuously monitor individual objects and can thus only infer which organism attacked a prey object from the attack marks left in the model prey material when subsequently observed. This simultaneously presents the main advantage (e.g. Howe et al., 2009) and the main disadvantage of this method: we know nothing about the circumstances under which animals attack plasticine prey. We showed that object shape is less important than expected and thus estimates of predation pressure derived from model prey need to be interpreted with caution. This also applies to our result of variation in predation with time of the day. However, the precise identification of animals attacking model prey and their behaviour before and during an attack (which may allow for a better interpretation of context) would require a sophisticated camera set-up for each object

that captures attacks at night and day at a macro-scale but is placed in a way to not disturb predation by larger animals (i.e. hindering access of birds or bats). Even for a comparatively small study like ours this is financially and logistically unfeasible.

A second caveat comes from the lack of spatially independent replicates of two aspects of our study, namely trichomes (only in the island site and between two congener species) and model prey size (only in the mainland site). Thus, our results with regards to plant surface structure and model prey size need to be considered as exploratory and would require further studies to allow for more general conclusions.

5.6 CONCLUSIONS

Our results suggest that variation in predation with daytime may be locality specific, as predation was higher at night than during the day in one but not the other forest site. We find no evidence of enemy-free space being provided by the trichomes of *Cupania rufescens* plants. Further studies including a larger set of forest sites and plant species are needed to determine the generality of our findings.

Caterpillar- and humanoid-shaped objects got attacked with equal frequency. The fact that the shape of plasticine prey had no effect on attack rates by invertebrate predators is novel and striking. Our results challenge the idea that the visual resemblance of model prey alone is sufficient to deceive predators, question the nature of attacks on artificial prey, and imply that further studies are needed to explain how predators use prey-derived cues to decide for or against an attack. While model prey studies have their value in our quest to understand complex multitrophic interactions, we urge greater caution when interpreting their results.

Chapter 6: Discussion and Conclusion

The overall aim of this thesis was to increase our understanding of the impact of plant-microbial and plant-insect interactions on the dynamics of secondary and mature tropical rainforests. The specific objectives were to:

1) Explore the potential of biotic plant-soil feedbacks (PSF) to differentially affect tree species performance at different stages of succession (i.e. in soils from forests that differ in their recovery time) and reveal how such effects may alter the rate and direction of tree species turnover during secondary succession of tropical rainforests.

2) Determine the variation in effects of microbes and insect herbivores on seedling performance along a rainfall gradient within the distribution of a tree species.

3) Test the diurnal and spatial variation in predation pressure on insect herbivores, and thus provide evidence for the contribution of tri-trophic interactions in shaping plant diversity.

4) Assess the validity of a common ecological method and thereby promote the reliability of scientific results.

These objectives were addressed in four data chapters.

Chapter 2 assessed PSF variation with tree species identity, successional stage, species' association with successional stages (with species being associated with the stage where they occur in great abundance), and light. ***Chapter 3*** looked at variation in PSF with phylogenetic distance of soil conditioning and successor species. Together, Chapters 2 and 3 aimed to shed light on the role of PSF during secondary succession and addressed objective 1.

Chapter 4 focused on variation in the effects of insect herbivores and fungi with plant vigour along a natural rainfall gradient within the distributional range of a widespread tropical tree species, addressing objective 2.

Chapter 5 investigated the presence of temporal and spatial enemy-free space for insect herbivores by comparing attack marks on model prey between day and night and between glabrous and pubescent host plants, thereby addressing objective 3. Additionally, it tested the importance of model prey shape on attack rates and addressed objective 4.

The following paragraphs discuss the results of the data chapters and their implications in more detail.

6.1 MAIN RESULTS AND IMPLICATIONS

6.1.1 Positive and negative PSF are pervasive in tropical secondary succession

The results of this thesis provide evidence that PSF have pervasive effects on successional plant communities of Panamanian tropical rainforest. PSF affected six out of seven tree species in the first greenhouse experiment (Chapter 2) and all three tree species in the second experiment (Chapter 3) and acted at all four stages of succession that were tested. These findings provide evidence that PSF may affect many tree species throughout secondary succession of tropical rainforests.

While the experimental design does not enable a definite determination of the causal agents, the presence of species-specific net negative and net positive PSF (Chapters 2 and 3) suggests that both microbial pathogens and mutualists can be host species-specific. The evolutionary arms-race between host plants and pathogens has been suggested to cause a higher species-specificity of pathogens than mutualists (Bennett & Klironomos, 2019) and the community composition of soil microbial pathogens has been shown to be more closely associated with plant community composition than that of mutualists (Schroeder et al., 2019). The results of this thesis, however, support other studies that found effects of tropical tree identity on the composition of mutualist soil fungi (Mangan, Herre, et al., 2010) and suggest that microbial mutualists might be more species-specific than typically assumed.

Light level affected the magnitude, and in some cases even the direction, of PSF effects on seedling performance, yet the effect of light varied among tree species and successional

stages (Chapter 2). Together with large interspecific variation in PSF, this complicates drawing general conclusions from the results. Nonetheless, some interesting trends emerged.

6.1.2 PSF may affect successional trajectories

The results of this thesis provided evidence for three pathways by which PSF may affect the successional trajectories of tropical rainforests. First, PSF effects on seedling emergence were overall more positive at "home" successional stages, i.e. in soils extracted from secondary forests of the successional stage at which a tree species is naturally abundant (Chapter 2). Seedling mortality in tropical rainforests can be extremely high (Augspurger, 1984; Solé et al., 2019) and even small positive PSF effects on emergence could provide a tree species with an important advantage and promote rapid colonization and ultimately abundance at a specific successional stage. Thereby, PSF could present a third force – in addition to competition and environmental filtering of tree species (summarized in van Breugel et al., 2013) – that affects successional trajectories. Positive PSF are expected to promote dominance of individual tree species (Klironomos, 2002) and thereby stall successional turnover of species. However, context-dependency of PSF, with mutualistic microbes having a reduced positive effect when carbohydrate supplies from plants decrease (Kiers et al., 2011) and thus supporting a tree species only as long as it is very vigorous, may still drive successional turnover and amplify the effects of environmental filtering of tree species. Further research will however be needed to test this extrapolation.

Second, the two late-successional tree species tested in this thesis were less affected by PSF than the five tree species that are associated with earlier stages of succession (Chapter 2). This result supports the idea postulated in the growth-defence trade-off (Wright et al., 2010) and is in accordance with research from temperate grasslands that found less-defended, fast-growing species, which are abundant at the early stages of succession, to be more negatively affected by PSF (Kardol et al., 2006; van de Voorde et al., 2011). If this result can be translated into a general pattern of tree species that are abundant at earlier stages of succession having stronger PSF effects on emergence and survival, PSF could contribute to explaining the rate of tree species turnover during succession.

Third, a clear phylogenetic signal in heterospecific PSF (Chapter 3) could affect the relative success of different tree species in the turnover occurring during secondary succession. A previous study found reduced seedling performance in soils conditioned by adult trees of the same species than other species (Mangan, Schnitzer, et al., 2010). This thesis extends beyond that, and shows, for the first time, that negative effects of microbial legacies of tropical trees decreased with phylogenetic distance between heterospecific predecessor and successor species (Chapter 3). The observed variation in conspecific PSF between positive and negative (Chapter 3) indicates that phylogenetic effects of microbiota may promote tree diversity at high taxonomic levels. If phylogenetic signals in PSF are common, they may favour the establishment of unrelated successors over close relatives. PSF could thereby contribute to divergence during turnover of tree species and explain the observed transition from phylogenetic clustering to dispersion (Purschke et al., 2013) along secondary succession of tropical rainforests.

6.1.3 Pest effects vary with plant vigour along a rainfall gradient

By comparing the effects of partial and full pest exclusion on seedling performance, this thesis found strong intraspecific variation in seedling vigour and pest effects on the growth and biomass of *L. aggregatum* along a rainfall gradient (Chapter 4). Both insect herbivores and fungal pathogens had the strongest negative effects on the performance of *L. aggregatum* seedlings at the sites where they were most vigorous, providing support for the plant vigour hypothesis (Price, 1991). Similar and additive effects of insects and pathogens on growth and biomass suggest that both pests importantly affect seedling performance.

Effects of pest exclusion existed despite the early termination of the experiment and one year after the last pesticide applications (due to restricted access to the field sites because of the COVID pandemic). This adds to existing evidence (Norghauer & Newbery, 2013) that pest damage during the initial stage of seedling establishment can have persistent effects on plant performance at later life stages with potential implications for species distribution and plant community composition. Variation in rainfall and seasonality has previously been shown to affect seedling performance and pest damage at the community level (e.g. Baltzer & Davies, 2012; Brenes-Arguedas et al., 2009). This thesis adds to this knowledge by showing that

environmentally-driven intraspecific variation in plant vigour may contribute to the effects of pest pressure along a rainfall gradient. Our result that pest effects were strongest where plant vigour was highest indicates that it is unlikely that pests are a major factor restricting the distributional range of this species (in which case negative pest effects would be expected to be most severe in sites where plants are least vigorous at their distributional extremes). However, these results may support the notion that both insect and pathogen pests have a role in mediating plant competition and affecting local abundance of tropical tree species (Bagchi et al., 2014; Connell, 1971; Janzen, 1970). Understanding the relative contribution of pest damage and plant vigour on plant performance in different environmental contexts will improve our ability to predict how the projected changes in rainfall patterns (Pokhrel et al., 2021) will affect species distributions and ultimately plant community composition.

6.1.4 Weak evidence for variation in predation pressure

This thesis, by assessing diurnal and spatial variation in predation, found little evidence for the existence of enemy-free space provided by the trichomes of *Cupania rufescens*. Further, predation was higher at night than during the day in one forest site but not the other (Chapter 5). Further studies including a larger set of forest sites and plant species are needed to determine the generality of these findings.

6.1.5 Common methods are not necessarily good methods!

Predation of tropical insect herbivores is difficult to observe directly, as it often happens quickly and predators leave no traces of the consumed prey. Thus, artificial model prey is often used to estimate predation pressure (Howe et al., 2009; Lövei & Ferrante, 2017). This thesis found that caterpillar-shaped and superhero-shaped plasticine objects are attacked with equal frequency (Chapter 5). In combination with earlier reports of attack marks by non-predators in plasticine prey (Pfennig et al., 2007) this finding indicates that model prey shape resembling that of real prey may not be sufficient to deceive predators. Instead, attacks on plasticine prey may include a large variety of animal responses to a novel object in the forest

and do not necessarily represent predation events. This finding highlights the need for further studies exploring how predators perceive their environment and use prey-derived cues to decide for or against an attack. While model prey studies have their value in our quest to understand complex multitrophic interactions, the striking finding of this study indicates the need for greater caution when interpreting their results.

6.2 LIMITATIONS

The complexity of tropical rainforest systems and the dynamic character of species interactions and secondary succession, together with temporal, logistic, and monetary limitations of a PhD project result in several limitations to the findings of this thesis that must be considered.

Most importantly, this thesis focused on eight tree species, while more than 3,000 tree species have been documented in Panama (Condit et al., 2020), most of which grow in rainforests. Species selection for greenhouse studies like the ones described in this thesis is biased, because a large number of seeds are needed to obtain statistically powerful results and thus only relatively abundant tree species are considered. However, the majority of tropical tree species are rare (Slik et al., 2015). This problem is further strengthened by the enormous inter-and intra-specific variation of PSF effects found in this thesis as well as in earlier studies (McCarthy-Neumann & Kobe, 2008). Thus, generalizations from the findings presented in this thesis must be made with caution.

Second, the findings on the role of PSF in secondary succession are based on a chronosequence. Chronosequences substitute space for time and cannot fully control for variation among sites (Powers & Marín-Spiotta, 2017). Together with a strong impact of local factors on successional trajectories (e.g. Poorter et al., 2021) this complicates the generalization of findings from one site to more general ecological patterns.

Third, while this thesis aimed to include important environmental variables, e.g. the effect of variation in light along secondary succession, many other factors will affect realized species

interactions in the field (De Long et al., 2019). One example are successional changes in soil pH and nutrients, which have been shown to affect bacterial communities (Yu et al., 2021) but to explain only a small proportion of variation in microbial community composition in Panamanian rainforests (Barberán et al., 2015). Given the limitations of a PhD project, I decided to include light as one of the most important factors affecting (tropical) plant community dynamics (e.g. Leigh et al., 2004) and with a shown impact on PSF (Bennett & Klironomos, 2019; Mangan, Herre, et al., 2010), the main focus of this thesis. To minimize potential artefacts from unmeasured edaphic variables however, my greenhouse experiments used a small amount of inoculum and 90% of the soil was identical among plants.

Fourth, PSF measured in greenhouse experiments have been criticised as overestimates of field PSF (Forero et al., 2019). While the mechanisms that may drive such overestimations are unclear (Forero et al., 2019), the methods employed in this thesis were designed to minimize experimental artefacts, e.g. due to pot size limitation, short experimental duration, and nutrient pulses in soils through sterilization.

A last important caveat is that the studies described in this thesis cannot determine the causal agents behind the measured effects of experimental soil sterilization and pest exclusion on plant performance with successional stage and along the rainfall gradient. While the experimental design makes it likely that microbial communities have cause the PSF measured here, a causal link can ultimately be made only by laboratory methods (e.g. time-consuming staining of mycorrhizas in roots) or increasingly by genomic analyses. We preserved field soil samples and samples of soils collected at the begging and the end of each experiment (Chapter 2 and 3; for all species x treatment combinations) and planned to sequence soil microbial communities to complement the analyses of plant performance. Grouping soil microfauna into operational taxonomic units into functional groups would have allowed us to describe changes in the relative abundance of these groups with forest successional stage, light level, and plant species identity. The COVID-19 pandemic and associated logistic and temporal limitations made it impossible to finish these analyses within the timeframe of this thesis. This is unfortunate as it greatly restricts the potential for us to determine the mechanisms causing the treatment effects on plant performance and thus the

impact of our findings. However, we are still determined to analyse the preserved samples as soon as possible and combine these datasets to shed light on the soil microbial "black box" and analyse, for the first time, the link between PSF effects and microbial community composition during secondary succession of tropical rainforests.

6.3 CONCLUSIONS

The results provided in this thesis strongly indicate that plant-microbe and plant-insect interactions affect the seedling performance of tree species in secondary and mature tropical rainforests in Panama. This provides the first assessment of the impact of plant-soil feedbacks (PSF) on the rate and direction of tree species turnover during secondary succession. It further showed notable variation in the effects of insect herbivores and fungi on seedling performance across a rainfall gradient. It also found good evidence of the unreliability of predation estimates derived from model prey studies. The results of this thesis contribute to close the outlined knowledge gap and thus the aim of this thesis was fulfilled.

The following main conclusions can be drawn.

1) Species-specific variation in PSF effects on tropical tree species is large, with further complexity added by modulating effects of abiotic variables and successional stage.

2) PSF are likely to have species-specific effects on tree establishment at different stages of secondary succession in tropical rainforests. Some evidence is presented that PSF may affect the rate and direction of successional tree species turnover by three mechanisms: more positive PSF occurring in the successional stages that a tree species is naturally abundant at, a phylogenetic signal in heterospecific PSF, and a lower susceptibility to PSF in tree species that are abundant at later stages of succession than those abundant at earlier stages.

3) Both, microbial mutualists and pathogens are likely to contribute to these PSF and variation in their relative strength can drive both net negative and net positive PSF.

4) Intraspecific variation in seedling vigour along a rainfall gradient affects the strength of pest effects on plant performance.

5) Estimates of predation pressure derived from plasticine model prey may include a variety of animal responses to these artificial objects and so not exclusively represent predation events. This highlights the need to assess the results of research using such models more critically.

6.4 OUTLOOK

This thesis provided a pioneering insight into the role of plant-microbial and plant-insect interactions in structuring tropical rainforest dynamics. However, the precise mechanisms and causal agents of the effects reported here remain to be discovered.

Further research can contribute to filling this knowledge gap via three options: First, the soil samples I collected at each crucial stage of the greenhouse experiments (Chapters 2 and 3) remain a valuable resource. Metagenomic analyses of these samples is planned and will allow us to link the patterns of plant performance described in this thesis to changes in microbial community composition. We will thereby be able to investigate causal links between the observed PSF and soil microbes. By opening the soil "black box" we might indeed be able to pinpoint the microbial drivers of PSF and provide valuable information to guide restoration efforts.

Second, by combining my measurements of *L. aggregatum* seedling performance along the rainfall gradient with measurements of pest damage on these seedlings that have been made by the masters student Lydia Martin, we will be able to evaluate the relative contribution of variation in pest damage levels and plant vigour on plant performance along the rainfall gradient. This will constitute the first study to combine variation in seedling vigour, pest damage and pest effects on seedling performance in several sites along a natural rainfall gradient within the distributional range of a species. This is interesting from a fundamental science perspective and may improve our ability to predict how the predicted changes in

rainfall regimes (Pokhrel et al., 2021) may affect tree species distributions and species interactions.

Lastly, only a subset of *L. aggregatum* seedlings were harvested in this thesis, with around ¾ of seedlings remaining in the field. We found effects on plant performance after more than 1 year after treatments had been terminated (due to the COVID pandemic). Together with findings of an earlier study (Norghauer & Newbery, 2013), this suggests that differences in pest pressure during the early stages of seedling establishment may persist, as differences in plant performance were detected at much later stages of the seedlings' development. The remaining seedlings in the field will allow us to return to the sites and test for long-term effects of pesticide exclusion during the initial four months of seedling establishment on plant performance. In this sense it will become a long-term "pulse" treatment experiment.

6.5 RECOMMENDATIONS FOR FUTURE RESEARCH

The enormous diversity and complexity of tropical rainforests makes these ecosystems so interesting and astounding. However, it also results in many research gaps. For instance, we know close to nothing about the context-dependent expression of pathogenicity, modulation of PSF by abiotic factors and plant competition, or the role of bacterial soil communities in driving PSF. Further, the PSF caused by other soil biota, such as nematodes and protists that have been shown to affect the health of temperate plants directly and indirectly through top-down control of plant antagonists (protists: Chandarana & Amaresan, 2022; nematodes: Wilschut et al., 2019; Wilschut & Geisen, 2021), and by alterations of available soil nitrogen with cascading effects on root architecture that in turn affect protist abundance (protists: Chandarana & Amaresan, 2022; Gao et al., 2019), remain largely unexplored in tropical rainforests. Understanding host-specificity in microbial pathogens and mutualists is crucial, as their combined effects, i.e. net PSF, will determine the degree to which a tree affects the performance of subsequently growing conspecifics relative to other species. Thereby, plant-microbial interactions may contribute to the maintenance of tropical diversity, as has been proposed by Janzen (1970) and Connell (1971) more than 50 years ago. Understanding the interplay of soil microbial pathogens and mutualists in shaping net PSF would massively

promote our knowledge on the fundamental processes that determine tropical tree species diversity and community composition.

Perhaps the most common characteristic of empirical studies on the mechanisms driving patterns of tropical diversity is the contradictory results they produce. Given the complexity of interacting factors, the enormous species richness of tropical forest ecosystems and the huge interspecific variation and context-dependency in PSF (which this thesis provides further evidence of) this may not be surprising. However, the alarming rate at which tropical forests are disappearing and the projected catastrophic effects of this loss (Boulton et al., 2022; Malhi et al., 2014) call for large-scale collaborative projects to reveal the mechanisms that drive their diversity. It is only by highly-replicated studies with a pre-defined and consistent methodology, which span various sites within the paleotropics and neotropics and include a large number of species, that we will be able to surmount the contradictory findings of small studies and ultimately may be able to determine the interplay of different factors that determine the capacity for rainforest recovery. This will be key to identify what needs to be done to accelerate the natural rate of recovery. While some progress has been made, e.g. with the Paris Climate Change Agreement and the formulation of the Sustainable Development Goals, reliable data are needed to close knowledge gaps and direct policies.

From the viewpoint of fundamental science, it would be interesting to cross disciplinary boundaries and test whether newly discovered mechanisms contributing to the maintenance of biodiversity in tropical rainforests may apply to other systems, such as coral reefs, building on the pioneering approach of Joseph Connell (Connell, 1978). It will now be timely to test whether the striking parallels between the fundamental mechanisms influencing species diversity in these systems also extend to their resilience. Potentially even more so than tropical rainforests, coral reefs are suffering from extremely severe and accelerating degradation. While the actors may differ, coral reefs just like rainforests depend on the interaction between long-lived, immobile and short-lived, highly mobile organisms and show remarkably similar temporal patterns of fast recovery of biomass and slow recovery of composition (McWilliam et al., 2020).

References

Anacker, B. L., Klironomos, J. N., Maherali, H., Reinhart, K. O., & Strauss, S. Y. (2014). Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. *Ecology Letters*, *17*(12), 1613–1621. https://doi.org/10.1111/ele.12378

Anderson-Teixeira, K. J., Davies, S. J., Bennett, A. C., Gonzalez-Akre, E. B., Muller-Landau, H. C., Joseph Wright, S., Abu Salim, K., Almeyda Zambrano, A. M., Alonso, A., Baltzer, J. L., Basset, Y., Bourg, N. A., Broadbent, E. N., Brockelman, W. Y., Bunyavejchewin, S., Burslem, D. F. R. P., Butt, N., Cao, M., Cardenas, D., … Zimmerman, J. (2015). CTFS-ForestGEO: A worldwide network monitoring forests in an era of global change. *Global Change Biology*, *21*(2), 528–549. https://doi.org/10.1111/gcb.12712

Arnold, A. E., & Asquith, N. M. (2002). Herbivory in a fragmented tropical forest: Patterns from islands at Lago Gatu´ n, Panama. *Biodiversity and Conservation*, *11*, 1663–1680.

Augspurger, C. K. (1984). Seedling Survival of Tropical Tree Species: Interactions of Dispersal Distance, Light-Gaps, and Pathogens. *Ecology*, *65*(6), 1705–1712. https://doi.org/10.2307/1937766

Bagchi, R., Gallery, R. E., Gripenberg, S., Gurr, S. J., Narayan, L., Addis, C. E., Freckleton, R. P., & Lewis, O. T. (2014). Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*, *506*(7486), 85–88. https://doi.org/10.1038/nature12911

Baltzer, J. L., & Davies, S. J. (2012). Rainfall seasonality and pest pressure as determinants of tropical tree species' distributions. *Ecology and Evolution*, *2*(11), 2682–2694. https://doi.org/10.1002/ece3.383

Barberán, A., McGuire, K. L., Wolf, J. A., Jones, F. A., Wright, S. J., Turner, B. L., Essene, A., Hubbell, S. P., Faircloth, B. C., & Fierer, N. (2015). Relating belowground microbial

composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. *Ecology Letters*, *18*(12), 1397–1405. https://doi.org/10.1111/ele.12536

Barlow, J., França, F., Gardner, T. A., Hicks, C. C., Lennox, G. D., Berenguer, E., Castello, L., Economo, E. P., Ferreira, J., Guénard, B., Gontijo Leal, C., Isaac, V., Lees, A. C., Parr, C. L., Wilson, S. K., Young, P. J., & Graham, N. A. J. (2018). The future of hyperdiverse tropical ecosystems. *Nature*, *559*(7715), 517–526. https://doi.org/10.1038/s41586-018-0301-1

Bateman, P. W., Fleming, P. A., & Wolfe, A. K. (2017). A different kind of ecological modelling: The use of clay model organisms to explore predator-prey interactions in vertebrates. *Journal of Zoology*, *301*(4), 251–262. https://doi.org/10.1111/jzo.12415

Benítez-Malvido, J., Rodríguez-Alvarado, G., Álvarez-Añorve, M., Ávila-Cabadilla, L. D., del-Val, E., Lira-Noriega, A., & Gregorio-Cipriano, R. (2021). Antagonistic Interactions Between Fusaria Species and Their Host Plants Are Influenced by Host Taxonomic Distance: A Case Study From Mexico. *Frontiers in Ecology and Evolution*, *9*, 615857. https://doi.org/10.3389/fevo.2021.615857

Bennett, J. A., & Klironomos, J. (2019). Mechanisms of plant–soil feedback: Interactions among biotic and abiotic drivers. *New Phytologist*, *222*(1), 91–96. https://doi.org/10.1111/nph.15603

Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M., & Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, *355*(6321), 181–184. https://doi.org/10.1126/science.aai8212

Benning, J. W., Eckhart, V. M., Geber, M. A., & Moeller, D. A. (2019). Biotic Interactions Contribute to the Geographic Range Limit of an Annual Plant: Herbivory and

Phenology Mediate Fitness beyond a Range Margin. *The American Naturalist*, *193*(6), 786–797. https://doi.org/10.1086/703187

Berger, D., & Gotthard, K. (2008). Time stress, predation risk and diurnal–nocturnal foraging trade-offs in larval prey. *Behavioral Ecology and Sociobiology*, *62*(10), 1655–1663. https://doi.org/10.1007/s00265-008-0594-4

Berger, J. R., & Wirth, R. (2004). Predation-mediated Mortality of Early Life Stages: A Field Experiment with Nymphs of an Herbivorous Stick Insect (Metriophasma diocles). *Biotropica*, *36*(3), 424–428. https://doi.org/10.1111/j.1744-7429.2004.tb00337.x

Bever, J. D., Mangan, S. A., & Alexander, H. M. (2015). Maintenance of Plant Species Diversity by Pathogens. *Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 305–325. https://doi.org/10.1146/annurev-ecolsys-112414-054306

Boege, K., & Dirzo, R. (2004). Intraspecific variation in growth, defense and herbivory in Dialium guianense (Caesalpiniaceae) mediated by edaphic heterogeneity. *Plant Ecology Formerly `Vegetatio'*, *175*(1), 59–69. https://doi.org/10.1023/B:VEGE.0000048092.82296.9a

Boulton, C. A., Lenton, T. M., & Boers, N. (2022). Pronounced loss of Amazon rainforest resilience since the early 2000s. *Nature Climate Change*, *12*(3), 271–278. https://doi.org/10.1038/s41558-022-01287-8

Bradley, D. J., Gilbert, G. S., & Martiny, J. B. H. (2008). Pathogens promote plant diversity through a compensatory response. *Ecology Letters*, *11*(5), 461–469. https://doi.org/10.1111/j.1461-0248.2008.01162.x

Brenes-Arguedas, T., Coley, P. D., & Kursar, T. A. (2009). Pests vs. Drought as determinants of plant distribution along a tropical rainfall gradient. *Ecology*, *90*(7), 1751–1761. https://doi.org/10.1890/08-1271.1

Brown, J. H. (2014). Why are there so many species in the tropics? *Journal of Biogeography*, *41*(1), 8–22. https://doi.org/10.1111/jbi.12228

Bullock, D. G. (1992). Crop rotation. *Critical Reviews in Plant Sciences*, *11*(4), 309–326. https://doi.org/10.1080/07352689209382349

Burnham, K. P., & Anderson, D. R. (2004). Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociological Methods & Research*, *33*(2), 261–304. https://doi.org/10.1177/0049124104268644

Chandarana, K. A., & Amaresan, N. (2022). Soil protists: An untapped microbial resource of agriculture and environmental importance. *Pedosphere*, *32*(1), 184–197. https://doi.org/10.1016/S1002-0160(21)60066-8

Chanthorn, W., Caughlin, T., Dechkla, S., & Brockelman, W. Y. (2013). The Relative Importance of Fungal Infection, Conspecific Density and Environmental Heterogeneity for Seedling Survival in a Dominant Tropical Tree. *Biotropica*, *45*(5), 587–593. https://doi.org/10.1111/btp.12044

Chaparro, J. M., Sheflin, A. M., Manter, D. K., & Vivanco, J. M. (2012). Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils*, *48*(5), 489–499. https://doi.org/10.1007/s00374-012-0691-4

Chazdon, R. L., Broadbent, E. N., Rozendaal, D. M. A., Bongers, F., Zambrano, A. M. A., Aide, T. M., Balvanera, P., Becknell, J. M., Boukili, V., Brancalion, P. H. S., Craven, D., Almeida-Cortez, J. S., Cabral, G. A. L., de Jong, B., Denslow, J. S., Dent, D. H., DeWalt, S. J., Dupuy, J. M., Durán, S. M., … Poorter, L. (2016). Carbon sequestration potential of second-growth forest regeneration in the Latin American tropics. *Science Advances*, *2*(5), e1501639. https://doi.org/10.1126/sciadv.1501639

Chen, W., Jiao, S., Li, Q., & Du, N. (2020). Dispersal limitation relative to environmental filtering governs the vertical small-scale assembly of soil microbiomes during restoration. *Journal of Applied Ecology*, *57*(2), 402–412. https://doi.org/10.1111/1365-2664.13533

Chesson, P., & Kuang, J. J. (2008). The interaction between predation and competition. *Nature*, *456*(7219), 235–238. https://doi.org/10.1038/nature07248

Clare, E. L., & Holderied, M. W. (2015). Acoustic shadows help gleaning bats find prey, but may be defeated by prey acoustic camouflage on rough surfaces. *ELife*, *4*, e07404. https://doi.org/10.7554/eLife.07404

Coley, P. D., & Barone, J. A. (1996). HERBIVORY AND PLANT DEFENSES IN TROPICAL FORESTS. *Annual Review of Ecology and Systematics*, *27*(1), 305–335. https://doi.org/10.1146/annurev.ecolsys.27.1.305

Coley, P. D., & Kursar, T. A. (2014). On Tropical Forests and Their Pests. *Science*, *343*(6166), 35–36. https://doi.org/10.1126/science.1248110

Condit, R. (1998). Tropical Forest Census Plots. In *Tropical Forest Census Plots*. Springer-Verlag and R.G. Landes Company. https://doi.org/10.1007/978-3-662-03664-8

Condit, R., Aguilar, S., & Pérez, R. (2020). Trees of Panama: A complete checklist with every geographic range. *Forest Ecosystems*, *7*(1), 42. https://doi.org/10.1186/s40663-020-00246-z

Condit, R., Engelbrecht, B. M. J., Pino, D., Pérez, R., & Turner, B. L. (2013). Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proceedings of the National Academy of Sciences*, *110*(13), 5064–5068. https://doi.org/10.1073/pnas.1218042110

Condit, R., Lao, R., Pérez, S., Aguilar, R. B., Foster, S. P., & Hubbell, S. P. (2019). In *Complete data from the Barro Colorado 50-ha plot: 423617 trees, 35 years, v3.* DataONE Dash, Dataset. https://doi.org/10.15146/5xcp-0d46

Condit, R., Pérez, R., & Daguerre, N. (2010). *Trees of Panama and Costa Rica* (Vol. 74). Princeton University Press.

Connell, J. H. (1971). On the Role of Natural Enemies in Preventing Competitive Exclusion in Some Marine Animals and in Rain Forest Trees. In P. J. den Boer & G. R. Gradwell (Eds.), *Dynamics of Populations* (pp. 298–310). Pudoc.

Connell, J. H. (1978). Diversity in Tropical Rain Forests and Coral Reefs: High diversity of trees and corals is maintained only in a nonequilibrium state. *Science*, *199*(4335), 1302–1310. https://doi.org/10.1126/science.199.4335.1302

Cornelissen, T., Fernandes, G. W., & Vasconcellos-Neto, J. (2008). Size Does Matter: Variation in Herbivory between and within Plants and the Plant Vigor Hypothesis. *Oikos*, *117*(8), 1121–1130.

Cornell, H. V., & Hawkins, B. A. (1995). Survival Patterns and Mortality Sources of Herbivorous Insects: Some Demographic Trends. *The American Naturalist*, *145*(4), 563–593. https://doi.org/10.1086/285756

Corrales, A., Henkel, T. W., & Smith, M. E. (2018). Ectomycorrhizal associations in the tropics – biogeography, diversity patterns and ecosystem roles. *New Phytologist*, *220*(4), 1076–1091. https://doi.org/10.1111/nph.15151

Cortois, R., Schröder-Georgi, T., Weigelt, A., Putten, W. H., & De Deyn, G. B. (2016). Plant–soil feedbacks: Role of plant functional group and plant traits. *Journal of Ecology*, *104*(6), 1608–1617. https://doi.org/10.1111/1365-2745.12643

Craven, D., Hall, J. S., Berlyn, G. P., Ashton, M. S., & van Breugel, M. (2015). Changing gears during succession: Shifting functional strategies in young tropical secondary forests. *Oecologia*, *179*(1), 293–305. https://doi.org/10.1007/s00442-015-3339-x

Croat, T. B. (1978). *Flora of Barro Colorado Island*. Stanford University Press.

Cronin, J. P., Tonsor, S. J., & Carson, W. P. (2010). A simultaneous test of trophic interaction models: Which vegetation characteristic explains herbivore control over plant community mass? *Ecology Letters*, *13*(2), 202–212. https://doi.org/10.1111/j.1461-0248.2009.01420.x

Cummings, P. (2019). *Analysis of Incidence Rates*. CRC Press.

Davidson, D. W., Snelling, R. R., & Longino, J. T. (1989). Competition Among Ants for Myrmecophytes and the Significance of Plant Trichomes. *Biotropica*, *21*(1), 64. https://doi.org/10.2307/2388444

De Long, J. R., Fry, E. L., Veen, G. F., & Kardol, P. (2019). Why are plant–soil feedbacks so unpredictable, and what to do about it? *Functional Ecology*, *33*(1), 118–128. https://doi.org/10.1111/1365-2435.13232

Derroire, G., Balvanera, P., Castellanos-Castro, C., Decocq, G., Kennard, D. K., Lebrija-Trejos, E., Leiva, J. A., Odén, P.-C., Powers, J. S., Rico-Gray, V., Tigabu, M., & Healey, J. R. (2016). Resilience of tropical dry forests—A meta-analysis of changes in species diversity and composition during secondary succession. *Oikos*, *125*(10), 1386–1397. https://doi.org/10.1111/oik.03229

Eck, J. L., Stump, S. M., Delavaux, C. S., Mangan, S. A., & Comita, L. S. (2019). Evidence of within-species specialization by soil microbes and the implications for plant community diversity. *Proceedings of the National Academy of Sciences*, *116*(15), 7371–7376. https://doi.org/10.1073/pnas.1810767116

Eichhorn, M. P., Nilus, R., Compton, S. G., Hartley, S. E., & Burslem, D. F. R. P. (2010). Herbivory of tropical rain forest tree seedlings correlates with future mortality. *Ecology*, *91*(4), 1092–1101.

Engelbrecht, B. M. J., Comita, L. S., Condit, R., Kursar, T. A., Tyree, M. T., Turner, B. L., & Hubbell, S. P. (2007). Drought sensitivity shapes species distribution patterns in tropical forests. *Nature*, *447*(7140), 80–82. https://doi.org/10.1038/nature05747

FAO. (2020). *Global Forest Resources Assessment 2020 – Key findings*. https://doi.org/10.4060/ca8753en

Ferrante, M., Barone, G., Kiss, M., Bozóné-Borbáth, E., & Lövei, G. L. (2017). Ground-level predation on artificial caterpillars indicates no enemy-free time for lepidopteran larvae. *Community Ecology*, *18*(3), 280–286. https://doi.org/10.1556/168.2017.18.3.6

Ferriss, R. S. (1984). Effects of microwave oven treatment on microorganisms in soil. *Phytopathology*, *74*(1), 121–126.

Fitzpatrick, C. R., Gehant, L., Kotanen, P. M., & Johnson, M. T. J. (2017). Phylogenetic relatedness, phenotypic similarity and plant-soil feedbacks. *Journal of Ecology*, *105*(3), 786–800. https://doi.org/10.1111/1365-2745.12709

Foley, J. A., Asner, G. P., Costa, M. H., Coe, M. T., DeFries, R., Gibbs, H. K., Howard, E. A., Olson, S., Patz, J., Ramankutty, N., & Snyder, P. (2007). Amazonia revealed: Forest degradation and loss of ecosystem goods and services in the Amazon Basin. *Frontiers in Ecology and the Environment*, *5*(1), 25–32. https://doi.org/10.1890/1540-9295(2007)5[25:ARFDAL]2.0.CO;2

Fordyce, J. A., & Agrawal, A. A. (2001). *The BlackwellScienceLtd role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail*. 9.

Forero, L. E., Grenzer, J., Heinze, J., Schittko, C., & Kulmatiski, A. (2019). Greenhouse- and Field-Measured Plant-Soil Feedbacks Are Not Correlated. *Frontiers in Environmental Science*, *7*, 184. https://doi.org/10.3389/fenvs.2019.00184

Fortunel, C., Paine, C. E. T., Fine, P. V. A., Mesones, I., Goret, J., Burban, B., Cazal, J., & Baraloto, C. (2016). There's no place like home: Seedling mortality contributes to the habitat specialisation of tree species across Amazonia. *Ecology Letters*, *19*(10), 1256–1266. https://doi.org/10.1111/ele.12661

Gallery, R. E., Moore, D. J. P., & Dalling, J. W. (2010). Interspecific variation in susceptibility to fungal pathogens in seeds of 10 tree species in the neotropical genus Cecropia: Cecropia seed pathogens. *Journal of Ecology*, *98*(1), 147–155. https://doi.org/10.1111/j.1365-2745.2009.01589.x

Gao, Z., Karlsson, I., Geisen, S., Kowalchuk, G., & Jousset, A. (2019). Protists: Puppet Masters of the Rhizosphere Microbiome. *Trends in Plant Science*, *24*(2), 165–176. https://doi.org/10.1016/j.tplants.2018.10.011

Garcia, L. C., & Eubanks, M. D. (2019). Overcompensation for insect herbivory: A review and meta-analysis of the evidence. *Ecology*, *100*(3). https://doi.org/10.1002/ecy.2585

García-Guzmán, G., & Benítez-Malvido, J. (2003). Effect of litter on the incidence of leaf-fungal pathogens and herbivory in seedlings of the tropical tree nectandra ambigens. *Journal of Tropical Ecology*, *19*(2), 171–177. https://doi.org/10.1017/S0266467403003195

Garrett, K. A., Dendy, S. P., Frank, E. E., Rouse, M. N., & Travers, S. E. (2006). Climate Change Effects on Plant Disease: Genomes to Ecosystems. *Annual Review of Phytopathology*, *44*(1), 489–509. https://doi.org/10.1146/annurev.phyto.44.070505.143420

Garwood, N. C. (1983). Seed germination in a Seasonal Tropical Forest in Panama: A Community Study. *Ecological Monographs*, *53*(2), 159–181.

Gaviria, J., Turner, B. L., & Engelbrecht, B. M. J. (2017). Drivers of tree species distribution across a tropical rainfall gradient. *Ecosphere*, *8*(2), e01712. https://doi.org/10.1002/ecs2.1712

Geipel, I., Jung, K., & Kalko, E. K. V. (2013). Perception of silent and motionless prey on vegetation by echolocation in the gleaning bat *Micronycteris microtis*. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1754), 20122830. https://doi.org/10.1098/rspb.2012.2830

Geipel, I., Steckel, J., Tschapka, M., Vanderelst, D., Schnitzler, H.-U., Kalko, E. K. V., Peremans, H., & Simon, R. (2019). Bats Actively Use Leaves as Specular Reflectors to Detect Acoustically Camouflaged Prey. *Current Biology*, *29*(16), 2731-2736.e3. https://doi.org/10.1016/j.cub.2019.06.076

Gely, C., Laurance, S. G. W., & Stork, N. E. (2020). How do herbivorous insects respond to drought stress in trees? *Biological Reviews*, *95*(2), 434–448. https://doi.org/10.1111/brv.12571

Gentry, A. H. (1988). Changes in Plant Community Diversity and Floristic Composition on Environmental and Geographical Gradients. *Annals of the Missouri Botanical Garden*, *75*(1), 1. https://doi.org/10.2307/2399464

Gilbert, G. S., Briggs, H. M., & Magarey, R. (2015). The Impact of Plant Enemies Shows a Phylogenetic Signal. *PLOS ONE*, *10*(4), e0123758. https://doi.org/10.1371/journal.pone.0123758

Gilbert, G. S., & Webb, C. O. (2007). Phylogenetic signal in plant pathogen-host range. *Proceedings of the National Academy of Sciences*, *104*(12), 4979–4983. https://doi.org/10.1073/pnas.0607968104

Givnish, T. J. (1999). On the causes of gradients in tropical tree diversity. *Journal of Ecology*, *87*(2), 193–210. https://doi.org/10.1046/j.1365-2745.1999.00333.x

Gorb, S. N., & Gorb, E. V. (2019). Frequency of plant visits by the generalist ant Lasius niger depends on the surface microstructure of plant stems. *Arthropod-Plant Interactions*, *13*(2), 311–320. https://doi.org/10.1007/s11829-019-09681-4

Gougherty, A. V., & Davies, T. J. (2021). Towards a phylogenetic ecology of plant pests and pathogens. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1837), 20200359. https://doi.org/10.1098/rstb.2020.0359

Greeney, H., Dyer, L., & Smilanich, A. (2012). Feeding by lepidopteran larvae is dangerous: A review of caterpillars' chemical, physiological, morphological, and behavioral defenses against natural enemies. *Invertebrate Survival Journal*, *9*(1), 7–34.

Guariguata, M. R., & Ostertag, R. (2001). Neotropical secondary forest succession: Changes in structural and functional characteristics. *Forest Ecology and Management*, *148*(1–3), 185–206. https://doi.org/10.1016/S0378-1127(00)00535-1

Habersetzer, J., & Vogler, B. (1983). Discrimination of surface-structured targets by the echolocating batMyotis myotis during flight. *Journal of Comparative Physiology*, *152*(2), 275–282. https://doi.org/10.1007/BF00611192

Hamann, E., Blevins, C., Franks, S. J., Jameel, M. I., & Anderson, J. T. (2021). Climate change alters plant–herbivore interactions. *New Phytologist*, *229*(4), 1894–1910. https://doi.org/10.1111/nph.17036

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., & Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, *8*(4), 157–178. https://doi.org/10.1016/j.ppees.2007.01.001

Harrison, M. L. K., & Banks-Leite, C. (2020). Edge effects on trophic cascades in tropical rainforests. *Conservation Biology*, *34*(4), 977–987. https://doi.org/10.1111/cobi.13438

Heinrich, B. (1979). Foraging strategies of caterpillars: Leaf damage and possible predator avoidance strategies. *Oecologia*, *42*(3), 325–337. https://doi.org/10.1007/BF00346597

Howe, A., Lövei, G. L., & Nachman, G. (2009). Dummy caterpillars as a simple method to assess predation rates on invertebrates in a tropical agroecosystem. *Entomologia Experimentalis et Applicata*, *131*(3), 325–329. https://doi.org/10.1111/j.1570-7458.2009.00860.x

Husband, R., Herre, E. A., Turner, S. L., Gallery, R., & Young, J. P. W. (2002). Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology*, *11*(12), 2669–2678. https://doi.org/10.1046/j.1365-294X.2002.01647.x

Ichie, T., Inoue, Y., Takahashi, N., Kamiya, K., & Kenzo, T. (2016). Ecological distribution of leaf stomata and trichomes among tree species in a Malaysian lowland tropical rain forest. *Journal of Plant Research*, *129*(4), 625–635. https://doi.org/10.1007/s10265-016-0795-2

Janos, D. P. (1980). Mycorrhizae Influence Tropical Succession. *Biotropica*, *12*(2), 56–64.

Janzen, D. H. (1970). Herbivores and the Number of Tree Species in Tropical Forests. *The American Naturalist*, *104*(940), 501–528. https://doi.org/10.1086/282687

Jones, P. L., & Agrawal, A. A. (2019). Beyond preference and performance: Host plant selection by monarch butterflies, *Danaus plexippus*. *Oikos*, *128*(8), 1092–1102. https://doi.org/10.1111/oik.06001

Kalka, M. B., & Kalko, E. K. V. (2006). Gleaning bats as underestimated predators of herbivorous insects: Diet of *Micronycteris microtis* (Phyllostomidae) in Panama. *Journal of Tropical Ecology*, *22*(1), 1–10. https://doi.org/10.1017/S0266467405002920

Kalka, M. B., Smith, A. R., & Kalko, E. K. V. (2008). Bats Limit Arthropods and Herbivory in a Tropical Forest. *Science*, *320*(5872), 71–71. https://doi.org/10.1126/science.1153352

Kaplan, I., Bokulich, N. A., Caporaso, J. G., Enders, L. S., Ghanem, W., & Ingerslew, K. S. (2020). Phylogenetic farming: Can evolutionary history predict crop rotation via the soil microbiome? *Evolutionary Applications*, *13*(8), 1984–1999. https://doi.org/10.1111/eva.12956

Kardol, P., Martijn Bezemer, T., & van der Putten, W. H. (2006). Temporal variation in plant-soil feedback controls succession: Plant-soil feedback controls succession. *Ecology Letters*, *9*(9), 1080–1088. https://doi.org/10.1111/j.1461-0248.2006.00953.x

Kariyat, R. R., Smith, J. D., Stephenson, A. G., De Moraes, C. M., & Mescher, M. C. (2017). Non-glandular trichomes of *Solanum carolinense* deter feeding by *Manduca sexta* caterpillars and cause damage to the gut peritrophic matrix. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1849), 20162323. https://doi.org/10.1098/rspb.2016.2323

Katan, J. (2017). Diseases caused by soilborne pathogens: Biology, management and challenges. *Journal of Plant Pathology*, *99*(2), 305–315. http://dx.doi.org/10.4454/jpp.v99i2.3862

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., Bago, A., Palmer, T. M., West, S. A., Vandenkoornhuyse, P., Jansa, J., & Bücking, H. (2011). Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. *Science*, *333*(6044), 880–882. https://doi.org/10.1126/science.1208473

Klironomos, J. N. (2002). *Feedback with soil biota contributes to plant rarity and invasiveness in communities*. *417*, 4.

Kobe, R. K., & Vriesendorp, C. F. (2011). Conspecific density dependence in seedlings varies with species shade tolerance in a wet tropical forest: Shade and density-dependent mortality. *Ecology Letters*, *14*(5), 503–510. https://doi.org/10.1111/j.1461-0248.2011.01612.x

Kottke, I., & Haug, I. (2004). The significance of mycorrhizal diversity of trees in the tropical mountain forest of southern Ecuador. *Lyonia*, *7*(December), 49–56.

Kreft, H., & Jetz, W. (2007). Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences*, *104*(14), 5925–5930. https://doi.org/10.1073/pnas.0608361104

Krishnadas, M., Bagchi, R., Sridhara, S., & Comita, L. S. (2018). Weaker plant-enemy interactions decrease tree seedling diversity with edge-effects in a fragmented tropical forest. *Nature Communications*, *9*(1), 4523. https://doi.org/10.1038/s41467-018-06997-2

Leigh, E. G. (2019). Tropical Seasonal Forest. In *Encyclopedia of Ecology* (pp. 684–692). Elsevier. https://doi.org/10.1016/B978-0-12-409548-9.10921-2

Leigh, E. G., Davidar, P., Dick, C. W., Terborgh, J., Puyravaud, J.-P., Steege, H., & Wright, S. J. (2004). Why Do Some Tropical Forests Have So Many Species of Trees? *Biotropica*, *36*(4), 447–473. https://doi.org/10.1111/j.1744-7429.2004.tb00342.x

Lekberg, Y., Bever, J. D., Bunn, R. A., Callaway, R. M., Hart, M. M., Kivlin, S. N., Klironomos, J., Larkin, B. G., Maron, J. L., Reinhart, K. O., Remke, M., & Putten, W. H. (2018). Relative importance of competition and plant–soil feedback, their synergy, context dependency and implications for coexistence. *Ecology Letters*, *21*(8), 1268–1281. https://doi.org/10.1111/ele.13093

Leponce, M., Corbara, B., Delabie, J. H. C., Orivel, J., Aberlenc, H.-P., Bail, J., Barrios, H., Campos, R. I., do Nascimento, I. C., Compin, A., Didham, R. K., Floren, A., Medianero, E., Ribeiro, S. P., Roisin, Y., Schmidl, J., Tishechkin, A. K., Winchester, N. N., Basset, Y., & Dejean, A. (2021). Spatial and functional structure of an entire ant assemblage in a lowland Panamanian rainforest. *Basic and Applied Ecology*, *56*, 32–44. https://doi.org/10.1016/j.baae.2021.06.007

Letunic, I., & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, *49*(W1), W293–W296. https://doi.org/10.1093/nar/gkab301

Levin, D. A. (1973). The Role of Trichomes in Plant Defense. *The Quarterly Review of Biology*, *48*(1, Part 1), 3–15. https://doi.org/10.1086/407484

Liu, X., Wang, Z., Huang, C., Li, M., Bibi, F., Zhou, S., & Nakamura, A. (2020). Ant assemblage composition explains high predation pressure on artificial caterpillars during early night. *Ecological Entomology*, *45*(3), 547–554. https://doi.org/10.1111/een.12826

Liu, Y., Fang, S., Chesson, P., & He, F. (2015). The effect of soil-borne pathogens depends on the abundance of host tree species. *Nature Communications*, *6*, 7. https://doi.org/10.1038/ncomms10017

Louda, S. M., & Collinge, S. K. (1992). Plant Resistance to Insect Herbivores: A Field Test of the Environmental Stress Hypothesis. *Ecology*, *73*(1), 153–169. https://doi.org/10.2307/1938728

Lövei, G. L., & Ferrante, M. (2017). A review of the sentinel prey method as a way of quantifying invertebrate predation under field conditions: Measuring predation pressure by sentinel prey. *Insect Science*, *24*(4), 528–542. https://doi.org/10.1111/1744-7917.12405

Lovelock, C. E., Andersen, K., & Morton, J. B. (2003). Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. *Oecologia*, *135*(2), 268–279. https://doi.org/10.1007/s00442-002-1166-3

Low, P. A., Sam, K., McArthur, C., Posa, M. R. C., & Hochuli, D. F. (2014). Determining predator identity from attack marks left in model caterpillars: Guidelines for best practice. *Entomologia Experimentalis et Applicata*, *152*(2), 120–126. https://doi.org/10.1111/eea.12207

Macarthur, R. H. (1965). PATTERNS OF SPECIES DIVERSITY. *Biological Reviews*, *40*(4), 510–533. https://doi.org/10.1111/j.1469-185X.1965.tb00815.x

Malhi, Y., Gardner, T. A., Goldsmith, G. R., Silman, M. R., & Zelazowski, P. (2014). Tropical Forests in the Anthropocene. *Annual Review of Environment and Resources*, *39*(1), 125–159. https://doi.org/10.1146/annurev-environ-030713-155141

Mangan, S. A., Herre, E. A., & Bever, J. D. (2010). Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant–soil feedback. *Ecology*, *91*(9), 2594–2603. https://doi.org/10.1890/09-0396.1

Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I., & Bever, J. D. (2010). Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, *466*(7307), 752–755. https://doi.org/10.1038/nature09273

McCarthy-Neumann, S., & Kobe, R. K. (2008). Tolerance of soil pathogens co-varies with shade tolerance across species of tropical tree seedlings. *Ecology*, *89*(7), 1883–1892. https://doi.org/10.1890/07-0211.1

McCarthy-Neumann, S., & Kobe, R. K. (2019). Site Soil-Fertility and Light Availability Influence Plant-Soil Feedback. *Frontiers in Ecology and Evolution*, *7*, 383. https://doi.org/10.3389/fevo.2019.00383

McClure, T. T., & Robbins, W. R. (1942). Resistance of Cucumber Seedlings to Damping-Off as Related to Age, Season of Year, and Level of Nitrogen Nutrition. *Botanical Gazette*, *103*(4), 684–697.

McDonald, J. H. (2009). *Handbook of biological statistics* (Vol. 2). sparky house publishing.

McWilliam, M., Pratchett, M. S., Hoogenboom, M. O., & Hughes, T. P. (2020). Deficits in functional trait diversity following recovery on coral reefs. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192628. https://doi.org/10.1098/rspb.2019.2628

Miller, E. C., Perron, G. G., & Collins, C. D. (2019). Plant-driven changes in soil microbial communities influence seed germination through negative feedbacks. *Ecology and Evolution*, *9*(16), 9298–9311. https://doi.org/10.1002/ece3.5476

Molleman, F., Remmel, T., & Sam, K. (2016). Phenology of Predation on Insects in a Tropical Forest: Temporal Variation in Attack Rate on Dummy Caterpillars. *Biotropica*, *48*(2), 229–236. https://doi.org/10.1111/btp.12268

Muehleisen, A. J., Engelbrecht, B. M. J., Jones, F. A., Manzané-Pinzón, E., & Comita, L. S. (2020). Local adaptation to herbivory within tropical tree species along a rainfall gradient. *Ecology*, *101*(11). https://doi.org/10.1002/ecy.3151

Norghauer, J. M., & Newbery, D. M. (2013). Herbivores equalize the seedling height growth of three dominant tree species in an African tropical rain forest. *Forest Ecology and Management*, *310*, 555–566. https://doi.org/10.1016/j.foreco.2013.08.029

Novotny, V., Basset, Y., Auga, J., Boen, W., Dal, C., Drozd, P., Kasbal, M., Isua, B., Kutil, R., & Manumbor, M. (1999). Predation risk for herbivorous insects on tropical vegetation: A search for enemy-free space and time. *Austral Ecology*, *24*(5), 477–483. https://doi.org/10.1046/j.1440-169x.1999.00987.x

Packer, A., & Clay, K. (2004). Development of negative feedback during successive growth cycles of black cherry. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1536), 317–324. https://doi.org/10.1098/rspb.2003.2583

Peay, K. G., Baraloto, C., & Fine, P. V. (2013). Strong coupling of plant and fungal community structure across western Amazonian rainforests. *The ISME Journal*, *7*, 1852–1861.

Pfennig, D. W., Harper, G. R., Brumo, A. F., Harcombe, W. R., & Pfennig, K. S. (2007). Population differences in predation on Batesian mimics in allopatry with their model: Selection against mimics is strongest when they are common. *Behavioral Ecology and Sociobiology*, *61*(4), 505–511. https://doi.org/10.1007/s00265-006-0278-x

Pizano, C., Mangan, S. A., Graham, J. H., & Kitajima, K. (2017). Host-specific effects of soil microbial filtrates prevail over those of arbuscular mycorrhizae in a fragmented

landscape. *Ecological Applications*, *27*(6), 1946–1957. https://doi.org/10.1002/eap.1579

Pokhrel, Y., Felfelani, F., Satoh, Y., Boulange, J., Burek, P., Gädeke, A., Gerten, D., Gosling, S. N., Grillakis, M., Gudmundsson, L., Hanasaki, N., Kim, H., Koutroulis, A., Liu, J., Papadimitriou, L., Schewe, J., Müller Schmied, H., Stacke, T., Telteu, C.-E., … Wada, Y. (2021). Global terrestrial water storage and drought severity under climate change. *Nature Climate Change*, *11*, 226–233. https://doi.org/10.1038/s41558-020-00972-w

Poorter, L., Craven, D., Jakovac, C. C., van der Sande, M. T., Amissah, L., Bongers, F., Chazdon, R. L., Farrior, C. E., Kambach, S., Meave, J. A., Muñoz, R., Norden, N., Rüger, N., van Breugel, M., Almeyda Zambrano, A. M., Amani, B., Andrade, J. L., Brancalion, P. H. S., Broadbent, E. N., … Hérault, B. (2021). Multidimensional tropical forest recovery. *Science*, *374*(6573), 1370–1376. https://doi.org/10.1126/science.abh3629

Posa, M. R. C., Sodhi, N. S., & Koh, L. P. (2007). Predation on artificial nests and caterpillar models across a disturbance gradient in Subic Bay, Philippines. *Journal of Tropical Ecology*, *23*(1), 27–33. https://doi.org/10.1017/S0266467406003671

Powers, J. S., & Marín-Spiotta, E. (2017). Ecosystem Processes and Biogeochemical Cycles in Secondary Tropical Forest Succession. *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 497–519. https://doi.org/10.1146/annurev-ecolsys-110316-022944

Price, P. W. (1991). The Plant Vigor Hypothesis and Herbivore Attack. *Oikos*, *62*(2), 244. https://doi.org/10.2307/3545270

Pringle, E. G., Álvarez-Loayza, P., & Terborgh, J. (2007). Seed characteristics and susceptibility to pathogen attack in tree seeds of the Peruvian Amazon. *Plant Ecology*, *193*(2), 211–222. https://doi.org/10.1007/s11258-006-9259-4

Purschke, O., Schmid, B. C., Sykes, M. T., Poschlod, P., Michalski, S. G., Durka, W., Kühn, I., Winter, M., & Prentice, H. C. (2013). Contrasting changes in taxonomic, phylogenetic and functional diversity during a long-term succession: Insights into assembly processes. *Journal of Ecology*, *101*(4), 857–866. https://doi.org/10.1111/1365-2745.12098

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. www.R-project.org

Remmel, T., & Tammaru, T. (2009). Size-dependent predation risk in tree-feeding insects with different colouration strategies: A field experiment: Size-dependent predation of insect larvae. *Journal of Animal Ecology*, *78*(5), 973–980. https://doi.org/10.1111/j.1365-2656.2009.01566.x

Riddick, E. W., & Simmons, A. M. (2014). Do plant trichomes cause more harm than good to predatory insects?: Plant trichomes and predators. *Pest Management Science*, *70*(11), 1655–1665. https://doi.org/10.1002/ps.3772

Rodríguez-Trelles, F., Tarrío, R., & Ayala, F. J. (2001). Erratic overdispersion of three molecular clocks: GPDH, SOD, and XDH. *Proceedings of the National Academy of Sciences*, *98*(20), 11405–11410. https://doi.org/10.1073/pnas.201392198

Román, F., Sautu, A., Hall, J. S., Sautu, A., Pérez, R., Liones, R. De, Paton, S., Hernández, A., Guerra, M., & Galdames, C. (2012). *Guía para la propagacion de 120 Árboles nativos de Panama y el Neotropico*.

Romero, F., Cazzato, S., Walder, F., Vogelgsang, S., Bender, S. F., & Heijden, M. G. A. (2022). Humidity and high temperature are important for predicting fungal disease outbreaks worldwide. *New Phytologist*, *234*(5), 1553–1556. https://doi.org/10.1111/nph.17340

Rozendaal, D. M. A., Bongers, F., Aide, T. M., Alvarez-Dávila, E., Ascarrunz, N., Balvanera, P., Becknell, J. M., Bentos, T. V., Brancalion, P. H. S., Cabral, G. A. L., Calvo-Rodriguez, S., Chave, J., César, R. G., Chazdon, R. L., Condit, R., Dallinga, J. S., De Almeida-Cortez, J. S., De Jong, B., De Oliveira, A., … Poorter, L. (2019). Biodiversity recovery of Neotropical secondary forests. *Science Advances*, *5*(3). https://doi.org/10.1126/sciadv.aau3114

Santiago, L. S., & Mulkey, S. S. (2005). Leaf productivity along a precipitation gradient in lowland Panama: Patterns from leaf to ecosystem. *Trees*, *19*(3), 349–356. https://doi.org/10.1007/s00468-004-0389-9

Sarmiento, C., Zalamea, P.-C., Dalling, J. W., Davis, A. S., Stump, S. M., U'Ren, J. M., & Arnold, A. E. (2017). Soilborne fungi have host affinity and host-specific effects on seed germination and survival in a lowland tropical forest. *Proceedings of the National Academy of Sciences*, *114*(43), 11458–11463. https://doi.org/10.1073/pnas.1706324114

Schroeder, J. W., Martin, J. T., Angulo, D. F., Arias-Del Razo, I., Barbosa, J. M., Perea, R., Sebastián-González, E., & Dirzo, R. (2019). Host plant phylogeny and abundance predict root-associated fungal community composition and diversity of mutualists and pathogens. *Journal of Ecology*, *107*(4), 1557–1566. https://doi.org/10.1111/1365-2745.13166

Schuldt, A., Hönig, L., Li, Y., Fichtner, A., Härdtle, W., Oheimb, G., Welk, E., & Bruelheide, H. (2017). Herbivore and pathogen effects on tree growth are additive, but mediated by tree diversity and plant traits. *Ecology and Evolution*, *7*(18), 7462–7474. https://doi.org/10.1002/ece3.3292

Segnitz, R. M., Russo, S. E., Davies, S. J., & Peay, K. G. (2020). Ectomycorrhizal fungi drive positive phylogenetic plant–soil feedbacks in a regionally dominant tropical plant family. *Ecology*, *101*(8). https://doi.org/10.1002/ecy.3083

Seifert, C. L., Schulze, C. H., Dreschke, T. C. T., Frötscher, H., & Fiedler, K. (2016). Day vs. Night predation on artificial caterpillars in primary rainforest habitats—An experimental approach. *Entomologia Experimentalis et Applicata*, *158*(1), 54–59. https://doi.org/10.1111/eea.12379

Silva, C. V. de J., Santos, J. R. dos, Galvão, L. S., Silva, R. D. 'agnol da, & Moura, Y. M. (2016). Floristic and structure of an Amazonian primary forest and a chronosequence of secondary succession. *Acta Amazonica*, *46*(2), 133–150. https://doi.org/10.1590/1809-4392201504341

Simamora, A. V., Stukely, M. J. C., Barber, P. A., Hardy, G. E. StJ., & Burgess, T. I. (2017). Age-related susceptibility of *Eucalyptus* species to *Phytophthora boodjera*. *Plant Pathology*, *66*(3), 501–512. https://doi.org/10.1111/ppa.12592

Slik, J. W. F., Arroyo-Rodríguez, V., Aiba, S.-I., Alvarez-Loayza, P., Alves, L. F., Ashton, P., Balvanera, P., Bastian, M. L., Bellingham, P. J., van den Berg, E., Bernacci, L., da Conceição Bispo, P., Blanc, L., Böhning-Gaese, K., Boeckx, P., Bongers, F., Boyle, B., Bradford, M., Brearley, F. Q., … Venticinque, E. M. (2015). An estimate of the number of tropical tree species. *Proceedings of the National Academy of Sciences*, *112*(24), 7472–7477. https://doi.org/10.1073/pnas.1423147112

Solé, R., Gripenberg, S., Lewis, O. T., Markesteijn, L., Barrios, H., Ratz, T., Ctvrtecka, R., Butterill, P. T., Segar, S. T., Metz, M. A., Dahl, C., Rivera, M., Viquez, K., Ferguson, W., Guevara, M., & Basset, Y. (2019). The role of herbivorous insects and pathogens in

the regeneration dynamics of Guazuma ulmifolia in Panama. *Nature Conservation*, *32*, 81–101. https://doi.org/10.3897/natureconservation.32.30108

Soudzilovskaia, N. A., Vaessen, S., Barcelo, M., He, J., Rahimlou, S., Abarenkov, K., Brundrett, M. C., Gomes, S., Merckx, V., Martinez-Suz, L., & Tedersoo, L. (2022). *Taxon occurrence data for the FungalRoot database*. PlutoF. https://doi.org/10.15468/a7ujmj

Spear, E. R., & Broders, K. D. (2021). Host-generalist fungal pathogens of seedlings may maintain forest diversity via host-specific impacts and differential susceptibility among tree species. *New Phytologist*, *231*(1), 460–474. https://doi.org/10.1111/nph.17379

Spear, E. R., Coley, P. D., & Kursar, T. A. (2015). Do pathogens limit the distributions of tropical trees across a rainfall gradient? *Journal of Ecology*, *103*(1), 165–174. https://doi.org/10.1111/1365-2745.12339

Speir, T., Cowling, J., Sparling, G., West, A., & Corderoy, D. (1986). Effects of microwave radiation on the microbial biomass, phosphatase activity and levels of extractable N and P in a low fertility soil under pasture. *Soil Biology and Biochemistry*, *18*(4), 377–382. https://doi.org/10.1016/0038-0717(86)90041-6

Styrsky, J. D., Kaplan, I., & Eubanks, M. D. (2006). Plant trichomes indirectly enhance tritrophic interactions involving a generalist predator, the red imported fire ant. *Biological Control*, *36*(3), 375–384. https://doi.org/10.1016/j.biocontrol.2005.10.003

Swinfield, T., Lewis, O. T., Bagchi, R., & Freckleton, R. P. (2012). Consequences of changing rainfall for fungal pathogen-induced mortality in tropical tree seedlings: Fungal Pathogen-Induced Mortality in Tropical Tree Seedlings. *Ecology and Evolution*, *2*(7), 1408–1413. https://doi.org/10.1002/ece3.252

Szefer, P., Molem, K., Sau, A., & Novotny, V. (2020). Impact of pathogenic fungi, herbivores and predators on secondary succession of tropical rainforest vegetation. *Journal of Ecology*, *108*(5), 1978–1988. https://doi.org/10.1111/1365-2745.13374

ter Steege, H., Pitman, N., Sabatier, D., Castellanos, H., Silveira, M., Phillips, O., Andel, T. V., Duivenvoorden, J., Oliveira, A. A. D., Ek, R., Thomas, R., Maas, P., Mori, S., Terborgh, J., & Vargas, N. N. E. (2003). A spatial model of tree a-diversity and tree density for the Amazon. *Biodiversity and Conservation*, *12*, 2255–2277.

Thomas, P., & Upreti, R. (2014). Influence of Seedling Age on the Susceptibility of Tomato Plants to <i>Ralstonia solanacearum</i> during Protray Screening and at Transplanting. *American Journal of Plant Sciences*, *05*(12), 1755–1762. https://doi.org/10.4236/ajps.2014.512190

Trevors, J. T. (1996). Sterilization and inhibition of microbial activity in soil. *Journal of Microbiological Methods*, *26*(1–2), 53–59. https://doi.org/10.1016/0167-7012(96)00843-3

Trotter, R. T., Cobb, N. S., & Whitham, T. G. (2007). Arthropod community diversity and trophic structure: A comparison between extremes of plant stress. *Ecological Entomology*, *33*(1), 1–11. https://doi.org/10.1111/j.1365-2311.2007.00941.x

Tvardikova, K., & Novotny, V. (2012). Predation on exposed and leaf-rolling artificial caterpillars in tropical forests of Papua New Guinea. *Journal of Tropical Ecology*, *28*(4), 331–341. https://doi.org/10.1017/S0266467412000235

Uribe-Mú, C. A., & Quesada, M. (2006). Preferences, patterns and consequences of branch removal on the dioecious tropical tree *Spondias purpurea* (Anacardiaceae) by the insect borer *Oncideres albomarginata chamela* (Cerambycidae). *Oikos*, *112*(3), 691–697. https://doi.org/10.1111/j.0030-1299.2006.13957.x

van Breugel, M., Hall, J. S., Craven, D., Bailon, M., Hernandez, A., Abbene, M., & van Breugel, P. (2013). Succession of Ephemeral Secondary Forests and Their Limited Role for the Conservation of Floristic Diversity in a Human-Modified Tropical Landscape. *PLoS ONE*, *8*(12), e82433. https://doi.org/10.1371/journal.pone.0082433

van Breugel, M., Martínez-Ramos, M., & Bongers, F. (2006). Community Dynamics during Early Secondary Succession in Mexican Tropical Rain Forests. *Journal of Tropical Ecology*, *22*(6), 663–674.

van de Voorde, T. F. J., van der Putten, W. H., & Martijn Bezemer, T. (2011). Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession: Intra- and interspecific plant-soil interactions. *Journal of Ecology*, *99*(4), 945–953. https://doi.org/10.1111/j.1365-2745.2011.01815.x

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. N., Van de Voorde, T. F. J., & Wardle, D. A. (2013). Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology*, *101*(2), 265–276. https://doi.org/10.1111/1365-2745.12054

Vermeij, G. J. (2015). Plants that lead: Do some surface features direct enemy traffic on leaves and stems? *Biological Journal of the Linnean Society*, *116*(2), 288–294. https://doi.org/10.1111/bij.12592

Weissflog, A., Markesteijn, L., Lewis, O. T., Comita, L. S., & Engelbrecht, B. M. J. (2018). Contrasting patterns of insect herbivory and predation pressure across a tropical rainfall gradient. *Biotropica*, *50*(2), 302–311. https://doi.org/10.1111/btp.12513

Williams-Guillen, K., Perfecto, I., & Vandermeer, J. (2008). Bats Limit Insects in a Neotropical Agroforestry System. *Science*, *320*(5872), 70–70. https://doi.org/10.1126/science.1152944

Wilschut, R. A., & Geisen, S. (2021). Nematodes as Drivers of Plant Performance in Natural Systems. *Trends in Plant Science*, *26*(3), 237–247. https://doi.org/10.1016/j.tplants.2020.10.006

Wilschut, R. A., van der Putten, W. H., Garbeva, P., Harkes, P., Konings, W., Kulkarni, P., Martens, H., & Geisen, S. (2019). Root traits and belowground herbivores relate to plant–soil feedback variation among congeners. *Nature Communications*, *10*(1), 1564. https://doi.org/10.1038/s41467-019-09615-x

Wise, M. J., & Abrahamson, W. G. (2008). Applying the Limiting Resource Model to Plant Tolerance of Apical Meristem Damage. *The American Naturalist*, *172*(5), 635–647. https://doi.org/10.1086/591691

Woodson, R. E., Schery, R. W., & Croat, T. B. (1976). Flora of Panama. Part VI. Family 108. Sapindaceae. *Annals of the Missouri Botanical Garden*, *63*(3), 419. https://doi.org/10.2307/2395282

Wright, S. J., Kitajima, K., Kraft, N. J. B., Reich, P. B., Wright, I. J., Bunker, D. E., Condit, R., Dalling, J. W., Davies, S. J., Díaz, S., Engelbrecht, B. M. J., Harms, K. E., Hubbell, S. P., Marks, C. O., Ruiz-Jaen, M. C., Salvador, C. M., & Zanne, A. E. (2010). Functional traits and the growth–mortality trade-off in tropical trees. *Ecology*, *91*(12), 3664–3674. https://doi.org/10.1890/09-2335.1

Xi, N., Bloor, J. M. G., & Chu, C. (2020). Soil microbes alter seedling performance and biotic interactions under plant competition and contrasting light conditions. *Annals of Botany*, *126*(6), 1089–1098. https://doi.org/10.1093/aob/mcaa134

Yu, Z., Liang, K., Huang, G., Wang, X., Lin, M., Chen, Y., & Zhou, Z. (2021). Soil Bacterial Community Shifts Are Driven by Soil Nutrient Availability along a Teak Plantation Chronosequence in Tropical Forests in China. *Biology*, *10*(12), 1329. https://doi.org/10.3390/biology10121329

Zangaro, W., Ansanelo, A. P., Lescano, L. E. A. M., de Almeida Alves, R., Rondina, A. B. L., & Nogueira, M. A. (2012). Infection intensity, spore density and inoculum potential of arbuscular mycorrhizal fungi decrease during secondary succession in tropical Brazilian ecosystems. *Journal of Tropical Ecology*, *28*(5), 453–462. https://doi.org/10.1017/S0266467412000399

Zhang, Y., Ni, J., Tang, F., Jiang, L., Guo, T., Pei, K., Sun, L., & Liang, Y. (2017). Diversity of root-associated fungi of Vaccinium mandarinorum along a human disturbance gradient in subtropical forests, China. *Journal of Plant Ecology*, *10*(1), 56–66. https://doi.org/10.1093/jpe/rtw022

Zhou, X., Liu, J., & Wu, F. (2017). Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. *Plant and Soil*, *415*(1–2), 507–520. https://doi.org/10.1007/s11104-017-3181-5

Zhou, Z., Wang, C., Jiang, L., & Luo, Y. (2017). Trends in soil microbial communities during secondary succession. *Soil Biology & Biochemistry*, *115*, 92–99. http://dx.doi.org/10.1016/j.soilbio.2017.08.014

Supplement Chapter 2

Data preparation and further details on statistical analyses

We excluded one *Faramea occidentalis* pot (under 5% light with live soil) from the analysis as it was forgotten when sowing seeds.

Biomass. – Young plants that emerged during the last quarter of the experimental time period created outliers with large leverage so we excluded all plants that were ≤ 105 days old. The 43 excluded plants (Table S2.3) had an average dry weight of 0.01 g while that of the remaining plants was 6.32 g. Further, we excluded two outlier values in the successional stage analysis (both *Siparuna pauciflora*) and five outliers in the association analysis (three *Vismia baccifera*, one *Gustavia superba* and one *Apeiba membranacea*) with Cook's distances > 0.5.

For each model, we assessed the proportion of variance explained by the position of the plant in the greenhouse and the number of plants growing in its pot by dividing the variance of the random effect by the total variance. A random effect was included into a model if it explained more than 5% of the variance. We added plant position for *V. baccifera* (40% light, successional stage and association analyses), number of plants per pot for *S. pauciflora* (40% light, successional stage and association) and *A. membranacea* (40% light, successional stage and association), and both random effects for *A. membranacea* (5% light, association).

Root and leaf biomass fractions

We assessed the partitioning of plant biomass into roots and leaves by dividing their dry weight by the total dry weight of the plant.

Statistical analyses. – We analysed root and leaf biomass fractions of all plants that were ≥ 105 days old with separate beta regression models for each species (betareg). Betaregression models of root and leaf biomass fractions against height included inoculum and successional stage/ association and their two-way interaction as fixed effects. For *G. superba* and *F. occidentalis* we harvested enough plants growing in sterilized and live soils in both light conditions and thus included light and the interaction of successional stage/ association x light as fixed effects to their models. Betaregression is sensitive to missing observations, leading to the exclusion of several treatments (Table S2.3). We could not analyse the effect of successional stage for *V. baccifera* and present only results of the association analysis. Further, models for *S. pauciflora* included only inoculum as a fixed effect.

Five of the 746 harvested plants had a leaf fraction of 0 and were excluded from the leaf fraction analysis (though they were included in the root fraction analysis). We excluded all species x treatment combinations with less than three plants (Table S2.3) to ensure a meaningful analysis of regression slopes. The sensitivity of

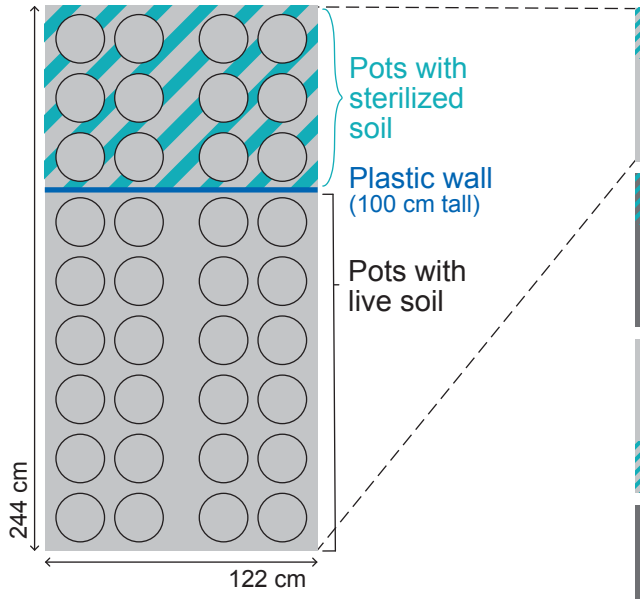
beta regression to missing levels of factors in interaction terms and the absence of harvested plants in either sterilized or live soil required us to exclude the following species x treatment combinations from the successional stage models: *G. superba* (40% light, 15 yrs), *S. pauciflora* (40% light, 15 yrs), and *V. baccifera* (all treatment combinations) (Table S2.3). No groups needed to be excluded from the association analysis.

We performed Anova type III equivalent analyses of the model coefficients (joint_tests in emmeans). We assessed PSF as Tukey-adjusted pairwise comparisons between regression slopes of sterilized vs live soils in each successional stage and association (emtrends in emmeans). For *G. superba* and *F. occidentalis* we calculated PSF for the two light levels separately. Subsequently, we calculated contrasts between PSF within each species x light combination to assess variation of PSF among successional stages/ association.

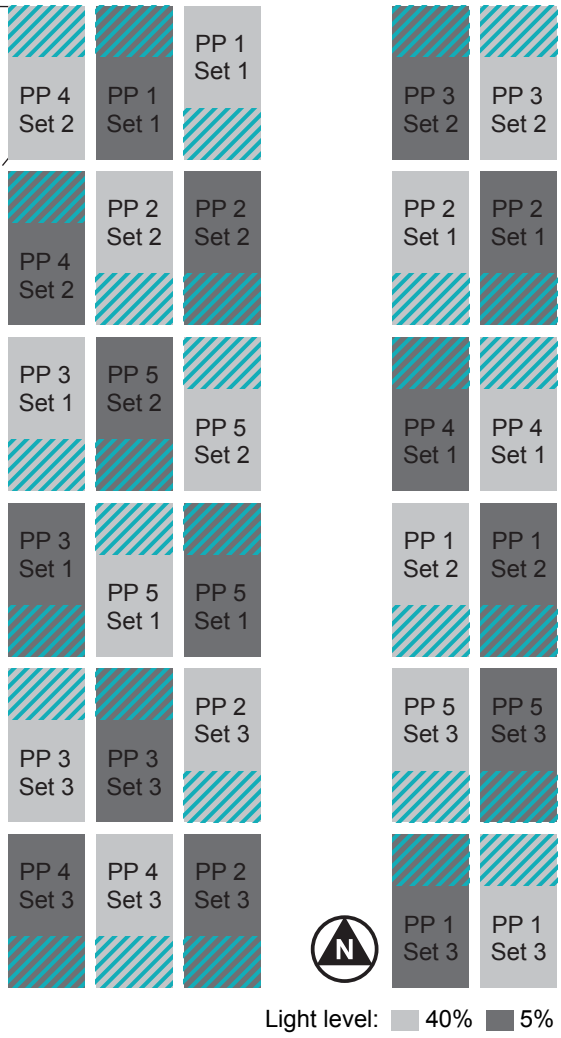
Results. – We found significant PSF on root biomass fraction in one of our species (inoculum in *F. occidentalis* across light levels and under 5% light; Table S2.2) and a significant interaction of PSF with successional stage on root biomass fraction for two species (inoculum x successional stage: *X. frutescens*, *G. superba* under 40% light; Table S2.2). Leaf biomass fraction was significantly affected by PSF in four species and by the interaction of PSF x successional stage in one species (inoculum, and inoculum x successional stage in Table S2.2). The effect of PSF on root biomass fraction was significant for F. occidentalis in one successional stage under 40% light and two successional stages under 5% light (Fig. S2.2). We found no consistent pattern of variation in PSF on neither root nor leaf biomass fraction with successional stage or association (Table S2.2, Fig. S2.2 – S2.5).

For the two species for which the effect of light could be analysed, we found that light level significantly affected the root biomass fraction in both species and the leaf biomass fraction in one species. We found no significant two-way interaction between PSF and successional stage, association, or light level in either species for either fraction (Table S2.2). Given the inconsistency of variation in PSF effects on root and leaf biomass fractions, we focused our discussion on the effects of PSF on survival, emergence, and biomass.

i) Topview of one of 30 tables

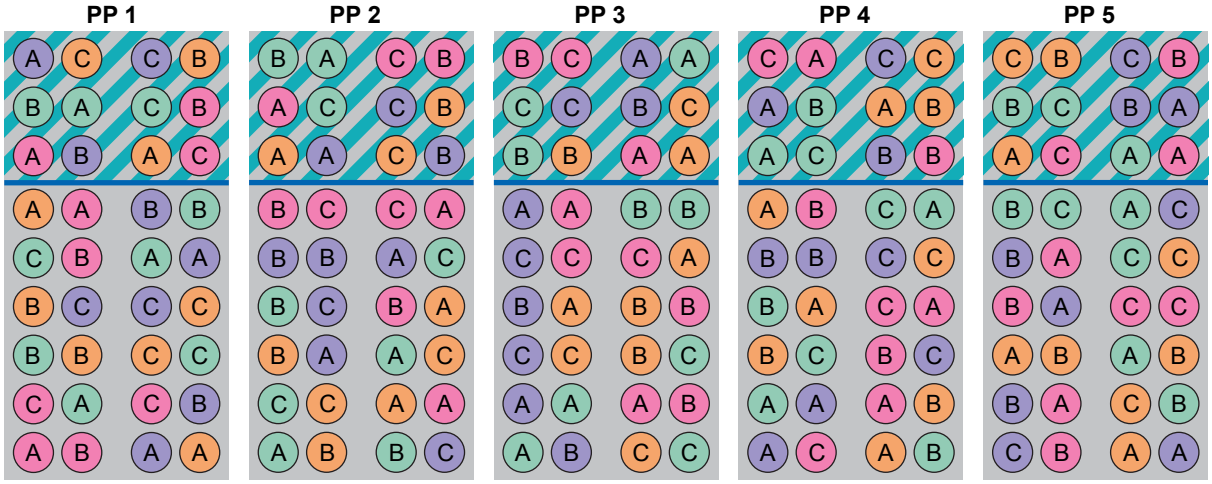


ii) Topview of greenhouse



iii) Greenhouse photographs

iv) Overview of pot positions (PP)



Species sets

Pot ID	Set 1	Set 2	Set 3
A	*Faramea occidentalis*	*Xylopia frutescens*	*Apeiba membranacea*
B	*Psychotria grandis*	*Vismia baccifera*	*Gustavia superba*
C	*Siparuna pauciflora*		

Soil successional stage:
0 yrs 15 yrs
25 yrs 115 yrs

Figure S2.1. Overview of experimental set-up in the greenhouse. Shown is **i)** a topview of one of 30 experimental tables, on which 36 experimental pots (circles) were placed. Each table was divided into a section of pots filled with sterilized soil (turquoise shaded area) and a section of pots filled with live (i.e. including microbes) soil. These sections were separated by a 100 cm tall plastic wall (dark blue line). **ii)** Thirty experimental tables were arranged in a greenhouse, systematically alternating between tables exposed to the high-light (40% ambient light, pale grey) and tables exposed to the low-light (5% ambient light, dark grey) treatment. Individual table IDs provide information about the arrangement of pots (pot position: PP 1-5) and the identity of tree seedling species (Set 1-3) grown on each table. We show **iii)** photographs of experimental tables in the greenhouse showing experimental pots, the separating plastic wall, and shade-cloth used to create the two different light treatments. We provide an **iv)** overview of the arrangement of experiment pots on the tables. The experiment included seedlings of seven three species, that were divided into three species sets (bottom left table). Seedlings were grown in soils from four successional stages (0, 15, 25, and 115 years of recovery) as indicated by different colors (bottom right legend). For each species x successional stage combination, we had ten pot replicates with live soil and five pot replicates with sterilized soil for each, the high-light and the low-light treatment. We created a template of pot positions randomizing species x successional stage treatments on five tables (only restriction: maximum two identical species x treatment combinations per table) as shown as PP1-5. This template was then applied to the five high-light and then five low-light tables of each species set (Set 1-3; see ii)).

Caption refers to the figure printed on the previous page.

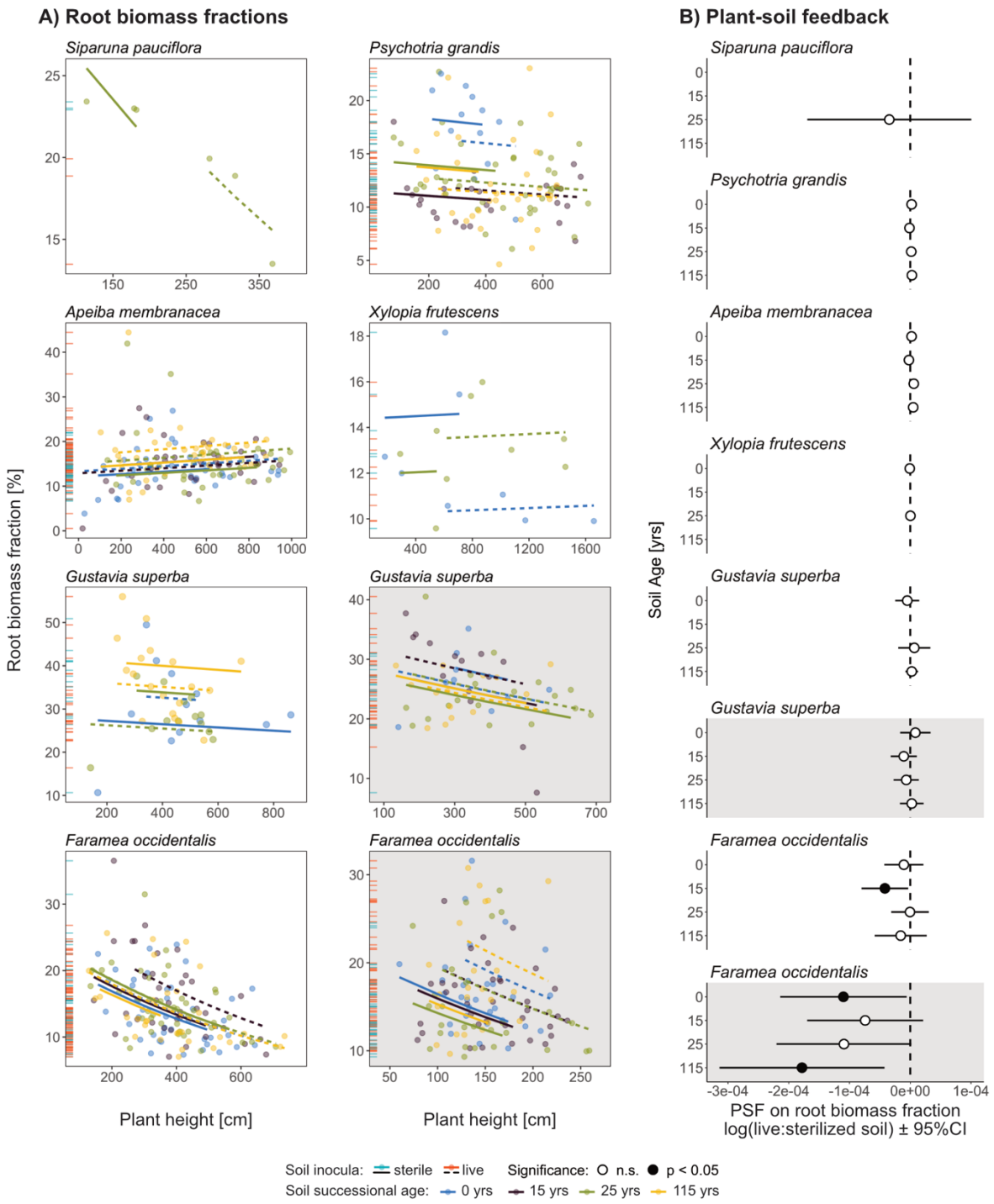


Figure S2.2. Root biomass fractions in six tropical tree species. We show **A) Root biomass fractions** plotted against plant height and the predicted betaregression curves for seedlings grown in live (= including soil microbial communities) and sterilized soil of four successional stages (0, 15, 25, 115 years of forest recovery) at 40% light (white panels) and 5% light (grey panels). Rugs on the left side indicate whether measurements were taken in sterilized or live soils. **B) Plant-soil feedback** (PSF) as the logarithmic odds ratio of estimated marginal mean root fractions between live and sterilized soil. Filled circles indicate significant PSF ($p < 0.05$).

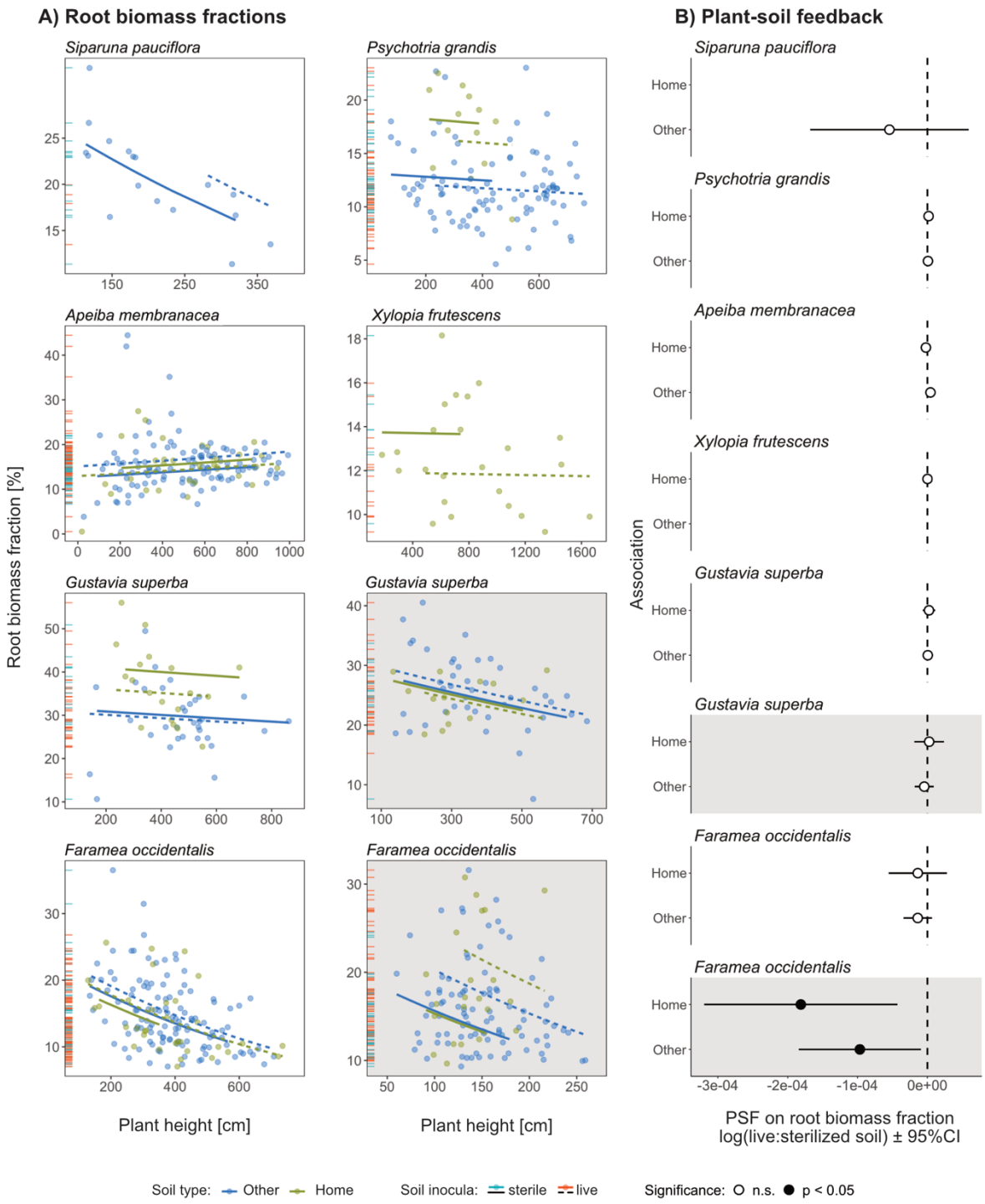


Figure S2.3. Root biomass fractions in six tropical tree species at "home". We show **A) Root biomass fractions** plotted against plant height and the predicted betaregression curves for seedlings grown in live (= including soil microbial communities) and sterilized soil of successional stages that a species is naturally abundant at ("home") and soils of other successional stages ("other) at 40% light (white panels) and 5% light (grey panels). Rugs on the left side indicate whether measurements were taken in sterilized or live soils. **B) Plant-soil feedback** (PSF) as the logarithmic odds ratio of estimated marginal mean root fractions between live and sterilized soil. Filled circles indicate significant PSF ($p < 0.05$).

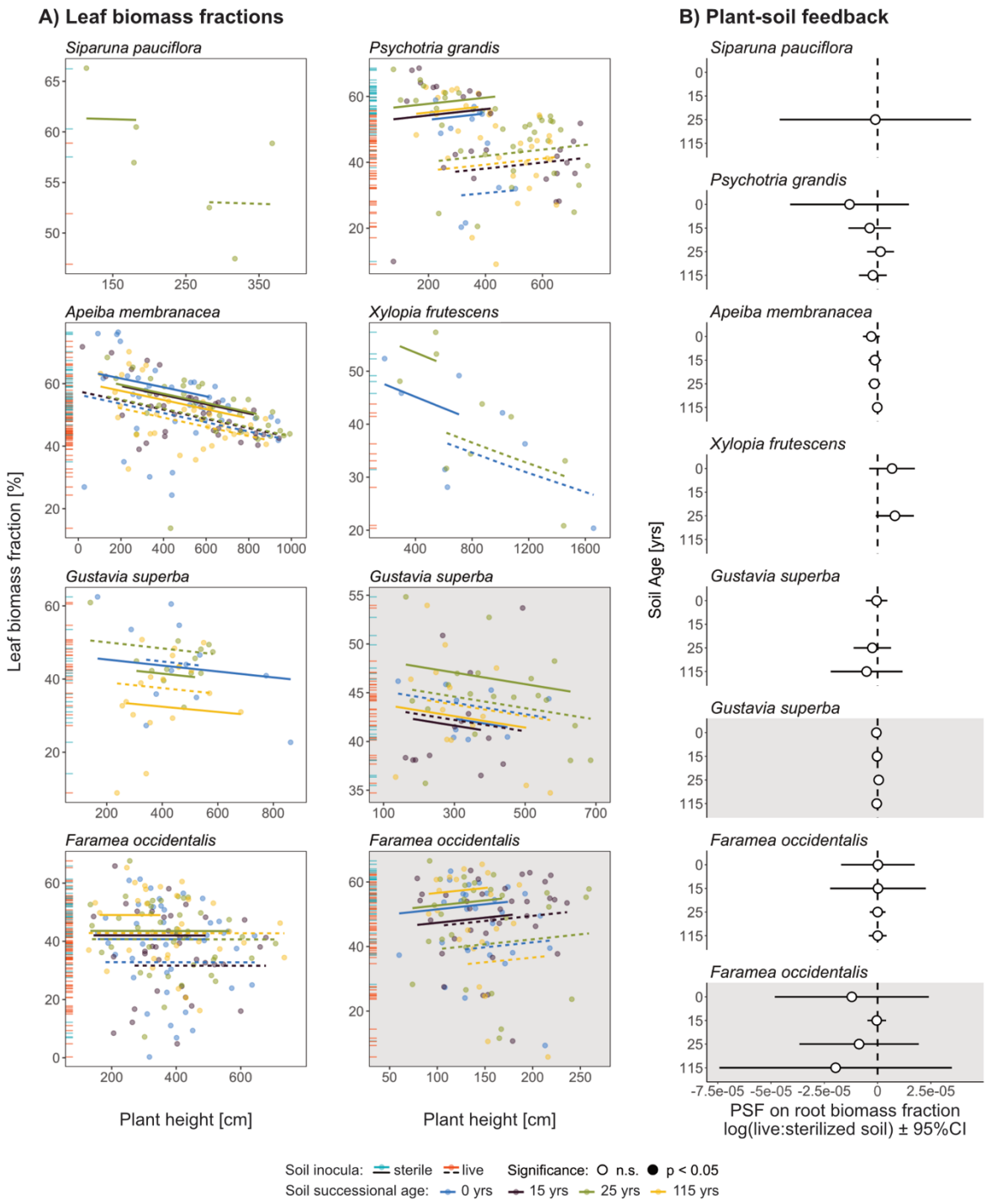


Figure S2.4. Leaf biomass fractions in six tropical tree species. We show **A) Root biomass fractions** plotted against plant height and the predicted betaregression curves for seedlings grown in live (= including soil microbial communities) and sterilized soil of four successional stages (0, 15, 25, 115 years of forest recovery) at 40% light (white panels) and 5% light (grey panels). Rugs on the left side indicate whether measurements were taken in sterilized or live soils. **B) Plant-soil feedback** (PSF) as the logarithmic odds ratio of estimated marginal mean root fractions between live and sterilized soil. Filled circles indicate significant PSF ($p < 0.05$).

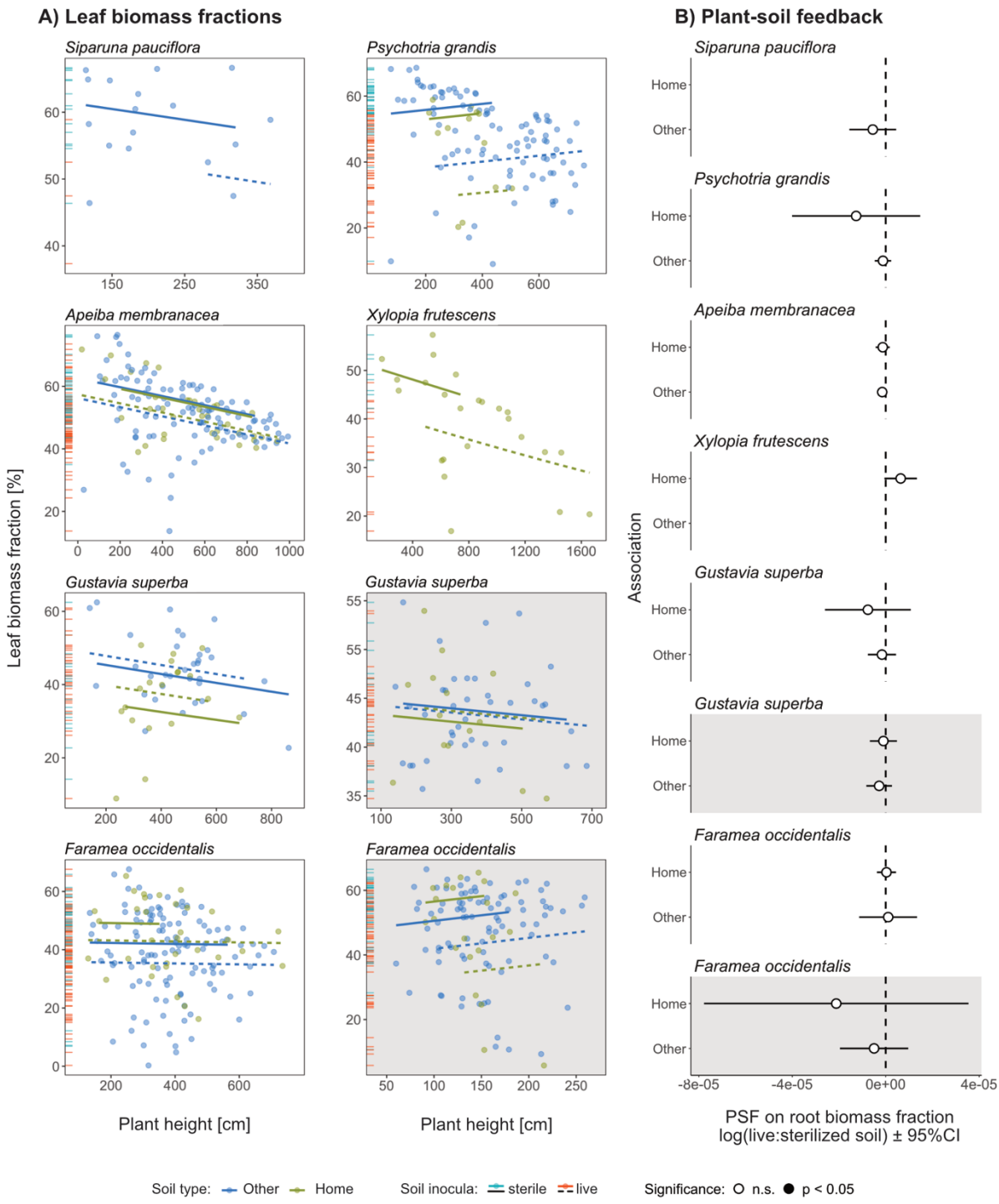


Figure S2.5. Leaf biomass fractions in six tropical tree species at "home". We show **A) Root biomass fractions** plotted against plant height and the predicted betaregression curves for seedlings grown in live (= including soil microbial communities) and sterilized soil of successional stages that a species is naturally abundant at ("home") and soils of other successional stages ("other) at 40% light (white panels) and 5% light (grey panels). Rugs on the left side indicate whether measurements were taken in sterilized or live soils. **B) Plant-soil feedback** (PSF) as the logarithmic odds ratio of estimated marginal mean root fractions between live and sterilized soil. Filled circles indicate significant PSF ($p < 0.05$).

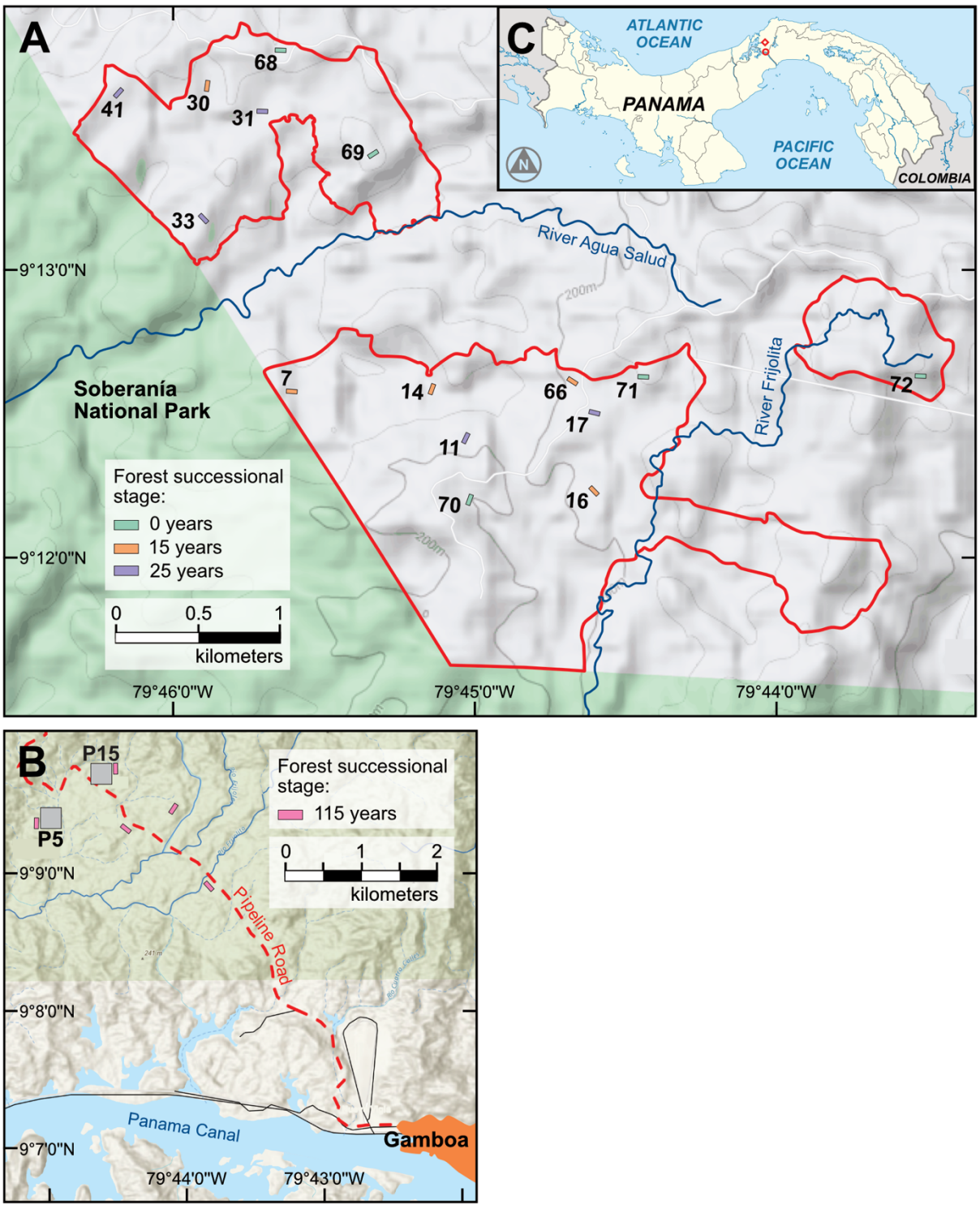


Figure S2.6. Maps showing field soil collection sites. We collected soils from five replicate sites for each of (**A**) three younger successional stages (0, 15, 25 years of forest recovery) in the Agua Salud chronosequence (numbering of sites is based on Agua Salud data) and (**B**) one older successional stage (115 years) along Pipeline Road. The location of two ForestGeo long-term sampling sites (P5 and P15) is added to facilitate orientation. (**C**) A country map shows the location of the Agua Salud sites (diamond) and the Pipeline Road sites (circle) in Central Panama.

Maps are strongly modified from (**A**) Google, (**B**) the Smithsonian Tropical Research Institute GIS portal, and (**C**) creative commons by Alexrk, CC BY-SA 3.0: https://commons.wikimedia.org/w/index.php?curid=6909551.

Table S2.1. Background data on seven tropical tree species. Species for our experiment were selected based on their abundance in our four successional stages (0, 15, 25, 115 years of recovery since agricultural abandonment). A species was associated to a successional stage, if it was among the 20 most abundant species in that successional stage. We calculated abundance as the importance value IV (=relative density plus relative basal area divided by two). IV can range from 0-1 with a higher value indicating higher abundance of a species at a respective successional stage. Seeds were collected from ≥ 6 adult trees per species ("n maternal trees"). Further, dates for seed collection, soil collection, and thinning are shown. Due to interspecific variation in the timing of seed production, experimental start was staggered: species in set 1 were sown on the 6.1.2018 and harvested between 29.7.-26.8.2020, while experimental duration was 24.-26.1.2019 to 30.7.-19.8.2020 for set 2, and 14.6.2019 to 3.8-7.8.2020 for set 3.

			IV per successional stage				n	Dates		
Set	Species	Family	0 yrs	15 yrs	25 yrs	115 yrs	maternal trees	Seed collection	Soil collection	Thinning
1	*Siparuna pauciflora* (Beurl.) A.DC.	Monimiaceae	0.04				8	17.10.-29.10.2018	29.10.-1.11.2018	7.-8.06.2019
1	*Psychotria grandis* Sw.	Rubiaceae	0.02				18	18.10.-1.11.2018	29.10.-1.11.2018	7.-8.06.2019
2	*Vismia baccifera* (L.) Triana & Planch.	Hypericaceae	0.10	0.03			12	30.12.2018 – 15.01.19	10.1.-15.01.2019	13.09.2019
3	*Apeiba membranacea* Spruce ex Benth.	Tiliaceae		0.03			6	1.02.-28.02.2019	23.05.-31.05.2019	21.01.2020
2	*Xylopia frutescens* Aubl.	Annonaceae	0.07	0.11	0.11		> 20	30.12.2018-15.01.19	10.1.-15.01.2019	13.09.2019
3	*Gustavia superba* (Kunth) O.Berg	Lecythidiaceae				0.06	19	15.05.-14.06.2019	23.05.-31.05.2019	21.01.2020
1	*Faramea occidentalis* (L.) A.Rich.	Rubiaceae				0.91	40	15.10.-30.10.2018	29.10.-1.11.2018	7.-8.06.2019

Table S2.2. Summary statistics on i) root and ii) leaf biomass fractions of six tropical tree species. Seedlings grew under high (40% light) or low light (5% light; "Light") in soils that either contained the living microbial community or had been sterilized ("Inoculum") and were collected from forests of four successional stages ("Successional stage"). We calculated the root and leaf fractions by dividing the dry weight of roots or leaves by the total dry weight of a plant. We show results of Anova-equivalent analyses following betaregression across four **A) Successional stages** and separate analyses on **B) Association**, which groups the four successional stages into "home", i.e. pooling all successional stages that a respective tree species is naturally abundant at, and "other". Significant values ($p < 0.05$) are printed in bold.

	Siparuna pauciflora		*Psychotria grandis*		*Apeiba membranacea*		*Xylopia frutescens*		*Gustavia superba*						*Faramea occidentalis*					
	40% light		40% light		40% light		40% light		40% light		5% light		Across light		40% light		5% light		Across light	
	F-ratio	*p*	F-ratio	*p*	F-ratio	*p*	F-ratio	*p*	F-ratio	*p*	F-ratio	*p*	F-ratio	*p*	F-ratio	*p*	F-ratio	*p*	F-ratio	*p*
i) ROOT BIOMASS FRACTION																				
A) Successional stage																				
Inoculum	0.38	0.540	0.90	0.342	2.97	0.085	1.35	0.245	1.05	0.306	0.09	0.767	<0.01	0.972	3.50	0.061	**30.17**	**<0.001**	**16.58**	**<0.001**
Successional stage			**7.62**	**<0.001**	1.93	0.123	0.21	0.650	**5.47**	**0.004**	0.61	0.608	2.13	0.095	1.93	0.123	2.22	0.084	0.24	0.868
Light													**25.67**	**<0.001**					**14.68**	**<0.001**
Inoculum x Successional stage			1.00	0.394	1.34	0.260	**12.09**	**0.001**	**3.32**	**0.036**	0.65	0.583	1.21	0.303	1.22	0.302	1.56	0.197	0.38	0.770
Inoculum x Light													1.19	0.275					2.46	0.117
Height	**6.58**	**0.010**	0.53	0.468	2.61	0.106	0.04	0.839	0.26	0.607	**5.33**	**0.021**	2.05	0.152	**53.03**	**<0.001**	**14.75**	**<0.001**	**68.05**	**<0.001**
B) Association																				
Inoculum	1.42	0.234	0.99	0.321	0.36	0.551	2.44	0.118	1.38	0.241	0.03	0.865	0.67	0.412	1.44	0.230	**30.30**	**<0.001**	**13.89**	**<0.001**
Association			**17.81**	**<0.001**	0.11	0.736			**10.25**	**0.001**	0.63	0.429	**4.89**	**0.027**	1.22	0.270	2.98	0.084	<0.01	0.981
Light													**26.71**	**<0.001**					**13.68**	**<0.001**
Inoculum x Association			0.21	0.646	3.15	0.076			0.74	0.391	0.32	0.572	0.80	0.370	<0.01	0.983	**4.00**	**0.046**	0.83	0.362
Inoculum x Light													1.45	0.228					2.69	0.101
Height	**13.76**	**<0.001**	0.31	0.580	2.58	0.108	<0.01	0.932	0.34	0.562	**6.39**	**0.012**	3.29	0.070	**46.11**	**<0.001**	**15.00**	**<0.001**	**66.63**	**<0.001**
ii) LEAF BIOMASS FRACTION																				
A) Successional stage																				
Inoculum	0.74	0.391	**38.25**	**<0.001**	**12.87**	**<0.001**	3.17	0.075	1.77	0.183	0.03	0.865	1.25	0.263	**5.48**	**0.020**	**16.65**	**<0.001**	**15.85**	**<0.001**
Successional stage			1.94	0.121	0.63	0.593	2.13	0.145	**4.47**	**0.011**	1.63	0.179	**3.74**	**0.011**	2.42	0.064	0.14	0.939	1.69	0.167
Light													2.36	0.125					**6.20**	**0.013**
Inoculum x Successional stage			0.33	0.807	0.29	0.835	0.84	0.359	0.24	0.786	0.54	0.654	0.10	0.959	0.42	0.737	**3.06**	**0.027**	0.60	0.613
Inoculum x Light													2.09	0.149					0.81	0.370
Height	<0.01	0.965	1.00	0.316	**19.34**	**<0.001**	3.06	0.080	0.50	0.478	1.51	0.219	1.32	0.251	<0.01	0.987	0.68	0.410	<0.01	0.989
B) Association																				
Inoculum	2.20	0.138	**34.08**	**<0.001**	**8.55**	**0.003**	3.35	0.067	1.45	0.228	0.05	0.823	2.45	0.118	2.59	0.108	**19.65**	**<0.001**	**12.82**	**<0.001**
Association			3.80	0.051	0.03	0.857			**8.78**	**0.003**	0.15	0.694	**4.90**	**0.027**	3.69	0.055	0.16	0.690	2.89	0.089
Light													0.14	0.709					**6.53**	**0.011**
Inoculum x Association			1.03	0.311	0.22	0.642			0.16	0.693	0.26	0.612	<0.01	0.951	0.01	0.919	**5.07**	**0.024**	0.73	0.393
Inoculum x Light													0.15	0.700					0.66	0.416
Height	0.77	0.380	0.95	0.330	**19.61**	**<0.001**	2.20	0.138	1.76	0.184	0.55	0.460	3.68	0.055	0.03	0.871	0.78	0.377	<0.01	0.986

Table S2.3. Sample sizes for biomass and biomass fraction analyses. Seedlings of seven tree species were grown under high (40% light) and low light level (5% light) in sterilized and live (i.e. including the microbial community) soils that were collected in forests of four successional stages (0, 15, 25, 115 yrs of forest recovery after agricultural abandonment). We present an overview of the number of plants that were harvested at the end of the greenhouse experiment and included in the analyses of biomass and biomass fractions per species x treatment combination. Numbers in parentheses show the number of plants that were ≤ 105 days old and were excluded from analysis. Red numbers indicate species x treatment combinations that were omitted from the analyses of biomass fractions because they had less than three observations making the calculation of regression slopes meaningless.

	40% light								5% light							
	0 yrs		15 yrs		25 yrs		115 yrs		0 yrs		15 yrs		25 yrs		115 yrs	
	sterile	live	sterile	live	sterile	live	sterile	live	sterile	live	sterile	live	sterile	live	sterile	live
Siparuna pauciflora	2	1	6	0	3	3 (1)	5	1	0	1	0	1	0	3	0	0
Psychotria grandis	8	5	12	14	13	25	10	22	0	6	0	8	0	9	0	0
Vismia baccifera	0	3	1	3	0	4	1	1	0	0	0	0	0	0	0	0
Apeiba membranacea	15	22 (1)	11 (1)	24 (1)	15	19 (2)	13 (1)	23	2 (4)	5 (7)	1 (3)	9 (3)	4 (5)	2 (6)	1 (3)	4 (5)
Xylopia frutescens	4	4	2	5	3	6	4	1	0	0	0	0	0	0	0	0
Gustavia superba	5	7	0	9	5	8	7	12	3	6	5	9	5	14	6	8
Faramea occidentalis	15	25	15	21	15	27	6	27	14	16	11	24	11	16	10	14

Table S2.4. Overview of plant-soil feedbacks (PSF) and PSF contrasts on performance in seven tree species. PSF compare performance between sterilized and live soils as logarithmic odds ratio (log(OR):emergence, biomass, leaf and root biomass fractions) and hazard ratio (HR: survival) with 1 standard error (se). Seven tropical tree species were grown at 40% and 5% ambient light. We show pairwise **contrasts** between PSF at four successional stages and soils from associated ("home" = pooling all successional stages a species is naturally abundant at) and "other" stages (pooling all successional stages at which a species is absent).

	Emergence				Biomass				Leaf biomass fraction				Root biomass fraction				Survival			
	40% light		5% light		40% light		5% light		40% light		5% light		40% light		5% light		40% light		5% light	
Siparuna pauciflora																				
	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR)± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	HR ± se	*p*	HR ± se	*p*
0	-0.23 ± 0.29	0.430	-0.13 ± 0.32	0.678	0.90 ± 0.44	0.070	–	–	–	–	–	–	–	–	–	–	0.55 ± 0.33	0.316	1.80 ± 1.02	0.295
15	0.47 ± 0.31	0.127	0.05 ± 0.32	0.883	–	–	–	–	–	–	–	–	–	–	–	–	0.19 ± 0.17	0.060	0.98 ± 0.58	0.975
25	-0.15 ± 0.30	0.615	0.29 ± 0.31	0.360	0.95 ± 0.62	0.151	–	–	-1.04E-06 ± 2.29E-05	0.964	–	–	-3.45E-05 ± 6.88E-05	0.616	–	–	0.66 ± 0.44	0.539	0.97 ± 0.58	0.954
115	-0.08 ± 0.32	0.805	0.07 ± 0.31	0.825	0.23 ± 0.57	0.696	–	–	–	–	–	–	–	–	–	–	0.38 ± 0.24	0.128	2.47 ± 1.38	0.107
Home	-0.26 ± 0.31	0.397	-0.12 ± 0.34	0.730	0.98 ± 0.54	0.093	–	–	–	–	–	–	–	–	–	–	0.58 ± 0.34	0.355	1.88 ± 1.05	0.260
Other	0.10 ± 0.18	0.592	0.13 ± 0.18	0.472	0.98 ± 0.28	0.004	–	–	-5.54E-06 ± 5.11E-06	0.278	–	–	-5.44E-05 ± 5.79E-05	0.347	–	–	0.41 ± 0.20	0.066	1.42 ± 0.57	0.382
Contrasts	**z**	***p***	**z**	***p***	**t**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***
0-15	1.69	0.092	0.40	0.686	–	–	–	–	–	–	–	–	–	–	–	–	1.13	0.257	0.85	0.396
0-25	0.20	0.844	0.95	0.344	0.06	0.957	–	–	–	–	–	–	–	–	–	–	-0.26	0.796	0.85	0.393
0-115	0.35	0.723	0.46	0.647	-1.16	0.275	–	–	–	–	–	–	–	–	–	–	0.48	0.633	-0.45	0.655
15-25	-1.45	0.147	0.54	0.589	–	–	–	–	–	–	–	–	–	–	–	–	-1.33	0.185	0.02	0.982
15-115	-1.24	0.216	0.05	0.961	–	–	–	–	–	–	–	–	–	–	–	–	-0.70	0.483	-1.32	0.185
25-115	0.16	0.872	-0.50	0.617	-0.70	0.499	–	–	–	–	–	–	–	–	–	–	0.69	0.491	-1.31	0.191
Other - home	1.00	0.316	0.64	0.520	<.01	0.996	–	–	–	–	–	–	–	–	–	–	-0.57	0.568	-0.48	0.628
Psychotria grandis																				
	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR)± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	HR ± se	*p*	HR ± se	*p*
0	-0.56 ± 0.37	0.128	0.12 ± 0.39	0.752	-1.09 ± 0.51	0.035	–	–	-1.32E-05 ± 1.42E-05	0.354	–	–	2.18E-06 ± 3.67E-06	0.552	–	–	7.73 ± 4.99	0.002	1.55 ± 0.60	0.259
15	-0.82 ± 0.41	0.047	-1.04 ± 0.44	0.018	0.44 ± 0.36	0.233	–	–	-3.71E-06 ± 5.09E-06	0.466	–	–	-1.29E-06 ± 3.58E-06	0.718	–	–	1.53 ± 0.80	0.419	2.00 ± 0.75	0.065
25	-0.84 ± 0.42	0.047	-0.91 ± 0.43	0.035	0.54 ± 0.30	0.077	–	–	1.38E-06 ± 3.22E-06	0.668	–	–	1.66E-06 ± 1.85E-06	0.370	–	–	1.36 ± 0.67	0.532	2.68 ± 1.11	0.017
115	-0.39 ± 0.38	0.305	-0.44 ± 0.39	0.259	0.31 ± 0.34	0.353	–	–	-2.21E-06 ± 3.31E-06	0.504	–	–	2.69E-06 ± 3.24E-06	0.406	–	–	4.61 ± 3.79	0.063	5.20 ± 3.00	0.004
Home	-0.67 ± 0.42	0.112	0.23 ± 0.41	0.579	-1.06 ± 0.50	0.038	–	–	-1.27E-05 ± 1.40E-05	0.365	–	–	1.74E-06 ± 3.49E-06	0.618	–	–	7.74 ± 5.00	0.002	1.56 ± 0.61	0.253
Other	-0.64 ± 0.24	0.008	-0.79 ± 0.24	0.001	0.47 ± 0.19	0.018	–	–	-1.18E-06 ± 1.78E-06	0.508	–	–	8.23E-07 ± 9.48E-07	0.385	–	–	1.82 ± 0.60	0.069	2.70 ± 0.76	<.001
Contrasts	**z**	***p***	**z**	***p***	**t**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***
0-15	-0.49	0.622	-2.02	0.044	2.51	0.014	–	–	0.85	0.394	–	–	-0.54	0.593	–	–	1.97	0.049	-0.52	0.604
0-25	-0.53	0.599	-1.81	0.071	2.80	0.006	–	–	0.90	0.369	–	–	-0.15	0.880	–	–	2.16	0.031	-1.06	0.287
0-115	0.32	0.749	-1.04	0.299	2.32	0.023	–	–	0.86	0.389	–	–	0.16	0.873	–	–	0.50	0.619	-1.86	0.064
15-25	-0.04	0.971	0.22	0.826	0.24	0.812	–	–	0.70	0.483	–	–	0.63	0.531	–	–	0.16	0.870	-0.58	0.561
15-115	0.76	0.448	1.03	0.305	-0.25	0.801	–	–	0.34	0.737	–	–	0.63	0.530	–	–	-1.14	0.253	-1.48	0.139
25-115	0.79	0.431	0.81	0.419	-0.52	0.606	–	–	-0.66	0.507	–	–	0.35	0.727	–	–	-1.28	0.199	-1.00	0.317
Other - home	0.05	0.960	-2.14	0.033	-2.89	0.005	–	–	-0.87	0.382	–	–	0.29	0.771	–	–	-2.02	0.044	1.31	0.189

Table S2.4 continued (1/3).

	Emergence				Biomass				Leaf fraction				Root fraction				Survival			
	40% light		5% light		40% light		5% light		40% light		5% light		40% light		5% light		40% light		5% light	
Vismia baccifera																				
	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	HR ± se	*p*	HR ± se	*p*
0	0.83 ± 0.21	<.001	1.79 ± 0.29	<.001	–	–	–	–	–	–	–	–	–	–	–	–	0.70 ± 0.13	0.056	0.59 ± 0.17	0.072
15	0.18 ± 0.20	0.375	0.68 ± 0.22	0.002	–	–	–	–	–	–	–	–	–	–	–	–	0.69 ± 0.13	0.042	0.46 ± 0.09	<.001
25	-0.10 ± 0.20	0.622	-0.03 ± 0.20	0.884	–	–	–	–	–	–	–	–	–	–	–	–	0.77 ± 0.13	0.130	0.24 ± 0.04	<.001
115	0.62 ± 0.21	0.004	0.08 ± 0.22	0.702	–	–	–	–	–	–	–	–	–	–	–	–	0.63 ± 0.12	0.019	0.21 ± 0.04	<.001
Home	0.48 ± 0.15	0.001	1.15 ± 0.17	<.001	1.84 ± 0.45	0.005	–	–	–	–	–	–	–	–	–	–	0.69 ± 0.09	0.007	0.49 ± 0.08	<.001
Other	0.26 ± 0.15	0.082	0.01 ± 0.15	0.960	2.81 ± 0.57	0.001	–	–	–	–	–	–	–	–	–	–	0.68 ± 0.09	0.004	0.23 ± 0.03	<.001
Contrasts	**z**	***p***	**z**	***p***	**t**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***
0-15	-2.28	0.022	-3.08	0.002	–	–	–	–	–	–	–	–	–	–	–	–	0.09	0.931	0.70	0.482
0-25	-3.26	0.001	-5.19	<.001	–	–	–	–	–	–	–	–	–	–	–	–	-0.36	0.720	2.69	0.007
0-115	-0.73	0.467	-4.73	<.001	–	–	–	–	–	–	–	–	–	–	–	–	0.39	0.694	2.84	0.004
15-25	-0.98	0.329	-2.40	0.017	–	–	–	–	–	–	–	–	–	–	–	–	-0.45	0.654	2.66	0.008
15-115	1.50	0.134	-1.93	0.054	–	–	–	–	–	–	–	–	–	–	–	–	0.32	0.751	2.85	0.004
25-115	2.45	0.014	0.38	0.704	–	–	–	–	–	–	–	–	–	–	–	–	0.75	0.452	0.38	0.704
Other - home	-1.10	0.273	-5.00	<.001	-1.19	0.267	–	–	–	–	–	–	–	–	–	–	-0.11	0.916	-3.79	<.001
Apeiba membranacea																				
	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	HR ± se	*p*	HR ± se	*p*
0	-0.43 ± 0.17	0.011	0.10 ± 0.17	0.567	-0.24 ± 0.34	0.479	-0.39 ± 0.40	0.335	-2.95E-06 ± 2.01E-06	0.142	–	–	2.16E-06 ± 3.05E-06	0.479	–	–	0.55 ± 0.17	0.049	0.68 ± 0.13	0.048
15	0.13 ± 0.16	0.416	0.01 ± 0.17	0.975	-0.37 ± 0.36	0.301	-0.27 ± 0.51	0.606	-1.29E-06 ± 1.53E-06	0.399	–	–	-2.20E-06 ± 3.52E-06	0.532	–	–	0.73 ± 0.18	0.206	1.12 ± 0.18	0.489
25	-0.20 ± 0.18	0.256	0.17 ± 0.17	0.256	0.08 ± 0.35	0.826	-0.28 ± 0.44	0.533	-1.52E-06 ± 1.44E-06	0.292	–	–	5.79E-06 ± 4.33E-06	0.181	–	–	0.60 ± 0.15	0.042	0.80 ± 0.14	0.191
115	-0.17 ± 0.17	0.312	0.08 ± 0.17	0.633	-0.48 ± 0.34	0.161	1.34 ± 0.53	0.020	-1.56E-07 ± 1.08E-06	0.885	–	–	4.83E-06 ± 4.06E-06	0.234	–	–	0.52 ± 0.16	0.039	0.90 ± 0.15	0.535
Home	0.14 ± 0.16	0.390	<.01 ± 0.17	1.000	-0.34 ± 0.35	0.331	-0.42 ± 0.62	0.508	-1.27E-06 ± 1.52E-06	0.403	–	–	–	–	–	–	0.73 ± 0.18	0.206	1.12 ± 0.18	0.483
Other	-0.27 ± 0.10	0.007	0.12 ± 0.10	0.228	-0.12 ± 0.21	0.572	0.27 ± 0.26	0.309	-1.49E-06 ± 8.87E-07	0.094	8.18E-06 ± 4.56E-05	0.858	–	–	–	–	0.57 ± 0.10	0.002	0.80 ± 0.09	0.052
Contrasts	**z**	***p***	**z**	***p***	**t**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***
0-15	2.40	0.016	-0.38	0.703	-0.26	0.796	0.20	0.847	0.67	0.505	–	–	-0.89	0.375	–	–	-0.74	0.462	-2.13	0.033
0-25	0.97	0.332	0.31	0.755	0.68	0.495	0.20	0.843	0.59	0.556	–	–	0.76	0.449	–	–	-0.21	0.831	-0.67	0.504
0-115	1.06	0.287	-0.06	0.950	-0.52	0.603	2.61	0.017	1.22	0.222	–	–	0.58	0.564	–	–	0.13	0.897	-1.19	0.233
15-25	-1.38	0.166	0.69	0.492	0.88	0.381	-0.01	0.989	-0.11	0.912	–	–	1.26	0.209	–	–	0.58	0.562	1.55	0.121
15-115	-1.29	0.196	0.32	0.752	-0.23	0.820	2.18	0.042	0.60	0.548	–	–	1.16	0.247	–	–	0.86	0.392	1.00	0.317
25-115	0.09	0.927	-0.37	0.710	-1.18	0.240	2.35	0.030	0.75	0.453	–	–	-0.22	0.823	–	–	0.35	0.728	-0.53	0.593
Other - home	-2.14	0.032	0.60	0.548	-0.53	0.598	-1.05	0.304	0.12	0.901	–	–	–	–	–	–	-0.86	0.391	-1.93	0.054

Table S2.4 continued (2/3).

	Emergence				Biomass				Leaf fraction				Root fraction				Survival			
	40% light		5% light		40% light		5% light		40% light		5% light		40% light		5% light		40% light		5% light	
Xylopia frutescens																				
	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	HR ± se	*p*	HR ± se	*p*
0	-1.45 ± 0.33	<.001	-0.93 ± 0.34	0.006	1.96 ± 0.73	0.014	–	–	6.80E-06 ± 5.45E-06	0.213	–	–	-8.18E-07 ± 4.21E-06	0.846	–	–	0.22 ± 0.18	0.058	4.45 ± 1.61	<.001
15	-1.30 ± 0.40	0.001	-1.30 ± 0.39	0.001	0.46 ± 0.85	0.594	–	–	–	–	–	–	–	–	–	–	0.17 ± 0.19	0.110	2.30 ± 0.87	0.027
25	-1.70 ± 0.41	<.001	-1.60 ± 0.44	<.001	1.30 ± 0.71	0.083	–	–	8.12E-06 ± 4.55E-06	0.074	–	–	2.75E-07 ± 1.22E-06	0.821	–	–	1.57 ± 1.29	0.578	1.15 ± 0.50	0.753
115	-2.56 ± 0.53	<.001	-2.50 ± 0.55	<.001	2.33 ± 1.21	0.068	–	–	–	–	–	–	–	–	–	–	1.08 ± 0.84	0.926	1.94 ± 1.07	0.230
Home	-1.49 ± 0.23	<.001	-1.22 ± 0.23	<.001	1.37 ± 0.42	0.003	–	–	6.41E-06 ± 3.55E-06	0.071	–	–	1.51E-07 ± 1.71E-06	0.930	–	–	0.48 ± 0.24	0.144	2.72 ± 0.67	<.001
Other	-2.59 ± 0.56	<.001	-2.53 ± 0.57	<.001	2.31 ± 1.17	0.060	–	–	–	–	–	–	–	–	–	–	1.14 ± 0.93	0.869	2.01 ± 1.11	0.207
Contrasts	**z**	***p***	**z**	***p***	**t**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***
0-15	0.29	0.772	-0.72	0.469	-1.36	0.189	–	–	–	–	–	–	–	–	–	–	0.20	0.845	1.30	0.195
0-25	-0.48	0.631	-1.20	0.229	-0.65	0.521	–	–	0.19	0.853	–	–	0.20	0.840	–	–	-1.72	0.085	2.40	0.016
0-115	-1.82	0.068	-2.47	0.014	0.27	0.788	–	–	–	–	–	–	–	–	–	–	-1.42	0.155	1.27	0.205
15-25	-0.69	0.490	-0.50	0.619	0.76	0.459	–	–	–	–	–	–	–	–	–	–	-1.62	0.105	1.21	0.226
15-115	-1.90	0.058	-1.79	0.074	1.30	0.209	–	–	–	–	–	–	–	–	–	–	-1.36	0.173	0.26	0.796
25-115	-1.29	0.198	-1.29	0.197	0.74	0.468	–	–	–	–	–	–	–	–	–	–	0.34	0.736	-0.75	0.453
Other - home					-0.77	0.446	–	–	–	–	–	–	–	–	–	–	0.97	0.333	-0.50	0.616
Gustavia superba																				
	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	HR ± se	*p*	HR ± se	*p*
0	-0.14 ± 0.56	0.801	-0.05 ± 0.64	0.938	0.48 ± 0.33	0.154	0.19 ± 0.47	0.691	-4.49E-07 ± 2.57E-06	0.861	-5.59E-07 ± 1.22E-06	0.646	-4.95E-06 ± 1.01E-05	0.623	8.45E-06 ± 1.27E-05	0.507	–	–	–	–
15	1.59 ± 0.87	0.066	0.06 ± 0.66	0.924	–	–	0.19 ± 0.37	0.604	–	–	-2.34E-07 ± 1.13E-06	0.836	–	–	-1.07E-05 ± 1.09E-05	0.325	–	–	–	–
25	-0.25 ± 0.63	0.695	0.65 ± 0.63	0.303	0.18 ± 0.32	0.578	0.16 ± 0.35	0.645	-2.47E-06 ± 4.47E-06	0.580	5.30E-07 ± 5.74E±07	0.356	6.71E-06 ± 1.34E-05	0.618	-6.61E-06 ± 1.05E-06	0.530	–	–	–	–
115	-0.38 ± 0.59	0.516	-0.43 ± 0.63	0.495	-0.04 ± 0.26	0.887	-0.37 ± 0.40	0.357	-5.15E-06 ± 8.59E-06	0.549	-4.02E-07 ± 9.27E-07	0.664	2.42E-06 ± 5.25E-06	0.646	2.44E-06 ± 9.92E-06	0.805	–	–	–	–
Home	-0.43 ± 0.63	0.500	-0.44 ± 0.66	0.504	-0.04 ± 0.26	0.884	-0.28 ± 0.37	0.450	-7.63E-06 ± 9.36E-06	0.415	-2.22E-07 ± 6.19E-07	0.719	2.29E-06 ± 4.53E-06	0.613	2.53E-06 ± 1.08E-05	0.815	–	–	–	–
Other	0.24 ± 0.43	0.575	0.26 ± 0.40	0.517	0.32 ± 0.22	0.161	0.21 ± 0.21	0.312	-1.61E-06 ± 3.08E-06	0.601	7.83E-08 ± 3.03E-07	0.796	5.72E-07 ± 2.34E-06	0.807	-4.48E-06 ± 6.96E-06	0.520	–	–	–	–
Contrasts	**z**	***p***	**z**	***p***	**t**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***
0-15	1.84	0.066	0.13	0.899	–	–	0.01	0.994	–	–	0.20	0.838	–	–	-1.07	0.284	–	–	–	–
0-25	-0.13	0.893	0.81	0.420	-0.66	0.515	-0.05	0.962	-0.42	0.676	0.75	0.454	0.51	0.612	-0.86	0.390	–	–	–	–
0-115	-0.32	0.752	-0.44	0.660	-1.23	0.226	-0.89	0.380	-0.55	0.585	0.11	0.912	0.49	0.622	-0.37	0.708	–	–	–	–
15-25	-1.75	0.081	0.64	0.521	–	–	-0.06	0.948	–	–	0.57	0.569	–	–	0.30	0.766	–	–	–	–
15-115	-1.92	0.055	-0.54	0.587	–	–	-1.04	0.305	–	–	-0.12	0.903	–	–	0.88	0.376	–	–	–	–
25-115	-0.16	0.875	-1.22	0.224	-0.52	0.604	-1.00	0.322	-0.40	0.689	-0.77	0.440	-0.48	0.629	0.62	0.535	–	–	–	–
Other - home	0.87	0.383	0.91	0.364	-1.04	0.303	-1.15	0.255	-0.66	0.509	-0.42	0.677	0.40	0.690	0.54	0.589	–	–	–	–

Table S2.4 continued (3/3).

	Emergence				Biomass				Leaf fraction				Root fraction				Survival			
	40% light		5% light		40% light		5% light		40% light		5% light		40% light		5% light		40% light		5% light	
Faramea occidentalis																				
	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	log(OR ± se	*p*	log(OR) ± se	*p*	log(OR) ± se	*p*	HR ± se	*p*	HR ± se	*p*
0	-0.73 ± 0.36	0.043	-1.16± 0.44	0.009	0.67 ± 0.22	0.003	0.17 ± 0.19	0.372	1.46E-07 ± 8.80E-06	0.987	-1.21E-05 ± 1.84E-05	0.512	-1.06E-05 ± 1.64E-05	0.518	-1.10E-04 ± 5.31E-05	0.038	–	–	–	–
15	-0.44 ± 0.45	0.333	-0.16 ± 0.40	0.701	0.49 ± 0.23	0.032	0.55 ± 0.19	0.004	1.90E-07 ± 1.14E-05	0.987	-4.05E-07 ± 2.25E-06	0.857	-4.17E-05 ± 1.96E-05	0.033	-7.42E-05 ± 4.87E-05	0.127	–	–	1.78 ± 2.52	0.684
25	-0.61 ± 0.50	0.221	-0.79 ± 0.43	0.070	0.34 ± 0.21	0.116	0.05 ± 0.21	0.808	3.12E-08 ± 1.88E-06	0.987	-8.68E-06 ± 1.43E-05	0.543	-4.41E-07 ± 1.58E-05	0.978	-1.09E-04 ± 5.66E-05	0.054	–	–	1.70 ± 2.40	0.709
115	0.03 ± 0.36	0.945	1.49 ± 0.42	<.001	0.54 ± 0.30	0.074	-0.13 ± 0.22	0.538	3.53E-08 ± 2.13E-06	0.987	-1.97E-05 ± 2.78E-05	0.479	-1.58E-05 ± 2.18E-05	0.470	-1.78E-04 ± 6.92E-05	0.010	–	–	1.98 ± 2.80	0.629
Home	<0.01± 0.37	1.000	1.56 ± 0.43	<.001	0.60 ± 0.31	0.055	0.27 ± 0.11	0.016	3.42E-07 ± 2.08E-06	0.870	-2.12E-05 ± 2.89E-05	0.463	-1.37E-05 ± 2.13E-05	0.518	-1.81E-04 ± 7.06E-05	0.010	–	–	1.97 ± 2.78	0.632
Other	-0.58 ± 0.26	0.028	-0.71 ± 0.26	0.006	0.53 ± 0.13	<.001	-0.13 ± 0.22	0.544	1.05E-06 ± 6.33E-06	0.868	-4.98E-06 ± 7.46E-06	0.505	-1.38E-05 ± 1.04E-05	0.182	-9.67E-05 ± 4.46E-05	0.030	–	–	0.61 ± 0.50	0.547
Contrasts	**z**	***p***	**z**	***p***	**t**	***p***	**t**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***	**z**	***p***
0-15	0.53	0.595	1.71	0.087	-0.57	0.567	1.41	0.162	0.02	0.987	0.67	0.501	-1.32	0.186	0.73	0.463	–	–	–	–
0-25	0.21	0.834	0.62	0.537	-1.08	0.284	-0.43	0.671	-0.02	0.987	0.25	0.803	0.46	0.649	0.02	0.987	–	–	–	–
0-115	1.52	0.128	4.41	<.001	-0.33	0.741	-1.05	0.295	-0.02	0.987	-0.38	0.702	-0.20	0.843	-1.26	0.208	–	–	–	–
15-25	-0.26	0.797	-1.06	0.287	-0.48	0.632	-1.79	0.077	-0.02	0.987	-0.62	0.536	1.71	0.087	-0.69	0.492	–	–	0.02	0.981
15-115	0.80	0.423	2.81	0.005	0.15	0.881	-2.38	0.019	-0.02	0.987	-0.72	0.472	0.97	0.334	-1.85	0.065	–	–	-0.05	0.958
25-115	1.03	0.301	3.76	<.001	0.56	0.579	-0.62	0.534	0.02	0.987	-0.51	0.611	-0.58	0.561	-1.26	0.209	–	–	-0.08	0.939
Other - home	-1.26	0.207	-4.51	<.001	0.20	0.840	-1.67	0.097	-0.17	0.869	-0.67	0.506	<.01	0.996	-1.74	0.082	–	–	-0.72	0.474

Supplement Chapter 3

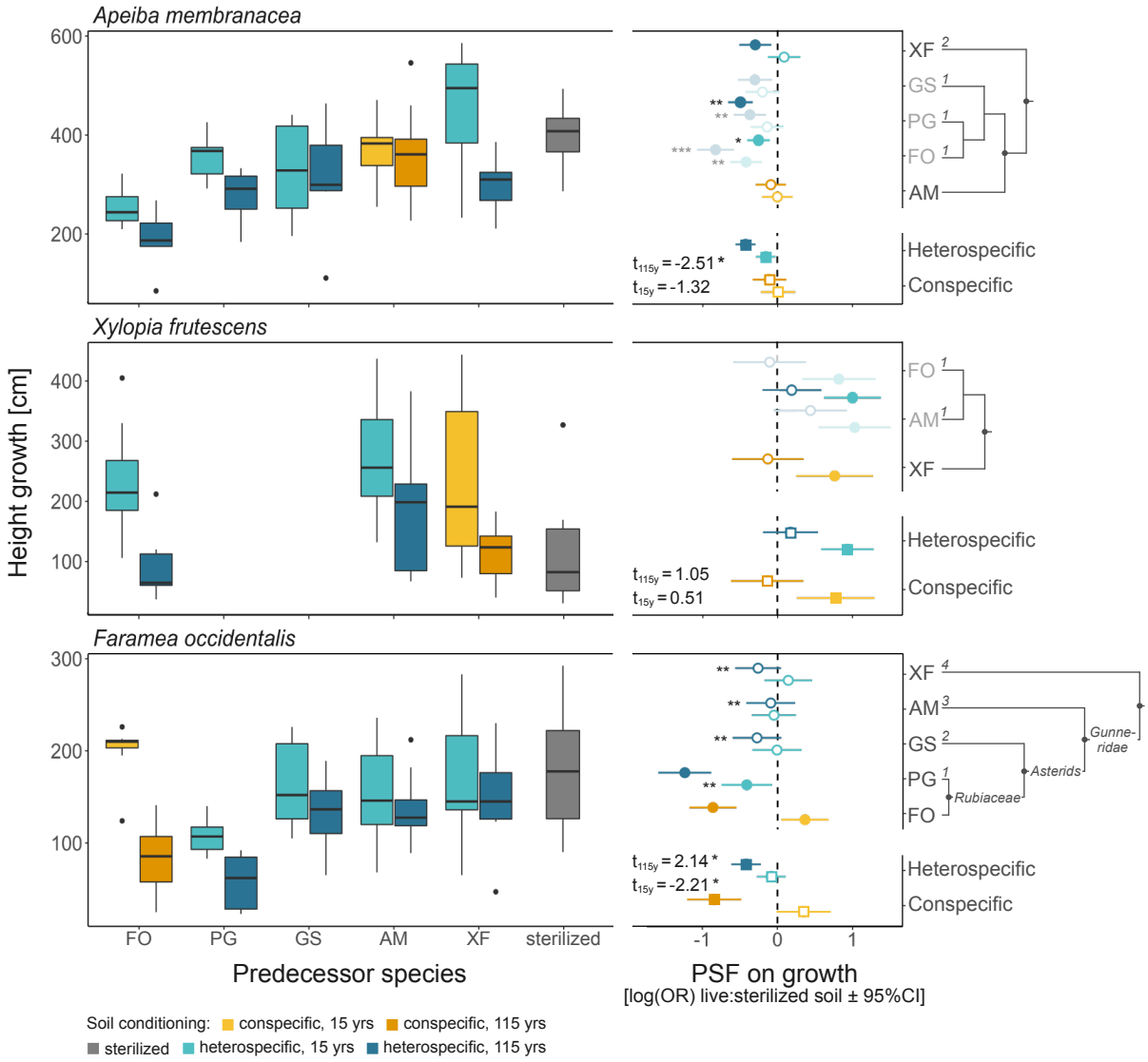


Figure S3.1. Effects of predecessor species and soil successional stage on height growth of three successor species. The left side of the panels show seedling height growth (difference between seedling aboveground height at transplantation and harvest after 7 months) of successor species grown in early- (15 years of recovery) or late- (115 years) successional soils that were sterilized or conditioned by conspecifics and heterospecific species. The centre of the panels shows plant-soil feedbacks (PSF) as the logarithmic odds ratio of the height growth of seedlings in live vs sterilized soil ± 95% confidence intervals. The vertical dashed line denotes an effect size of zero at which the conditioning species had no impact on height growth relative to sterilized soil. Positive PSF represent a higher height growth in conditioned than sterilized soils. PSF averaged over all heterospecific predecessors and conspecific predecessor (squares) and separately for each predecessor species (circles) are shown. Cladograms on the right side of the panels illustrate the phylogenetic relatedness between successor and predecessor species. We grouped heterospecific predecessors into phylogenetic categories (1-4), based on their last common ancestor with the respective successor species. Where several predecessor species were pooled into a phylogenetic category, the category's PSF is plotted on top of the PSF of each individual predecessor species (faded). Filled symbols represent significant PSF ($p < 0.05$). Stars indicate significant differences between PSF of conspecifics and a heterospecific or a phylogenetic category (p < 0.001*** < 0.01** < 0.05*). Results of pairwise contrasts between conspecific and overall heterospecific PSF are provided for early- and late-successional soils. FO = *Faramea occidentalis*, PG = *Psychotria grandis*, GS = *Gustavia superba*, AM = *Apeiba membranacea*, XF = *Xylopia frutescens*.

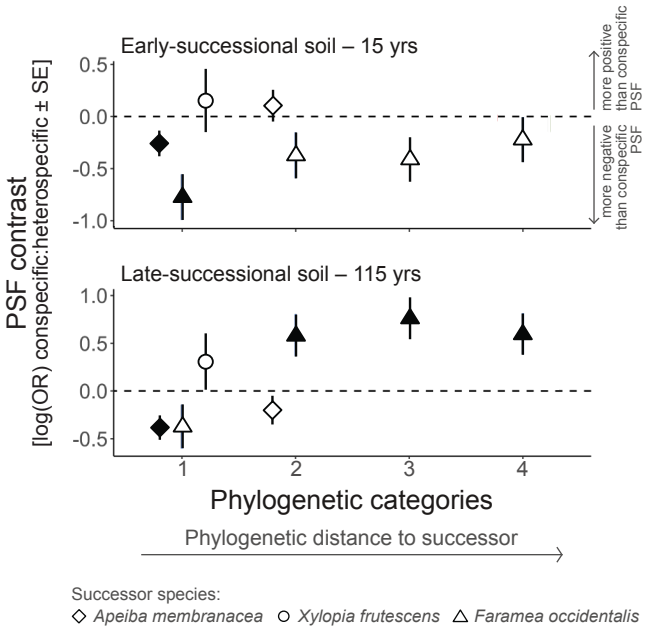


Figure S3.2. Phylogenetic signal in plant-soil feedback (PSF) on seedling height growth. We show contrasts between heterospecific relative to conspecific predecessor-successor pairs against their respective phylogenetic distance. PSF were calculated as logarithmic odds ratio of the height growth of seedlings of three successor species growing in soils conditioned by conspecific versus heterospecific predecessors. Positive values, above the dashed line, indicate a better performance in heterospecific than conspecific conditioned soils and negative values indicate the opposite. Error bars represent one standard error. Phylogenetic distance between predecessor and successor species was measured as a categorical variable based on cladograms (see Fig. S3.1). Filled symbols represent significant PSF ($p < 0.05$).

Table S3.1. Plant-soil-feedbacks (PSF) on seedling A) biomass and B) height growth of three successor tree species. PSF were calculated as the logarithm of the odds ratio (log(OR)) between the performance of seedlings grown in soils including microbial communities (live) and sterilized soil. PSF in early- (15 yrs of recovery) and late- (115 years) successional soils that have been conditioned by three to five **predecessor species**, as well as the pooled heterospecific PSF are shown. Further, we grouped predecessors into phylogenetic categories that represent their **phylogenetic distance** from the respective successor species, based on taxonomic hierarchies (see Fig. 2 for cladograms). Note that for X. frutescens all predecessors were grouped into the same phylogenetic category (i.e., phylogenetic PSF equals overall heterospecific PSF). For F. occidentalis each predecessor represented a distinct phylogenetic group (i.e., phylogenetic PSF are reordered predecessor PSF). Significant values ($p < 0.05$) are printed in bold.

	Successor species											
	Apeiba membranacea				*Xylopia frutescens*				*Faramea occidentalis*			
	15 yrs		115 yrs		15 yrs		115 yrs		15 yrs		115 yrs	
A) BIOMASS	log(OR) ± SE	*p*	log(OR) ± SE	*p*	log(OR) ± SE	*p*	log(OR) ± SE	*p*	log(OR) ± SE	*p*	log(OR) ± SE	*p*
Predecessor species												
Apeiba membranacea	-0.94 ± 1.02	0.359	-1.29 ± 1.04	0.219	1.97 ± 0.51	**<0.001**	1.48 ± 0.51	**0.005**	-0.76 ± 0.21	**<0.001**	-0.66 ± 0.23	**0.005**
Faramea occidentalis	-3.58 ± 1.13	**0.002**	-6.35 ± 1.31	**<0.001**	1.48 ± 0.51	**0.005**	-0.16 ± 0.51	0.757	-0.03 ± 0.23	0.891	-2.36 ± 0.23	**<0.001**
Gustavia superba	-1.93 ± 1.15	0.097	-3.65 ± 1.19	**0.003**	–	–	–	–	-0.84 ± 0.24	**0.001**	-1.18 ± 0.23	**<0.001**
Psychotria grandis	-2.00 ± 1.13	0.079	-4.86 ± 1.11	**<0.001**	–	–	–	–	-1.79 ± 0.24	**<0.001**	-2.01 ± 0.25	**<0.001**
Xylopia frutescens	3.35 ± 1.12	**0.003**	-4.61 ± 1.13	**<0.001**	1.46 ± 0.57	**0.012**	0.08 ± 0.51	0.877	-0.21 ± 0.23	0.370	-0.75 ± 0.22	**0.001**
Heterospecific	-0.91 ± 0.77	0.244	-4.64 ± 0.81	**<0.001**	1.73 ± 0.38	**<0.001**	0.67 ± 0.39	0.089	-0.83 ± 0.14	**<0.001**	-1.08 ± 0.15	**<0.001**
Phylogenetic distance												
1	–	–	–	–	–	–	–	–	-1.79 ± 0.24	**<0.001**	-2.01 ± 0.25	**<0.001**
2	–	–	–	–	–	–	–	–	-0.84 ± 0.24	**0.001**	-1.18 ± 0.23	**<0.001**
3	-2.35 ± 0.73	**0.002**	-4.72 ± 0.78	**<0.001**	–	–	–	–	-0.76 ± 0.21	**<0.001**	-0.66 ± 0.23	**0.005**
4	3.35 ± 1.12	**0.003**	-4.61 ± 1.13	**<0.001**	1.73 ± 0.38	**<0.001**	0.67 ± 0.39	0.089	-0.21 ± 0.23	0.370	-0.75 ± 0.22	**0.001**
B) HEIGHT GROWTH	log(OR) ± SE	*p*	log(OR) ± SE	*p*	log(OR) ± SE	*p*	log(OR) ± SE	*p*	log(OR) ± SE	*p*	log(OR) ± SE	*p*
Predecessor species												
Apeiba membranacea	<.01 ± 0.10	0.972	-0.09 ± 0.10	0.379	1.03 ± 0.24	**<0.001**	0.44 ± 0.24	0.073	-0.05 ± 0.15	0.743	-0.09 ± 0.16	0.580
Faramea occidentalis	-0.42 ± 0.11	**<0.001**	-0.83 ± 0.12	**<0.001**	0.82 ± 0.24	**0.001**	-0.10 ± 0.24	0.684	0.36 ± 0.16	**0.023**	-0.86 ± 0.16	**<0.001**
Gustavia superba	-0.20 ± 0.12	0.081	-0.30 ± 0.12	**0.009**	–	–	–	–	-0.01 ± 0.17	0.959	-0.27 ± 0.16	0.096
Psychotria grandis	-0.14 ± 0.11	0.206	-0.37 ± 0.11	**0.001**	–	–	–	–	-0.41 ± 0.17	**0.017**	-1.23 ± 0.18	**<0.001**
Xylopia frutescens	0.09 ± 0.11	0.431	-0.30 ± 0.11	**0.007**	0.77 ± 0.26	**0.004**	-0.12 ± 0.24	0.607	0.14 ± 0.16	0.378	-0.26 ± 0.15	0.094
Heterospecific	-0.15 ± 0.07	**0.022**	-0.42 ± 0.07	**<0.001**	0.93 ± 0.18	**<0.001**	0.18 ± 0.18	0.344	-0.08 ± 0.10	0.401	-0.42 ± 0.10	**<0.001**
Phylogenetic distance												
1	–	–	–	–	–	–	–	–	-0.41 ± 0.17	**0.017**	-1.23 ± 0.18	**<0.001**
2	–	–	–	–	–	–	–	–	-0.01 ± 0.17	0.959	-0.27 ± 0.16	0.096
3	-0.24 ± 0.07	**<0.001**	-0.47 ± 0.07	**<0.001**	–	–	–	–	-0.05 ± 0.15	0.743	-0.09 ± 0.16	0.580
4	0.13 ± 0.12	0.280	-0.28 ± 0.11	**0.015**	0.93 ± 0.18	**<0.001**	0.18 ± 0.18	0.344	0.14 ± 0.16	0.378	-0.26 ± 0.15	0.094

Table S3.2. Summary statistics on height growth of three tree species. Results of analyses of variance (type III) and estimated coefficients of determination (R^2) of three separate linear mixed effects regression models for the height growth (difference between seedling aboveground height at start and end of the seven month greenhouse experiment) of three successor tree species. All analyses included the fixed effects inoculum (soils including microbiota vs sterilized soil) and successional stage of the soil (Stage: 15 years vs 115 years of forest recovery). Additionally, we assessed the effects of soil conditioning predecessor species by analysing **A)** soil conditioning species into the binary variable con-hetero (conspecific predecessor vs all heterospecific predecessors pooled), **B)** the identity of soil conditioning species (predecessor: conspecific and 2-4 heterospecific tree species), and **C)** predecessors grouped into phylogenetic categories as a measure of phylogenetic distance between the successor and the predecessor species. Note that for X. frutescens all predecessors were grouped into the same phylogenetic category (i.e., phylogenetic analysis equals con- vs. heterospecific analysis). For F. occidentalis each predecessor represented a distinct phylogenetic group (i.e., phylogenetic analysis equals predecessor species analysis). Significant values ($p < 0.05$) are highlighted in bold. If degrees of freedom (df) were identical for all three species only one value is given, if they differed, df are ordered like the species.

	Successor species						
	Apeiba membranacea		*Xylopia frutescens*		*Faramea occidentalis*		
A) CON- vs. HETEROSPECIFIC	**X^2**	***p***	**X^2**	***p***	**X^2**	***p***	***df***
Inoculum	0.84	0.361	0.32	0.574	21.45	**<0.001**	1
Con/Hetero	11.63	**0.001**	1.97	0.160	8.43	**0.004**	1
Stage	1.00	0.318	12.57	**<0.001**	43.43	**<0.001**	1
Inoculum X Con/Hetero	6.32	**0.012**	1.11	0.292	4.59	**0.032**	1
Inoculum X Stage	0.52	0.470	7.14	**0.008**	23.16	**<0.001**	1
Con/Hetero X Stage	1.43	0.232	0.25	0.620	17.39	**<0.001**	1
Inoculum X Con/Hetero X Stage	0.76	0.383	0.14	0.709	9.42	**0.002**	1
Conditional R^2	0.48		0.63		0.50		
B) PREDECESSOR SPECIES	**X^2**	***p***	**X^2**	***p***	**X^2**	***p***	***df***
Inoculum	0.79	0.375	3.37	0.067	0.31	0.575	1
Predecessor	37.14	**<0.001**	7.32	**0.026**	58.70	**<0.001**	4, 2, 4
Stage	0.71	0.398	5.81	**0.016**	0.07	0.787	1
Inoculum X Predecessor	23.22	**<0.001**	3.89	0.143	35.04	**<0.001**	4, 2, 4
Inoculum X Stage	0.38	0.540	3.18	0.075	0.04	0.842	1
Predecessor X Stage	7.31	0.121	1.08	0.583	34.52	**<0.001**	4, 2, 4
Inoculum X Predecessor X Stage	4.15	0.386	0.60	0.740	18.91	**0.001**	4, 2, 4
Conditional R^2	0.59		0.64		0.64		
C) PHYLOGENETIC DISTANCE	**X^2**	***p***	**X^2**	***p***	**X^2**	***p***	***df***
Inoculum	0.55	0.459	0.32	0.574	0.31	0.575	1
Phylogeny	16.90	**<0.001**	1.97	0.160	58.70	**<0.001**	2, 1, 4
Stage	0.84	0.361	12.57	**<0.001**	0.07	0.787	1
Inoculum X Phylogeny	9.63	**0.008**	1.11	0.292	35.04	**<0.001**	2, 1, 4
Inoculum X Stage	0.44	0.507	7.14	**0.008**	0.04	0.842	1
Phylogeny X Stage	3.92	0.141	0.25	0.620	34.52	**<0.001**	2, 1, 4
Inoculum X Phylogeny X Stage	2.10	0.349	0.14	0.709	18.91	**0.001**	2, 1, 4
Conditional R^2	0.54		0.63		0.64		

Table S3.3. Contrasts between plant-soil feedbacks (PSF) on seedling A) biomass and B) height growth. Seedlings of three successor species were grown in early- (15 years of forest recovery) and late- (115 years) successional conditioned by one of five predecessor species. First, we calculated PSF as the logarithmic odds ratio of seedling biomass in each conditioned soil versus sterilized soil and pooled for all heterospecific conditioned soils versus sterilized soil ("heterospecific"). For each **predecessor species**, we then calculated pairwise contrasts (t-ratios) between heterospecific PSF and conspecific PSF for each predecessor-successor pair and for the pooled heterospecific PSF. Further, we grouped predecessors into phylogenetic categories that represent their **phylogenetic distance** from the respective successor species (see Fig. 2 for cladograms) and calculated PSF for each phylogenetic category. We then contrasted PSF of each phylogenetic category against conspecific PSF. Note that for X. frutescens all predecessors were grouped into the same phylogenetic category (i.e., phylogenetic contrast equals overall heterospecific contrast). For F. occidentalis each predecessor represented a distinct phylogenetic group (i.e., phylogenetic contrasts are reordered predecessor contrasts). Negative t-ratios indicate a more negative PSF in conspecific PSF as compared to heterospecific PSF. Significant values ($p < 0.05$) are highlighted in bold.

	Successor species											
	Apeiba membranacea				*Xylopia frutescens*				*Faramea occidentalis*			
	15 yrs		115 yrs		15 yrs		115 yrs		15 yrs		115 yrs	
A) BIOMASS	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*
Predecessor species												
Apeiba membranacea	–	–	–	–	0.67	0.506	1.97	0.053	-2.39	**0.018**	5.44	**<0.001**
Faramea occidentalis	-1.86	0.066	-3.28	**0.001**	0.02	0.984	-0.34	0.738	–	–	–	–
Gustavia superba	-0.69	0.491	-1.62	0.108	–	–	–	–	-2.57	**0.011**	3.81	**<0.001**
Psychotria grandis	-0.74	0.463	-2.48	**0.015**	–	–	–	–	-5.65	**<0.001**	1.09	0.278
Xylopia frutescens	3.05	**0.003**	-2.40	**0.018**	–	–	–	–	-0.58	0.562	5.25	**<0.001**
Heterospecific	0.07	0.947	-2.57	**0.011**	0.28	0.778	0.92	0.361	-2.78	**0.006**	3.96	**<0.001**
Phylogenetic distance												
1	–	–	–	–	–	–	–	–	-5.65	**<0.001**	1.09	0.278
2	–	–	–	–	–	–	–	–	-2.57	**0.011**	3.81	**<0.001**
3	-1.36	0.175	-3.07	**0.003**	–	–	–	–	-2.39	**0.018**	5.44	**<0.001**
4	3.18	**0.002**	-2.39	**0.018**	0.28	0.778	0.92	0.361	-0.58	0.562	5.25	**<0.001**
B) HEIGHT GROWTH	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*
Predecessor species												
Apeiba membranacea	–	–	–	–	0.77	0.447	1.70	0.093	-1.94	0.055	3.49	**0.001**
Faramea occidentalis	-2.91	**0.004**	-4.70	**<0.001**	0.16	0.872	0.07	0.943	–	–	–	–
Gustavia superba	-1.36	0.178	-1.42	0.157	–	–	–	–	-1.70	0.093	2.66	**0.009**
Psychotria grandis	-0.93	0.355	-1.89	0.062	–	–	–	–	-3.53	**0.001**	-1.62	0.107
Xylopia frutescens	0.63	0.530	-1.46	0.146	–	–	–	–	-1.03	0.304	2.77	**0.006**
Heterospecific	-1.32	0.191	-2.51	**0.013**	0.51	0.611	1.05	0.298	-2.21	**0.029**	2.14	**0.035**
Phylogenetic distance												
1	–	–	–	–	–	–	–	–	-3.53	**0.001**	-1.62	0.107
2	–	–	–	–	–	–	–	–	-1.70	0.093	2.66	**0.009**
3	-2.11	**0.037**	-3.05	**0.003**	–	–	–	–	-1.94	0.055	3.49	**0.001**
4	0.70	0.487	-1.34	0.184	0.51	0.611	1.05	0.298	-1.03	0.304	2.77	**0.006**

Table S3.4. Contrasts between plant-soil feedbacks (PSF) on seedling height growth in late- versus early-successional soils. Seedlings of three successor species were grown in early- (15 years of forest recovery) and late- (115 years) successional soils conditioned by one of five predecessor species. We calculated PSF as the logarithmic odds ratio of seedling biomass in each conditioned soil versus sterilized soil and pooled for all heterospecific conditioned soils versus sterilized soil ("heterospecific"). We then calculated pairwise contrasts (t-ratios) between late- and early-successional forest soils for each predecessor-successor pair and for the pooled heterospecific PSF. Negative t-ratios indicate a more negative PSF in late- compared to early-successional sites. Significant values ($p < 0.05$) are highlighted in bold.

	Successor species					
	Apeiba membranacea		*Xylopia frutescens*		*Faramea occidentalis*	
Predecessor species	t-ratio	*p*	t-ratio	*p*	t-ratio	*p*
Apeiba membranacea	-0.61	0.542	-1.77	0.081	-0.20	0.843
Faramea occidentalis	-2.63	**0.010**	-2.79	**0.007**	-5.71	**<0.001**
Gustavia superba	-0.63	0.528	–	–	-1.16	0.249
Psychotria grandis	-1.52	0.131	–	–	-3.41	**0.001**
Xylopia frutescens	-2.62	**0.010**	-2.62	**0.011**	-1.84	0.068
Heterospecific	-3.18	**0.002**	-3.23	**0.002**	-2.61	**0.010**

Table S3.5. Summary statistics on seedling biomass of three tree species. Results of analyses of variance (type III) and estimated coefficients of determination (R^2) of three separate linear mixed effects regression models for the biomass of seedlings each of three successor tree species. All analyses included the fixed effects inoculum (soils including microbiota vs sterilized soil) and successional stage of the soil (stage: 15 years vs 115 years of forest recovery). Additionally, we assessed the effects of soil conditioning species by analysing **A)** transforming soil conditioning species into the binary variable con-hetero (conspecific predecessor vs overall heterospecific predecessors) and **B)** predecessor species grouped into phylogenetic categories as a measure of phylogenetic distance between predecessor and successor species. Note that for X. frutescens all predecessors were grouped into the same phylogenetic category (i.e., phylogenetic analysis equals con- vs. heterospecific analysis). For F. occidentalis each predecessor represented a distinct phylogenetic group (i.e., phylogenetic analysis equals predecessor species analysis shown in Table 1 in the main text). Significant values ($p < 0.05$) are highlighted in bold. If degrees of freedom (df) were identical for all three species only one value is given, if they differed, df are ordered like the species.

	Successor species						
	Apeiba membranacea		*Xylopia frutescens*		*Faramea occidentalis*		
A) CON- vs. HETEROSPECIFIC	**X^2**	***p***	**X^2**	***p***	**X^2**	***p***	***df***
Inoculum	1.28	0.257	0.02	0.879	68.87	**<0.001**	1
Con/Hetero	12.11	**0.001**	1.61	0.205	28.96	**<0.001**	1
Stage	0.09	0.760	6.49	**0.011**	67.08	**<0.001**	1
Inoculum X Con/Hetero	6.64	**0.010**	0.86	0.355	15.77	**<0.001**	1
Inoculum X Stage	0.05	0.823	3.64	0.057	36.16	**<0.001**	1
Con/Hetero X Stage	6.67	**0.010**	0.33	0.568	41.62	**<0.001**	1
Inoculum X Con/Hetero X Stage	3.58	0.058	0.18	0.670	22.64	**<0.001**	1
Conditional R^2	0.55		0.45		0.78		
B) PHYLOGENETIC DISTANCE	**X^2**	***p***	**X^2**	***p***	**X^2**	***p***	***df***
Inoculum	1.28	0.258	0.02	0.879	8.17	**0.004**	1
Phylogeny	18.19	**<0.001**	1.61	0.205	80.78	**<0.001**	2, 1, 4
Stage	0.12	0.726	6.49	**0.011**	0.17	0.678	1
Inoculum X Phylogeny	10.07	**0.007**	0.86	0.355	46.36	**<0.001**	2, 1, 4
Inoculum X Stage	0.07	0.797	3.64	0.057	0.09	0.759	1
Phylogeny X Stage	31.99	**<0.001**	0.33	0.568	70.34	**<0.001**	2, 1, 4
Inoculum X Phylogeny X Stage	17.54	**<0.001**	0.18	0.670	38.95	**<0.001**	2, 1, 4
Conditional R^2	0.67		0.45		0.86		

Supplement Chapter 4

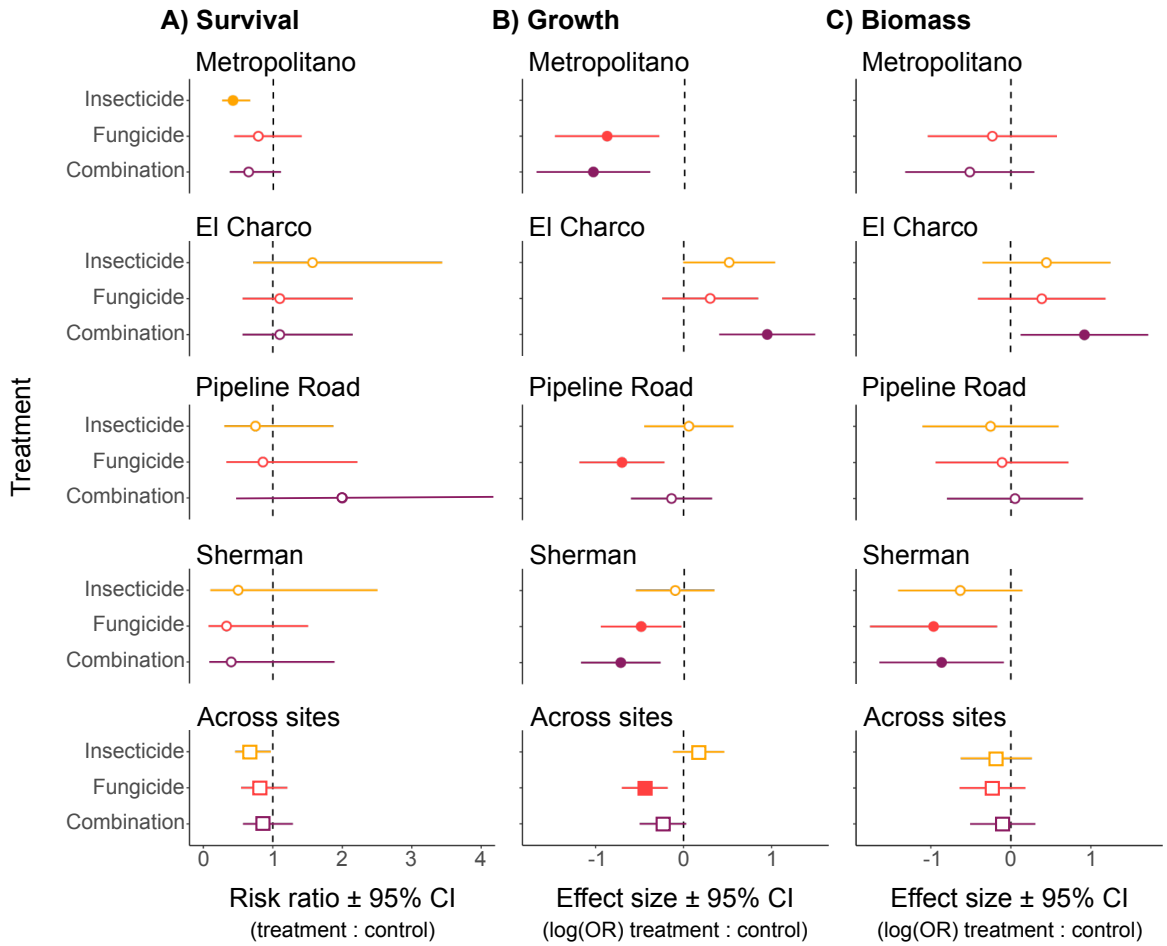


Figure S4.1. Effect of pesticide application on seedling performance in four forest sites. We measured **A)** survival, **B)** height growth (stem base to apex), and **C)** biomass (dry weight of roots, stems, and leaves) of seedlings of the widespread tree species *Lacistema aggregatum* that were planted to four tropical forest sites along a Panamanian rainfall gradient (from driest, Metropolitano, to wettest, Sherman). We calculated pairwise contrasts between each of three pesticide treatments (insecticide, fungicide, and a combination of insecticide plus fungicide application) and a water-sprayed control treatment as **A)** Risk ratios comparing the probability of survival, and **B+C)** logarithmic odds ratios comparing estimated marginal mean height growth and biomass. Pesticide effects are shown for each site (circles) and averaged across sites (squares). Filled symbols represent pesticide effects on seedling performance that are statistically different ($p < 0.05$) from the control treatments (dashed line). Ratios to the left of the dashed lines can be interpreted as a negative effect of the respective pesticide treatment on plant performance as compared with the water-sprayed control.

Table S4.1. Overview of seedling performance data. Seedlings of *Lacistema aggregatum* were planted to four tropical forest sites along a natural rainfall gradient (driest to wettest from left to right). In each site, we measured survival, height growth ("Growth", stem base to apex) and biomass (dry weight of roots, stems, and leaves) of seedlings that were grown in a water-sprayed control, insecticide application, fungicide application, or a combination application of insecticide plus fungicide. We report the number of seedlings that died over the 16 month experiment (n_{dead}), the number of seedlings that were not found at the end of the experiment (NA), and the percentage of seedlings that survived when considering NA as dead, i.e. as Survival = 1-(n_{dead}+NA)/ 25, and in brackets when excluding NAs, i.e. as Survival=1-n_{dead}/(25-NA). The total number of seedlings per treatment-site combination was 25. Overall is the average across treatments. Mean seedling height growth in cm and biomass in g are reported with one standard error.

	Metropolitano					El Charco					Pipeline Road					Sherman				
	n_{dead}	NA	Survival	Growth ± se	Biomass ± se	n_{dead}	NA	Survival	Growth ± se	Biomass ± se	n_{dead}	NA	Survival	Growth ± se	Biomass ± se	n_{dead}	NA	Survival	Growth ± se	Biomass ± se
Control	5	5	60 (75.0)	41.6 ± 3.5	0.36 ± 0.11	9	1	60 (62.5)	17.9 ± 2.3	0.28 ± 0.05	2	3	80 (90.9)	39.0 ± 5.6	0.33 ± 0.04	0	1	96 (100.0)	23.6 ± 2.4	0.42 ± 0.07
Insecticide	11	14	0 (0.0)	–	–	4	2	76 (82.6)	33.7 ± 5.2	0.45 ± 0.11	6	1	72 (75.0)	36.9 ± 4.7	0.25 ± 0.05	3	0	88 (88.0)	20.5 ± 1.8	0.24 ± 0.05
Fungicide	10	3	48 (54.5)	21.8 ± 5.0	0.31 ± 0.09	6	3	64 (72.7)	24.1 ± 3.4	0.40 ± 0.08	4	2	76 (82.6)	19.3 ± 2.4	0.30 ± 0.05	5	0	80 (80.0)	13.8 ± 1.3	0.15 ± 0.03
Combination	15	1	36 (37.5)	14.8 ± 2.1	0.19 ± 0.01	6	3	64 (72.7)	44.3 ± 5.1	0.70 ± 0.10	1	1	92 (95.8)	28.0 ± 1.9	0.35 ± 0.07	3	1	84 (87.5)	11.4 ± 1.3	0.17 ± 0.02
Overall	41	23	36 (41.8)	28.3 ± 3.0	0.28 ± 0.05	25	9	66 (72.6)	30.3 ± 2.5	0.46 ± 0.05	13	7	80 (85.0)	30.5 ± 2.1	0.31 ± 0.03	11	2	87 (88.9)	17.6 ± 1.0	0.24 ± 0.03

Table S4.2. Summary statistics of survival excluding seedlings that could not be found at the end of the experiment. Seedlings of *Lacistema aggregatum* were grown in four tropical forest sites ("Site") spanning a steep rainfall gradient along the Isthmus of Panama. In each site, 25 seedlings each were exposed to four different treatments ("Treatment"): water-sprayed control, insecticide application, fungicide application, and a combination of insecticide plus fungicide application. Chi-square values and p-values of a type-III Anova and the variance explained (conditional R^2) are shown. Statistically significant values ($p < 0.05$) are highlighted in bold.

	SURVIVAL	
	χ^2	*p*
Treatment	16.96	**< 0.001**
Site	12.19	**0.007**
Treatment x Site	22.45	**0.008**
Conditional R^2	0.18	

Table S4.3. Variation in seedling performance between forest sites within each of four treatments. We measured performance of *Lacistema aggregatum* seedlings that were planted into four tropical forest sites (Metropolitano, El Charco, Pipeline Road, Sherman) along the Panamanian rainfall gradient. In each site, seedlings were subject to four treatments: water-sprayed control, insecticide application, fungicide application, and a combination treatment with insecticide plus fungicide application. We measured percentage survival, height growth (difference in height from the beginning of the experiment; stem base to apex) and biomass (dry weight of roots, stems, and leaves) after 16 months. We show the **A)** risk ratios for the percentage of survival between sites with p-values derived from multiple pairwise comparisons (emmeans based on logistic regression). A risk ratio of 1 means identical survival in the compared sites and risk ratios >1 indicate higher survival in the second site. **B+C)** logarithmic odds ratios between sites of estimated marginal height growth and biomass with one standard error. Statistically significant differences between sites are highlighted in bold ($p < 0.05$).

	Control		Insecticide		Fungicide		Combination	
A) SURVIVAL	**RR ± se**	***p***	**RR ± se**	***p***	**RR ± se**	***p***	**RR ± se**	***p***
Metropolitano – El Charco	1.00 ± 0.35	1.000	3.71 ± 1.30	**0.001**	1.40 ± 0.46	0.695	1.70 ± 0.52	0.235
Metropolitano – Pipeline Road	1.83 ± 0.88	0.469	3.25 ± 1.03	**0.001**	2.00 ± 0.82	0.218	5.67 ± 3.89	**0.002**
Metropolitano – Sherman	5.50 ± 5.39	**0.048**	6.50 ± 3.44	**<0.001**	2.33 ± 1.05	0.122	3.40 ± 1.64	**0.008**
El Charco – Pipeline Road	1.83 ± 0.88	0.469	0.88 ± 0.44	0.990	1.43 ± 0.65	0.818	3.33 ± 2.49	0.147
El Charco – Sherman	5.50 ± 5.39	**0.048**	1.75 ± 1.20	0.748	1.67 ± 0.83	0.638	2.00 ± 1.09	0.440
Pipeline Road – Sherman	3.00 ± 3.29	0.458	2.00 ± 1.32	0.570	1.17 ± 0.66	0.989	0.60 ± 0.53	0.873
B) HEIGHT GROWTH	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***
Metropolitano – El Charco	0.93 ± 0.23	**<0.001**	–	–	-0.25 ± 0.24	0.734	-1.05 ± 0.26	**0.001**
Metropolitano – Pipeline Road	0.24 ± 0.21	0.677	–	–	0.07 ± 0.23	0.992	-0.65 ± 0.25	**0.044**
Metropolitano – Sherman	0.65 ± 0.21	**0.010**	–	–	0.26 ± 0.23	0.674	0.33 ± 0.25	0.543
El Charco – Pipeline Road	-0.69 ± 0.21	**0.008**	-0.23 ± 0.21	0.695	0.31 ± 0.21	0.460	0.40 ± 0.20	0.215
El Charco – Sherman	-0.28 ± 0.21	0.522	0.33 ± 0.20	0.322	0.51 ± 0.21	0.080	1.38 ± 0.21	**<0.001**
Pipeline Road – Sherman	0.41 ± 0.19	0.142	0.57 ± 0.21	**0.032**	0.19 ± 0.20	0.770	0.99 ± 0.19	**<0.001**
C) BIOMASS	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***
Metropolitano – El Charco	0.15 ± 0.32	0.969	–	–	-0.48 ± 0.32	0.460	-1.29 ± 0.33	**0.001**
Metropolitano – Pipeline Road	0.01 ± 0.34	1.000	–	–	-0.11 ± 0.32	0.985	-0.56 ± 0.32	0.322
Metropolitano – Sherman	-0.22 ± 0.32	0.906	–	–	0.51 ± 0.32	0.394	0.13 ± 0.32	0.977
El Charco – Pipeline Road	-0.14 ± 0.33	0.975	0.57 ± 0.33	0.323	0.36 ± 0.32	0.677	0.74 ± 0.32	0.111
El Charco – Sherman	-0.37 ± 0.33	0.677	0.72 ± 0.32	0.128	0.99 ± 0.33	**0.019**	1.42 ± 0.33	**<0.001**
Pipeline Road – Sherman	-0.23 ± 0.34	0.910	0.15 ± 0.33	0.967	0.63 ± 0.33	0.228	0.69 ± 0.32	0.152

Table S4.4. Pairwise comparison of seedling performance between treatments within four forest sites. We measured performance of *Lacistema aggregatum* seedlings that were planted into four tropical forest sites (Metropolitano, El Charco, Pipeline Road, Sherman) along the Panamanian rainfall gradient. In each site, seedlings were subject to four treatments: water-sprayed control, insecticide application, fungicide application, and a combination treatment with insecticide plus fungicide application. We measured percentage survival, height growth (difference from the height at the beginning of the experiment; stem base to apex) and biomass (dry weight of roots, stems, and leaves) after 16 months. We show pairwise comparisons between treatments within each of the four forest sites and averaged across the sites. We show the **A)** risk ratios of the percentage survival between treatments with p-values derived from multiple pairwise comparisons (emmeans based on logistic regression), and **B+C)** logarithmic odds ratios between treatments of estimated marginal height growth and biomass with one standard error. For height growth and biomass the compared treatments have equal means at log(OR)=0, while for survival, a risk ratio of 1 indicates identical means. Values larger than log(OR)=0 and RR=1 indicate a higher performance to survive in the first treatment compared with the second treatment. Statistically significant differences between treatments are highlighted in bold ($p < 0.05$).

	Metropolitano		El Charco		Pipeline Road		Sherman		Across sites	
A) SURVIVAL	**RR ± se**	***p***	**RR ± se**	***p***	**RR ± se**	***p***	**RR ± se**	***p***	**RR ± se**	***p***
Control : Insecticide	0.67 ± 0.10	**0.005**	1.57 ± 0.69	0.660	0.75 ± 0.40	0.926	0.50 ± 0.61	0.830	0.67 ± 0.13	0.063
Control : Fungicide	0.79 ± 0.25	0.846	1.10 ± 0.40	0.992	0.86 ± 0.48	0.989	0.33 ± 0.37	0.458	0.81 ± 0.17	0.545
Control : Combination	0.65 ± 0.19	0.367	1.10 ± 0.40	0.992	2.00 ± 1.69	0.704	0.40 ± 0.46	0.641	0.86 ± 0.18	0.903
B) HEIGHT GROWTH	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***
Control : Insecticide	–	–	-0.52 ± 0.22	0.084	-0.06 ± 0.21	0.992	0.10 ± 0.18	0.947	–	–
Control : Fungicide	0.87 ± 0.24	**0.002**	-0.30 ± 0.23	0.547	0.70 ± 0.20	**0.003**	0.49 ± 0.19	0.052	0.44 ± 0.11	**<0.001**
Control : Combination	1.03 ± 0.27	**0.001**	-0.95 ± 0.23	**<0.001**	0.14 ± 0.19	0.891	0.72 ± 0.19	**0.001**	0.23 ± 0.11	0.147
Fungicide : Insecticide	–	–	-0.22 ± 0.21	0.743	-0.76 ± 0.21	**0.002**	-0.39 ± 0.19	0.194	–	–
Insecticide : Combination	–	–	-0.43 ± 0.21	0.184	0.20 ± 0.20	0.769	0.62 ± 0.19	**0.008**	–	–
Fungicide : Combination	0.16 ± 0.28	0.944	-0.65 ± 0.22	**0.021**	-0.56 ± 0.19	**0.021**	0.23 ± 0.20	0.641	-0.21 ± 0.11	0.262
C) BIOMASS	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***	**log(OR) ± se**	***p***
Control : Insecticide	–	–	-0.45 ± 0.33	0.516	0.26 ± 0.34	0.879	0.63 ± 0.31	0.195	–	–
Control : Fungicide	0.23 ± 0.33	0.893	-0.39 ± 0.32	0.626	0.11 ± 0.33	0.987	0.97 ± 0.32	**0.021**	0.23 ± 0.17	0.520
Control : Combination	0.51 ± 0.33	0.403	-0.93 ± 0.32	**0.030**	-0.05 ± 0.34	0.999	0.87 ± 0.31	**0.039**	0.10 ± 0.17	0.928
Fungicide : Insecticide	–	–	-0.06 ± 0.33	0.998	0.14 ± 0.33	0.971	-0.33 ± 0.32	0.720	–	–
Insecticide : Combination	–	–	-0.48 ± 0.33	0.473	-0.31 ± 0.32	0.777	0.23 ± 0.31	0.874	–	–
Fungicide : Combination	0.28 ± 0.32	0.809	-0.53 ± 0.33	0.369	-0.16 ± 0.32	0.957	-0.10 ± 0.32	0.989	-0.13 ± 0.16	0.852

Table S4.5. Variation in pesticide effects on seedling performance among forest sites. We planted seedlings of *Lacistema aggregatum* to four tropical forest sites along a tropical rainfall gradient. In each site, seedlings were exposed to four treatments (water-sprayed control, insecticide, fungicide, and a combination of insecticide plus fungicide). After 16 months, we measured **A)** percentage of seedlings that survived, **B)** height growth (height difference between the start and end of the experiment) and **C)** biomass (dry weight of roots, stems, and leaves). We assessed the effect of a pesticide application by first contrasting seedling performance in one of the three pesticide treatments (insecticide, fungicide, combination) with the seedling performance in water-sprayed control. We then compared these contrasts between pairs of each of our four forest sites. Statistically significant differences between sites are highlighted in bold ($p < 0.05$).

	Insecticide : Control		Fungicide : Control		Combination : Control	
A) SURVIVAL	**z-ratio**	***p***	**z-ratio**	***p***	**z-ratio**	***p***
Metropolitano – El Charco	-3.47	**0.001**	-0.77	0.440	-1.34	0.179
Metropolitano – Pipeline Road	-2.58	**0.010**	-0.29	0.770	-1.82	0.068
Metropolitano – Sherman	-2.01	**0.045**	0.80	0.423	0.13	0.895
El Charco – Pipeline Road	1.24	0.216	0.42	0.672	-0.71	0.480
El Charco – Sherman	1.34	0.181	1.38	0.166	1.15	0.252
Pipeline Road – Sherman	0.35	0.725	0.99	0.321	1.60	0.111
B) HEIGHT GROWTH	**t-ratio**	***p***	**t-ratio**	***p***	**t-ratio**	***p***
Metropolitano – El Charco	–	–	-3.53	**< 0.001**	-5.65	**< 0.001**
Metropolitano – Pipeline Road	–	–	-0.55	0.580	-2.73	**0.007**
Metropolitano – Sherman	–	–	-1.26	0.210	-0.96	0.338
El Charco – Pipeline Road	1.51	0.133	3.32	**0.001**	3.67	**< 0.001**
El Charco – Sherman	2.17	**0.031**	2.67	**0.008**	5.68	**< 0.001**
Pipeline Road – Sherman	0.58	0.564	-0.77	0.441	2.17	**0.031**
C) BIOMASS	**t-ratio**	***p***	**t-ratio**	***p***	**t-ratio**	***p***
Metropolitano – El Charco	–	–	-1.36	0.179	-3.10	**0.003**
Metropolitano – Pipeline Road	–	–	-0.26	0.797	-1.20	0.237
Metropolitano – Sherman	–	–	1.60	0.115	0.78	0.439
El Charco – Pipeline Road	1.51	0.136	1.10	0.277	1.90	0.063
El Charco – Sherman	2.41	**0.020**	2.99	**0.004**	3.95	**< 0.001**
Pipeline Road – Sherman	0.80	0.425	1.84	0.071	1.95	0.056

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Figure S5.1. Attack marks on plasticine model prey. We show **(a-c)** arthropod mandible marks, **(d-g)** arthropod mandible marks plus chew marks, **(h-j)** deep and **(k-l)** shallow chew marks, and **(m-n)** dental impressions of a small mammal. Further, plasticine material was exposed to specific insects, and we show bite marks caused by **(o)** a cockroach (Blattidae), and **(p)** a short-horned grasshopper (*Coscineuta coxalis*, Acrididae). Scale bars indicate 5 mm length for each image.

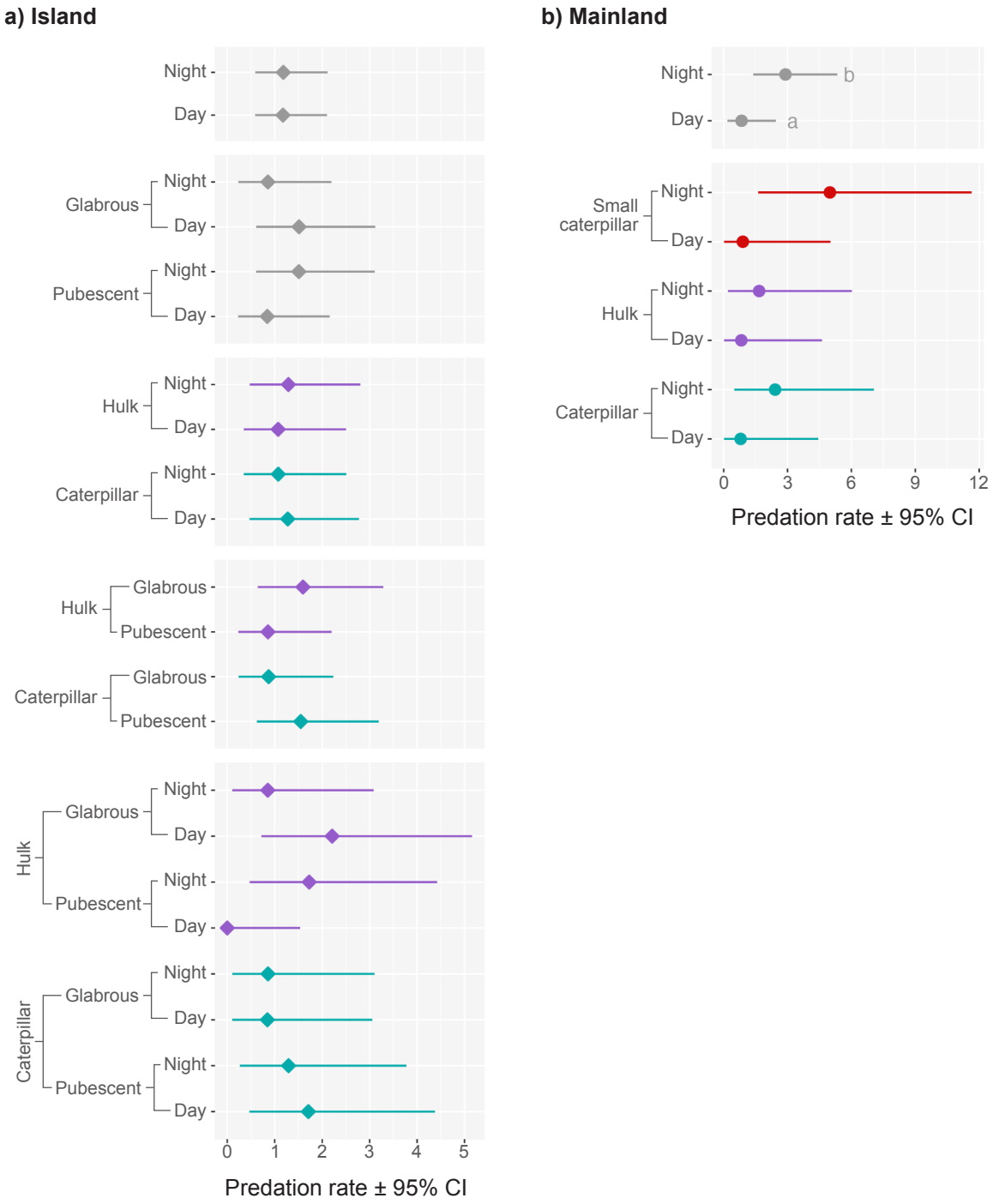

Figure S5.2. Mean predation rates on model prey in two Panamanian rainforests. Mean predation rate with 95% confidence intervals averaged over all objects per day and night (uppermost panels) and for all possible combinations of object, daytime, and plant type in the **(a)** island and **(b)** mainland experiment. Different letters indicate the statistically significant difference between diurnal and nocturnal predation in the mainland experiment.

Table S5.1. Results of pairwise two-sided Fisher's tests. comparing predation risk among object, pubescence, daytime, and the combination of these effects for the island and mainland experiment. Further, we compared predation risk between the experiments for caterpillars and hulks that were used in both experiments and as an overall predation risk across all objects. P-values are Holm-adjusted to correct for multiple comparisons.

Contrast	n	Odds ratio	95 % CI	*p*
ISLAND EXPERIMENT				
Caterpillar – Hulk				
Total	199	0.99	0.37 – 2.66	1
Pubescent	100	1.86	0.44 – 9.30	1
Glabrous	99	0.52	0.10 – 2.24	1
Day	199	1.20	0.29 – 5.15	1
Night	199	0.82	0.19 – 3.34	1
Pubescent X day	100	Inf	–	1
Pubescent X night	100	0.74	0.10 – 4.62	1
Glabrous X day	99	0.37	0.03 – 2.40	1
Glabrous X night	99	0.98	0.07 – 14.02	1
Day – Night				
Total	398	1.00	0.38 – 2.61	1
Caterpillar	200	1.21	0.30 – 5.20	1
Hulk	198	0.82	0.19 – 3.37	1
Pubescent	200	0.56	0.12 – 2.27	1
Glabrous	198	1.80	0.44 – 8.68	1
Caterpillar X pubescent	100	1.36	0.22 – 9.78	1
Caterpillar X glabrous	100	1.00	0.07 – 14.31	1
Hulk X pubescent	100	0.00	0.00 – 1.48	1
Hulk X glabrous	98	2.64	0.41 – 29.14	1
Pubescent – glabrous				
Total	199	0.99	0.37 – 2.66	1
Caterpillar	100	1.86	0.44 – 9.30	1
Hulk	99	0.52	0.10 – 2.24	1
Day	199	0.55	0.11 – 2.24	1
Night	199	1.78	0.44 – 8.59	1
Caterpillar X day	100	2.07	0.28 – 23.94	1
Caterpillar X night	100	1.52	0.17 – 19.03	1
Hulk X day	99	0.00	0.00 – 1.03	0.748
Hulk X night	99	2.03	0.28 – 23.46	1
MAINLAND EXPERIMENT				
Hulk – caterpillar				
Total	53	0.75	0.10 – 5.01	1
Day	53	1.04	0.01 – 84.68	1
Night	53	0.67	0.05 – 6.42	1
Hulk – small caterpillar				
Total	50	0.40	0.06 – 2.18	0.843
Day	50	0.92	0.01 – 75.28	1
Night	50	0.32	0.03 – 2.25	1
Caterpillar – small caterpillar				
Total	51	0.53	0.10 –2.62	0.970
Day	51	0.89	0.01 – 72.38	1
Night	51	0.48	0.07 – 2.85	1
Day – Night				
Total	154	0.27	0.05 – 1.12	0.079
Hulk	52	0.49	0.01 – 9.92	1
Caterpillar	54	0.31	0.01 – 4.22	1
Small caterpillar	48	0.17	0.00 – 1.72	1
Island – Mainland experiment				
Hulk	125	0.96	0.23 – 5.79	1
Caterpillar	127	0.71	0.19 – 3.35	1
Day	276	1.44	0.37 – 8.27	1
Night	276	0.39	0.14 – 1.08	0.266
Total	276	0.61	0.28 – 1.41	0.226

Table S5.2. Pairwise comparisons of predation rate. Comparisons of estimated point differences among predation rates on three plasticine model prey objects (Caterpillar, Small caterpillar, Hulk) that were exposed to predators in two Panamanian rainforest sites (island and mainland experiment). Shown are differences among objects averaged across the 96 hours experimental duration (Object), differences between diurnal and nocturnal predation rates (Day – Night) per object, as total average across objects and for two kinds of host plants (pubescent and glabrous), and difference between predation rates on the two host plant types (Pubescent – Glabrous) within each object type. Significant values ($p < 0.05$) are printed in bold.

	Island experiment		Mainland experiment	
	Est. point difference	*p*	Est. point difference	*p*
Object				
Caterpillar - Hulk	-1e-05	0.988	0.0004	0.743
Small caterpillar – Hulk			0.0018	0.220
Small caterpillar – Caterpillar			0.0014	0.344
Day – Night				
Hulk	-0.0002	0.761	-0.0008	0.561
Caterpillar	2e-04	0.778	-0.0016	0.314
Small caterpillar			-0.0041	0.089
Total	-1e-05	0.988	**-0.0021**	**0.047**
Hulk X pubescent	**-0.0017**	**0.046**		
Hulk X glabrous	0.0014	0.242		
Caterpillar X pubescent	0.0004	0.713		
Caterpillar X glabrous	-1e-05	0.988		
Pubescent	-0.0007	0.348		
Glabrous	0.0007	0.358		
Pubescent – glabrous				
Hulk	-0.0007	0.320		
Caterpillar	0.0007	0.354		
Total	-3e-05	0.958		