

UNDERSTANDING THE EFFECTS OF FUNGAL
SPILLOVER ON TROPICAL FOREST SEEDLING
COMMUNITIES

By

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UNDERSTANDING THE EFFECTS OF FUNGAL
SPILLOVER ON TROPICAL FOREST
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Abstract: Deforestation rates of tropical forest have led to a massive increase in forest edge habitats globally. One of the foremost fragmentation types of concern is agriculture, which can introduce new pathogenic fungi into tropical forests via spillover. The long-term effects these spillovers will have on tropical forests are largely unknown but have the potential to influence overall community diversity. This study utilizes both a theoretical model that uses a modified Lotka-Volterra equation and an empirical study in Costa Rican forests to investigate how pathogenic fungal spillover will affect tree seedling diversity. Theoretically, spillover had various effects on plants, depending on a species' competitive strength, palatability to the pathogen, and overall pathogen strength. In all cases, coexistence of species was inhibited by spillover at the forest edge, however it promoted more instances of coexistence at the interior and rescued weaker plant species when targeting the superior competitor. The study in Costa Rica found that fungal pathogens had no effect on survival but varying effects on overall diversity. This indicates that fungal spillover has the potential to weaken or strengthen mechanisms driving diversity and may be forest-specific, no longer defined by traditional diversity hypotheses. Overall, this study highlights the importance of understanding the effects of fungal spillover and how it may potentially influence the growth and survival of tree seedlings in fragmented tropical forests across the globe.

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CHAPTER I

INTRODUCTION

In the last few decades there has been a markable increase in deforestation across the globe (Fischer et al. 2021), leading to a rapid rise in the number and size of fragmented forests (Rand et al. 2006; Fischer et al. 2021). Currently, deforestation is the largest threat to tropical forests (Laurance 2013; Nguyen & Kanemoto 2021), which are home to the most biodiverse ecosystems in the world (Gibson et al. 2011). The fragmentation of tropical forests not only directly eliminates usable habitats (Wilson et al. 2016), it alters the abiotic environment at the forest edge, including increases in light, exposure to wind and rain, and changes in soil moisture (Benítez-Malvido & Lemus-Albor 2005; Bachelot & Kobe 2013; Bever et al. 2015; Krishnadas & Comita 2018) and can vary depending on the type of fragmentation (Collins et al. 2017). These abiotic changes modify the growing conditions of tropical tree seedlings which can affect plant community diversity (Krishnadas & Stump 2021) as well as promote different types of natural enemies that influence this diversity (Bachelot & Kobe 2013; Bever et al. 2015; Krishnadas & Comita 2018; Krishnadas et al. 2018).

Specifically, in the neotropics, we are concerned about tropical forest fragmentation by coffee agriculture as recent evidence has shown pathogenic fungi being transferred from coffee agriculture to nearby tropical forests (Aldrich Wolfe). The effects that this fungal pathogen

spillover has on the forest understory community are unknown, however, they have the potential to disrupt the mechanisms driving tropical tree diversity (Bagchi et al. 2010, 2014; Krishnadas & Stump 2021). To better understand the effects of this spillover, I investigated this topic both theoretically and empirically. I developed a two prey – one predator Lotka-Volterra model that tested the effects of spillover on a plant coexistence. In Costa Rica, I set up an eight-week study on leaf fungal pathogen effects on tree seedling survival and growth in four forests near coffee plantations.

Previous theoretical models show that fungal pathogen spillover can have a strong influence on plant community structure (Cantrell et al. 2001; Rand et al. 2006; Mordecai 2013b; see reviews in Tylianakis & Morris 2017). These studies also show that these effects are largely impacted by a plant species' competitive ability and the ability of the spillover to permeate past the forest edge (Cantrell et al. 2001; Rand et al. 2006, Mordecai 2013b). Theoretical studies that investigate non-spillover effects of fungal pathogens have found that fungal pathogens can influence plant communities in a variety of ways, especially depending on spillover host-specificity. However, to our knowledge, there is a lack of theoretical work that examines the effects of fungal spillover onto a system that is already regulated by a density-dependent enemy. In this study, we analyzed the effects of the introduction of a new enemy via diffusion onto a one-predator two-prey Lotka-Volterra system.

More specifically we hypothesized that (1) the introduced pathogen would have varying effects on each plant species depending on their competitive ability; (2) the degree of the introduced pathogen's host-specificity will either rescue the weaker competing plant species or

drive it to extinction depending on plant competitive ability; and (3) increased velocity of the new pathogen spillover will increase its effect at the edge and spread further into the plant community.

Previous empirical studies have noted an increase in negative fungal pathogen effects at the tropical forest edge (Meiners et al. 2002; Bell et al. 2006; Krishnadas & Comita 2018), which can potentially be heightened with the addition of fungal spillover (Power & Mitchell 2004; Rand et al. 2006, Krishnadas et al. 2018). Because fungal pathogens are important drivers of seedling demographics, they can either strength of weaken the diversity driving mechanism, referred to as negative density dependence, in these forests, which can either lead to increased diversity or local extinction events (Bachelot & Kobe 2013; Bagchi et al. 2014). The effects of forest fragmentation, specifically fungal pathogen spillover from agriculture, has been largely understudied. Thus, the aim of this study was to examine the effects of fungal pathogens on negative density-dependence and overall tree seedling community composition in forests fragmented by coffee plantations.

Specifically, we hypothesize (1) that there will an increase in pathogen infection and decrease in survival, growth, and diversity near forest edge due to high exposure to fungal pathogen spillover. We hypothesize (2) that there will be a concurrent increase in fungal pathogen presence in the soil at the forest edge. If spillover is important near the edge of the forest, we also hypothesis that (3) NDD will be stronger at the edge than in the interior of the forest, specifically for Rubiaceae plants if spillover pathogens preferential targets this family. We also hypothesis (4) if coffee management practices affect spillover and sun coffee plantations experience higher spillover of fungal pathogens, changes in NDD with distance from coffee plantations should be more pronounced in sun than in shade coffee plantations.

CHAPTER II

FUNGAL SPILLOVER INFLUENCES PLANT COMPETITIVE ABILITY AND AFFECTS SPECIES' COEXISTENCE

Abstract

Tropical forests are the most biodiverse ecosystems in the world and are critical to global ecosystem functions. Human-mediated disturbance has increased forest fragmentation, yet the effects on the mechanisms maintaining tropical tree diversity are widely unknown. Fragmentation by agriculture has the potential to introduce new interactions into tropical forests via spillover, particularly pathogenic fungi. Existing theory has mainly focused on transmission dynamics however there is evidence that pathogen spillover can influence plant community composition. To investigate the effects of spillover on tropical forest communities, we created a theoretical model representing one predator (fungi) and two prey (plant) populations in coexistence and introduced a new fungal pathogen via spillover. We tested how host-specificity and intensity of spillover would alter plant coexistence outcomes, when plant competition levels differ. Results show that while spillover drove the system into extinction at the forest edge, spillover has the ability to promote coexistence, rescue weaker competitors, or drive plant populations into extinction, dependent on the plant species' competitive ability. These effects are also heightened with the addition of wind. In the field, the effects of fungal spillover on forests are unknown, and this model provides insight on the influences of fungal pathogen spillover on plant community composition and diversity.

Introduction

Habitat fragmentations are the leading causes of biodiversity loss and ecosystem disruptions (Fahrig 2003; Haddad et al. 2015; Wilson et al. 2016; Luo et al. 2022). While fragmentation directly affects habitat loss, there are significant indirect effects on biotic community structures (Wilson et al. 2016), for instance, fragmentation can alter plant community composition in a variety of ways over time, depending on the environment and type of fragmentation (Collins et al. 2017). By creating new edges between habitats, fragmentation can also lead to the movement of new organisms into these habitats, a process known as spillover (Power & Mitchell 2004; Rand et al. 2006; Blitzer et al. 2012). The intensification of agriculture has not only increased habitat fragmentation, but the monoculture style of planting has led to an influx of the movement of organisms, such as herbivores, pollinators and fungi, from agriculture into natural areas (Power & Mitchell 2004; Blitzer et al. 2012; Geslin et al. 2017; Velasquez et al. 2018). Specifically, the movement of fungal pathogens has become concerning, since these organisms can readily travel via wind, water or via the movements of other biotic organisms (Power & Mitchell 2004; Borremans et al. 2019).

Hence, we are interested in “fungal pathogen spillover” which is defined following Power and Mitchell (2004) as the movement of a fungal pathogen from a “reservoir host”, where the fungal pathogen is maintained at a high density, to a “non-reservoir host”. This non-reservoir host will experience a higher degree of infection due to the spillover (throughout denoted as “fungal pathogen spillover”). While there have been empirical studies on the spillover effects of fungal pathogens from natural areas onto agriculture, there is very limited studies to understand the spillover effects from

agriculture onto natural habitats (Power & Mitchell 2004; Blitzer et al. 2012; Krishnadas et al. 2018). This knowledge gap is very important to understand because high densities of domesticated plants can sustain pathogens over a long period of time (Power & Mitchell 2004), there is a rising concern for agriculture to act as a new reservoir of fungal pathogens for natural habitats. Therefore, with increasing risk for spillover, there is a need to investigate how spillover might disrupt natural communities.

Theory about fungal pathogen spillover has typically focused on transmission dynamics (Borremans et al. 2019; Greenspoon & Wadhawan 2021) but less on direct impacts on host community composition itself (Power & Mitchell 2004). Few theoretical studies have, nonetheless, shown that specifically fungal pathogen spillover can greatly influence plant population composition and diversity (Cantrell et al. 2001; Rand et al. 2006; Mordecai 2013b; see reviews in Tylianakis & Morris 2017). For example, Cantrell et al. (2001) used a Lotka-Volterra model with a type II functional response and found that pathogen spillover can reduce population survival and overall patch size. However, this study ignores resident fungal pathogens and solely focuses on the effects of the three dynamics of two prey species and one introduced pathogen. This introduced pathogen's population was also limited by prey availability, which does not facilitate true "spillover" by definition (Cantrell et al. 2001).

Alternatively, Rand et al. (2006) used simple conceptual models to demonstrate spillover. Despite these models being broadly applicable, this study also failed to incorporate the community response of plants and focused on enemy resource use and how that changed over time (Rand et al. 2006). Mordecai (2013b) showed that the effects of pathogen spillover on the coexistence of two cheatgrass species varied with plant

species' competition levels. However, similar to Cantrell et al. (2001) this study only modeled the dynamics of three populations: the two plant species and the introduced pathogen, ignoring the presence of resident pathogens. In the absence of spillover, the two cheatgrass species were only regulated by competition, which resulted in competitive exclusion (Mordecai 2013b). Additionally, the author parameterized the model to a specific study system (cheatgrass, Mordecai 2013a), limiting its broader applications (Mordecai 2013b). To our knowledge, we are still lacking a theoretical framework where pathogen spillover is introduced into a system already regulated by a density-dependent enemy.

Theoretical studies without spillover have highlighted that relative host specificity of a plant natural enemy, acting in a density-dependent (Nathan & Casagrandi 2004; Schroeder et al. 2020) and distant-dependent (Adler & Mueller-Landau 2005; Sedio & Ostling 2013) manner, can influence species composition in a variety of ways (Stump & Chesson 2015; Greenspoon & Wadhawan 2021). These theoretical models focus on the Janzen-Connell (JC) hypothesis, which suggests that seedlings occurring at high conspecific densities are more likely to undergo attacks from natural enemies and is referred to as negative density dependence (NDD) (Connell 1971; Janzen 1970). Even though these models do not investigate the added effects of the introduction of a new pathogen via spillover, their findings regarding host-specificity might expand to the introduction of a new pathogen (Stump & Chesson 2015; Greenspoon & Wadhawan 2021). Specifically, we can assume that the impact of spillover on plant communities will also depend on host specificity. For example, if these spillover pathogens are community density responsive instead of host specific, it can lead to population instability and reduce

forest understory diversity (Freckleton & Lewis 2006), especially depending on a plant species' susceptibility (Jiang et al. 2020). If the spillover of pathogen has a narrow range of host specificity, it could lead to local extinction or rescue some plant species (Mordecai 2013b; Sedio & Ostling 2013; Jiang et al. 2020; Schroeder et al. 2020).

In our study, we analyzed the effects of the introduction of a new enemy via diffusion onto a one-predator two-prey Lotka-Volterra system (Figure 1). More specifically we hypothesized that (1) the introduced pathogen would have varying effects on each plant species depending on their competitive ability; (2) the degree of the introduced pathogen's host-specificity will either rescue the weaker competing plant species or drive it to extinction depending on plant competitive ability; and (3) increased velocity of the new pathogen spillover will increase its effect at the edge and spread further into the plant community.

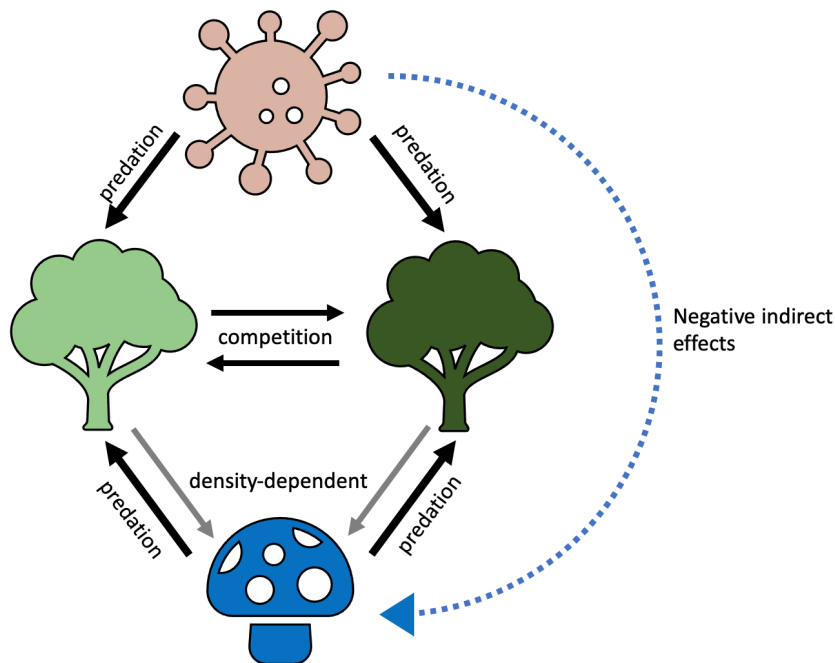


Figure 1. Schematic diagram of the predicted relationships of spillover introduced into a two-prey, one-predator system.

Methods

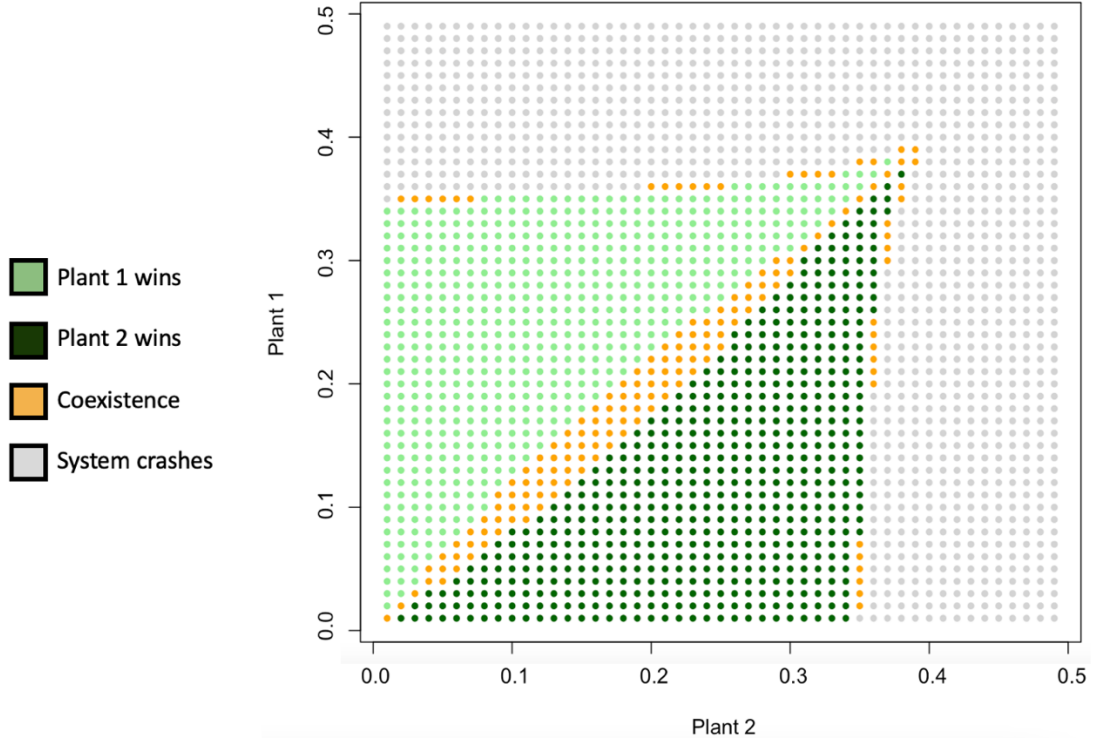


Figure 2. Preliminary system: No spillover is introduced, only allowing species to coexist when competition rates are almost equal, otherwise plants exclude each other as their competition rates increase. Initial states are equal ($x_1=5$, $x_2=5$, $y_1=5$, $y_2=0$). Growth rates and intraspecific competition for plants are equal ($A_{1,2}=3$; $\alpha_{11,22}=0.5$) and both are targeted equally by predator 1 ($\beta_{11,21}=0.5$; $e_{11,21}=0.1$) and predator 1 is limited by net prey gain and death ($\delta_{1,2}=0.1$; $m=0.5$).

First, we simulated a community of two plant species interacting with one enemy without spillover using a Lotka-Volterra model (Figure 2). We assumed the two plant species (x_1 and x_2) (Eqns. 1 & 2) are competing with one another and that the enemy (y_1) (Eqn. 3) is attacking each of them equally in a density-dependent manner. Plant population dynamics are controlled by their growth rates (A_1 and A_2), intra- (a_{11} , a_{22}) and interspecific (a_{12} , a_{21}) competition, and enemy attacks.

$$\frac{dx_1(t)}{dt} = x_1(t) \left(A_1 - a_{11}x_1(t) - a_{12}x_2(t) - \frac{\beta_{11}y_1(t)}{1+e_{11}x_1(t)} \right) \quad (\text{Eqn. 1})$$

$$\frac{dx_2(t)}{dt} = x_2(t) \left(A_2 - a_{22}x_2(t) - a_{21}x_1(t) - \frac{\beta_{21}y_1(t)}{1+e_{21}x_2(t)} \right) \quad (\text{Eqn. 2})$$

$$\frac{dy_1(t)}{dt} = y_1(t) \left(\frac{\delta_1\beta_{11}x_1(t)}{1+e_{11}x_1(t)} + \frac{\delta_2\beta_{21}x_2(t)}{1+e_{21}x_2(t)} \right) - m \quad (\text{Eqn. 3})$$

For the enemy attacks, we used a type II functional response to simulate enemy density-dependence on plant populations (Krivan 2008). The effects of the enemy on plant dynamics are regulated by two species-specific coefficients, the rate of enemy ability to find plants (β_{11} and β_{21}) and infection rate (e_{11} and e_{21} ; Eqn. 3). Enemy population dynamics are regulated by their numerical responses to plant attacks (where delta represents enemy energy gained) and their death rate (m).

Our model includes a realistic non-linear functional response of the predator on the two prey. Such Lotka-Volterra one predator – two prey models with a type II functional response have previously been studied (Klebanog and Hasting 1994). A common dynamical outcome of this model is chaos, which can be stabilized with low functional response of the predator on the prey (Klebanog and Hasting 1994). Based on previous theoretical studies we know that in a three-species Lotka-Volterra system, if intraspecific competition is greater than interspecific competition and a predator attacks both prey equally in a linear fashion, that depending on a plant's competitive ability, predation can promote coexistence (Parrish & Sailer 1970). Hence, we will not re-analyze the dynamics of this simple model and will directly modify it to introduce spillover. Due to the lack of literature, we estimated parameters for the equations to the best of our ability (Table 1).

| Equation Parameters | | Range of values |
|----------------------------|---|-------------------------------------|
| x_1, x_2 | Plant (prey) populations | 5 |
| y_1 | Predator population | 5 |
| A_1, A_2 | Growth rates for plant populations | 3 |
| α_{11}, α_{22} | Intraspecific competition | 0.5 |
| α_{12}, α_{21} | Interspecific competition | 0.0-0.5 |
| β | Enemy predation rate | 0 or 0.3 |
| δ | Enemy gain from predation | 0.1 |
| $e_{11,21}$ | Enemy infection rate (Pathogen 1) | 0.1 |
| $e_{22,12}$ | Enemy infection rate (Spillover) | 0.5 |
| m | Death | 0.5 |
| D | Diffusion coefficient | 10 |
| v | Velocity (movement from agriculture towards the forest) | 0, 0.25 or randomized from (0.01-2) |
| r | Constant source from agriculture | 2 |

Table 1. Parameters used for the model system.

The second fungal pathogen was introduced via spillover using a one-dimensional advection – diffusion - reaction transport process. Specifically, we assumed spillover occurs in one dimension from a constant source (agriculture) into a new system (forest) via the processes of diffusion and advection. The spillover mechanism assumes this pathogen is unregulated by plant population size or death and is defined by the diffusion coefficient (D), the velocity (v) representing transport by constant wind, and a constant source (r) (Eqn. 7).

$$\frac{\partial x_1(t,l)}{\partial t} = x_1(t,l) \left(A_1 - a_{11}x_1(t,l) - a_{12}x_2(t,l) - \frac{\beta_{11}y_1(t,l)}{1+e_{11}x_1(t,l)} - \frac{\beta_{12}y_2(t,l)}{1+e_{12}x_1(t,l)} \right) \quad (\text{Eqn. 4})$$

$$\frac{\partial x_2(t,l)}{\partial t} = x_2(t,l) \left(A_2 - a_{22}x_2(t,l) - a_{21}x_1(t,l) - \frac{\beta_{21}y_1(t,l)}{1+e_{21}x_2(t,l)} - \frac{\beta_{22}y_2(t,l)}{1+e_{22}x_2(t,l)} \right) \quad (\text{Eqn. 5})$$

$$\frac{\partial y_1(t,l)}{\partial t} = y_1(t,l) \left(\frac{\delta_1\beta_{11}x_1(t,l)}{1+e_{11}x_1(t,l)} - \frac{\delta_2\beta_{21}x_2(t,l)}{1+e_{21}x_2(t,l)} \right) - m \quad (\text{Eqn. 6})$$

$$\frac{\partial y_2(t,l)}{\partial t} = D \frac{\partial^2 y_2(t,l)}{\partial^2 l} - v \frac{\partial y_2(t,l)}{\partial l} + r \quad (\text{Eqn. 7})$$

Spillover was first introduced as ‘slow diffusion’ ($r=2$, moving from the source without velocity $v=0$) where the concentration was highest at the forest edge and increased its spread into the forest over time. (1) To answer our hypothesis about the effect of plant competitive ability on the outcome of spillover, we first investigated the parameter space defined by the two coefficients of interspecific competition bounded by intraspecific coefficient value. Specifically, both interspecific competition coefficients increased incrementally by 0.01 between 0.01 and 0.5 (intraspecific competition). We simulated the outcome of spillover on coexistence for all combinations of the two coefficients of interspecific competition. We compared these results to the ones obtained by varying plant interspecific competition parameters in the absence of spillover, similar to previous studies examining Lotka-Volterra models with one-predator and two-prey species (Cantrell et al. 2001; Rand et al. 2006; Mordecai 2013b).

(2) To investigate the effect of host preference of the introduced pathogen, we created two host preference scenarios, generalist and specialist. As a generalist spillover, targeted both plant populations equally ($\beta_{12,22}=0.3$). As a specialist, spillover only targeted Plant 1 (x_1) ($\beta_{12}=0.3$; $\beta_{21}=0$). We then simulated the impact of spillover for the two host-specificity scenarios on the varied interspecific competition coefficients from the first simulation (1).

(3) To test the effects of simulated wind, we added in the velocity parameter ($v=0.25$) to the spillover equation (Eqn. 7) and repeated the above simulations for both types of spillover host-specificities. In addition, we simulated erratic wind behavior by creating turbulence, using a vector with randomized velocities from 0.1-2, randomly assigning a number to each grid space, instead of velocity as a singular value.

For each of these analyses, we concluded plants were able to coexist when their population remain above 1 throughout the simulations. We assumed a plant was going extinct when its population size dropped below 1. Finally, we said the system crashed in scenarios where

the population size of the two plants species dropped below 1, or the resident pathogen population was driven to extinction.

Simulations were performed using the ReacTran (v1.4.3.1; Soetaert & Meysman 2012) and deSolve (v1.30; Soetaert et al. 2010) packages in R. ReacTran was used to create a spatial grid for the diffusion-advection equation and deSolve for the time integration and solving the partial differential equations (Eqns 1-7). The simulations without spillover (Eqns 1-3) were solved using the ODE function and with spillover (Eqns 4-7) using the ODE.1D function.

Results

Competitive ability and host specificity influences the outcomes of spillover

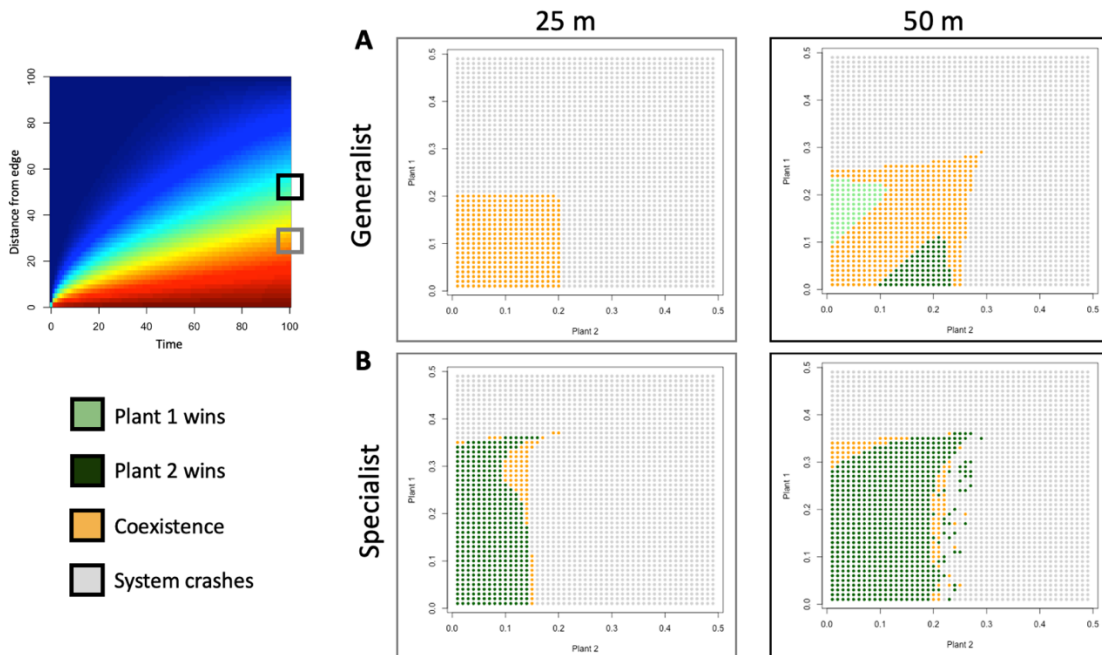


Figure 3. Spillover with wind: Generalist spillover promotes coexistence between species and specialist spillover excludes Plant 1, regardless of competitive ability. Initial states are equal ($x_1=5$, $x_2=5$, $y_1=5$) and diffusion spreads over time ($D=10$; $r=2$; $v=0.25$). Growth rates and intraspecific competition for both plants are equal ($A_{1,2}=3$; $\alpha_{11,22}=0.5$). Both are targeted equally

by predator 1 ($\beta_{11,21} = 0.3$; $e_{11,21} = 0.1$) and predator 1 is limited by net prey gain and death ($\delta_{1,2} = 0.1$; $m = 0.5$). The effect of spillover on both plants, whenever targeted, is equal ($\beta_{12,22} = 0.3$; $e_{12,22} = 0.3$).

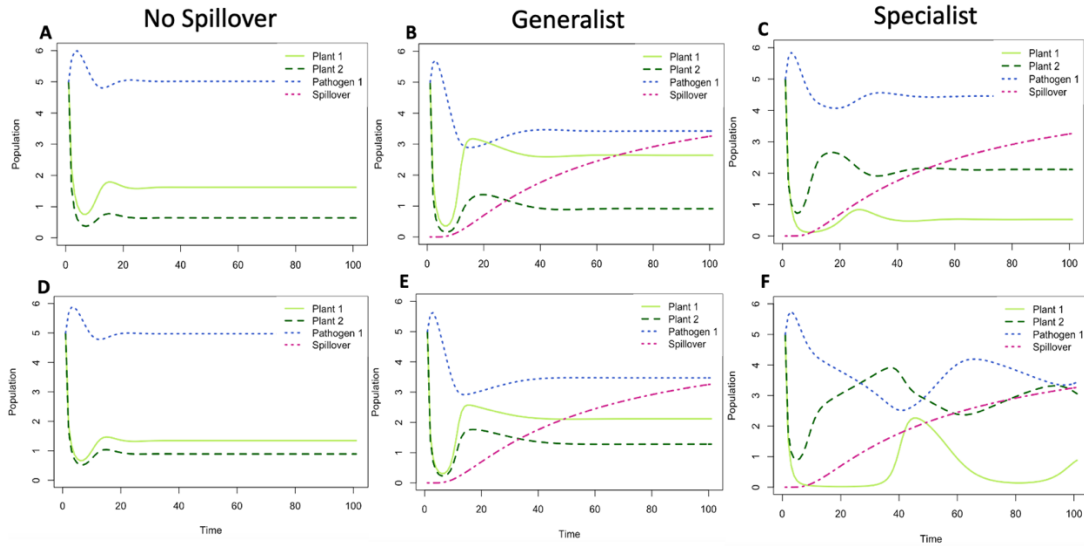


Figure 4. The effects of spillover over time at 50 m into the forest with different levels of competition when Plant 2 is excluded over time ($\alpha_{12} = 0.05$; $\alpha_{21} = 0.2$; A-C), or in coexistence ($\alpha_{12} = 0.15$; $\alpha_{21} = 0.22$; D-F) interspecific competition levels without spillover (A & D). First, generalist spillover drives Plant 2 into extinction quickly whereas specialist spillover rescues Plant 2 and excludes Plant 1 (A-C). When competition rates are similar, but Plant 1 is still stronger, generalist spillover helps achieve coexistence, and specialist once again excludes Plant 1. The effect of spillover on both plants, whenever targeted, is equal ($\beta_{12,22} = 0.3$; $e_{12,22} = 0.3$). Initial states are equal ($x_1 = 5$, $x_2 = 5$, $y_1 = 5$), growth rates and intraspecific competition for plants are equal ($A_{1,2} = 3$; $\alpha_{11,22} = 0.5$), both are targeted equally by predator 1 ($\beta_{11,21} = 0.5$; $e_{11,21} = 0.1$) and predator 1 is limited by net prey gain and death ($\delta_{1,2} = 0.1$; $m = 0.5$).

Host-specificity and plant competitive ability influenced the coexistence outcomes when spillover was introduced. When the spillover pathogen was a generalist, targeting both plant species equally, it reduced the range of competitive ability over which plants could competitively exclude each other (Figure 3A). In other words, generalist spillover enabled plants to coexist over a wider range of competitive abilities than they would in the absence of spillover (Figure 3A). In particular, when plant competition rates were different, generalist spillover was able to rescue the weaker plant and promote coexistence (Figure 4E). However, if the stronger competitor's competition was significantly higher, than generalist spillover drove the weaker species into extinction more quickly than without spillover (Figure 4B).

When the spillover pathogen was a specialist, most of the outcomes resulted in the competitive exclusion of the species targeted by the spillover pathogen, from now on referred to as the "target species" (Figure 3B). In other words, the weaker competing plant species was able to exclude the stronger competitor when it was also the target species. In the absence of spillover, the stronger competing plant species would have excluded the weaker plant species, and therefore spillover reversed the competitive outcome (Figure 4F). Only when the target species had a significantly greater competition rate than the non-target species that coexistence can be achieved (Figure 4C).

Wind influences the outcome of spillover

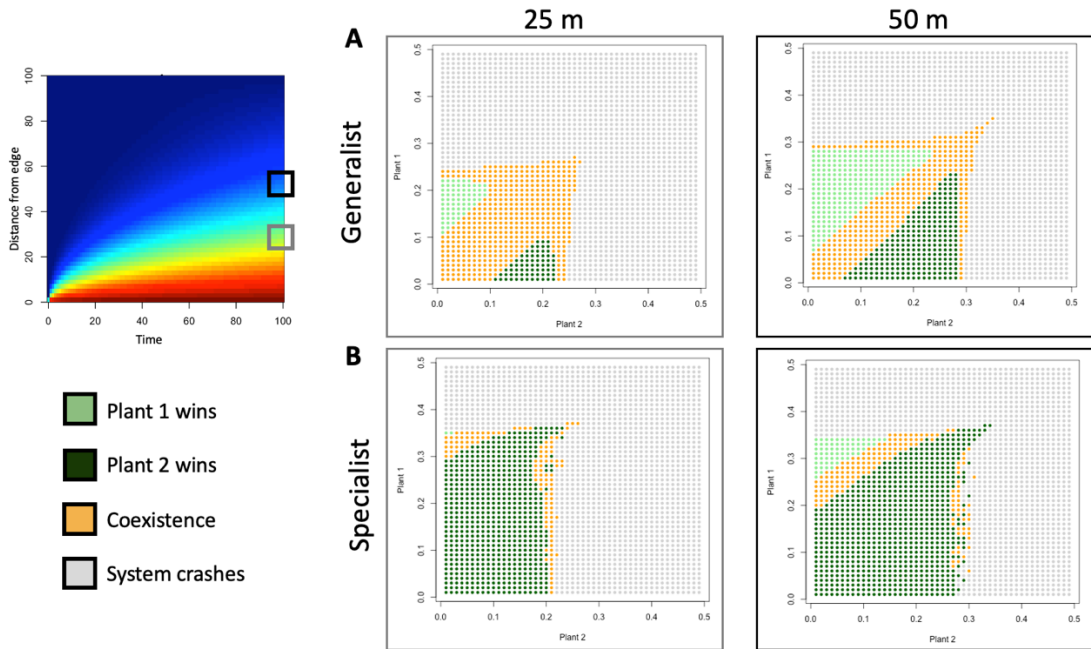


Figure 5. Spillover with no wind: Generalist spillover promotes coexistence outcomes while specialist spillover allows Plant 2 to competitively exclude Plant 1. Initial states are equal ($x_1=5$, $x_2=5$, $y_1=5$), diffusion spreads slowly over time ($D=10$; $r=2$; $v=0$). Growth rates and intraspecific competition for plants are equal ($A_{1,2}=3$; $\alpha_{11,22}=0.5$), both are targeted equally by predator 1 ($\beta_{11,21}=0.5$; $e_{11,21}=0.1$) and predator 1 is limited by net prey gain and death ($\delta_{1,2}=0.1$; $m=0.5$). The effect of spillover on both plants, whenever targeted, is equal ($\beta_{12,22}=0.3$; $e_{12,22}=0.3$).

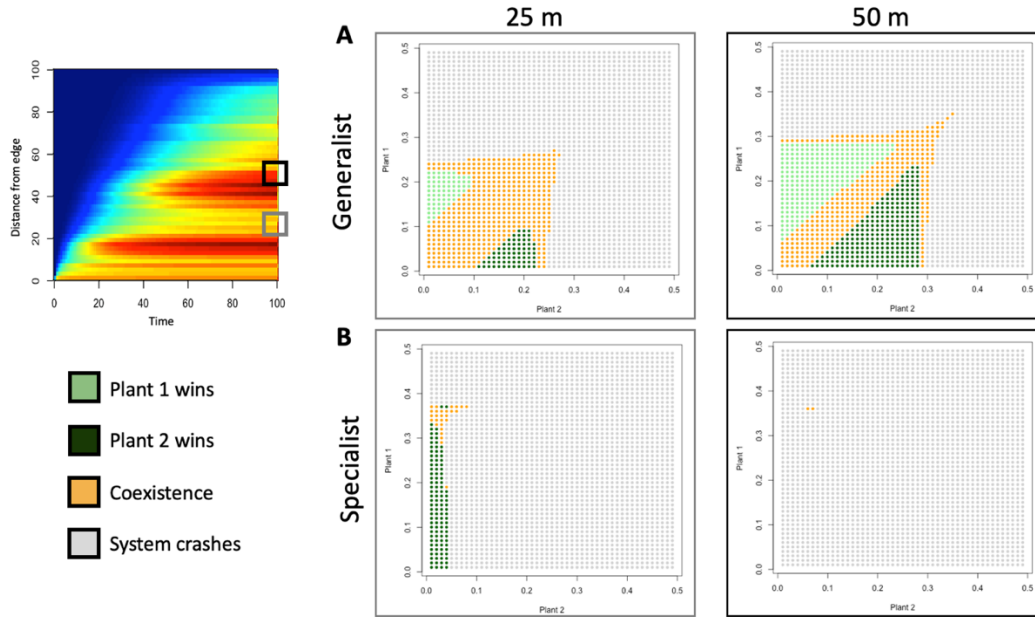


Figure 6. Spillover with turbulence: Species outcomes are unpredictable with distance but similar outcomes are reflected when spillover strengths are similar to nonturbulent models. Generalist spillover promotes coexistence and specialist spillover allows Plant 2 to exclude Plant 1. Initial states are equal ($x_1=5$, $x_2=5$, $y_1=5$), diffusion spreads erratically over time ($D=10$; $r=2$). Turbulence is added through a randomized vector with velocity strength between 0.1 and 2 at each location. Growth rates and intraspecific competition for plants are equal ($A_{1,2}=3$; $\alpha_{11,22}=0.5$), both are targeted equally by predator 1 ($\beta_{11,21}=0.5$; $e_{11,21}=0.1$) and predator 1 is limited by net prey gain and death ($\delta_{1,2}=0.1$; $m=0.5$). The effect of spillover on both plants, whenever targeted, is equal ($\beta_{12,22}=0.3$; $e_{12,22}=0.3$).

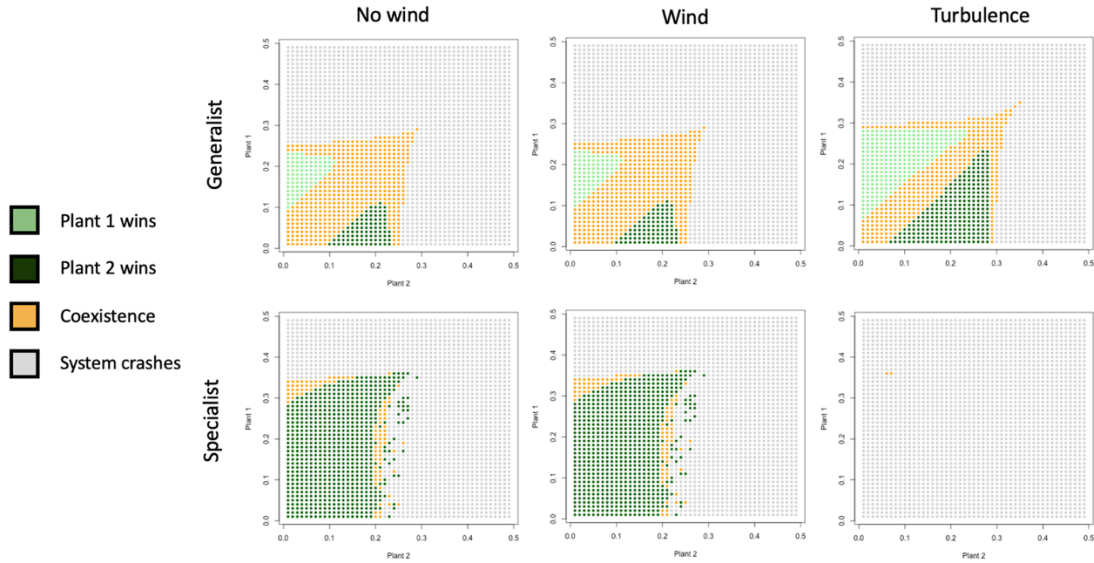


Figure 7. Spillover in the middle of the forest (50 m) with wind types. Wind implementation increases crash outcomes with increasing interspecific competition. Turbulence creates erratic outcomes that very dependent on spillover strength. Initial states are equal ($x_1=5, x_2=5, y_1=5$). Growth rates and intraspecific competition for plants are equal ($A_{1,2}=3; \alpha_{11,22}=0.5$), both are targeted equally by predator 1 ($\beta_{11,21}=0.5; e_{11,21}=0.1$) and predator 1 is limited by net prey gain and death ($\delta_{1,2}=0.1; m=0.5$). The effect of spillover on both plants, whenever targeted, is equal ($\beta_{12,22}=0.3; e_{12,22}=0.3$).

At the forest edge, spillover restricted all possible coexistence and exclusion outcomes, causing the system to crash. When the system crashed, both plant populations and/or the original predator were driven into extinction. This outcome extended into the forest at various distances, depending on the wind strength influencing spillover. Interestingly, the addition of wind influenced plant populations in a similar manner than without wind (Figure 5), however, crash outcomes were elongated further from the distance of the spillover source than without wind (Figure 3). Because of this,

coexistence is promoted further into the forest interior, at the loss of species existence at the forest edge (Figure 7).

When we simulated turbulence, the effects of spillover became highly variable (Figure 6). When turbulence mimicked similar spillover strengths as the non-turbulent system, the plant responses were similar, however this variability made the effects of spillover erratic and unpredictable (Figure 7).

Discussion

Competitive limitations are relaxed with spillover

The success of a plant species was largely reliant on their competitive abilities. In outcomes where competitive strengths differed, the weaker species was typically excluded (Figure 2) except in the presence of spillover (Figure 3). Additionally, coexistence and exclusion outcomes were limited by interspecific competition. If any interspecific competition rate was greater than 70% of the intraspecific competition rate, it led the system to crash in almost all outcomes. This is similar to other theoretical studies that found intraspecific competition to be significantly greater than interspecific competitions in one-predator two-prey models for species to exist (Mordecai 2013b, Jiang et al. 2020). This also reinforces the importance of intraspecific competition as an equalizing mechanism of coexistence (Chesson 2000; Godwin et al. 2020).

However, when spillover was added, this limitation was further limited with proximity to the edge (Figure 3), destabilizing the system. Specifically, as spillover strength increased, the instability of the system increased as well, reducing intraspecific competition and subsequently limiting the range of interspecific competitive ability. This correlation in limitation for both intra and interspecific competition reinforces coexistence mechanisms as defined by modern coexistence theory (Chesson 2000). Empirically, studies have found that co-infection of plants by

fungal pathogens leads to an increased pathogen load on the plant, which is greater than pathogen loads with single infections (Laine & Mäkinen 2018). This is particularly problematic because this drastically shifts the resource allocation of plants under infection to enemy defense, which can dilute its efforts towards competition and self-limitation (see reviews in Hiezel & Lay 2008 and Dutt et al. 2022). The results of our study are novel and show that spillover can disrupt the equalizing mechanisms that drive species coexistence (Chesson 2000; Godwin et al. 2020).

Host-specificity rescues weaker plants

The coexistence outcomes of spillover varied depending on host specificity. When the spillover pathogen was a specialist, the targeted plant species was competitively excluded, until the forest interior when spillover becomes negligible (Figure 3B). In the same respect, when spillover targeted the stronger competitor, it was able to rescue the weaker competitor from exclusion (Figure 3B). The outcome of this scenario in our model acts as a stabilizing mechanism of coexistence, as noted by Chesson (2000), suggesting that in certain scenarios our introduction of spillover can reduce competitive exclusion of weaker species. Similarly, an empirical study on cheatgrass also found that an invasive grass was limited and negatively influenced by host-specific spillover due to a low infection tolerance (Mordecai 2013a). The native grass was able to outcompete the invasives in most outcomes, due to the invasives' susceptibility to its own pathogen and the native's resistance (Mordecai 2013a). This suggests that a pathogen's target species' tolerance or susceptibility to infection can influence the overall effects of pathogen spillover. It is also important to note that when host-specificity targeted the weaker species, it was excluded from the system (Figures 3B). Indicating that pathogen spillover, as shown in our models, could either support a species' ability to recover from a stronger competitor, promote coexistence, or suppress its ability to survive long-term (as described in Flory et al. 2018). There were a few outcomes when the targeted species' competitive ability was significantly higher than the non-target, that coexistence could be achieved, but never exclusion of the non-target species

until spillover was negligible at the interior (Figure 3B). This result shows that pathogen specificity can limit a stronger competitor's abundance and therefore promote coexistence in plant communities. This result is similar to non-spillover theory models that studied pathogen host-specificity (Mordecai 2011; Mordecai 2013b; Schroder et al 2020), which emphasizes the impact that pathogen spillover can have on a pre-existing system.

Generalist spillover promotes coexistence

As predicted, the effects of generalist spillover contrasted those of specialist spillover. Specifically, generalist spillover promoted more coexistence outcomes regardless of a species' competitive strength (Figure 3A), acting as an equalizer and reducing species' ability to exclude each other. This is referred to as an equalizing mechanism, in which the effects of interspecific competitive abilities are reduced (Chesson 2000). In the Mordecai (2013a) cheatgrass study noted above, spillover also promoted coexistence between native and invasive plants as a result of generalist spillover. Because the native grass was less susceptible, in scenarios where growth rates of the invasive were typically higher, spillover diluted this effect and promoted coexistence (Mordecai 2013a). This, along with our results, suggests that spillover can act as an equalizing mechanism of coexistence further promoting the balance of species' competitive abilities (Letten et al. 2017; Chesson 2000, Godwin et al. 2020).

In contrast, an empirical study on edge effects of tropical tree seedlings found that generalist enemies near the forest edge reduced negative density dependence. The reduction of this diversity promoting mechanism in this study suggests general spillover could promote the competitive exclusion of weaker species and negatively affect coexistence (Krishnadas et al. 2018). We also found similar instances of competitive exclusion in our models with unequal competitive abilities of plants. Because generalist spillover attacked both strong and weak species, the weaker species was unable to recover and was excluded (Figure 4C). This implies

that generalist spillover can also disrupt coexistence, dependent on plant species tolerance and competitive strengths.

Spillover disrupts ecosystem dynamics at the forest edge

It is important to note that in all cases with spillover in our model, the system crashes from the forest edge (0 m) until about a few meters or more into the forest interior before any outcomes can be achieved. This shows that to prevent total extinction, spillover needs to exist at extremely low levels, due to high pathogen accumulation near the forest edge (Rand et al. 2006). This is critical because it highlights that although spillover can have some positive effects on the system towards the forest interior, it can also lead to population extinction at the edge (Figure 3 at 0m) and disrupt the equalizing mechanisms of coexistence (Chesson 2000). It also expands upon preexisting theoretical knowledge about the potential danger fungal spillover poses to ecosystems (Cantrell et al. 2001; Power & Mitchell 2004; Rand et al. 2006; Mordecai 2013b). Empirically, this outcome would be detrimental to plant community diversity as well as overall tropical forest ecosystem dynamics.

Our findings emphasize the importance of distance from the source of spillover influencing species dynamics, following the Janzen-Connell hypothesis on distance-dependence (Janzen 1970; Connell 1971; Stump and Chesson 2013). However, for our research, distance is referred to as distance from the source of spillover, rather than from conspecific adults. This spillover is unlike the traditional factors that drive diversity in tropical forests because it has the potential to either support the preexisting mechanisms or disrupt the entire ecosystem. In addition, prior empirical studies of edge effects on forest communities found that seedling survival decreases in tropical forests near the edge (Krishnadas et al. 2018; Krishnadas & Stump 2021). However, edge effects in addition to fungal spillover has been largely understudied. In addition to the multitude of mostly negative edge effects, the variable yet strong influences of

spillover on the plant community highlights the importance of further investigation into this phenomenon both theoretically and empirically.

Wind increases the reach of spillover and varies coexistence outcomes

With the introduction of spillover with wind, the area in which spillover crashed the system spread further into the forest (Figure 7). When spillover was a specialist, due to its increased reach into the forest interior, weaker species were able to maintain exclusion outcomes further into the forest (Figure 7). This also resulted in slightly more coexistence outcomes than without wind (Figure 7), which could further support the claim that spillover can act as a mechanism of coexistence and population stabilization in certain scenarios, as it weakens the targeted specie's competitive ability (Mordecai 2013b). It has been found that edge effects can infiltrate up to 150 meters into natural areas as a result of wind and weather patterns (Laurence 2000), suggesting our wind model more accurately depicts the possible effects of spillover into the forest community (Figure 3). The examination of wind patterns due to edge effects has been previously noted empirically to fluctuate over time, which can have sporadic effects (Laurence et al. 2007). Similarly, we found that when we added turbulence to spillover, there was an erratic effect of the spillover pathogen on coexistence outcomes (Figure 6). This variability can lead to population instability and vulnerability that extends beyond the reach of edge effects on community composition (Faust et al. 2018). It can also make understanding pathogen spillover effects unpredictable in some areas with intense wind shifts.

Our model highlights the variability of spillover effects onto plant ecosystems and the fluctuation of these effects depending on the plants distance from spillover source, wind intensity, and plant species' competitive abilities. We emphasize claims by earlier studies that suggest a strong relationship between land conversion and forest fragmentation (Power & Mitchell 2004; Rand et al. 2006; Faust et al. 2018), by demonstrating a strong negative effect of fungal pathogen

spillover, that changes over time. Our study also stresses the importance of studying the effects of the addition of a distant dependent enemy on a pre-existing density dependent system. Although spillover increased coexistence in certain parts of the forest, it also drove many areas near the forest edge into extinction. This further supports that the mechanisms driving seedling diversity and ecosystem coexistence can be disrupted or enhanced when exposed to pathogen spillover, and that these effects vary greatly (Rand et al. 2006; Blitzer et al. 2012; Mordecai 2011, 2013a, 2013b).

It is also important to note that the competition models were conducted at the last time step to demonstrate long-term exposure, but that spillover effects are less pronounced in the short term (Figure 4). From a conservation stand-point, this study stresses the need to address pathogen spillover events early on, to try and mitigate potential long-term damage and local extinction events to tropical plant communities. Our findings set the framework for this pathogen spillover model to be expanded to other ecosystems and incorporate specific species traits and potential plant mutualists (Greenhalgh & Haque 2007; Holland et al. 2013; Mordecai 2013a; Bachelot et al. 2015), to better understand the effects of fungal pathogen spillover in addition to edge effects, in various endangered ecosystems that have not yet been addressed.

CHAPTER III

THE EFFECTS OF FUNGAL PATHOGENS ON TROPICAL TREE SEEDLINGS IN COSTA RICAN FORESTS NEAR COFFEE PLANTATIONS

Abstract

Tropical forests, the most biodiverse ecosystems in the world, are facing increased amounts of human-mediated disturbance in the form of fragmentation, especially as a result of agriculture. While the effects of fragmentation on diversity driving mechanisms in tropical forests are widely unknown, agricultural expansion has the potential to introduce new biotic interactions into tropical forests via spillover, particularly by pathogenic fungi. Due to the variability in coffee management practices, we chose to examine two forests near sun and two forests near shade coffee plantations. This study aimed to test the effects of fungal pathogens on the tree seedling community in the tropical wet premontane forests of Costa Rica, fragmented by coffee farms. In general, fungal pathogens had negative effects on forest plant diversity, limited effects on negative density dependence and various effects on tree seedling growth. These effects also varied across the four forests that were studied over a two-month period. These findings highlight the variability of fungal pathogens at the forest edge and into the forest interior, which has potentially negative long-term implications on overall tropical forest biodiversity in Costa Rica.

Introduction

Tropical forests are one of the most diverse ecosystems and are critical to the functioning of the biosphere (Gibson et al. 2011). Continual rates of deforestation have led to an increase in fragmented forests globally (Rand et al. 2006) and is currently the number one threat to tropical forests (Laurance 2013). These newly created edges, coupled with a rise in global temperatures (Krishnadas & Comita 2018), have resulted in a loss of biodiversity in tropical forests (Gibson et al. 2011). Biodiversity loss has been particularly pronounced for tree species, but the mechanisms behind this negative edge effect remain unknown (Krishnadas et al. 2018; Krishnadas & Stump 2021). Potential mechanisms could include pathogen spillover from domesticated plants to natural areas (Power & Mitchell 2004), which can potentially drive changes in the forest understory (Krishnadas et al. 2018; Krishnadas & Stump 2021). Understanding the implications of such spillover is crucial as habitat loss continues and fragmentation introduces new species interactions that may disrupt the processes that maintain species diversity (Blitzer et al. 2012).

With increasing forest fragmentation, spillover from agriculture into natural areas, and vice versa, have become increasingly common (Blitzer et al. 2012; Power & Mitchell 2004). Here, spillover is defined following Power and Mitchell (2004)'s definition as the movement of pathogen from a "reservoir host", where the pathogen is maintained at a high density, to a "non-reservoir host", where this host will experience a higher degree of infection due to the spillover. As the number of fragmented ecoregions is increased, proximity to agricultural edges increases vulnerability to spillover effects (Rand et al. 2006). Such effects, including local extinction events (Rand et al. 2006), can have a strong effect on the forest understory (Bagchi et al. 2010, 2014; Krishnadas & Stump 2021). While there have been studies on the spillover effects of fungal pathogens from natural areas onto agriculture, there have been minimal studies to understand the spillover effects from agriculture onto natural habitats (Power & Mitchell 2004; Rand et al. 2006; Blitzer et al. 2012; Krishnadas et al. 2018). Moreover, fungal pathogens have been found to have

a reduced effect on seedling diversity near forest edges (Krishnadas & Comita 2018; Krishnadas et al. 2018) which could be further exaggerated with the addition of fungal spillover. Previous studies on cheatgrass exposed to fungal pathogen spillover from agriculture led to high infection rates of non-reservoir hosts with complex effects on plant survivorship (Beckstead et al. 2010; Mordecai 2013a; 2013b). Due to the fact that high densities of domesticated plants can sustain pathogens over a long period of time (Power & Mitchel 2004), there is a rising concern for agricultural areas to act as a new reservoir of fungal pathogens for natural habitats, including for tropical forests.

Although the concept of tropical forests as biodiversity hot spots has been widely accepted, the mechanisms that maintain this diversity have been largely debated (Bagchi et al. 2014). One of the foremost hypotheses explaining tropical forest diversity is the Janzen-Connell hypothesis, which suggests that seeds/ seedlings occurring at high conspecific densities or in close proximity to adult conspecifics, are more likely to undergo attacks from natural enemies, such as fungal pathogens and herbivores (Janzen 1970; Connell 1971). This negative density/distance dependence (NDD) rescues rare plant species and prevents one species from becoming too dominant (Janzen 1970; Connell 1971). Importantly, this mechanism could promote coexistence if conspecific density limits a species more strongly than heterospecific density (Bell et al. 2006; Bever et al. 2015). Studies have repeatedly found that seedling recruitment decreases, and mortality increases with high conspecific seedling densities (Clark & Clark 1984; Comita et al. 2014). NDD plays an important role in maintaining species diversity in tropical forest understories by regulating species abundance (Bagchi et al. 2014; Comita et al. 2010; Connell 1971; Janzen 1970; Bachelot et al. 2017; Krishnadas & Comita 2018; Krishnadas et al. 2018). Lower seedling recruitment at high conspecific densities has been attributed to increasing pressure from natural enemies (Bachelot et al. 2015), especially from fungal pathogens (Freckleton & Lewis 2006; Liu et al. 2012; Hazelwood et al. 2021). However, the impact of

fungal pathogens on plant community diversity might depend on pathogen host specificity. Increased NDD at high conspecific densities leads to high diversity rates among seedlings, however increased NDD at high overall plant densities leads to a reduction in diversity. The impact of host specificity might also extend to spillover pathogens; if the spillover pathogen has a narrow range of host specificity, it could lead to local extinction (Beckstead et al. 2010; see reviews in Rand et al. 2006) or rescue some plant species (Mordecai 2013a; 2013b). Moreover, if the spillover pathogen is a generalist instead of host specific, it can lead to population instability and reduce forest understory diversity (Freckleton & Lewis 2006). Overall, it is expected spillover will either dilute or strengthen negative density dependence (NDD), depending on host community and pathogen host specificity, which can affect the diversity of understory species that exist in these forests.

Historically, the Janzen-Connell hypothesis proposed that host specialization of natural enemies was a key element to drive patterns of NDD (Janzen 1970; Connell 1971). However, empirical and theoretical work has relaxed this requirement, and highlighted the importance of host preference instead (Benítez et al. 2013; Hanski 1981; Odegaard et al. 2005; Sedio and Ostling 2013). Rather than just considering conspecific and heterospecific species, it might be useful to also consider the phylogenetic relatedness across species present at a given location (Liu et al. 2012). Natural enemies have been found to select host plants with phylogenetically correlated functional traits and similar evolutionary histories (Paine et al. 2012; but see Song et al. 2021). Specifically, pathogenic fungi within the same genus have been found to infect closely related species across genera and families (Liu et al. 2016). This has led to an increase in mortality when seedlings are located near other species with similar phylogenetic histories (Bever et al. 2015; Liu et al. 2012; Paine et al. 2012). Similarly, fungal spillovers could also have distinct effects on forest understory depending on phylogeny. It has been suggested that the phylogenetic relatedness between domesticated and wild plants could result in similar instances of infection

and mortality (Blitzer et al. 2012; Liu et al. 2012; Power & Mitchell 2004). Further, as edge effects may increase the susceptibility of plant communities to pathogen spillover (Bever et al. 2015; Krishnadas & Comita 2018; Rand et al. 2006), it is important to note the variability in the pathogen host and to study the phylogenetic distance between domesticated plants and plants in natural areas and the implications it may have on the forest understory.

Besides introducing new fungal pathogens, forest fragmentation can directly influence plant species diversity (Krishnadas & Stump 2021) and fungal pathogens by altering the environmental conditions of the forests (Bachelot & Kobe 2013; Bever et al. 2015), but these affects are not widely studied (Krishnadas & Comita 2018; Krishnadas et al. 2018). Increasing forest edge habitat can affect important abiotic factors that are necessary to seedling recruitment by increasing light availability, decreasing soil moisture and humidity, and increasing exposure to wind and rain (Benítez-Malvido & Lemus-Albor 2005; Krishnadas & Comita 2018). This resulting edge effect, along with pathogen spillover, has the potential to change the composition of the forest community.

Finally agricultural practices have been suggested to influence the amount of fungal spillover experienced by adjacent natural areas (Blitzer et al. 2012; Rand et al. 2006). Specifically, in Costa Rica, montane tropical forests have experienced an increase in forest fragmentation due to coffee plantations (Aldrich-Wolfe; Toledo & Moguel 2012). Coffee plantations are managed along a continuum of two extremes that can influence the degree of fungal spillover: Shade or sun coffee management. Shade management is the traditional method of growing coffee, that stems from indigenous practices, where coffee is planted into the forest understory and original tree cover is maintained. This practice involves little to no chemical pesticide or fertilizer use to produce low-yield, high quality coffee beans. It has been documented to mitigate runoff,

store carbon, and prevent soil erosion while maintaining higher species richness and diversity, compared to sun coffee management. Sun coffee management involves the removal of all surrounding canopy trees and using large amounts of chemical pesticides and fertilizers to maximize coffee yield at high densities. Since coffee plantations in Costa Rica are run almost entirely by small scale farmers, there is significant variation in management methods that can have a variety of effects on the level of fungal spillover (Toledo & Moguel 2012). In addition, many forest understory species reside in the same family as coffee, Rubiaceae. This relationship between domesticated and non-domesticated plants, has the potential to result in increased infections of forest species in the Rubiaceae family, in line with host-preference of pathogens as described earlier (Bever et al. 2015; Liu et al. 2012, 2016; Paine et al. 2012).

In this study, we focus on the seedlings stage because it is a bottleneck in tree life cycle (Freckleton & Lewis 2006). Seedlings are highly sensitive to biotic interactions and so the processes that influence seedling communities, influence the future of forest composition (Silva Matos et al. 1999; Bell et al. 2006). Most pathogen related mortality also occurs in the first few weeks of seedling germination (Augsburger 1984; Bagchi et al. 2010; Bagchi et al. 2014), and seedling susceptibility to fungal diseases decreases as seedlings age, potentially when other factors become more prevalent (e.g. light requirements) (Krishnadas & Comita 2018; Freckleton & Lewis 2006). By studying unmanipulated communities at the seedling stage, we can understand the effects of underlying processes over natural densities (Freckleton & Lewis 2006) that will aid in the understanding of the future forest composition (Freckleton & Lewis 2006; Silva Matos et al. 1999).

Specifically, we hypothesize (1) that there will be an increase in pathogen infection and decrease in survival, growth, and diversity near forest edge due to high exposure to fungal pathogen spillover. We hypothesize (2) if spillover is important near the edge of the forest, we also hypothesize that NDD will be stronger at the edge than in the interior of the forest, specifically for Rubiaceae plants if spillover pathogens preferentially target this family. We also hypothesize (3) if coffee management practices affect spillover and sun coffee plantations experience higher spillover of fungal pathogens, changes in NDD strength with distance from coffee plantations should be more pronounced in sun than in shade coffee plantations.

Methods



Figure 8. Aerial map of site locations in the Puntarenas region of Costa Rica.

Sites

Four Costa Rican forests adjacent to coffee farms were chosen, two with sun (Site 1 (8°50'47.90"N | 82°53'59.48"W) and Site 2 (8°54'22.13"N | 82°58'39.45"W)) and two with shade (Site 3 (8°53'12.86"N | 82°51'07.70"W) and Site 4 (8°58'25.62"N | 82°55'39.28"W)) coffee management (Figure 8). These forests are classified as tropical, wet, pre-montane forests. They are in southwest Costa Rica in the region of Puntarenas, with elevations between 950-1,100 m above sea level, an average temperature of 20.5°C and rainfall average of 5074 mm (Climate Data; World Topographic Map). These sites have been previously used to quantify the fungal spillover as described in the introduction; they are established research plots with connections to the coffee farmers (Aldrich-Wolfe, in prep).

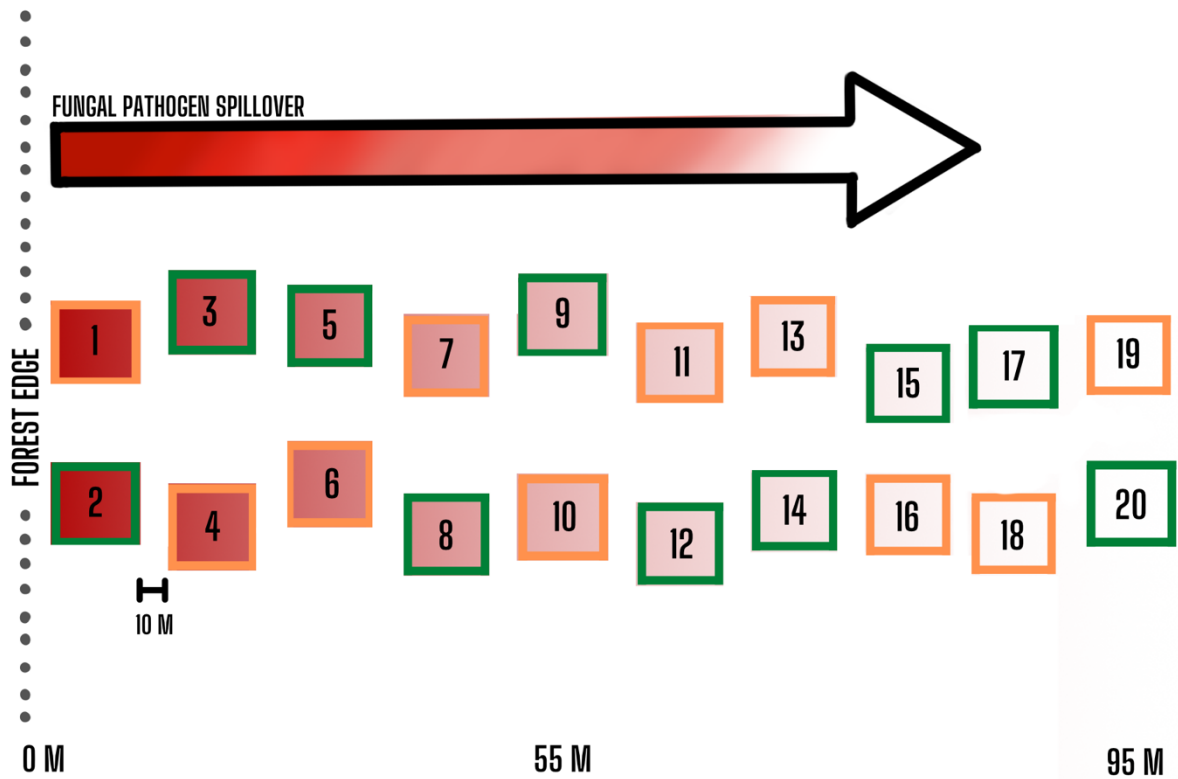


Figure 9. Experimental plot set-up and expected spillover effects. Plots began 5m from the true forest edge and were placed within 1m of its pair. Plot pairs were set up around 10 m from each other, from 5m- 95m into the forest. The hypothesized effect of fungal pathogen spillover is shaded in red. Fungicide treated plots= orange, control plots= dark green.

In late May 2022, twenty 1x1 m seedling plots were set up over the existing seedling community at each forest starting at 5 m from the forest edge (Figure 9). At initial set up, GPS coordinates and light canopy measurements were taken in the middle of each seedling plot. Two seedling plots were set up every 10 m starting 5 m from the forest edge, with 0.5-1 m distance between two plots at a given distance, to 105 m into the forest. Seedling plots at each distance were randomly assigned a treatment to be sprayed with either 10mL of leaf fungicide or sprayed with 10mL of water onto the plant foliage by hand, once a week for 7 weeks, starting one week after set-up. For the fungicide treatment, Amistar, which is a broad-spectrum preventative and curative fungicide, was used at 0.05g per 100mL (Sygenta). The data collection ran for 8 weeks total, from late May to mid-July 2022.

Seedling and tree survey

At initial set-up all woody plant species in the plot were individually tagged, identified to species, and measured for height (cm) and root collar diameter (mm). If more than 20 of any species were present in a given plot, all were counted but only a random 20 were tagged and measured bi-weekly. Distance and height (m) of adult trees within 10 m of each seedling plot were measured. During the experiment, any newly germinated seedlings were tagged and recorded, and tags were removed upon seedling mortality each week. Additionally, seedling measurements of height, root collar diameter and leaf fungal amounts were collected bi-weekly. The amount of leaf fungal damage was assessed as a visual proportion of total leaf damage to undamaged leaf for the entire seedling.

Abiotic factors

Sites were revisited each week to collect precipitation, wind, temperature, and humidity data. Precipitation was collected using rain collectors that were placed outside of the plots at 5

and 105 m and emptied each week after measuring. Temperature, wind, and humidity data were collected at forest edge (0m), 5m, 55m and 105m, using a weather meter (Kestral; Boothwyn, PA, USA). Canopy openness was measured in the center of each plot once at the beginning of the experiment using a densiometer.

Statistical analyses

We analyzed each forest individually, with 20 plots in each forest (t=80). First, we investigated changes at the plot level in diversity, species richness, growth, survival, and fungal damage with distance from forest edge. We used the Simpson diversity index to measure diversity at the plot level. Within forest, plot level changes in diversity, survival, growth, fungal damage and species richness were tested against the effects of treatment, distance, the interaction of treatment and distance, and light availability using a linear regression. Across forests, changes in these factors due to coffee management style, treatment, their interaction, distance and light were also tested using linear regression.

To investigate changes in negative density dependence due to distance from forest edge and fungicide treatment, in each forest we first pooled plots from 5m-55m and 60m-105m into two groups (forest edge and forest interior). We used a generalized linear mixed-effects model to predict seedling survival and growth (defined as changes in height in $\text{cm}\cdot\text{day}^{-1}$) as a function of conspecific and heterospecific densities, treatment, initial height, light availability, and relevant interactions. To account for differences across species and plots, we used species and plot as random effects. Finally, to account for repeated measurements, we also used individual seedling as random effect. For seedling survival, we used a binomial link function.

All the variables for each model were z-transformed. Highly skewed variables (conspecific density, height, diameter, fungal damage) were log-transformed and were then z-

transformed. Conspecific density was z-transformed within species to account for differences in life strategy among species.

Finally, we compared the results of these models across the four forests to investigate for trends in how forest management might influence spillover and seedling demographics.

All analyses were conducted in R (v4.1.1; R Core Team 2021) using the lme4 and vegan packages (v2.6.2; Okansen et al. 2022).

Results

Over the eight-week period, we recorded 978 woody seedlings (Site1= 329, Site 2= 310, Site 3=212, Site 4=127) totaling 83 different species (Site 1=50, Site 2=34, Site 3=36, Site 4=36) within the 80, 1x1 m plots associated with this study. In general, survival, growth, diversity and negative density dependence varied across forests and management styles in terms of distance and treatment.

Changes in survival, growth, diversity, and leaf fungal presence with distance from coffee plantations

Overall, there was no significant difference in survival between management groups or across individual forests. There was also no significant difference in survival at the plot level within each forest with distance or treatment.

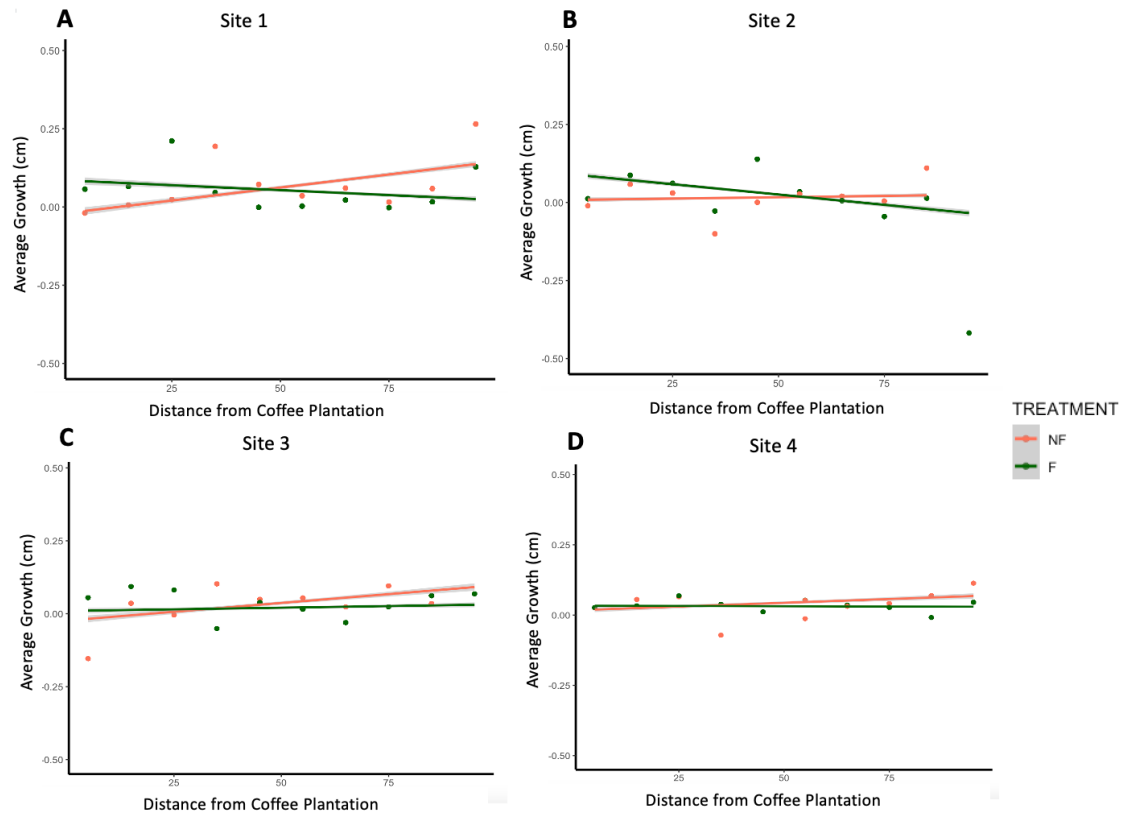


Figure 10. Average growth (cm) for each plot in each forest. Across the four forests, growth in fungicide treated plots increased with distance from the edge. In Sites 1 (A), 2 (B) and 4 (D), growth decreased with distance in control plots but slightly increased with distance in Site 3 (C).

However, when comparing seedling growth, there was a significant difference across the four forests ($F_3=81.45$, $p<0.001$). Specifically, seedling growth increased with distance ($F_1=83.66$, $p<0.001$) and was greater in plots treated with fungicide compared to the control plots ($F_1=34.2$, $p<0.001$). Additionally, there was a significant interaction among distance, treatment, and forest ($F_3=34.30$, 15.37 , $p<0.001$). Specifically, increases in growth were more pronounced in Sites 1 and 3 with distance, and plots treated with fungicide had a stronger response with distance than the control plots. Within this, Site 1 had the largest growth with distance in fungicide treated

plots over all of the forests, and Site 2 had the biggest decrease in growth with distance over all of the forests in control plots (Figure 10).

Although growth was different across forests, there was variability of seedling growth within forests as well. Fungicide treated plots in Site 1 had significantly higher growth rates at the forest interior but lower growth rates at the forest edge than control plots ($F_1=41.80$, $p<0.001$). As distance from the coffee plantation increased, the growth rate increased with treated plots and decreased with control plots ($F_1= 89.64$, $p<0.001$). In addition, there was a significant interaction with treatment and distance where treated plots had a higher change in growth rates with distance than control plots ($F_1= 248.05$, $p<0.001$; Figure 10A). Similarly, growth rates in Site 2 increased with distance in treated plots but decreased with distance for control plots ($F_1=10.962$, $p<0.001$). However, there was not a significant difference between treatments. This difference in growth rates was due to a significant interaction between treatments with distance where control plots had a more pronounced change in growth rates with distance than treated plots ($F_1= 171$, $p<0.011$; Figure 10B).

In Site 3, seedling growth increased across the plots with distance from the edge ($F_1= 77.69$, $p<0.001$). There was also significantly higher growth rates in treated plots ($F_1= 12.74$, $p<0.001$). In addition, there was an interaction between treatment and distance where growth rates across the plots were about the same at the forest edge, but were significantly higher in treated plots with distance than control plots ($F_1=77.69$, $p<0.001$; Figure 10C). Similar to the growth rates in Site 1, Site 4 also had an increase in growth rates in treated plots and a decrease in growth for control plots with distance ($F_1= 28.85$, $p<0.001$). At the forest edge, control plots had higher growth rates however, as distance from the edge increased, treated plots had higher growth rates ($F_1= 14.69$, $p<0.001$). Changes in growth were significantly more pronounced in treated plots

than in control plots as distance from the edge increased, due to a significant interaction with treatment and distance ($F_1= 41.43, p<0.001$; Figure 10D).

Overall, there was no significant difference in species richness across forests. However, species richness was significantly affected in Site 1, where species richness increased with distance ($F_1= 6.51, p=0.02$).

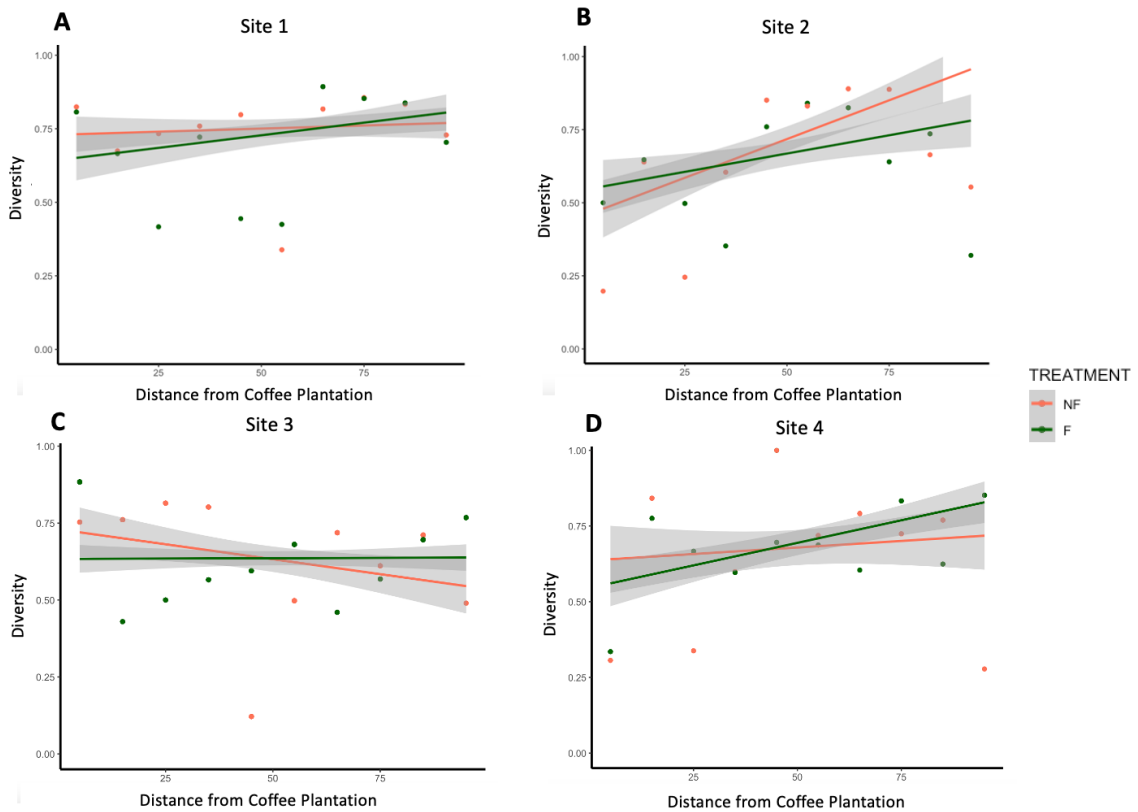


Figure 11. Diversity in each plot in individual forests. Overall, diversity increased with distance from the forest edge. The effects of treatment differed, where fungicide treatment negated fungal pathogen effects on diversity in Site 1 (A) and Site 4 (D) but decreased with distance in Site 3 (C) and increased more than control plots with distance in Site 2 (B).

In contrast, there was a significant difference in diversity across all four forests ($F_3=14.072$, $p<0.001$; Figure 11). In particular, diversity increased with distance ($F_1= 17.537$, $p<0.001$). Diversity with distance was also influenced by forest ($F_3= 14.924$, $p<0.001$), where Sites 1 and 4 had relatively similar diversity rates with distance but Site 2 had significantly high increases and Site 3 had significantly high decreases in diversity with distance. There was also a significant three-way interaction with forest, distance and treatment ($F_3= 3.955$, $p=0.008$). Specifically, control plots had greater increases in diversity with distance than treated plots. However, Site 2 had the greatest increase in diversity across forests and Site 3 had a decrease in diversity with distance for treated plots.

Within each forest, diversity significantly increased with distance across three out of the four forests (Site 1: ($F_1=6.027$, $p=0.015$; Figure 11A), Site 2: ($F_1= 38.208$, $p<0.001$; Figure 11B), Site 4: ($F_1= 10.99$, $p=0.0014$; Figure 11D)) which supports our first hypothesis. However, diversity significantly decreased with distance in Site 3 ($F_1= 3.015$, $p=0.02$; Figure 11C). Additionally in Site 2, treated plots had significantly higher diversity than control plots at the interior of the forest ($F_1= 4.072$, $p=0.046$), and more pronounced increases in diversity in treated plots with distance than control plots ($F_1= 4.703$, $p=0.032$; Figure 11B).

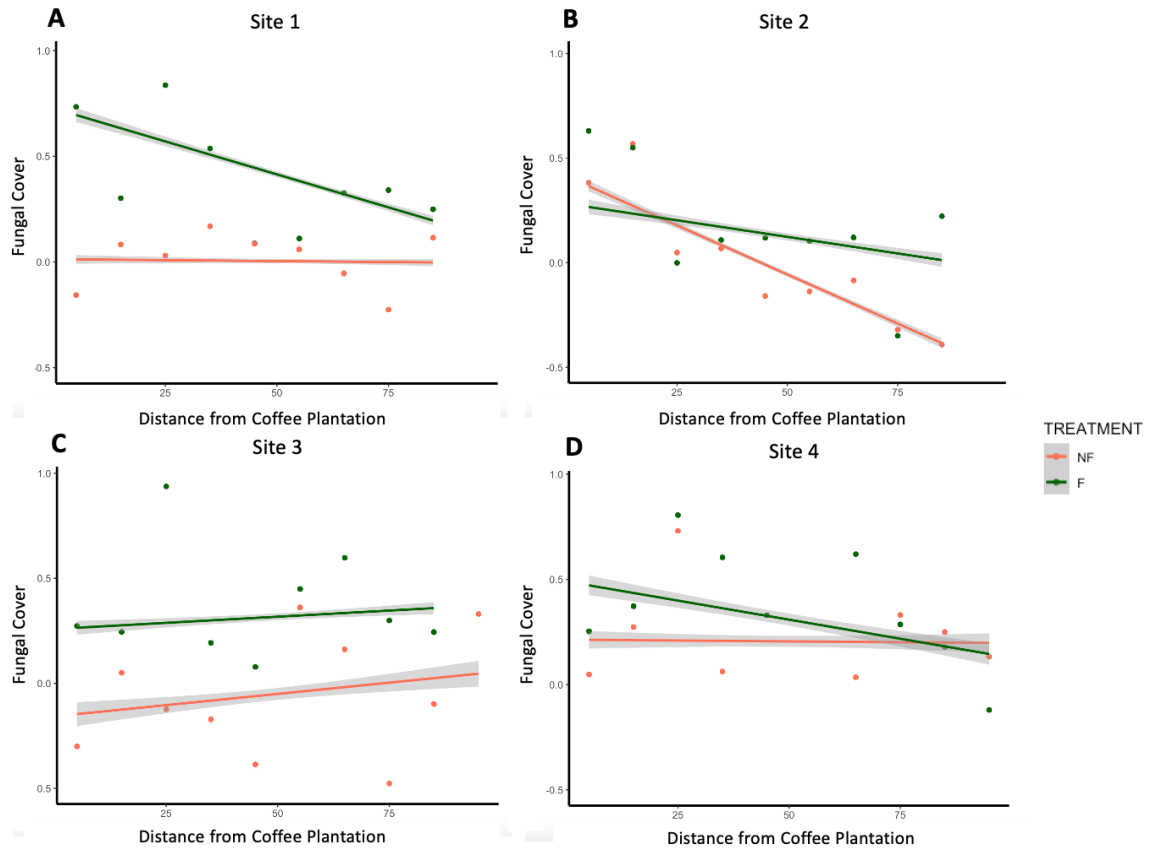


Figure 12. Change in leaf fungal amounts in all four forests with distance from the coffee plantation. Average leaf fungal amounts were lower in fungicide treated plots, however the effect of distance from the edge varied across forests.

The amount of leaf fungi on seedlings also varied between the four forests. In Site 1, leaf fungal amounts were significantly lower in treated plots ($F_1= 1267.78$, $p<0.001$) and decreased with distance from coffee plantations ($F_1= 63.01$, $p<0.001$). In addition, there was an interaction with treatment and distance where overall, treated plots had lower fungal amounts and which decreased at higher rates with distance than control plots ($F_1= 34.15$, $p<0.001$; Figure 12A). Similarly, in Site 2, fungal amounts were also lower in treated plots ($F_1= 358.56$, $p<0.001$) and decreased with distance ($F_1= 111.85$, $p<0.001$). However, the interaction with treatment and

distance was different than Site 1, in which both treated and control plots had similar fungal amounts at the edge, but the amount in treated plots decreased more rapidly with distance ($F_1=31.37$, $p<0.001$; Figure 12B). Overall, both forests had a decrease in fungal cover with distance, which was greater in treated plots, showing the effects of fungicide treatment and distance from the source of potential spillover. Although in Site 3, fungal amounts actually increased with distance from the coffee plantation ($F_1=24.7$, $p<0.001$), it was significantly lower overall in treated plots ($F_1=524.02$, $p<0.001$; Figure 12C). More interestingly, in Site 4, fungal amounts increased with distance in treated plots but decreased with distance for control plots ($F_1=12.173$, $p<0.001$). In addition, there was an interaction with distance and treatment, due to the directional change in fungal amounts as well as a higher fungal amounts in control plots than treated plots at the edge, but a lower coverage than treated plots at the interior ($F_1=19.295$, $p<0.001$; Figure 12D).

Changes in negative density dependence across individual forests

Contrary to our hypothesis, there was no correlation between conspecific density and survival in any of the forests when tested individually. There was, however, a significant positive correlation between root collar diameter growth and both conspecific and heterospecific density at the interior of Site 3 (95% CI [2.73e-03, 0.02], $p=0.007$). There was also a positive correlation between root collar diameter growth and the interaction between heterospecific density and fungicide at the interior of Site 4 (95% CI [6.92e-04, 0.05], $p=0.043$). Upon analysis, there were not enough individuals in the Rubiaceae family in any of the study plots within the four forests to be able to test the effects of NDD and are therefore omitted from the results.

Coffee plantation management effects on NDD, growth and diversity

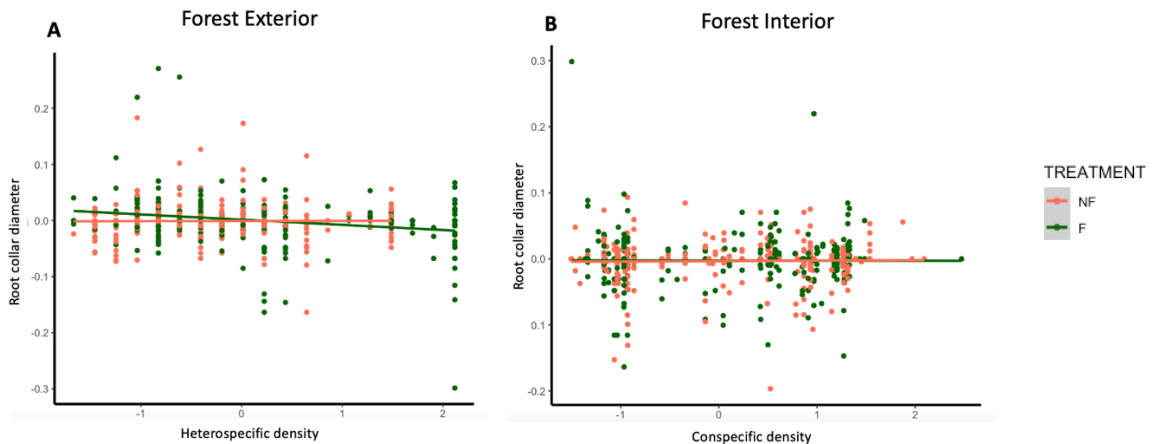


Figure 13. The effects of heterospecific density on root collar diameter (RCD) in shade forests. A) At the edge, RCD is positively correlated to conspecific density, but negatively correlated to the interaction of heterospecific density and treatment as shown by the change in slope from positive for fungicide plots to negative in control plots. B) At the forest interior RCD is negatively correlated to conspecific and heterospecific density, as shown by the negative slope for both fungicide and control plots.

When grouped by management type, shade forests had a positive correlation with seedling growth in root collar diameter (RCD) and conspecific density (95% CI [7.88e-05, 0.01], $p=0.47$) at the forest edge (Figure 13A). There was also a negative correlation with heterospecific density (95% CI [-0.01, -3.25e-03], $p<0.001$) but a positive correlation with the interaction heterospecific density and fungicide treatment (95% CI [0.01, 0.03], $p<0.001$) at the edge of shade forests (Figure 13A). At the interior of shade forests, there was a negative correlation with RCD and both conspecific (95% CI [-9.09e-3, -7.1e-4], $p=0.01$) and heterospecific density (95% CI [-0.01, -1.86e-03], $p=0.005$, Figure 13B). There were no correlations between conspecific density and either growth or survival in sun forests.

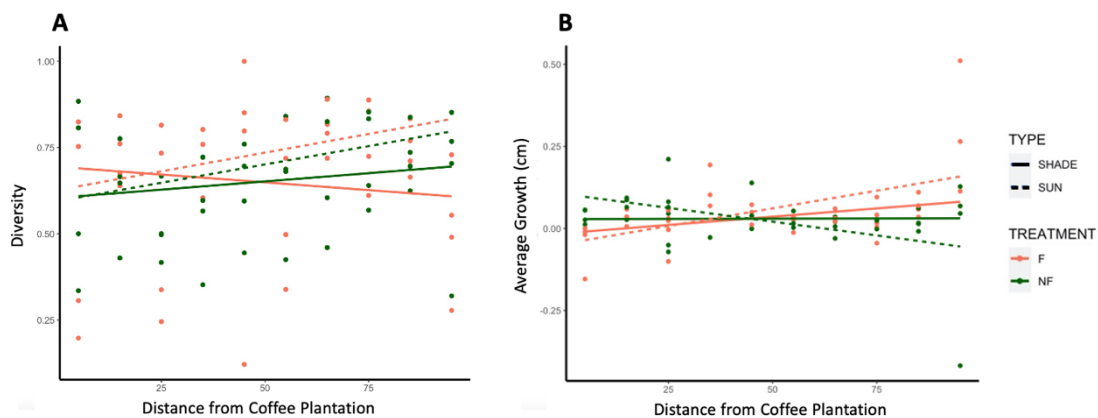


Figure 14. Diversity (A) and growth (B) across the two different management groups with distance from the forest edge. Diversity was significantly increased with distance from the edge in both management groups, however there is a higher increase in diversity in sun forests. While fungicide treated plots in sun forests had greater diversity with distance, treated plots in shade forests had decreased diversity with distance. Growth in both forest types was significantly different with distance and treatment. Growth in shade forests increased with distance and was not significantly different with treatment, but treated plots in sun forests had greater growth and control plots had decreased growth from the edge.

There was also significant differences in growth and diversity between management groups. In general, diversity increased with distance from coffee plantations ($F_1 = 17.534$, $p < 0.001$) however, sun management groups overall had higher diversity and more rapid increases in diversity with distance from the edge ($F_1 = 28.594$, $p < 0.001$; Figure 14A). In contrast, diversity decreased in with distance for treated plots in shade management groups. Interestingly, there was an interaction between management groups and distance, where treated plots in shade management had higher diversity at the forest edge than control plots in shade and both treated

and control sun groups. However, as distance from the edge increased, the diversity in treated shade management groups decreased while the others increased ($F_1 = 14.004$, $p < 0.001$).

In terms of growth, there was an increase across both management groups with distance, except for control plots in sun where growth decreased with distance, creating a significant interaction between distance and treatment ($F_1 = 12.796$, $p < 0.001$; Figure 14B). In addition, there was a three-way interaction between management group, treatment and distance ($F_1 = 4.499$, $p = 0.03$; Figure 14B). Specifically, the growth rates in the shade group increased similarly between treatments, however there was a more rapid increase in growth in the treated plots in the sun group and a conversely decreasing rate of growth in the control plots in the sun group.

Discussion

Our study is the first to attempt to link changes in seedling demographics at the edge of a tropical forests with potential fungal pathogen spillover from agriculture. Overall, given the evidence of fungal spillover from coffee plantations at these sites (Aldrich-Wolfe in prep), and the significant effect of fungicide on seedling communities, our results are consistent with a strong impact of fungal spillover on seedlings although the effect of distance from coffee plantation varied greatly across the study forests. While there were no significant differences in survival, the variability in growth and diversity suggests that the interactions between proximity to coffee and the tree seedling community in our study may have long-term effects of the forest understory community, also noted by Comita et al. (2014). Finally, our findings highlight the importance of considering management approaches, as different coffee plantations were associated with different spillover (Aldrich-Wolfe in prep) and seedling demographics in the adjacent forests. In particular, our study suggests that traditionally managed coffee plantations might lead to fungal spillover with stronger effects on adjacent forests.

Distance from coffee plantation is affecting growth, diversity and leaf fungal presence.

Contrary to our hypothesis, there was no significant difference in survival with distance from the edge or with fungicide treatment. In other words, along the transect seedling survival was not linearly influenced by distance from the edge. This is dissimilar to prior studies that observed a decrease in seedling survival at the forest edge (Meiners et al. 2002; Bell et al. 2006; Krishnadas & Comita 2018; Krishnadas et al 2018) but similar to one study in the Amazonian wet forest (Sizer & Tanner 1999). The lack of differences in survival could indicate that in the short-term, fungal pathogens are not affecting survival in these forests but they are affecting growth and diversity, which could perhaps influence survival over a longer period of time. It could also indicate the effects of other above and below-ground factors on seedling mortality that were not accounted for in this study, such as soil fungi, herbivory and long-term wind patterns (Bachelot & Kobe 2013; Krishnadas & Comita 2018).

Conversely, there were significant effects of both distance and fungicide treatment for seedling growth across the forests (Figure 10). In line with our hypothesis, there was increased growth across all forests in plots treated with fungicide. This supports fungal pathogens as important drivers of seedling demographics (Rand et al. 2006; Baghci et al. 2014; Krishnadas & Comita 2018). Something interesting to note is the unexpected decrease in growth with distance in control plots in Sites 1, 2, and 4 (Figure 10), which highlights variability in effects due to distance (Comita et al. 2014). This suggests that fungal effects on growth may be stronger at the forest interior than at the forest edge and were negated by the effects of fungicide treatment. This is similar to findings by Krishnadas and Comita (2018) that also suggested fungal pathogens had more negative effects on seedling recruitment gains at the forest interior. However, their study focused on pathogens at the edge of forests in India, that did not have instances of fungal pathogen spillover and are not fragmented by coffee agriculture.

Species richness was only found to significantly increase with distance in Site 1. The lack of change in species richness across forests suggests that that neither edge nor fungal pathogens

have a strong influence on species richness (Razafindratsima et al. 2017) but play a stronger role in driving overall diversity, which is reflected in our results.

In addition, seedling diversity was significantly different across all forests. In all but one forest (Site 3), there was an overall increase in diversity with distance from the edge (Figure 11A, B & D). There was also no significant difference between treatment groups in Sites 1 and 4 but a slight increase in diversity with fungicide treatment in Site 2. This trend suggests that other factors may be driving diversity in addition to fungal pathogens, which is more pronounced in Sites 1 and 4 but less so in Site 2, where fungicide did increase diversity. These factors could include changes in abiotic conditions besides light, such as soil type and nutrient availability (Razafindratsima et al. 2017; Krishnadas et al. 2018) or increases in other natural enemy presence, such as insects (Power & Mitchell 2004), as a result of edge effects. This also contrasts with a prior study that found higher diversity at the forest edge (Clark & Clark 1984). The differences in diversity at the edge could also be due different types of fragmentation for each forest (Alignier et al. 2014). Specifically, larger forest fragments have been found to exhibit less negative effects of diversity at the edge, which could be the case in our study, as forest fragment sizes differed (Krishnadas & Comita 2019; Viswanathan et al. 2020). The increase in diversity with distance from the edge is consistent with previous studies which found a decrease in diversity with proximity to the forest edge (Comita et al. 2010; Bagchi et al. 2014; Bachelot et al. 2017; Krishnadas & Comita 2018; Krishnadas et al. 2018). The change in diversity within Site 3 suggests that there is a similar effect of fungal pathogens driving diversity throughout the forest and does not change with edge effects. This is also reflected in this forest with the increase in growth with distance from the edge and fungicide treatment but the minimal change in growth with control plots (Figure 11C). These findings differ from a prior study that found fungicide treatments increased diversity at the forest interior (Krishnadas & Comita 2019). At this particular site, there was less of a distinct fragment between coffee plantation and forest, however there was

a large presence of leaf cutter ants in the forest interior, which have been found to decrease understory diversity when active (Garrettson et al. 1998).

Overall, leaf fungal coverage decreased with distance from the forest edge and was lower in fungicide treated plots, similar to prior studies (Benítez-Malvido, J. & Lemus-Albor 2005; Bagchi et al. 2014). This suggests that any changes in diversity and growth in this study can be largely, however not completely, attributed to fungal pathogens. In accordance with other studies on fungal pathogens, it also indicates that fungal densities are higher at forest edge (Gilbert et al. 2007; Hazelwood et al. 2021).

Negative density dependence

Surprisingly, we found very little, correlations of conspecific density and growth and no correlations for survival. Unlike previous studies in the tropics, who found that fungal pathogens are driving NDD at the forest edge (Clark & Clark 1984; Comita et al. 2014), our results indicate otherwise. Interestingly, the positive effect of conspecific density and subsequent negative effect of heterospecific density and treatment on root collar diameter in shade forests indicates the occurrence of positive density dependence, which has not been noted in previous studies (Figure 13). The negative effects of both conspecific and heterospecific density on root collar dynamics at the interior of shade forests highlights a weakening of NDD. This negative effect of heterospecific density, plus the non-significant correlations between conspecific density and growth in terms of height and in sun forests, emphasizes the ability of fungal pathogens in this forest to dilute the mechanisms maintaining diversity. The dilution of NDD on growth in these forests could be due to the reallocation of resources in order to combat pathogen infection (Freckleton & Lewis 2006; Krishnadas & Comita 2018).

There were no correlations between conspecific density and seedling survival in any of the forests. This emphasizes the ability of leaf fungal pathogens to dilute NDD at the forest edge

and the interior suggests a dilution of fungal pathogen effects as noted previously in non-fungal spillover studies (Benitez-Malvido & Lemus-Albor 2005; Bagchi et al. 2010, 2014; Comita et al. 2014; Bever et al. 2015). This also indicates the ability of edge effects at our sites to permeate into the forest interior (Danta de Paula et al. 2016; Faust et al. 2018). In addition, the absence of NDD at the edge of these sites is consistent with the hypothesis that forest fragmentation weakens NDD (Krishnadas et al. 2018; Viswanathan et al. 2019, 2020) and potentially favors generalist pathogens (Rand et al. 2006). However, the lack of correlation between survival and treatment suggests that this weakening could happen because of other effects. These include altered climate conditions like increased exposure to sunlight or weather (Bachelot & Kobe 2013; Viswanathan et al. 2020), increased exposure to new enemies, specifically fungal pathogens from coffee plantations (Power & Mitchell 2004), or a shift in favor of generalist enemies (Bagchi et al. 2014; Krishnadas et al. 2018). However, the increase in diversity with distance from the edge in some of these sites regardless of NDD, indicates that there are other, and possibly stronger, edge effects driving diversity in this forest than just fungal pathogens alone (Liu et al. 2012; Bagchi et al. 2014).

The lack of correlation between seedling survival and light availability at the edge in all four forests contrasts with prior studies that found negative effects of seedlings in the presence of high light at the forest edge (Kobe 1999; Krishnadas & Comita 2018). However, our findings are similar to a prior study by Augspurger (1984), who found a similar lack of relation between light and seedling survival.

The variability in effects of fungal pathogens on NDD in these forests could be largely attributed to the types of fungi present, since different fungi have been known to affect understory communities differently (Augspurger 1984; Brown & Heckman 2021) and have not yet been fully identified at our study sites. It has also been noted that the influence of NDD can vary depending on a plant species' life history (Liu et al. 2012, 2016; Paine et al. 2012) and shade tolerance

(Kobe & Vriesendorp 2011) in certain forests, which may be the case for our varied results or lack thereof.

In addition, when we examined specifically for species in the Rubiaceae family to test phylogenetic relatedness and the effects of NDD, we did not have enough seedlings to warrant any analyses, which could either be indicative of the long-term effects on this specific family (Liu et al. 2012, 2016; Paine et al. 2012; Bever et al. 2015) or simply their low population sizes in these specific forests.

Coffee management styles indicate seedling vulnerability at the forest edge.

Our hypothesis regarding coffee management types was only partially supported and had variable results in terms of growth and diversity. We originally hypothesized that NDD would be more present in sun than shade forests, however there was no effect of conspecific density on survival in either forest type. There was however a difference in diversity between the two forest types at the plot level. While there was an overall increase in diversity with distance from the forest edge, shade forests had higher diversity at the forest edge. There was a sharper increase in diversity with distance from the edge in sun forests, emphasizing the limitations of fungal pathogens on diversity at the forest edge (Figure 14A). The low diversity at the edge and sharp increase in diversity with distance in sun forests also supports our original hypothesis on the more pronounced effects of distance in sun forests. While the results of diversity vary slightly across individual forests, they fall in line with the individuality of coffee farming practices in Costa Rica and how they can have a variety of effects on the nearby forests (Toledo & Moguel 2012). The difference in diversity also emphasizes the multiple influences that may be driving diversity in these forests, which may shift from the edge to the interior (Comita et al. 2014; Krishnadas & Comita 2019) and warrants further investigation to gain a better understanding of the full effects of coffee plantations on the forest understory in this area.

The effects of NDD on growth was also diluted in sun forests and at shade forest interiors, but there was positive density dependence on growth at the edge in shade forests. Again, supporting the idea that fungal pathogens are negatively affecting NDD in these coffee fragmented forests. This builds on prior studies who found fungal pathogens negatively affect NDD (Freckleton & Lewis 2006; Liu et al. 2012; Hazelwood et al. 2021), with our forests being in close proximity and experiencing fungal introductions from coffee plantations. However, at the plot level, growth was significantly greater in treated plots within forests near sun coffee plantations, but significantly lower in nontreated plots (Figure 14B). This could suggest that energy resources are being allocated more towards defense rather than growth in these forests (Berger et al. 2007; see reviews in Schultz et al. 2013). It also further supports the evidence of sun coffee plantations retaining higher amounts of fungal pathogens (Toledo & Moguel 2012), which have a clear effect on the forest community. In addition, treatment in shade forest also had a positively affected growth, although it was less significant than in sun forests. This supports the evidence that eco-friendlier practices may lessen the negative edge effects than sun management (Toledo & Moguel 2012).

To conclude, while the overall results may be variable, there is a clear indication that proximity to the forest edge in these forests is disrupting NDD and may be changing the way that we understand how diversity in tropical forests maintained (Bagchi et al. 2014; Comita et al. 2010; Bachelot et al. 2017; Krishnadas & Comita 2018; Krishnadas et al. 2018; Krishnadas & Stump 2021). In other words, our results suggest that the mechanisms maintaining diversity may be different across forests, especially depending on fragmentation type (Alignier et al. 2014) and the agriculture practices nearby (Rand et al. 2006; Toledo & Moguel 2012). For example, in studies examining fungal spillover effects on cheatgrass, both Beckstead et al. (2010) and Mordecai (2013) also found there to be various effects on plant diversity and survival, especially when considering plant competition and tolerance to infection. However, other studies

investigating non-spillover fungal pathogen effects at the forest edge, found low survival rates at the edge (Rand et al. 2006; Bagchi et al. 2010, 2014; Krishnadas et al. 2018), which further supports our claim that spillover is shifting the traditional mechanisms of diversity in these forests. These inconsistent effects in fungal pathogens highlight the importance of the vulnerability of these forests and how that varies drastically between forests, further driving the need for more investigation.

CHAPTER IV

CONCLUSION

Theoretically, we have shown that the introduction fungal pathogen spillover can have a variety of effects on the dynamics of a system with two plant species and a density-dependent fungal pathogen. Due to its inability to be influenced by prey species population sizes, spillover acted as an equalizing mechanism that promoted species coexistence or fully disrupted population dynamics. These effects were dependent on the spillover pathogen's host-specificity in addition to the plant species' competitive ability. Specifically, generalist spillover promoted more coexistence outcomes between plant species, limiting one species from outcompeting the other. In addition, specialist spillover promoted the success of the nontargeted species, allowing for the nontargeted weaker plant species to gain a competitive advantage whilst limiting the target plant species regardless of its competitive ability. In general, spillover eliminated any possibility of species existence at the forest edge, and as it moved into the forest, its effects were diluted to eventually mimic outcomes of those without spillover. This effect was strengthened with wind and made unpredictable with turbulence. Overall, this model shows that fungal pathogen spillover can be unpredictable and have a myriad of both positive and negative effects on the plant community.

Empirically, the variety of effects due to fungal pathogens was also demonstrated in our four study forests in Costa Rica. Specifically, growth tended to increase with distance in

fungicide treated plots but had differing effects in control plots. Additionally, these pathogens had a negative effect on diversity at the forest edge, that diluted with distance and with fungicide treatment. Although leaf fungal amounts were typically lower in fungicide treated plots, they had differing effects with distance from the edge. The lack of effects of NDD on survival and growth indicate that fungal pathogens have a strong potential to dilute the mechanisms driving diversity.

Overall, the inconsistency in fungal pathogen spillover effects make it an important ecological issue that needs to be addressed in the tropical forest community. This also emphasizes the need to understand how the mechanisms that have been previously understood to maintain tropical forest biodiversity are changing in response to forest fragmentation and proximity to agriculture. By utilizing both theoretical and empirical data, we highlight effects that fungal pathogen spillover can have on plant community diversity. Our studies emphasize the need to further understand the effects of fungal pathogen spillover and how they vary geographically. Understanding the implications of fungal spillover will help us better plan for forest conservation and restoration efforts to maintain tropical forest biodiversity on a global scale.

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