MATURE TREE EFFECTS ON SEEDLING REGENERATION: PLANT-SOIL FEEDBACKS, THEIR LEGACIES, AND RESTORATION

By

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ABSTRACT

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Trees affect seedlings through many pathways, including via interactions with soil and surrounding vegetation, that can shape seedling recruitment. Plant-soil feedbacks (PSFs) occur when a plant modifies soil biota or abiotic factors in a way that influences the performance of subsequent or co-occurring plants. PSFs shape plant community dynamics by affecting species relative abundance. After a plant initiating PSFs dies, legacies of PSFs occurring as soil signatures that influence subsequent plants could persist for unknown duration.

In this dissertation, I investigated PSFs and PSF legacies of two species, *Prunus serotina* and *Acer rubrum*, and the effectiveness of a Hawaiian tree for forest restoration. To examine PSF legacies, my approach was to plant seedlings in soils collected around live trees and stumps of varying ages in greenhouse experiments. PSFs were measured in live tree soils and PSF legacies as the difference between seedling performance in live tree and stump soils.

For *P. serotina*, negative PSF legacies were short-lived, lasting up to 0.5 years after tree removal and occurred under 5% but not 30% full sun. Though restricted to low light, short-lived legacies of *P. serotina* PSFs could have lasting impacts on plant community dynamics during crucial post-disturbance regeneration by disfavoring *P. serotina* in small tree-fall gaps.

To examine how long soil pathogens outlive tree hosts in gap soils, I studied the presence of oomycetes in soils near live *Prunus serotina* trees, and 0.5 and 1.5-year-old stumps. I isolated five species of oomycetes from soils, two of which were pathogenic (*Pythium intermedium* and *Pythium irregulare*) to *P. serotina* and present in soils of all stump ages. Continued presence of

pathogens of *P. serotina* in gap soils demonstrates the potential for impacts on conspecific regeneration after tree death, though pairing this finding with improved seedling survival after the first growing season suggests that pathogen effects may weaken with time.

For *A. rubrum*, I found positive PSFs and PSF legacies lasting > 8 years in conspecific soils. These results demonstrate that escaping soil enemies is not a mechanism contributing to the historical rise of *A. rubrum* abundance in many forests of eastern North America. Rather, positive PSFs and multi-year legacies will promote *A. rubrum* seedling recruitment near conspecific trees and reinforce the growing dominance of *A. rubrum* across many forests.

In a restoration context, I examined mature tree effects on seedling regeneration through a study of mechanisms of grass suppression and facilitation by planted trees; grass suppression is a crucial condition needed to create opportunities for seedling recruitment. Using stands of a native N-fixer, *Acacia koa* (koa), on Hawai‘i Island, I evaluated whether koa suppresses invasive grasses and if so, by which mechanisms. I found consistent effects of grass suppression by koa via shading and litter accumulation, but importantly, total grass suppression rarely occurred. Grass persistence under koa canopies may be driven by a shift in composition to more shade tolerant species. If complete grass suppression and/or more diverse forest are desired, then further management interventions, like diverse understory plantings, could amplify mechanisms of grass suppression and thereby create seedling recruitment opportunities.

Taken together, these findings demonstrate the relevance of PSF legacies for forest community dynamics and how PSF legacies can vary in duration and direction depending upon the tree species involved. When planting trees to suppress invasive grasses, multiple mechanisms are involved with a net effect of suppressing grasses, but they may be insufficient to meet goals.

To all who inspired and guided me,
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

The tree seedling stage is a critical bottleneck in forest population dynamics with implications for future forest composition (Canham and Murphy 2016); mature trees influence the regeneration of seedlings beneath their canopies by altering conditions above- and below-ground (Zinke 1962; Holl et al. 2000). Aboveground effects of trees include reduced light and rainfall, and a moderated microclimate (Nepstad et al. 1996; Holl 1999; Gómez-Aparicio et al. 2005; Rehm et al. 2021). Belowground, soil nutrient concentrations and cycling can be altered primarily through litter inputs, and soil biota, composed of antagonists and mutualists, are shaped through interactions with tree roots (Perakis et al. 2012; Waring et al. 2015; Bennett et al. 2017).

Plant-soil interactions which can lead to distinct outcomes depending upon the tree altering the soil and the seedling responding (Gustafson and Casper 2006; Bever et al. 2012; Hersh et al. 2012); such tree-soil-seedling interactions are plant-soil feedbacks (PSFs). PSFs occur when a plant modifies the soil in any way that influences the performance of subsequent or co-occurring plants (Bever et al. 1997); their involvement in plant community dynamics has been increasingly recognized in recent decades (Klironomos 2002; Ehrenfeld et al. 2005; Mangan et al. 2010; Bauer et al. 2015). PSFs can manifest as positive or negative effects on plants of the same species (conspecific PSFs) or different species (heterospecific PSFs) mediated through biotic or abiotic components of the soil (Van der Putten et al. 2013). Potential biotic aspects of PSFs encompass bacteria, nematodes, below-ground invertebrates, mycorrhizal fungi, and soil pathogens (Van Der Putten and Van Der Stoel 1998; Packer and Clay 2000; Kardol et al. 2007; Eisenhauer et al. 2011; Bennett et al. 2017). Abiotic soil effects can take the form of altered soil

nutrient concentrations, nutrient cycling, and chemical exudates with allelopathic effects (Stinson et al. 2006; Waring et al. 2015). Soil nutrient concentrations and cycling are primarily impacted through litter contributions with varying nutrient content and quality (Scott and Binkley 1997; Prescott 2002; Hobbie 2015). Though PSFs of trees are often studied in the context of individual trees and surrounding seedlings, PSFs scale up to influence whole forest composition. Positive conspecific PSFs can promote single species dominance (Smith and Reynolds 2012; Liang et al. 2020), while poorer performance in conspecific soils than heterospecific soils (negative PSFs) can enhance forest diversity (Mangan et al. 2010; Liang et al. 2016). Negative conspecific PSFs can be evident in patterns of negative conspecific density dependence and align with expectations of the Janzen-Connell hypothesis (being mediated through soil enemies) (Bagchi et al. 2010; Comita et al. 2014).

Most PSF work to-date has focused on effects occurring when trees are alive, healthy, and their roots actively interacting with soils (but see Gómez-Aparicio et al. 2017). After tree death, PSFs could also persist as soil signatures and continue shaping plant communities in forest gaps. Many components of soils modified by trees can persist as long-term alterations to soils. For example, altered soil nutrient concentrations associated with individual trees can persist more than 40 years near stumps in New Zealand rainforest (Wardle et al. 2008) or through fire and land-use transition of a Mexican dry forest (Døckersmith et al. 1999). Many types of soil biota (e.g., fungi, oomycetes, bacteria, and protists) can enter dormancy in the absence of a host and persist for years in soil as spores or cysts (Martin and Loper 1999; Adl and Gupta 2006; Raaijmakers et al. 2009; Nguyen et al. 2012), creating the potential for lasting PSF legacies.

How long soils signatures from PSFs influence subsequent plants is uncertain. In this dissertation, I focus on PSF legacies as soil signatures present after plant death and distinguish

PSF legacies as distinct from PSFs occurring when the plant influencing the soil is alive. PSF legacies in agricultural systems, such as recurring soil-borne disease, have long been avoided via crop rotation (Shipton 1977; Cook 1981; Mariotte et al. 2018). Yet in non-agricultural systems, few examples of PSF legacies exist and those that do exist suggest PSF legacy effect longevity may vary from a single growing season (Grman and Suding 2010) to multiyear effects (Kulmatiski and Beard 2011). Understanding how long PSF legacies persist after tree death could illuminate previously overlooked sources of soil variation and their influence on seedling regeneration. PSF legacies persisting long-term, especially after evidence of the tree initiating PSFs has decomposed, would introduce a nearly intractable source of variation into forest dynamics as 'ghosts of trees past'.

PSFs and their legacies are one pathway by which trees influence regeneration and thus future forest composition. However, PSFs and PSF legacies do not operate in isolation and in forest management and restoration contexts the totality of mature tree effects on vegetation beneath their canopies has to be considered. Trees may both facilitate and suppress vegetation (Callaway and Walker 1997) and these effects can be leveraged in restoration to reduce undesirable species and benefit desired recruits (Holl et al. 2000; Corbin and Holl 2012). In practice, the interplay between mechanisms of suppression and facilitation and site conditions could lead to varying restoration outcomes.

Overview of Chapters

This dissertation includes four chapters focusing on three different systems. The first three chapters are linked by their focus on PSF legacy effects and the fourth takes a broader approach in examining tropical forest restoration and mechanisms by which trees affect vegetation beneath their canopies. A short summary of each chapter follows.

Chapter 2: To determine if PSF legacy effects occur and how long they persist, I used the well-studied negative conspecific PSF of *Prunus serotina* (black cherry) as a model system (Packer and Clay 2000; Reinhart and Clay 2009). I leveraged single-tree selection harvests of *P. serotina* as isolated incidents of aboveground tree death to create a chronosequence of stump ages (or time since tree death). I evaluated PSFs and their legacies by growing *P. serotina* seedlings in a greenhouse in soils from live trees, stumps, and the surrounding forest matrix and comparing their survival and growth. To examine the influence of light on *P. serotina* PSFs and their legacies, I grew seedlings under two light levels corresponding to single tree fall gaps and larger gaps (5% and 30% full sun respectively) (Walters et al. 2016). I found that *P. serotina* PSF legacies were present for < 1.5 years and restricted to low light (5% full sun). Though short-lived, these PSF legacy effects could have lasting effects on the composition of post-disturbance regeneration by disfavoring *P. serotina* in small gaps.

Chapter 3: Building upon the prior chapter, I examined the persistence of soil biota, specifically pathogenic oomycetes in the genus *Pythium*, after *P. serotina* tree harvest through simultaneous greenhouse and culturing experiments. In a greenhouse set-up similar to chapter 2, I grew *P. serotina* seedlings in soils from live trees and stumps of two ages (0.5 and 1.5 years old) and monitored their survival and growth. At the same time, I isolated oomycetes from soils by baiting in two phases: 1) from the same field soils used in the greenhouse, and then 2) from the soils of symptomatic and asymptomatic *P. serotina* seedlings in the greenhouse. After sorting our isolates into groups by morphology, I identified representative isolates from each morphogroup by sequencing, and conducted a pathogenicity trial using the three most common oomycete species. I found that two species, *Pythium intermedium* and *Pythium irregulare*, are pathogenic to *P. serotina* and present in soils of all ages. The continued presence of these two

pathogens demonstrates the potential for pathogen damage to *P. serotina* seedlings regenerating in gaps, though slightly higher survival in the oldest stump soils (1.5 years old) suggests that the effects may weaken with time.

Chapter 4: To expand into evaluating heterospecific effects, I examined PSFs and PSF legacies across multiple species soil sources. Using methods similar to chapter 2, I evaluated *A. rubrum* seedling survival and growth in the greenhouse in soils collected around stumps and live trees at single-tree selection harvest sites sourced soil from multiple species, *A. rubrum* and 5 heterospecifics. Unexpectedly, I found higher survival and growth of *A. rubrum* seedlings in *A. rubrum* soils than heterospecific soils (positive PSFs) and multiyear (> 8 years) PSF legacies. I found limited variation among PSFs in heterospecific soils and could not resolve heterospecific PSF legacies. Overall, positive conspecific PSFs and longer-lasting PSF legacies suggest that beneficial conditions for *A. rubrum* recruitment persist after tree death. The contrast between multiyear (> 8 years) positive PSF legacy effects of *A. rubrum* and short-lived (< 1.5 years) negative PSF legacies of *P. serotina* demonstrates a potential range of variation in PSF legacy effects and variability between species, suggesting that further studies of PSF legacies could illuminate their importance in forest dynamics and succession.

Chapter 5: Broadening out beyond PSFs and PSF legacies and focusing on an applied context, I studied the effects of *Acacia koa* (koa) canopy trees on exotic grass suppression in Hawaiian restoration forests. Grass suppression is a critical prerequisite for restoration of a diverse forest because competition with grasses limits recruitment of woody species (Cabin et al. 2002a; Denslow et al. 2006). As an N-fixing tree, koa may facilitate grass growth through contributions to soil N (Yelenik 2017). By disentangling mechanisms of grass suppression and facilitation by koa I aimed to determine the efficacy of planting koa as a restoration tool for grass. At sites

ranging across varying rainfall and temperature conditions on Hawai‘i Island, I examined the influence of koa density on grass suppression and the various pathways by which koa affects grasses by measuring grass biomass, koa density, light levels, soil moisture, plant-available soil N, understory composition, and koa litter biomass. I found two paths by which koa suppresses grasses, shading and litter accumulation. Reducing soil moisture may also suppress grasses but was more closely related to the effects of site. Critically, complete grass suppression rarely occurred under koa monocultures. Mechanisms of grass suppression could be reinforced through additional management interventions, specifically planting additional species that cast deeper shade and/or produce litter that is slower to decompose. Such plantings could add on to the effects of koa canopies and create conditions more favorable for further woody species recruitment.

The final chapter is a synthesis of the main findings of the four research chapters.

CHAPTER 2

Short-lived legacies of *Prunus serotina*

plant-soil feedbacks

ABSTRACT

Plant-soil feedbacks (PSFs) are often involved in fundamental ecological processes such as plant succession and species coexistence. After a plant initiating PSFs dies, legacies of PSFs occurring as soil signatures that influence subsequent plants could persist for unknown duration. Altered resource environments following plant death (especially light availability) could affect whether legacy effects manifest and persist. To evaluate PSFs and their legacies, we obtained soils from a chronosequence of *Prunus serotina* harvests. In a greenhouse experiment, we planted conspecific seedlings under two light levels in these soils of varying time since the influence of live *Prunus serotina*, and compared seed/seedling survival in soils from live trees, stumps, and surrounding forest matrix within each site and across the chronosequence. PSF legacies were measured as the difference between seedling performance in live tree and stump soils within a site. Negative PSF legacies of *P. serotina* were short-lived, lasting up to 0.5 years after tree removal. These effects occurred under 5% but not 30% full sun. PSFs and their legacies manifested in seed/seedling survival, but not biomass. Though restricted to low light, short-lived legacies of *P. serotina* PSFs could have lasting impacts on plant community dynamics during post-disturbance regeneration by disfavoring *P. serotina* regeneration in small tree-fall gaps.

INTRODUCTION

Understanding the mechanisms by which plant species coexist and community succession occurs are fundamental themes of plant community ecology (Palmer 1994; Chesson 2000; Wright 2002). Plant-soil feedbacks (PSFs) are increasingly recognized as crucial to plant community functioning and key components of both species coexistence and succession (Bever 2003; Van der Putten et al. 2013). PSFs occur when plants modify soil biotic or abiotic factors, which in turn influence the growth and survival of other nearby or subsequent plants (Bever et al. 1997). PSFs affect species coexistence and increase diversity when conspecifics are disfavored in their own soil relative to heterospecifics (Bever 2003; Petermann et al. 2008; Bagchi et al. 2010; Crawford et al. 2019). During succession, PSFs drive shifts in plant species abundance that change in strength and direction with plant successional stage (Bauer et al. 2015).

The relevance of PSFs for community functioning could extend beyond the time when a plant is actively modifying the soil because alterations to the soil biota or abiotic factors could persist long after the plant has died or been harvested (Kardol et al. 2007; Wardle et al. 2008) and continue to shape performance of other plants as a PSF legacy effect. Here we focus on PSF legacies as soil signatures present after plant death, which are distinct from PSFs occurring when the plant initiating the feedback is alive. We investigate PSF legacies as plant responses to soil signatures manifesting months to years after the plant initiating PSFs was harvested by using a chronosequence of removal times of the plant initiating the PSFs.

PSF legacies, like PSFs, could operate through both soil biota and abiotic factors persisting in the absence of a plant host. Examples include lingering soil chemical signatures around tree stumps (Wardle et al. 2008) or soil biota (e.g., fungi, oomycetes) entering dormancy for years as spores or cysts (Martin and Loper 1999; Nguyen et al. 2012).

In many agricultural systems, avoiding the accumulation of soil disease (PSFs) and PSF legacies in the form of persistent soil disease has made crop rotation common practice (Shipton 1977; Cook 1981; Mariotte et al. 2018); yet in non-agricultural systems, the persistence of biotic-PSF legacies is underexplored and could be crucial to our understanding of plant communities. Short-term biotic PSF legacy effects have been found in a few instances, such as succession of herbaceous plant communities (Kardol et al. 2007) and interactions between native and exotic herbaceous plants (Grman and Suding 2010), but in another different herbaceous system multiple growing seasons were required to change the soil microbial community composition and overcome the prior soil legacy (Kulmatiski and Beard 2011). If PSF legacy effects persist long-term, after aboveground evidence of the influencing plant is gone, they could introduce additional complexity into seedling survivorship and growth, processes that are critical in plant community dynamics.

Forests provide particularly suitable systems for studying PSF legacies. Stumps persist for years after death, providing evidence of individual past trees, often identifiable to species. Being long-lived, trees modify soils over decades potentially leading to greater accumulation of abiotic changes and/or soil biota involved in PSFs, which could be more likely to persist after tree death as PSF legacies. In mature forests, succession occurs through gap dynamics whereby tree loss opens growing space and resources by forming a gap which creates conditions for juvenile trees to recruit to the canopy (Canham 1985; Gray and Spies 1996). Succession in gaps could be influenced by legacies of PSFs, especially during critical seedling establishment. Like PSFs, the outcome of PSF legacies could depend upon the species of both the past tree and responding seedling (Gustafson and Casper 2006; Bever et al. 2012; Hersh et al. 2012).

The loss of a forest tree initiating PSFs is unavoidably confounded with a shift in resources following gap creation. Tree death allows more light to reach the forest understory and PSFs detrimental to tree seedling survival are often found exclusively in low light (McCarthy-Neumann and Ibáñez 2013). Thus, the influx of light could improve seedling survival through hypothesized mechanisms of accelerated development through pathogen-vulnerable early seedling stages (Augspurger 1984), maintaining a more favorable carbon balance with mycorrhizal fungi (Smith and Read 2008), or greater expression of defense and recovery traits such as phenolics (Entry et al. 1991; Ichihara and Yamaji 2009) and nonstructural carbohydrate storage (Myers and Kitajima 2007; Kobe et al. 2010). Pathogen abundance may also be reduced in gaps or near dead trees (Reinhart et al. 2010a; Gómez-Aparicio et al. 2012). On the other hand, greater soil water content in gaps than under closed canopy during the first growing season following tree loss (Ritter et al. 2005) could lead to greater disease incidence (Erwin and Ribero 1996).

To better understand the lifespan of PSF legacies and elucidate their potential to affect plant community dynamics after the plant initiating PSFs has perished, we addressed two questions: (1) How long do PSF legacy effects last? (2) Is the duration of PSF legacies influenced by light availability? We used negative conspecific PSFs experienced by the temperate forest tree species, *Prunus serotina* Ehrh (Packer and Clay 2000; Reinhart and Clay 2009) as our model system. To evaluate PSFs, we compared *P. serotina* seed/seedling survival in soils collected 'near' and 'far' from live conspecific trees. To assess PSF legacies, we compared *P. serotina* seed/seedling survival between soils collected near live conspecific trees and stumps across a range of tree harvest ages.

METHODS

To determine the longevity of PSF legacies associated with *P. serotina*, we conducted a greenhouse study using field soils from sites forming a chronosequence of harvest times. We sought sites with selective harvests in order to have a defined time of aboveground tree loss. We used a chronosequence of four sites aged up to 15 years since harvest. To assess the possibility of soil nutrient based PSF legacies and ensure sites did not vary widely in soil nutrients, we measured base cations, soil N, and C:N ratios from soils near each stump and live tree.

Study Species

We selected *P. serotina*, black cherry, as a model species for its strong negative conspecific PSFs relative to other temperate forest trees (Packer and Clay 2003; Bennett et al. 2017). *Prunus serotina,* a shade intolerant canopy tree, is native to Eastern North America, parts of Mexico and Central America (Hough 1960); its regeneration is gap dependent. Negative PSFs associated with *P. serotina* have been linked to pathogenic oomycetes, specifically *Pythium* spp., which occur across the native range of *P. serotina* (Packer and Clay 2000; Reinhart et al. 2010b). *Pythium* affects all life stages but is most likely to cause pre- and post-emergence mortality in young seedlings and infect fine roots of older plants; it can also function saprophytically (Hendrix and Campbell 1973; Martin and Loper 1999). In the absence of a host, oomycetes can enter dormancy and persist for years in soil as oospores (e.g., 12 years for *Pythium ultimum* Hoppe 1966 as cited in Martin and Loper 1999), a mechanism which can lead to recurring disease and PSF legacy effects.

Study Sites

To evaluate the duration of PSF legacies, we used a chronosequence of harvest sites composed of *P. serotina* stumps and live trees from single-tree selection harvests in forests of

similar overstory composition in southwestern Michigan. Sites were oak-transition northern hardwood forests largely dominated by *Quercus rubra*, with components of *Acer saccharum*, *Prunus serotina*, and occasional *Quercus velutina*, *Quercus alba*, *Acer rubrum*, and *Juglans nigra*. Single-tree selection harvests mimic spatial patterns of isolated mortality events and allowed for selection of stumps and live trees in the same forest stands. We selected four sites with harvests spanning 0.5 to ~15 years prior: Russ Forest (RF) in Decatur, MI (harvested 0.5 years prior), Lux Arbor Reserve (LA) in Delton, MI (1.5 years), privately owned forest in DeWitt, MI (DW) (3.5 years), and Rose Dell Woodlot (RD) in Albion, MI (~15 years). At the most recent harvest site (RF), we randomly selected individuals for harvest. At all other sites, forest managers selected trees, some of which was motivated by storm damage at site RD.

At each harvest site, we sampled soils around stumps and live conspecific trees (Table 2.1). *Prunus serotina* stumps were identified by bark characteristics; for trees removed more than one year prior we selected stumps without sprouts. We removed sprouts from stumps less than one year old in the summer preceding soil sampling. Because we were focused on PSF legacies, we sampled soils around more stumps than live trees to capture variation in stump soils. Each focal stump or live tree was > 30 m from any other focal individual. All were dominant or codominant trees with at least 38 cm diameter at the top of stumps and 25 cm diameter at breast height (DBH) for trees. Live and removed trees included a range of sizes.

Soil cores were sampled in late August 2016 around each focal stump or tree in 7.6 cm diameter × 25.4 cm length PVC tubes to minimize soil disturbance. The PVC tubes were cleaned by soaking in 0.5 % NaOCl for 20 minutes and rinsing with water prior to use.

Table 2.1. *Prunus serotina* trees and stumps where soil was sampled.

Site	Samples	N[a]
RF	live trees	3
	0.5 yr. old stumps	5
LA	live trees	3
	1.5 yr. old stumps	6
DW	live trees	3
	3.5 yr. old stumps	5
RD	live trees	3
	~15 yr. old stumps	5

a) At each tree or stump, 16 soil cores were removed as detailed in Figure 2.1.

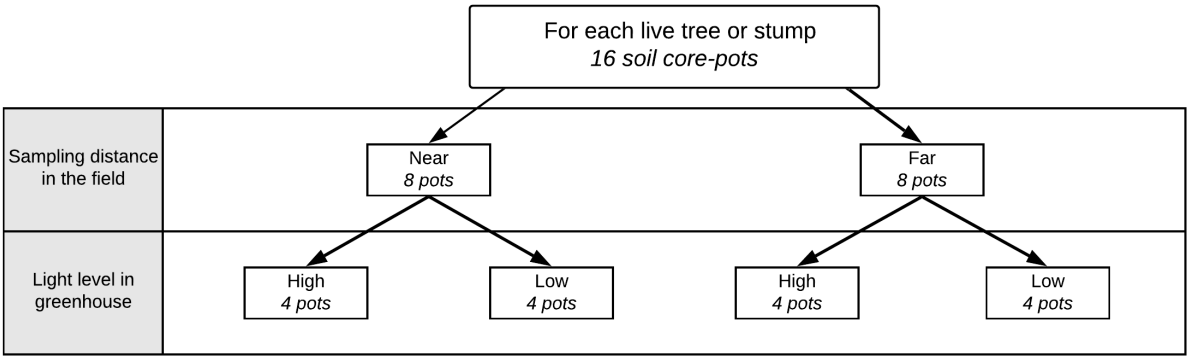

Figure 2.1 Diagram of soil sampling design and treatments applied to soil cores. Near cores were removed within 2 meters. Far cores were sampled at least 20 meters from the focal individual (live tree/stump), 5 meters from other *P. serotina* trees or stumps, and 4 meters from any trees greater than 5 cm diameter at breast height. Light levels were applied in the greenhouse using shade cloth to reduce light to 5 and 30 % full sun for low and high light, respectively

Soil Sampling Design

We collected soil cores near and far from each stump or tree (Fig. 2.1). Eight 'near' soil cores were removed within two meters of the tree or stump. Eight 'far' soil cores were collected at least 20 meters from the focal individual, 5 meters from any other *P. serotina* trees (reaching 1.37 m height) or stumps, and 4 meters from any trees greater than 5 cm DBH. Far soils represent background soil conditions beyond the influence of any large individual tree or canopy gaps.

Greenhouse Set-Up

Each soil core was converted into a pot by fixing mesh across the PVC tube's bottom. Soil within two meters of *P. serotina* trees or stumps contains roughly 80% of the tree's fine root biomass (Meinen et al. 2009), and thus has been cultured in the field by *P. serotina*. We did not condition soils as is common in many greenhouse studies of PSFs (Brinkman et al. 2010) to avoid overestimating legacy effects. We used field-cultured soils to mimic realistic conditions, even though these soils could be influenced by neighboring trees (Brinkman et al. 2010) and often have smaller effect sizes than greenhouse-trained soils (Kulmatiski et al. 2008). To reduce storage effects on soil biota, we transitioned the soil cores from the field to greenhouse within two weeks.

The soil core-pots were placed on four greenhouse benches in a randomized complete block design under two light levels (two benches each). We used shade cloth (Green-Tek®, BFG Supply Co., Burton, OH, USA) to approximate a set of field conditions under which PSF legacy effects might occur. Light was reduced to 5% or 30% full sun, approximating light in a small single tree-fall gap and a larger multiple tree gap (~1000-1200 m^2) respectively (Walters et al. 2016). While *P. serotina* is often classified as shade intolerant, as a seedling it occurs across a range of light levels (Burns and Honkala 1990). We used a total of 528 soil core-pots (33 stumps or live trees × 2 distances × 2 light levels × 4 pots (Table 2.1, Fig. 2.1)).

We weighed and planted a recently germinated *P. serotina* seed into each pot. If a seedling did not emerge within ~10 days, an additional seed with radicle was planted. If after 2–3 plantings no seedling emerged it was conservatively assumed that pre-emergence damping off had occurred. Seeds were sourced from Sheffield's Seed Co (Locke, New York, USA) and surface sterilized with 0.6% NaOCl for 10 minutes, rinsed with DI water, cold stratified in perlite

for > 120 days, washed with 0.06% NaOCl, rinsed with DI water, and then planted. We applied a selective larvicide, Gnatrol (Active Ingredient: *Bacillus thuringiensis* (37.4%); Nufarm Americas Inc., Alsip, IL), to all pots when watering to control fungus gnats, a common greenhouse pest that feed on decaying organic matter. The presence of fungus gnats should not have affected seedling mortality nor have led to *Pythium* transmission between pots. We applied Gnatrol at a rate of 1 Tbsp per 5 gallons water twice a week from the time we observed an outbreak of fungus gnats (~ 6 weeks) until the study's end. Fungus gnats are unlikely to kill seedlings and we did not observe any fungus gnat-caused mortality. However, they can wound roots thereby increasing vulnerability to infection and likely exacerbate the effects of pathogens (Graham and McNeill 1972). Vectoring *Pythium* spp. between pots by adult fungus gnats has not been reported in the literature and is unlikely because *Pythium* spp. do not produce aerial spore structures that adult fungus gnats would encounter (Braun et al. 2010). Fungus gnat larvae can vector soil-borne oomycetes, but the larval stage cannot travel between pots (Braun et al. 2012). Further, *Pythium* spp. do not survive in the gut of fungus gnats as they transition from the larval to adult stage (Braun et al. 2010).

We censused seedlings thrice weekly. At each census, we assessed emergence, survivorship, damage on live seedlings, and changes in seedling health. If seedling health was declining, we checked for and recorded symptoms associated with damping-off from soil pathogens, such as stem lesions at the soil line (Table A2.1). Each pot was watered twice weekly with 50 mL DI water. The study lasted 11.5 weeks, at which point we harvested surviving seedlings. We measured final seedling height, separated aboveground and belowground tissues, oven-dried the tissues at 65°C for at least 48 hours, and then weighed biomass.

Soil Nutrient Analyses

We measured soil base cations, inorganic soil N, and C:N ratio to assess relative differences in soil nutrient availability between live tree and stump soils within a site and determine whether there were differences in soil nutrient availability that may have been due to PSF legacies. In September 2016, we collected and combined loose soil samples (1–10 cm deep) from three equidistant points roughly 1 meter from each tree or stump included in the study. Only soils from the near sampling distance (Fig. 2.1) were analyzed because individual tree effects on soils and their potential PSF legacies were of greatest interest.

Soil samples were frozen and then air dried prior to analysis. All samples were passed through a 2 mm sieve and then finely ground using a ball mill (8000D Mixer/Mill, SPEX Sample Prep, Metuchen, NJ). Samples were analyzed via combustion to determine total C and N (vario MACRO cube Elemental Analyzer, Elementar Americas Inc., Ronkonkoma, NY). To assess plant available inorganic forms of nitrogen, 2M potassium chloride was used to extract soil nitrate and ammonium and analyzed colorimetrically using an ELx808 Absorbance Microplate Reader (BioTek Instruments, Inc, Winooski, VT). Base cations (K, Ca, Mg) and phosphate were extracted using Mehlich III solution (Mehlich 1984). Phosphate was analyzed colorimetrically with an Absorbance Microplate Reader and base cations were analyzed in AA3 AutoAnalyzer (SEAL Analytical Inc., Mequon, WI).

Statistical Analysis

Seed/Seedling Survival

Seed/seedling survival was analyzed with Cox Proportional Hazards regression models (Cox and Oakes 1984) using the R package ‘coxme’ (Therneau 2020). All surviving seedlings

were censored. At the pot level, the entire lifespan of seeds/seedlings from planting (with radicle protruding) to the study's end was used to account for pre- and post-emergence damping off.

To avoid confounding site and stump age effects in this chronosequence study, we used a nested term of soil origin (far soils, near stump soils, or near live tree soils nested within site). This nested term of soil origin limited comparisons to soils within a site (but not across sites). Within each site, we examined PSFs by comparing seed/seedling survival in far soils versus near live tree soils and PSF legacies by comparing seed/seedling survival in near live tree versus near stump soils.

We used a series of models to examine different facets of the data: (Model 1) To evaluate light level treatments, data from all pots were used, with light treatment as a fixed effect, and soil origin and greenhouse bench as random effects. (Models 2 & 3) To examine PSF legacies within each light level (Question 2), the dataset was split by light level, and analyzed separately in models with soil origin as the sole fixed effect and greenhouse bench as a random effect.

Biomass

Dry-weight biomass of harvested seedlings was analyzed in a linear model including seed mass, light treatment, and soil origin (far, near stump, or near live tree nested within site) as fixed effects and greenhouse bench as a random effect using the 'lme4' package in R (Bates et al. 2015).

Soil Nutrients

Base cations (K, Ca, Mg) were correlated and therefore treated as sum of base cations for analysis. Using the adonis in function in R's vegan package (Oksanen et al. 2019), we used a PERMANOVA with stump or live tree as a fixed effect and site as a random effect to evaluate whether soil nutrients differed at stumps and live trees.

All analyses were performed in R version 3.5.1 (R Core Team 2019).

RESULTS

Seed/Seedling Survival

Before assessing PSF legacy effects, we established the presence of PSFs based on distance effects by comparing seed/seedling survival in soils near live trees to far soils (Table 2.2). We present hazard ratios (HR), an integration of the hazard experienced by seeds/seedlings across the study's duration; HR < 1 represents decreased hazard relative to a baseline hazard. We also examined percent survival at the end of the experiment.

Negative PSFs consistently occurred at all sites only under low light (5% full sun), as demonstrated by lower seed/seedling survival in live tree soils compared to far soils (RF: HR = 0.398, LA: HR = 0.444, DW: HR = 0.549, RD: HR = 0.560; Fig. 2.2, Table 2.2). Seed/seedling survival was ~19.1 % greater in high light than low light ($p = 0.02$; Table 2.2). Under high light, negative PSFs were only present at site RD (~23.9% lower survival in live tree than far soils, HR = 0.374, $P < 0.01$) (Table 2.2, Fig. B2.1d). To evaluate PSF legacy effects over time, PSFs had to be present at multiple sites to provide stumps of varying ages; therefore, we limited our evaluation of PSF legacies to low light.

Under low light, PSF legacies were present for less than 1.5 years after the tree was removed. PSF legacies of < 1.5 years were supported by similar seed/seedling survival in soils from trees removed 0.5 years ago as for live trees (0% difference in final survival, HR = 1.068, $P = 0.86$ for survival curves from live tree versus 0.5-yr-old stump soils) and an improvement in final survival, indicating a release from negative PSF legacies, in soils from trees removed 1.5 years ago and longer (~15.8% higher; Fig. 2.2, Table 2.2). Seed/seedling survival in soils from

trees removed 1.5-yrs-ago (Site: LA) and ~ 15-yrs-ago (Site: RD) was greater than in live tree soils during the course of the study (HR = 0.502, p = 0.06 & HR = 0.512, p = 0.08 respectively). In soils from 3.5-yr-old stumps (Site: DW), seed/seedling survival did not differ from live tree soils (HR = 0.687, P = 0.33). This lack of statistical difference is likely due to the crossing of the survival curves, which obscured detection of treatment effects and violates the proportional hazards assumption of Cox survival analysis. However, greater final seed/seedling survival in soils from trees removed 1.5, 3.5, and ~15-yrs-ago in comparison to live tree soils from the same sites (12.5%, 20%, 15% respectively) supports a release from negative PSF legacies ≥ 1.5 years after tree loss.

Table 2.2. Hazard ratios from Cox proportional hazards survival model of *P. serotina* seedling survival in soils sourced from conspecific live trees and stumps, and far from *P. serotina* individuals. Seedlings were grown under two light levels in the greenhouse, 30 % full sun (middle column) and 5 % full sun (far right column). All models include a random effect for greenhouse bench. An asterisk denotes statistical significance ($p < 0.05$). Overall site effects are referenced against site RF. Full dataset model includes a random effect of far, stump, and live tree soils nested within site. Hazard ratios > 1 represent an increase in hazard for that parameter.

	Full dataset	High light subset	Low light subset
Parameter Name	*Hazard Ratio ± std. error (P-value)*	*Hazard Ratio ± std. error (P-value)*	*Hazard Ratio ± std. error (P-value)*
Light: Low vs High	1.458 ± 0.162 (0.02)*		
Overall site effect			
Site: LA		0.940 ± 0.500 (0.90)	1.990 ± 0.412 (0.095)
Site: DW		0.945 ± 0.500 (0.91)	1.346 ± 0.409 (0.47)
Site: RD		2.298 ± 0.467 (0.08)	1.897 ± 0.412 (0.12)
Far vs Live Trees (nested w/n site)-assessing PSFs			
Site: RF		0.566 ± 0.433 (0.19)	0.398 ± 0.366 (0.01)*
Site: LA		0.928 ± 0.408 (0.85)	0.444 ± 0.345 (0.02)*
Site: DW		1.082 ± 0.409 (0.85)	0.549 ± 0.347 (0.08)
Site: RD		0.374 ± 0.376 (< 0.01)*	0.560 ± 0.344 (0.09)
Stumps vs Live Trees (nested w/n site)-assessing PSF legacies			
Site: RF (0.5-yr-old stumps)		0.818 ± 0.438 (0.65)	1.068 ± 0.366 (0.86)
Site: LA (1.5-yr-old stumps)		0.929 ± 0.433 (0.86)	0.502 ± 0.365 (0.06)
Site: DW (3.5-yr-old stumps)		1.388 ± 0.434 (0.45)	0.687 ± 0.384 (0.33)
Site: RD (~15-yr-old stumps)		0.410 ± 0.405 (0.03)*	0.512 ± 0.379 (0.08)

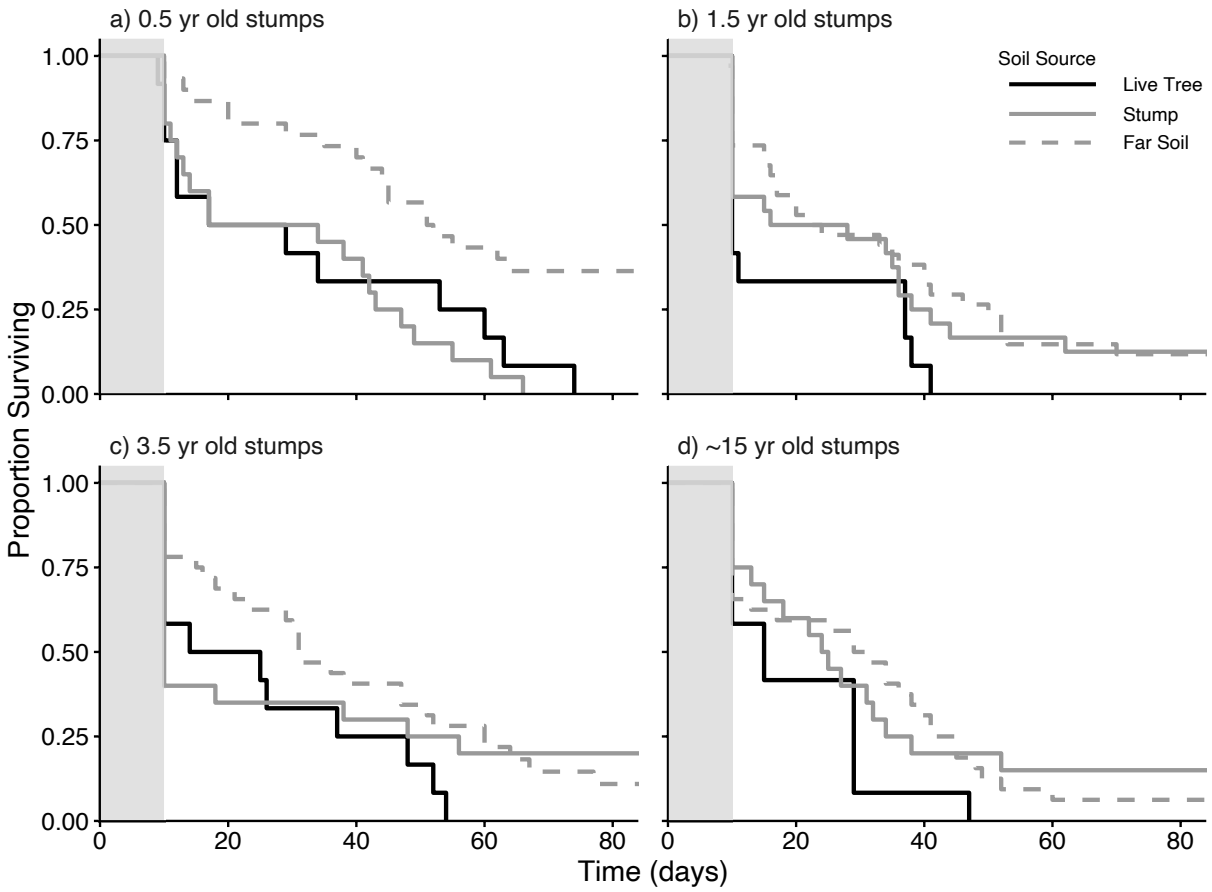

Figure 2.2 Survival curves of *Prunus serotina* seeds/seedlings in low light (5% full sun). Gray band (0-10 days) covers time between planting seed with radicle to aboveground emergence. Far soils represent background forest soil conditions without the influence of any individual tree. Comparing survival curves of far soils and live tree soils within a site evaluates PSFs, while comparing stump soil and live tree soil survival curves within a site assesses PSF legacy effects. Panels show different times since *P. serotina* tree removal: a) 0.5 yrs at Russ Forest (RF), b) 1.5 yrs at Lux Arbor (LA), c) 3.5 yrs at DeWitt (DW), and d) ~15 yrs at Rose Dell (RD).

Biomass

There were no differences in seedling dry-weight biomass between far and live tree soils, between the stump and live tree soils, or with greenhouse light level (Tables A2.2–2.3). The only influence on seedling dry-weight biomass was seed mass ($p < 0.001$).

Soil Nutrients

Soil nutrients varied minimally between stump and live tree soils within a site (Fig. B2.2). There were no consistent differences between nutrient concentrations in live tree and stump soils ($p = 0.35$; Table A2.4).

DISCUSSION

Plant-soil feedback legacies occur in soils surrounding *P. serotina* stumps but are short-lived (addressing Question 1) and restricted to low light (Question 2). Seeds/seedlings experienced negative PSF legacies in soils near 0.5-yr-old stumps similar to those near live trees at low light (Fig. 2.2, Table 2.2), but there was a marked improvement in survival (~15.8%) in soils from trees removed more than 0.5 years ago. Thus, legacies of negative PSFs, like those experienced by *P. serotina* seedlings, are not expected to last beyond the first growing season.

The shorter persistence of PSF legacies documented here simplifies the identification of mechanisms structuring communities. Longer PSF legacy persistence, after evidence of the tree initiating PSFs disappeared, would have introduced a virtually intractable source of variation into community dynamics.

The restriction of *P. serotina* PSF legacies to < 1.5 years could have important consequences for community dynamics, depending on the timing of regeneration. PSF legacies present for even a single growing season could influence community composition by

disadvantaging conspecific seedlings during the critical period of post-disturbance regeneration. We recognize that this study did not establish the performance of *P. serotina* seedlings relative to other species and thus our projections of gap dynamics are limited. However, our results do establish that *P. serotina* seedlings perform relatively poorly in the presence of a conspecific mature tree or in the soils of that tree for less than 1.5 years after tree harvest, relative to soils away from mature conspecifics.

Our results support that negative PSF legacies of *P. serotina* would occur in small, single tree gaps (~5% full sun, represented by our low light treatment in the greenhouse) but are unlikely in larger gaps with more light. Thus, *P. serotina* is less likely to regenerate in gaps formed by the loss of a single conspecific, at least for one growing season, but is released from negative PSF legacies in larger gaps. This temporary disfavoring of *P. serotina* in small gaps could create an opportunity for heterospecific replacement and thereby be a mechanism enhancing forest diversity (Bagchi et al. 2010; Crawford et al. 2019).

PSF legacy effects manifested in survival but not growth, consistent with previous studies that showed *P. serotina* PSFs with more pronounced survival than biomass responses (Packer and Clay 2000, 2004). We offer the caveat that detection of biomass responses may have been limited by sample size because only surviving seedlings could be measured (< 35% survival) (Table A2.3). Nevertheless, our data support the importance of characterizing survival responses in PSF studies, despite the prevalence of biomass as a measure of plant performance (Kulmatiski et al. 2008).

Soil biota likely drove negative conspecific PSFs and PSF legacy effects associated with *P. serotina*. Prior research has identified pathogenic oomycetes, specifically *Pythium* spp., as a mechanism for negative conspecific PSFs for *P. serotina* through various methods including soil

sterilization, isolation and inoculation with oomycetes (Packer and Clay 2003; Reinhart and Clay 2009). We have three lines of evidence that support soil biota as the mechanism for PSFs and PSF legacies here. First, we frequently observed damping off symptoms in dying seedlings, which is consistent with oomycete pathogens. Second, in a related study, we isolated *Pythium intermedium* and *Pythium irregulare* from declining seedlings with damping off symptoms and identified these oomycetes as pathogenic to *P. serotina* seedlings by completing Koch's postulates (see Chapter 3). Third, the original experiment included a (failed) soil sterilization treatment by microwaving (Ferriss 1984), which partially sterilized the soil cores (results not included); partial sterilization improved seedling survival ~21.3%, which could be due to a reduction in soil borne antagonists. However, we cannot exclude the possibility that microwaving increased soil nutrients (Troelstra et al. 2001), though microwave sterilization releases fewer nutrients than autoclaving (Ferriss 1984).

Furthermore, survival differences between stump and live tree soils are unlikely due to the soil nutrients we measured (NO_3^-, NH_4^+, PO_4^{3-}, Ca, Mg, K, C:N) because none varied consistently between stump and live tree soils (Table A2.4, Fig. B2.2). We did not test whether nutrient-based PSFs are operating (which requires a comparison of *P. serotina* live tree versus far soils) and therefore cannot exclude the possibility of long-term soil nutrient-based PSF legacies extending beyond the time frame of the study (e.g., Wardle et al. 2008).

If soil biota are responsible for the observed survival patterns, two compatible mechanisms may drive short-term legacies of *P. serotina* PSFs. First, root systems of recently harvested stumps may remain alive short-term. Root death does not instantaneously follow aboveground tree harvest and root systems slowly dying back may continue influencing soil microbes. Root lifespans could be further extended through re-sprouting, as is common for *P.*

serotina (Burns and Honkala 1990), but we minimized this by avoiding stumps with sprouts. A second potential mechanism is persistence of oomycetes as saprophytes on the tree's dying root system (Martin and Loper 1999). As saprophytes, *Pythium* spp. tend to be outcompeted (Martin and Loper 1999), which would curtail their longevity. The formation of oospores is an unlikely mechanism here, as oospore lifespans (Martin and Loper 1999) would support multi-year legacies. Short-term persistence of PSF legacies suggests that soil biota turn-over rapidly and seedlings respond to soil biotic communities that are largely shaped by the species of roots actively growing, not prior occupants.

The restriction to low light of negative conspecific PSFs and their legacies could arise from irradiance effects on both pathogens and seedlings. Cool and moist soil conditions favor *Pythium* growth (Martin and Loper 1999 and references therein). Under low light, seedlings often experience more negative PSFs (McCarthy-Neumann and Ibáñez 2013) and may be more susceptible to pathogen attack due to lower non-structural carbohydrates (Myers and Kitajima 2007; Kobe et al. 2010) and defensive compounds, such as phenolics (Entry et al. 1991; Ichihara and Yamaji 2009). Thus, poor low-light survivorship of *P. serotina* seedlings could arise from an interaction between seedling carbon limitation and pathogen pressure.

We observed frequent pre- and post-emergence damping-off in the greenhouse, which contributed to *P. serotina* PSFs and their legacies, consistent with numerous other studies supporting the importance of early life history stages in shaping forest communities (e.g., Kobe and Vriesendorp 2011). Our study suggests that mortality of germinated seeds before they emerge from the soil is also an important filter (Gallery et al. 2010; Bagchi et al. 2014). *Pythium* is a likely driver of both seed and seedling survival patterns (Martin and Loper 1999). Studying

seedlings only above a height threshold (e.g., Johnson et al. 2012, 2017) excludes important mortality processes in early seedling life.

While PSF legacies of *P. serotina* persist short-term, they may affect succession in forest gaps by lasting through a critical window for regeneration and impede conspecific recruitment in small/single-tree gaps. For *P. serotina*, negative PSF legacies make conspecific seedlings unlikely to recruit into single-tree gaps in the first growing season after disturbance; during this time, a flush of regeneration of other species could fill much of the growing space (Walters et al. 2016) and preempt further *P. serotina* seedlings from establishing. Thus, *P. serotina* is most likely to recruit in larger gaps, which introduce more light to the seedling layer and release seedlings from negative PSFs and their legacies.

Seed dormancy and dispersal might be key adaptations for escaping PSF legacy effects. Most *P. serotina* seed falls to the ground near parent trees (Hough 1960), leading to high densities of seedlings near conspecific trees experiencing negative PSFs (Packer and Clay 2003). By remaining dormant in the seed bank for 3-5 years (Marquis 1975), *P. serotina* seeds could escape PSF legacies in time and germinate in more favorable conditions for survival. Long-distance dispersal of *P. serotina* seeds (Hough 1960) also allows seeds/seedlings to avoid both negative PSFs and, preemptively, their legacy effects. Dispersal would be particularly important for species with long-lived PSF legacies as the legacy effect could exceed the duration of seed dormancy.

Caveats

Sudden tree death due to harvesting differs from naturally occurring senescence (Pedersen 1998) but could be similar to tree death by wind or other acute disturbances. A harvest system was advantageous experimentally to examine PSF legacy effects and create a

chronosequence because harvest creates a discrete timepoint of aboveground tree death. Healthy roots that remain after tree harvest would be expected to promote the persistence of PSF legacies more so than slow coordinated senescence above- and below- ground. Thus, even though our study system favored the detection of longer-lived PSF legacies, we found short-lived effects which suggests that rapid turn-over of soil biota occurs and depends upon the species of roots present.

Our findings are based on a single species, *P. serotina*. We chose *P. serotina* for its strong negative PSFs (Bennett et al. 2017), which we expected to have a more persistent legacy. Other tree species and causal agents of PSFs could be associated with longer lasting PSF legacy effects and more expansive impacts on community dynamics. For example, mycorrhizal fungi could manifest legacy effects by persisting as spores (Nguyen et al. 2012) and on the roots of neighboring plants. Abiotic-PSFs, driven by individual plant effects on soil nutrients (Waring et al. 2015), could leave a signature of tree harvests for 40 years (Wardle et al. 2008). Further research on PSF legacies could focus on additional components of PSFs and encompass conspecific and heterospecific PSF legacies to better resolve their importance in plant community dynamics.

Conclusions

PSF legacies of *P. serotina* are short-lived and restricted to low light. Though potential mechanisms of PSF legacies, such as soil biota or nutrients, can persist long-term after tree harvest, *P. serotina* seedlings were released from negative PSF legacy effects shortly (< 1.5 years) after conspecific adult trees were lost. In single tree-fall gaps, negative PSF legacies could affect succession by hampering *P. serotina* regeneration during the critical one year period

immediately after disturbance. Thus, despite being short-lived, *P. serotina* PSF legacies could have important impacts on conspecific regeneration, especially in smaller gaps.

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CHAPTER 3

Oomycetes associated with *Prunus serotina*

persist in soil after tree harvest

ABSTRACT

Soil-borne pathogens can shape forest communities by lowering seedling survivorship. Many soil pathogens can persist long-term as survival spores, but how long pathogens outlive tree hosts in gap soils and whether they continue to affect seedling survival is uncertain. We studied the presence of oomycetes and evaluated seedling performance in soils near live *Prunus serotina* trees, and 0.5 and 1.5-year-old stumps. We isolated five species of oomycetes from soils, two of which were pathogenic (*Pythium intermedium* and *Pythium irregulare*) to *Prunus serotina*. There was a non-significant ~10.5% increase in conspecific seedling survival in stumps versus live trees and pathogens were present in soils of all stump ages. The continued presence of pathogens of *Prunus serotina* in gap soils demonstrates the potential for impacts on conspecific regeneration after tree death, though the slight improvement in survival suggests that these effects may weaken with time.

INTRODUCTION

Pathogens can structure forest communities by causing seedling mortality or hampering growth of early seedling life stages (Bagchi et al. 2010; Mangan et al. 2010; Liu et al. 2015). Shared antagonists between trees and conspecific seedlings can lead to higher seedling mortality near conspecific adults and thereby maintain tree species diversity in forests (Janzen 1970; Connell 1971; Packer and Clay 2000; Comita et al. 2014). When a tree is harvested or falls, a

canopy gap is created, and the outcome of typically negative pathogen-seedling interactions could shift because of the loss of the tree host and altered light environment. Gap-forming disturbance is a pervasive part of temperate forest dynamics necessary for regeneration of many species, where the increase in resources creates opportunities for new individuals to reach the canopy (Runkle 1982; Gray and Spies 1996).

Regeneration dynamics unfolding in gaps could be shaped by pathogens, which can persist long-term via dormant survival spores in the soil (Agrios 2005). Pathogen loads can be diminished in gaps three years after disturbance (Reinhart et al. 2010a) and near dead trees and stumps of unknown age (Gómez-Aparicio et al. 2012), potentially allowing susceptible species to establish. Improved survival of *Prunus serotina* Ehrh (black cherry) seedlings 1.5 years after conspecific tree harvest (see Chapter 2) suggests that pathogenic oomycetes, which typically reduce *Pr. serotina* seedling survival near live trees (Packer and Clay 2000; Reinhart and Clay 2009), have relatively short-term effects after tree death. Diminished pathogen loads in gaps and improved seedling survivorship after the death of mature trees suggest the possibility that the presence of soil-borne oomycetes decreases after the death of the mature tree host.

Altered light levels and soil moisture in gaps influence pathogen-seedling interactions in the understory via synergistic effects on both pathogens and seedlings. The influx of light created by tree loss might allow seedlings to escape damage by aiding in the development of plant defenses (Roberts and Paul 2006; Ichihara and Yamaji 2009), which are especially important in early seedling stages (Augspurger 1990; Boege and Marquis 2005). In the first growing season after tree loss, greater soil moisture in gaps could favor oomycetes, although drier and warmer conditions in subsequent seasons could disfavor them (Erwin and Ribero 1996; Ritter et al. 2005). Though the response of tree seedlings to light has been well studied (Kobe 1999; Coates

2000; Seiwa 2007), shifts in pathogen presence following tree death may also influence which tree species successfully recruit after disturbance.

Oomycetes could persist in gap soils through multiple mechanisms (Martin and Loper 1999). Oomycetes survive long-term by producing thick-walled and pigmented "survival spores" such as oospores and sometimes chlamydospores (Erwin and Ribero 1996). Oospores remain viable for years (Martin and Loper 1999); in one instance, oospores of *Pythium ultimum* Trow were viable for 12 years (Hoppe 1966 as cited in Martin and Loper 1999). *Pythium* spp. can also persist for shorter time periods as saprophytes by colonizing dead and dying root systems, although as saprophytes *Pythium* spp. are often outcompeted (Hendrix and Campbell 1973). Another mechanism of oomycete persistence is through the continued survival of a stump's root system, providing a live food source for pathogens. In forests, this legacy of oomycetes in gap soils or the hidden signature of prior trees could negatively impact seedling regeneration.

To better understand the persistence of soil pathogens in the altered environment of gaps, we examined the presence of oomycetes in and how seedlings respond to soils in gaps, created through selective harvests of *Pr. serotina*. We used tree harvests as a model of sudden tree death, which also enabled establishment of a defined time of tree death. *Pr. serotina* is a temperate tree native to eastern North America, parts of Mexico, and Central America (Auclair and Cottam 1971). As a shade intolerant species, gap-forming disturbances are essential for *Pr. serotina* regeneration. Oomycetes, specifically *Pythium* spp., can cause substantial mortality of *Pr. serotina* seedlings near live conspecific trees, making *Pr. serotina* seedlings more likely to survive early life stages when distant from conspecific adults (Packer and Clay 2000; Reinhart and Clay 2009). Using soils collected around live *Pr. serotina* trees and stumps aged 0.5 and 1.5 years old, we asked two questions: (Q1) how does *Pr. serotina* seedling performance (survival

and growth) differ with time since tree death? (Q2) how does presence of oomycete pathogens shift with time since tree death?

METHODS

Sampling of Field Sites

We sampled soil from two stands with similar overstory composition in southwest Michigan (MI), USA: Russ Forest (RF) in Decatur and Rose Dell Woodlot (RD) in Albion. We selected *Pr. serotina* trees in spring 2016 and 2017, harvested a subset, and sampled soil in fall 2017 from live trees and 0.5-year-old stumps at both sites and 1.5-year-old stumps at site RF (Table 3.1). We used single-tree harvests to minimize variation in microclimate conditions between soils in gaps (near stumps) and near trees while allowing for selection of stumps and live trees within the same stand. Focal stumps and live trees were more than 30 meters distant from any other focal individual.

Table 3.1 *Prunus serotina* trees and stumps where soil was sampled.

Site	Samples	N
RD	live trees	7
	0.5 yr. old stumps	5
RF	live trees	8
	0.5 yr. old stumps	5
	1.5 yr. old stumps	5

Three loose soil samples (for oomycete baiting) and fourteen intact soil cores (for greenhouse experiment) were collected around live *Pr. serotina* trees and stumps of two ages (0.5 and 1.5 yrs. since harvest) between August 14–30, 2017. Loose soil samples (0–10 cm depth) were removed from three 1-meter equidistant points with a spade (surface disinfected with 70% ethanol between samples) around each stump or tree and then stored at 4°C for up to

three weeks. Intact soil cores were collected within 2 meters of each tree or stump in 7.6 × 25.4 cm PVC (polyvinyl chloride) tubes by pushing them into the ground with a tamper and removing them with a shovel. Prior to use in the field, the PVC tubes were disinfected by soaking in 0.5 % NaOCl for thirty minutes and then rinsing with water.

Q1-Seedling Performance

Greenhouse Study

Soil cores were transitioned from the field to greenhouse within two weeks of collection. We collected intact soil cores to minimize soil disturbance, compared to acquiring loose soil samples and blending with another medium. Each field-collected soil core was converted into a pot by fixing mesh across its bottom. We used a total of 420 soil core-pots (30 stumps or live trees × 14 pots) placed on two greenhouse benches in a randomized complete block design. In early September 2017, recently germinated *Pr. serotina* seeds were weighed and planted. Seeds were sourced from Sheffield's Seed Co (Locke, New York, USA) and cleaned with 0.6% NaOCl for 10 minutes prior to stratification in perlite for 120 days and soaked again in 0.06% NaOCl prior to planting.

The seedlings were grown under two layers of shade cloth (Green-Tek®, BFG Supply Co., Burton, OH, USA) to reduce light levels to ~5% full sun which approximates the lowest amount of light expected in a single tree-fall gap. Though often classified as shade intolerant, as a seedling *Pr. serotina* occurs across a range of light levels (Burns and Honkala 1990).

If no seedling emerged ~10 days after planting, pots were re-planted with an additional seed with radicle; if no seedling emerged after 2-3 plantings, we assumed pre-emergence damping off occurred in that pot. To control a common greenhouse pest, fungus gnats, we applied a selective larvicide, Gnatrol (Active Ingredient: *Bacillus thuringiensis* (37.4%); Nufarm

Americas Inc., Alsip, IL), when watering. We watered each pot twice a week with 50 mL deionized water. Seedlings were censused thrice weekly for emergence, survivorship, and seedling health. Symptoms, such as lesions near the soil line, were used to attribute declining seedling health to damping-off from soil pathogens. After 13 weeks, we harvested surviving seedlings, separated above- and belowground tissues, and dried the seedlings in an oven at 65°C for at least 48 hours before weighing.

We also compared soil nutrient availability between live tree and stump soils within our two sites to determine whether nutrient variation may have influenced seedling survival. We measured soil base cations (Ca, K, Mg, PO_4^{3-}), inorganic soil N (NO_3^-, NH_4^+), and C:N ratio. For these analyses we collected three additional loose soil samples (1-10 cm deep) in August 2017 from three equidistant points roughly 1 meter from each tree or stump and air-dried the samples.

Each sample was sieved (2mm) and finely ground using a ball mill (8000D Mixer/Mill, SPEX Sample Prep, Metuchen, NJ). Total C and N was determined via combustion (vario MACRO cube Elemental Analyzer, Elementar Americas Inc., Ronkonkoma, NY). We used 2M potassium chloride to extract soil nitrate and ammonium, which are plant available inorganic forms of nitrogen, and analyzed the extractions colorimetrically using an ELx808 Absorbance Microplate Reader (BioTek Instruments, Inc, Winooski, VT). Mehlich III solution was used to extract base cations (K, Ca, Mg) and phosphate (Mehlich 1984). An Absorbance Microplate Reader was used to for colorimetric assessment of phosphate. Base cations were analyzed with an AA3 AutoAnalyzer (SEAL Analytical Inc., Mequon, WI).

Q2-Oomycete Presence

To examine the presence of *Pythium* and *Phytophthora* in soils from *Pr. serotina* trees and stumps we used a multi-faceted approach. We first baited directly from loose field soils

collected around each tree or stump to establish the distribution of oomycete isolates. Concurrently with the greenhouse study examining seedling survival, we sampled pairs of symptomatic and asymptomatic seedlings grown in the same soil source in the greenhouse to examine associations between seedling health and oomycete presence. To determine pathogenicity of our three most common isolates, we conducted a pathogenicity trial.

Isolation and identification of oomycetes

Direct baiting from field soil

Pythium spp. and *Phytophthora* spp. were isolated from loose field soils by baiting using a technique modified from Erwin and Ribeiro (1996). Baiting from soil is the selective acquisition of potential pathogens on susceptible plant material suspended in soil samples flooded with water (Erwin and Ribero 1996). We baited from all soils within 5 weeks of field sampling. One hundred and fifty grams of each soil sample and 400 ml of deionized distilled water were placed in a small plastic container with lid. To target a variety of oomycetes, three types of bait material were floated on the water in each container.

The three types of bait material used were: *Agrostis stolonifera* L. (creeping bentgrass), *Secale cereale* L. (rye), and *Pieris japonica* cv. *'Brouwer's Beauty'* (Thunb.) D. Don ex G. Don (Japanese Pieris). *A. stolonifera* is effective at detecting *Pythium* in hydroponic systems (Watanabe et al. 2008), as is rye (https://plantpath.psu.edu/pythium). *Pi. japonica* has been used to detect *Phytophthora* spp. from the rhizosphere of hardwood and softwood trees showing root rot symptoms (C. M. Medina-Mora, unpublished work). When culturing from the bait materials, *A. stolonifera* was placed only on plates of PARB because in an earlier trial we found no hyphae growing from *A. stolonifera* blades on PARBhy.

Once water-soaked lesions were observed on bait plant tissue (after ~ 4–7 days), sections from the leading edge of lesions were plated onto two types of culture media: PARB (modified from PARP+B in Oudemans (1999) by using 15g/L Bacto™ Malt Extract (BD Biosciences) and 20g/L Bacto™ Agar (BD Biosciences) in place of corn meal agar and without pentachloronitrobenzene) and PARBhy (modified as above from Oudemans (1999) with the addition of 50 mg/L hymexazol). These two culture media were used to maximize detection of both *Pythium* and *Phytophthora*; hymexazol inhibits the growth of most *Pythium*, allowing *Phytophthora* to be detected on PARBhy. On PARB medium both *Pythium* and *Phytophthora* will grow, but *Pythium* grows faster, making *Phytophthora* detection difficult. After 1–7 days, sections of hyphae growing out of the bait leaf samples embedded on PARB and PARBhy media were transferred to Petri dishes (100mm x 15mm) of two other types of culture media: carrot agar (Brasier 1967; Erwin and Ribero 1996) and V8 agar (modified from Miller (1955); Erwin and Ribeiro (1996) by adding 16 g/L Bacto-Agar instead of 15 g/L and amended with 10 mg/L rifampicin and 250 mg/L ampicillin).

After approximately 7 days, when hyphal growth covered the entire dish, cultures grown on carrot medium were sorted into groups with similar morphologies based on typical characteristics of *Pythium* and *Phytophthora* (e.g., formation of rosette-like patterns and occurrence of aerial hyphae). Representative isolates from each morphological group were sub-cultured until pure cultures were obtained and stored on carrot agar slants overlaid with sterile mineral oil at room temperature.

Symptomatic and asymptomatic seedling baiting from greenhouse

To isolate potentially pathogenic oomycetes, we sampled pairs (same soil source) of symptomatic and asymptomatic seedlings grown in soil cores in the greenhouse study described previously.

Once seedlings began damping off (rapid drooping of leaves often accompanied by a dark lesion at the soil line extending through the width of seedling's stem), pairs of symptomatic and asymptomatic seedlings grown in soils from the same tree or stump were selected for baiting. Twenty-one pairs of seedlings were removed, which is a subset of the soils sourced from all trees and stumps present in the greenhouse study (N=30, Table 3.1). No more than one pair of seedlings was selected from each tree or stump soil source and our selection includes all combinations of site, live tree, and stump age (RD, live trees (N=6), 0.5-year-old stumps (N=4); RF, live trees (N=4), 0.5-year-old stumps (N=4), 1.5-year-old stumps (N=3) where N refers to a pair of seedlings). Baiting and isolation of pure cultures were conducted as described previously except that 30-50 g of soil plus the seedling root and 150 ml of deionized distilled water were used for baiting because the volume of soil the seedlings interacted with in the soil cores was smaller than the loose soil samples from the field. Thus, loose field soils (3 samples, 150 g soil each) were more thoroughly sampled for oomycetes than the greenhouse soil cores (1 sample, 30–50 g soil). The 3 loose field soil samples were removed from 3 different points around the base of a live tree or stump, which could have captured some of the variation in the soil community across space. In contrast, an individual soil core was removed from a smaller area (7.6 cm diameter) and spatial variation in pathogen populations (Burdon and Thrall 1999; Martin and Loper 1999; Reinhart and Clay 2009) could lead to varying amounts of inoculum depending

upon the soil core. Cultures from the soil cores were sorted into the previously established morphological groups.

Molecular Identification

A single isolate from each morphological group was grown on carrot agar overlaid with a disc of sterile cellophane for roughly 1 week at room temperature (approx. 20ºC); the mycelia was harvested and placed in 2.0 ml sterile microcentrifuge tubes. We amplified part of the internal transcribed spacer (ITS) region using the ITS4 (White et al. 1990) and ITS6F primers (Cooke et al. 2000). Using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA), DNA was extracted according to the manufacturer's protocol, except that 2% CTAB buffer (Hamelin et al. 2000) was used as the extraction solution.

Following DNA extraction, Polymerase Chain Reaction (PCR) was run on an ABI 2720 thermo cycler (Applied Biosystems, Foster City, CA, USA). The PCR reaction mixture contained: 5 µL of 5X GoTaq ® Flexi colorless buffer (Promega Corp., Madison, WI, USA), 4 µL of Mg^{2+}(25 mM) (Promega), 0.5 µL of dNTP (100 mM) (Invitrogen, Carlsbad, CA, USA), 1.0 µL of each primer (10 mM), 0.13µL of GoTaq ® Flexi (Promega; 5U μL^{-1}), DNA (50 ng μL^{-1}), and PCR grade water to reach a final volume of 25 µL. Thermal cycling conditions for the PCR reactions were: initial denaturation at 95˚C for 2 min, then 35 cycles of 95˚C for 20 s, 55˚C for 25 s and 72˚C for 50 s, with a final extension of 72˚C for 10 min and a holding temperature at 4˚C. Agarose gel (0.75%) electrophoresis was used to visualize PCR products.

To purify PCR products, the Qiagen PCR purification kit (Qiagen, Valencia, CA, USA) was used following manufacturer's suggestions. The PCR products were then sequenced by the MSU-Research Technology Support Facility (Michigan State University, https://rtsf.natsci.msu.edu/genomics/sequencing-services/sanger/) using an ABI 3730xl platform

(Applied Biosystems, Foster City, CA, USA) or by Psomagen Inc. USA (https://psomagen.com) using Next Generation Sequencing technology (NGS; Psomagen, Rockville, MD, USA). We aligned forward and reverse sequences of each isolate and manually edited using Geneious Pro (ver. 10.2.3) created by Biomatters (available from http://www.geneious.com/). Isolate identity was determined by sequence similarity from Blast searches in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) by matching sequences to specimens with ≥ 98% similarity. GenBank accession numbers are listed in Table 3.4.

Pathogenicity Trial

To test which oomycete isolates acquired from our baiting experiments cause disease, we conducted two pathogenicity trials in a greenhouse in June and November 2018, consisting of 10 and 8 replicates, respectively, per oomycete species, plus an uninoculated control. In both trials, we planted recently germinated *Pr. serotina* seeds with two true leaves in 4 in. pots with a blend of 50% sterile sand and 50% potting mix (Sunshine® Mix #8, SunGro®, Agawam, MA, USA). *Pr. serotina* seeds were surface disinfected (0.6% NaOCl for 10 minutes) prior to cold stratification for 120 days and briefly rinsed with 0.06% NaOCl prior to planting to reduce contamination. Seeds for the June 2018 trial were collected from one of the study sites, Rose Dell Woodlot in Albion, MI and were sourced from Louisiana Forest Seed Company (Lecompte, LA, USA) for the November 2018 trial. We used the three species of oomycetes most commonly isolated by baiting as inoculum. Generation of zoospores for inoculum was attempted by floating agar discs with mycelia in a 1.5% soil extract solution (modified from Jeffers and Aldwinckle 1987 by sterilizing), but could not be induced for all species; *Py. irregulare* and *Py. intermedium* both rarely produce zoospores in culture (van der Plaats-Niterink 1981). Thus, we produced oospores for inoculum by sub-culturing each species onto carrot agar and growing for 7 days. To

estimate oospore formation for each species, we removed an agar disc from each culture and estimated oospore counts by tallying oospores within 3 randomly selected squares of a gridded coverslip under 100x magnification. After estimating the number of oospores produced by each species, enough agar discs were collected and used to inoculate each seedling with a concentration of ~1×10^4 oospores per pot.

At the time of planting, the potting media was watered until saturated and carrot agar discs containing oospores were incorporated into the upper layer of potting media. Each pot contained a single seedling and was watered three times a week. Replicate pots within a treatment group were maintained on a single tray in the greenhouse to prevent cross contamination between pots inoculated with different oomycetes. When a seedling began to show signs of damping off, its roots and surrounding soil were used in the baiting procedure described previously to re-isolate the pathogen. During the November 2018 trial, baiting was conducted only with blades of *A. stolonifera* and *S. cereale*; no *Pi. Japonica* leaves were available. Isolates were then compared to representative cultures of each species to determine their identities and complete Koch's postulates.

Statistical Analyses

All analyses were performed in R version 3.5.1 (R Core Team 2019).

Survival

Seedling survival in the greenhouse study was analyzed using a Cox Proportional Hazards model (Cox and Oakes 1984) with the 'survival' package in R (Therneau and Grambsch 2000). Harvest age (live tree, 0.5-year-old stump, or 1.5-year-old stump) was nested within site (RD or RF) and used as the sole fixed effect in the survival model.

Biomass

Total dry-weight biomass of surviving seedlings was analyzed in a linear model with seed mass and harvest age nested within site as fixed effects.

Distribution of Oomycetes

To examine how the presence of oomycetes relates to harvest age, presence/absence data of oomycetes acquired via the two baiting experiments (direct field soil baiting and greenhouse seedling baiting) were combined and then analyzed with a separate model for each oomycete species using logistic regression. Data from both baiting experiments were combined because species occurrence trends were similar. Presence/absence data from the three loose field soil samples from each live tree or stump were combined (N=30). In each model, harvest age nested within site, and study (direct field soil baiting vs. greenhouse seedling baiting) were used as fixed effects.

Associations with Seedling Health in the Greenhouse

To assess how seedling damping off related to oomycete incidence, presence/absence data from the greenhouse grown seedlings in soil cores were analyzed with a logistic regression model using the 'lme4' package in R (Bates et al. 2015). Seedling status (symptomatic vs asymptomatic) was the response variable and presence of the three common oomycetes were fixed effects with seedling pair as a random effect.

Soil Nutrients

We used the sum of base cations (K, Ca, Mg) for analysis because their concentrations were correlated. Using a PERMANOVA with the 'pairwise.adonis2' function in the 'vegan' package in R (Martinez Arbizu 2020), we assessed whether soil nutrients differed based on

harvest age. This model included site as a random effect with harvest age (live tree, 0.5-year-old stump, or 1.5-year-old stump) as fixed effect.

Pathogenicity Trial

Seedling survival from the two pathogenicity trials was analyzed in a mixed effects logistic regression, using the 'lme4' package in R (Bates et al. 2015), where each oomycete inoculum was a fixed effect and trial was a random effect. To determine whether seedling survival varied between the two pathogenicity trials, the data were pooled and analyzed in a logistic regression using trial as a fixed effect. The two trials did not differ significantly from each other ($p = 0.09$). Means were compared using Tukey's HSD using the 'emmeans' package in R (Lenth 2020).

Oomycete Detection by Bait Material

To examine whether each bait type led to differing oomycete detection, counts of isolates from each bait were combined based on tree or stump soil source from both baiting studies (direct field soil baiting and greenhouse seedling baiting). Each species of oomycete was analyzed using a zero-inflated model with a type 1 negative binomial distribution, using the 'glmmTMB' package in R (Brooks et al. 2017), where the count of isolates for that species was the response, bait material was a fixed effect, and harvest age (live tree, 0.5-year-old stump, or 1.5-year-old stumps) nested within site was a random effect. One species (*Py. salpingophorum/conidiophorum*) was detected only once and could not be modelled. For another species (*Py. intermedium*) the model would not converge with a nested random effect, therefore only harvest age was used as a random effect. Baits were then compared to each other with a post-hoc test using Tukey's HSD using the 'emmeans' package in R (Lenth 2020).

RESULTS

Q1-Seedling Performance

Survival

At both sites (RD & RF), final seed/seedling survival tended to be higher, although not statistically different, in soils from stumps than live trees regardless of stump age (Table 3.2, Fig. 3.1). In evaluating survival, we present hazard ratios (HR) which integrate the hazard seeds/seedlings experience across the study's duration with HR < 1 representing a decreased hazard of mortality relative to a baseline hazard. At site RD, seed/seedling survival in soils from stumps aged 0.5 years old was not different from live tree soils (HR = 0.864, p = 0.50). At site RF, seed/seedling survival in soils from trees removed both 0.5 and 1.5 years ago was not different from live tree soils based on hazard ratios (HR = 0.676, p = 0.11 & HR = 0.750, p = 0.24 respectively). To look at general patterns, we compared all stump soils (combined across sites and ages) versus all live tree soils (combined across sites); this was justified because survival curves of sites nor stump age varied. Final survival is consistently higher (10.5%) in stump soils than live tree soils across both sites (HR = 0.77, p = 0.08), which could be biologically meaningful.

Table 3.2 Hazard ratios from a Cox proportional hazards model evaluating seedling survival in soils from varying harvest ages (live trees, 0.5-yr-old stumps, and 1.5-yr-old stumps). Hazard ratios < 1 represent a decrease in mortality hazard for that parameter.

Parameter Name	Hazard Ratio ± Std. Error (P-value)
Site: RF vs RD	0.733 ± 0.194 (0.11)
Site: RD (0.5-yr-old stumps)	0.864 ± 0.217 (0.50)
Site: RF (0.5-yr-old stumps)	0.676 ± 0.243 (0.11)
Site: RF (1.5-yr-old stumps)	0.750 ± 0.243 (0.24)

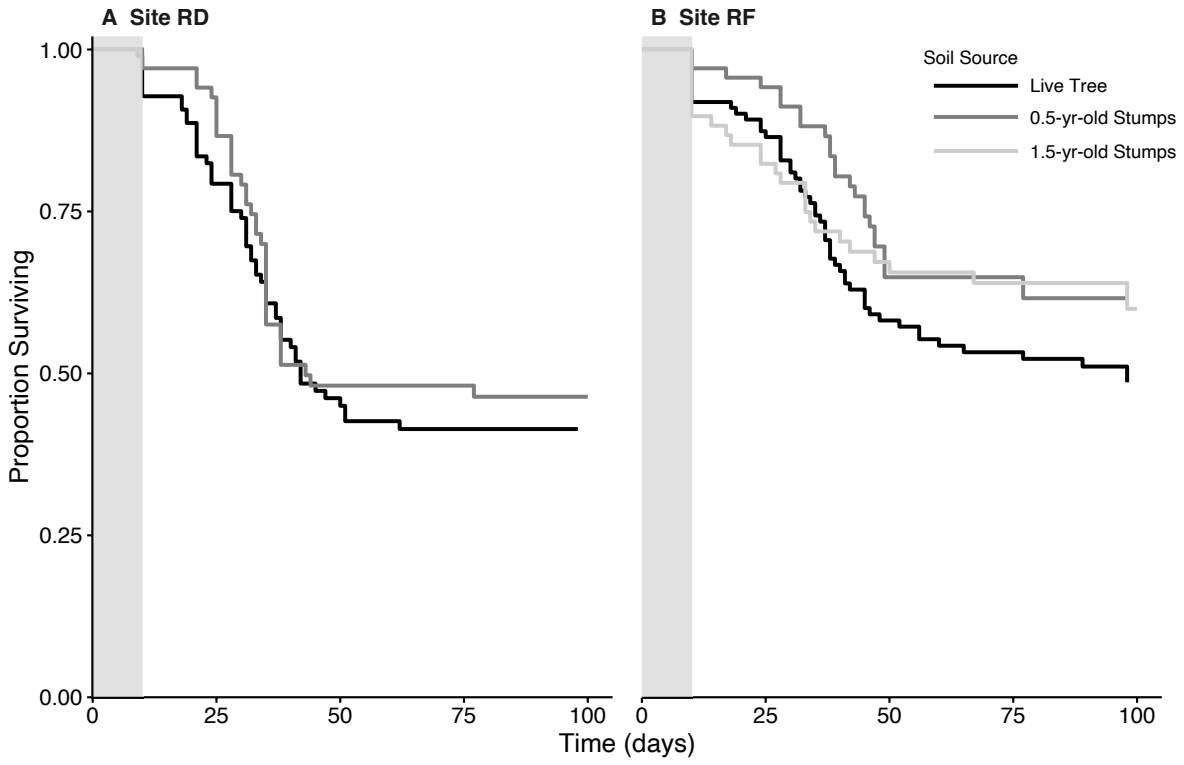

Figure 3.1 Survival curves of *P. serotina* seeds/seedlings grown in a greenhouse at 5% full sun. Gray band (0-10 days) covers time between planting seed with radicle to aboveground emergence. Panels show different sites with soils collected from varying stump ages compared against live tree soils: a) Rose Dell (RD) with 0.5-yr-old stumps, b) Russ Forest (RF) with 0.5 and 1.5-yr-old stumps.

Biomass

Seedlings grown in soils from 0.5-year-old stumps were larger than seedlings in live tree soils ($p = 0.05$) at site RF, but not different at site RD ($p = 0.88$) (Table 3.3 & A3.1). Biomass of seedlings in 1.5-year-old stump soils was not different from those in live tree soils at site RF ($p = 0.15$). Seedlings derived from seeds of greater mass grew larger ($p = 0.024$).

Table 3.3 Estimates from a linear model of oven-dried seedling biomass (g) of surviving seedlings. An asterisk denotes statistical significance.

	Estimate ± Std. Error (p-value)
Site: RF	0.004 ± 0.010 (0.70)
Seed Mass (g)	0.090 ± 0.039 * (0.024)
Stumps vs Live Trees (nested w/n site)-assessing PSF legacies	
Site: RD (0.5-yr-old stumps)	-0.002 ± 0.012 (0.88)
Site: RF (0.5-yr-old stumps)	0.020 ± 0.010 * (0.05)
Site: RF (1.5-yr-old stumps)	0.015 ± 0.010 (0.15)

Soil Nutrients

Soil nutrient availability (sum of base cations, phosphate, C:N ratio, and total inorganic N (ammonium + nitrate)) did not differ significantly between live trees and 1.5-year-old stump or 0.5-year-old stump soils ($p = 0.09$ and $p = 0.19$ respectively). However, soil nutrients did differ between the two stump ages at site RF ($p = 0.03$; Table A3.2), driven by slightly higher inorganic soil N and lower concentrations of base cations in 1.5-year-old stump soils (Fig. B3.1). Differences in soil nutrients are not associated with seedling survival and thus are not considered further.

Q2-Oomycete Presence

Identity of Oomycetes

Two hundred and ninety-eight isolates were obtained via direct baiting from the loose field soil samples (Table 3.4 & A3.6). At least one isolate came from each stump or live tree, except for two live trees at Russ Forest where no isolates were acquired. On average, there were roughly 10 isolates per stump or live tree. From seedlings grown in the greenhouse, one hundred and forty-eight isolates were obtained from twenty-one pairs of symptomatic and asymptomatic

seedlings grown in soil cores (Table A3.4 & A3.7). On average, 3.5 isolates were found per soil core.

Isolates from both baiting procedures were sorted into thirty morphological groups or were assigned a species designation once initial sequencing work was complete. Five species (*Pythium irregulare* Buisman, *Pythium intermedium* de Bary, *Phytopythium vexans* (de Bary) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque, *Phytophthora citricola* Saw., *Pythium salpingophorum*/*conidiophorum* Drechsler/Jokl) were identified based on 98% sequence similarity match in a blast search (Table 3.4). Three species, *Pythium irregulare*, *Pythium intermedium*, *Phytopythium vexans* (hereafter *Py. irregulare, Py. intermedium, Pp. vexans*), accounted for 10.1%, 14.1% and 71.8% of isolations from direct baiting field soils and 8.8%, 9.5% and 73.0% of isolations from symptomatic seedlings grown in the greenhouse.

Table 3.4 Oomycete species identified in this study by baiting from soil samples collected from the rhizosphere of *Prunus serotina* stumps and live trees (N=30) located in Russ Forest (RF) and Rose Dell Woodlot (RD) in Michigan, USA. Species presence and absence are noted by + and – respectively.

Species	**GenBank Accession No.**	**Percent Sequence Match**	**Matching GenBank Accession No.**	**Locations Observed**				
				Site: RF			Site: RD	
				live trees	0.5 yr-old stumps	1.5 yr-old stumps	live trees	0.5 yr-old stumps
Pythium irregulare	MT647271	99%	AY598702.2	+	+	+	+	+
Pythium intermedium	MT647270	99%	KU211482.1	+	+	+	+	+
Phytopythium vexans (group 1)	MT647272	99%	HQ643400.2	+	+	+	+	+
Phytopythium vexans (group 2)	MT647273	98%	HQ643400.2	+	+	–	+	+
Phytophthora citricola I[1]	MT647267	99%	FJ665234.1	+	+	–	–	–
Phytophthora citricola I/III[1]	MT647268	100%	FJ665234.1/ FJ392327.1	+	+	+	+	–
Pythium salpingophorum/ conidiophorum	MT647269	98%	AY598629.2/ AY598630.2	–	–	+	–	–

1) Subgroups based on Jung and Burgess, 2009

Distribution of Oomycetes

Presence of the three dominant oomycetes (*Py. irregulare, Py. intermedium, Pp. vexans*) did not vary with time since harvest (live tree, 0.5-year-old and 1.5-year-old stumps) (Table 3.4, Fig. 3.2). These oomycetes were present at both sites when directly baiting from field soil and baiting from symptomatic and asymptomatic greenhouse-grown seedlings in soil cores; we combined data from both baiting procedures for analysis. One isolate (*Py. intermedium*) occurred more frequently when baiting from field soils than greenhouse seedlings (OR = 9.25, p = 0.0003; Table A3.3).

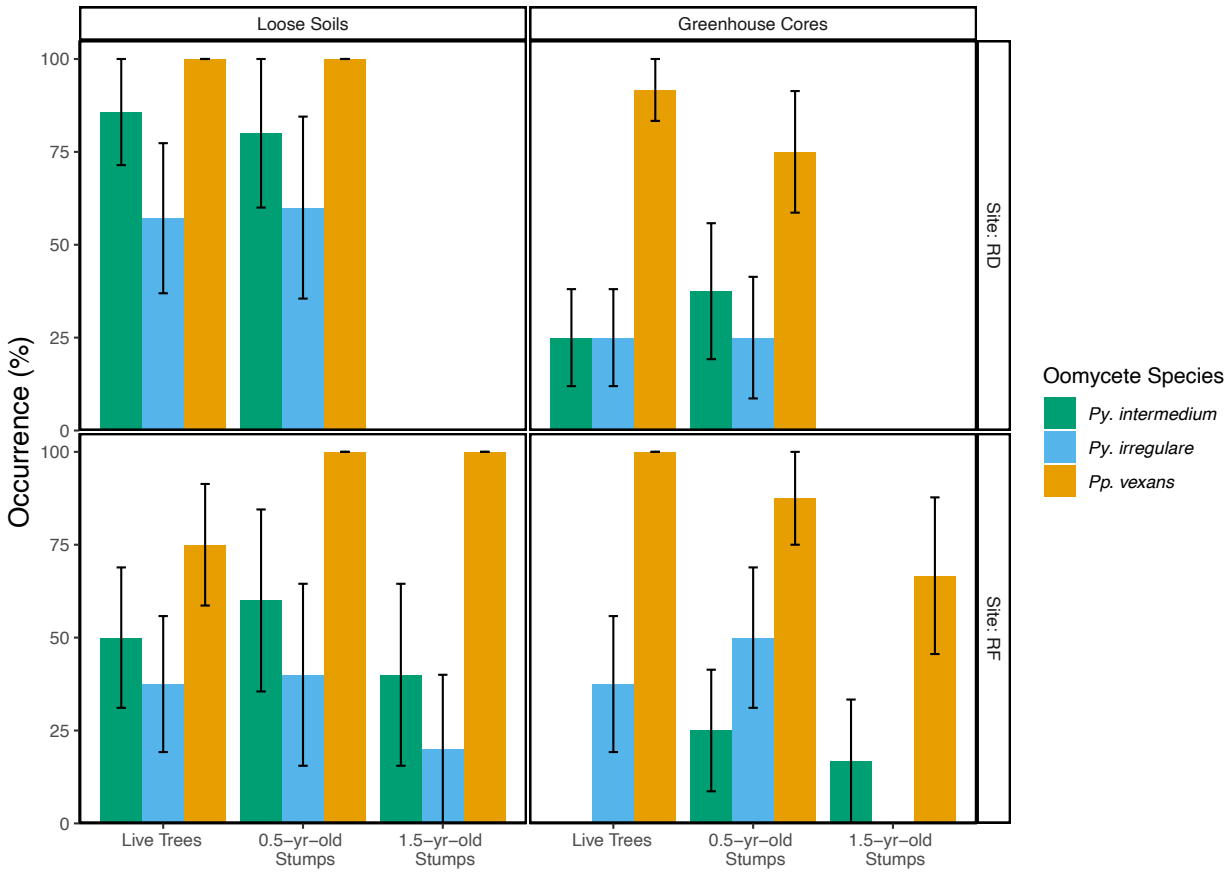

Figure 3.2 Percent of samples in which each of the three most common oomycetes occurred in loose soils and intact soil cores used in greenhouse collected from two sites (Rose Dell Woodlot (RD) and Russ Forest (RF)). No 1.5-year-old stumps were sampled at site RD. Error bars depict the standard error of the mean. *Py.* = *Pythium*, *Pp.* = *Phytopythium*.

Associations with Seedling Health in the Greenhouse

Seedling health status in the greenhouse also was not correlated with the presence of any of the oomycetes (Fig. 3.3). The odds of a seedling being symptomatic or asymptomatic were equivalent in the presence of *Py. irregulare* (OR = 1.36, p = 0.66), *Py. intermedium* (OR = 0.67, p = 0.70), or *Pp. vexans* (OR = 0.15, p = 0.11). *Py. irregulare* was not detected in any of the 1.5-year-old stump soils in the greenhouse and *Py. intermedium* was detected in a single sample of 1.5-year-old stump soil with a symptomatic seedling.

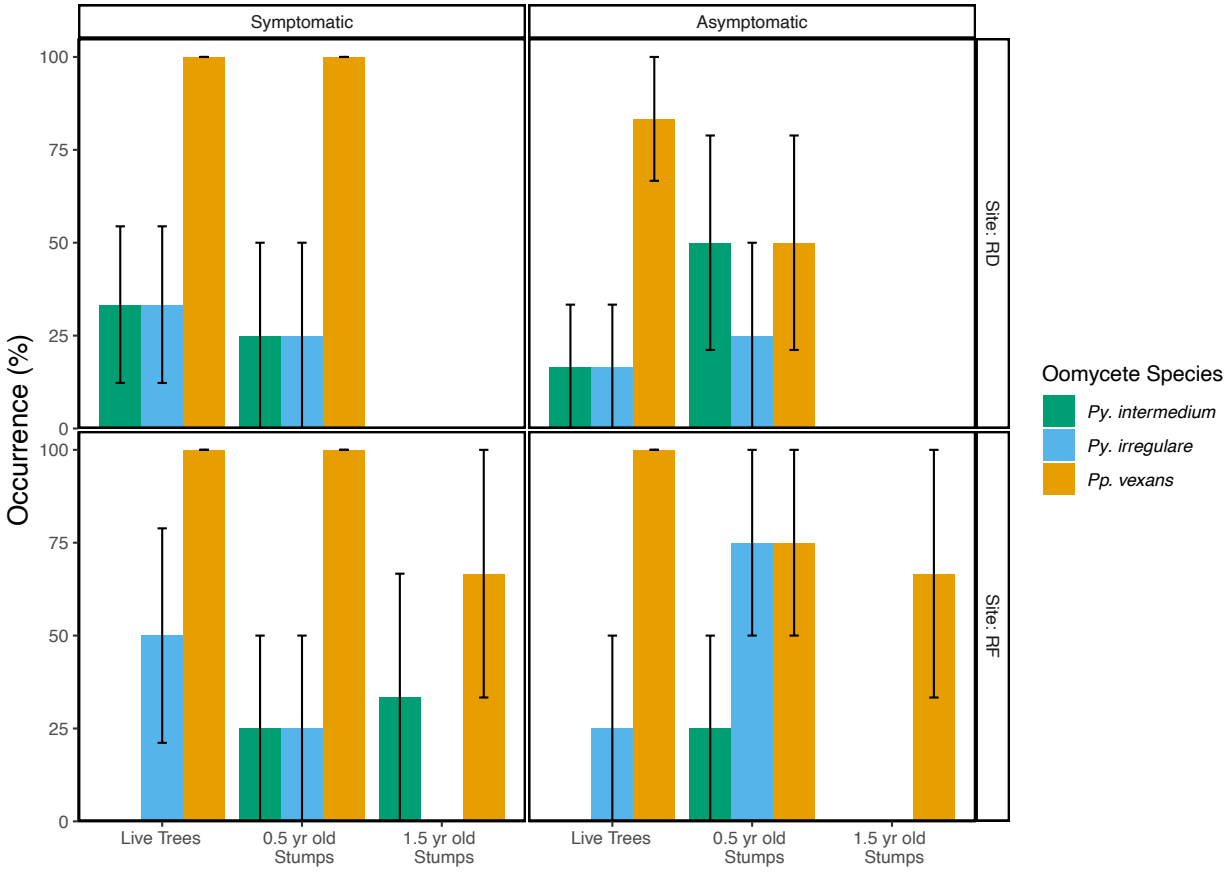

Figure 3.3 Percent of samples where each of the three most common oomycetes occurred in soils of greenhouse-grown symptomatic and asymptomatic seedlings. Seedlings were grown in the greenhouse and sampled in pairs (with and without disease symptoms) from the same soil source in soil cores collected from two sites (Rose Dell Woodlot (RD) and Russ Forest (RF)). No 1.5-year-old stumps were sampled at site RD. Error bars depict the standard error of the mean. *Py. = Pythium*, *Pp. = Phytopythium*.

Oomycete Detection by Bait Material

The three bait materials used (leaves from *A. stolonifera*, *S. cereale*, *Pi. Japonica*) each varied their ability to bait oomycetes (Fig. B3.2, Table A3.5). *S. cereale* leaves were colonized by members of all three genera detected in this study, *Pythium*, *Phytopythium*, and *Phytophthora*. *Pi. japonica*, a common bait for *Phytophthora*, was also effective for baiting *Pythium* spp. based upon its fairly frequent colonization by *Py. intermedium* and *Py. irregulare*. *A. stolonifera* was frequently colonized by *Py. intermedium* and *Py. irregulare*. *Pp. vexans* colonized leaves from all 3 plant species, which is reflected in the dominance of *Pp. vexans* among our isolated cultures and demonstrates the generalist tendencies of *Pp. vexans*. *Pp. vexans* was detected less frequently by *A. stolonifera* than *Pi. japonica* (OR = 0.53, p = 0.004) and *S. cereale* (OR = 0.44, p = 0.0001). *Py. irregulare* was detected more frequently by *S. cereale* than *Pi. japonica* (OR = 0.16, p = 0.005). *Py. intermedium* was detected equally well by *A. stolonifera* and *S. cereale* (OR = 1.41, p = 0.52), and significantly less frequently by *Pi. Japonica* than the other two bait materials. *Ph. citricola* was detected more frequently by *Pi. Japonica* than *S. cereale* (OR = 6.96, p = 0.02) and never detected by *A. stolonifera* because *A. stolonifera* was only plated onto media that inhibits *Phytophthora* growth (see methods). The single isolate of *Pythium salpingophorum*/*conidiophorum* was acquired using *S. cereale* as bait material.

Pathogenicity Trial

Within one to two weeks of inoculation, *Pr. serotina* seedlings began damping-off (sudden drooping of leaves accompanied by a lesion at the soil line) when inoculated with *Py. intermedium* or *Py. irregulare*. Root necrosis was also observed on these seedlings. These damping-off symptoms are consistent with those observed in *Pr. serotina* seedlings in the field and other greenhouse studies (Packer and Clay 2000; Reinhart and Clay 2009).

Seedling survival varied significantly with oomycete species (Fig. 3.4). *Py. intermedium* and *Py. irregulare* exhibited the greatest virulence leading to 78.8% and 94.5% mortality of inoculated seedlings, respectively. Survival of seedlings inoculated with *Py. intermedium* and *Py. irregulare* was significantly different from the control ($p = 0.003$ for *Py. intermedium*; $p < 0.001$ for *Py. irregulare*), but not from each other ($p = 0.526$). Survival of *Pp. vexans* inoculated seedlings did not differ significantly from the control ($p = 1.00$).

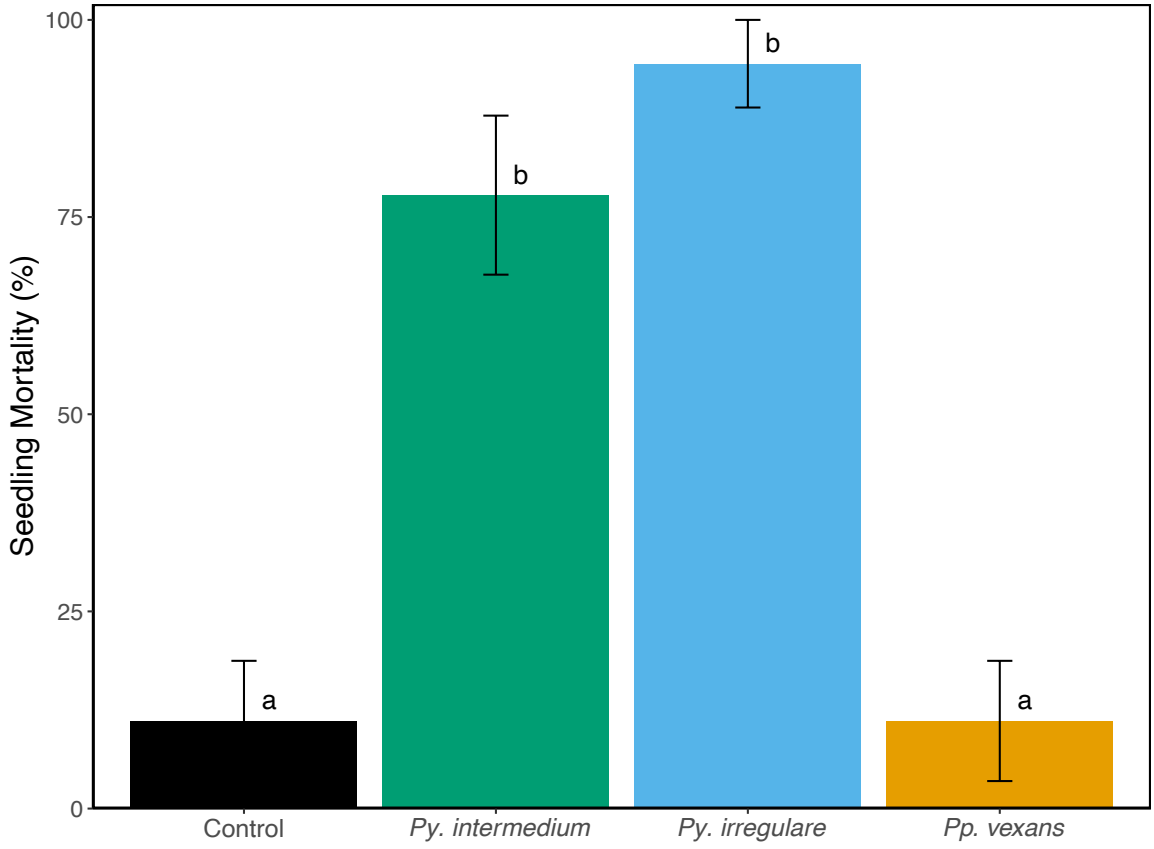

Figure 3.4 Percent mortality of *Prunus serotina* seedlings from two pathogenicity trials (June and November 2018). Seedlings were planted in potting media once two true leaves had emerged and grown under shade cloth at 5% full sun in a greenhouse. Error bars depict the standard error of the mean and different letters near the error bars signify statistical differences ($p < 0.05$). *Py.*= *Pythium*, *Pp. = Phytopythium*, *Ph. = Phytophthora*.

DISCUSSION

Seedling performance, in terms of survival, did not differ significantly between *Pr. serotina* trees and stumps aged 0.5 and 1.5 years old (answering Q1). This finding differs from chapter 2 of this dissertation, where seedling survival improved 1.5 years after harvest; a trend of higher seedling survival in stump soils of the present study is consistent overall with chapter 2. Of the 5 oomycete species we isolated from *Pr. serotina* tree and stump soils (Table 3.4), the three most abundant species (*Py. irregulare, Py. intermedium, Pp. vexans*) were well distributed in soils from both study sites near live conspecific trees and both stump ages (Fig. 3.2, Table 3.4 & A3.3). Two species, *Py. intermedium* and *Py. irregulare*, were confirmed as pathogens of *Pr. serotina* seedlings by completing Koch's postulates. We found both pathogens of *Pr. serotina* present in gap soils 0.5 and 1.5 years following tree death (answering Q2).

Continuous pathogen presence after tree death creates the potential for impacts on seedlings regenerating in gaps. Pathogens of *Pr. serotina* (*Py. intermedium* and *Py. irregulare*) were present in soils from both stump ages (Fig. 3.2). Nevertheless, we observed a modest and nonsignificant (~10.5%) increase in seedling survival in stump soils across both sites, consistent with the results of Gómez-Aparicio et al. (2012) of lower pathogen densities in the absence of a live host. Our study evaluated only pathogen presence, not density; additional studies, over longer time periods using molecular methods (e.g., Spies et al. 2011b) would enable examination of pathogen density over time since tree loss.

The effects of soil pathogens on seedling performance may lessen with time since tree death based on biomass and survival trends. Biomass responses were variable but suggest a slight improvement in growth with time since harvest. At site RF, seedlings in 0.5 and 1.5 year-old stump soils were larger than in live tree soils, though only significantly larger in 0.5 year old

soils (Table 3.3). These modest effects on biomass are consistent with other studies of *Pr. serotina* interactions with oomycetes that showed stronger survival than biomass responses (Packer and Clay 2000, 2004). A 10.5% survival increase in both stump soil ages relative to live tree soils at both sites also suggests weakening effects of pathogens on seedlings after tree death (Fig. 3.1). In contrast, in a prior study, seedling survival was significantly greater in soils of 1.5-year-old stumps and older (see Chapter 2). While the present study and Chapter 2 of this dissertation suggest that seedling survival will improve with time since tree death, they differ in the timing of release from pathogen damage. Future studies could test the factors underlying variable persistence of oomycete effects on seedlings following tree death.

Factors other than pathogen presence likely affect *Pr. serotina* seedling mortality in the field, which may explain why seedling health was not correlated with the presence of *Py. intermedium* or *Py. irregulare* for seedlings grown in soil cores in the greenhouse (Table A3.4 & A3.7, Fig. 3.3). Even though the pathogenicity trial demonstrated the ability of *Py. intermedium* and *Py. irregulare* to cause mortality in *Pr. serotina* when pots were inundated with inoculum ($\sim 1 \times 10^4$ oospores per pot), the soil cores in which seedlings were grown (in the greenhouse) are more similar to field conditions because they contain a community of soil biota. The soil community in the cores could contain seedling mutualists that reduce the effects of pathogens (Borowicz 2001) or antagonists that suppress pathogens (Thrane et al. 2000). Variation in pathogen density, not simply pathogen presence alone, could also determine whether disease symptoms occur (Fraedrich et al. 1989). Additionally, seedling genetic variation could cause variation in their susceptibility to pathogens (Hammond-Kosack and Jones 1997).

Environmental conditions such as light level could also affect whether disease symptoms manifest (Martin and Loper 1999 and citations therein). All seedlings in this study were grown

under shade cloth at 5% full sun. This light level mimics the amount of light in small/single-tree forest gaps and reflects the conditions in the field where the soil cores were collected. Relatively low light could lead to negative carbon balance for seedlings (Myers and Kitajima 2007) and reduced production of defensive compounds, such as phenolics (Ichihara and Yamaji 2009), which may leave seedlings more susceptible to pathogen damage.

Many of the oomycetes we isolated are well distributed pathogens found commonly in soils and on plants, suggesting they are generalists. Four of the species (*Py. intermedium*, *Py. irregulare*, *Ph. citricola*, *Pp. vexans*) isolated have a wide geographic range and a variety of known hosts including woody species (van der Plaats-Niterink 1981; Jung and Burgess 2009). *Py. irregulare* is often a pathogen of seedlings (van der Plaats-Niterink 1981).

Our study establishes *Py. irregulare* as a pathogen of *Pr. serotina* seedlings and supports the prior finding of *Py. intermedium* pathogenicity (Reinhart et al. 2010b). *Py. intermedium* and *Pp. vexans* have been detected in prior studies on roots of *Pr. serotina* seedlings (Packer and Clay 2004; Reinhart et al. 2010b); of these two species, only *Py. intermedium* was previously evaluated in a pathogenicity trial and found to be pathogenic to *Pr. serotina* seedlings (Reinhart et al. 2010b). The identity of our fifth species *Py. salpingophorum/conidiophorum* could not be resolved based on our sequence data, which was equally similar (98% similarity) to specimens of both species. *Py. salpingophorum* and *Py. conidiophorum* are sister species (Lévesque and de Cock 2004); both are uncommon, but have been isolated from plants and soil and can cause damping-off (van der Plaats-Niterink 1981; Li et al. 2014).

Pp. vexans was the most common species isolated, yet despite a slight tendency to isolate *Pp. vexans* more frequently from symptomatic than asymptomatic seedlings in the greenhouse ($p = 0.11$; Table A3.4), we could not induce seedling mortality when inoculating seedlings with *Pp.*

vexans in the pathogenicity trial. *Pp. vexans* could be co-infecting seedlings along with the two pathogenic species (*Py. intermedium* and *Py. irregulare*) and creating an additive effect that does not manifest with inoculation by *Pp. vexans* alone, as is the case when *Abies fraseri* (Pursh) Poir. is inoculated with *Pp. vexans* and *Ph. citricola* (Shin, K, Medina-Mora, CM and Sakalidis ML, unpublished work). Alternatively, *Pp. vexans* may not be pathogenic and is simply ubiquitous in this system.

Pp. vexans also could be a species complex that requires further phylogenetic resolution (de Cock et al. 2015). We found two genetically different isolates of *Pp. vexans* which may vary in pathogenicity. *Pp. vexans* is associated with numerous root-disease systems, often in orchards, (Spies et al. 2011b, a; Tao et al. 2011; Benfradj et al. 2017; Polat et al. 2017), but does not consistently act as a pathogen and different isolates may vary in their ability to cause disease (Rodríguez-Padrón et al. 2018). At the time of our pathogenicity trials, we were unaware of the two genetically different *Pp. vexans* isolates among our isolates and therefore tested a single *Pp. vexans* isolate. Future pathogenicity trials could test these possibilities by using both *Pp. vexans* isolates and dual inoculation with other oomycetes.

After *Pr. serotina* trees are harvested, pathogenic *Pythium* spp. present in the soil could opportunistically colonize regenerating seedlings, particularly in suitable environments such as high soil moisture (Martin and Loper 1999). Our work shows that in small gaps (5% full sun or less) within 1.5 years after tree death soil pathogens are still present (Fig. 3.2), and this could have consequences for the community of seedlings that regenerate. *Pr. serotina* seedlings may experience significantly improved survival near stumps 1.5 years after harvest (see Chapter 2) or later, as the current study suggests. In either case, the persistence of pathogenic oomycetes in gap soils could be an important filter limiting *Pr. serotina* regeneration because in the first growing

season after tree harvest the understory of forest gaps often fills with a flush of vegetation newly released from light limitation (Walters et al. 2016).

In other systems where species susceptible to pathogenic oomycetes are expected to naturally regenerate or are introduced as seeds or young plants (such as in restoration), delayed planting to avoid persistent soil pathogens could be beneficial. In some coniferous forests the persistence of fungal inocula (*Heterobasidion annosum* (Fr.) Bref., *Armillaria ostoyae* (Romagn.) Herink, *Phellinus sulphurascens* Pilát) has led managers to remove stumps to reduce inoculum loads (Cleary et al. 2013). In our study system where seedlings are primarily affected, altering environmental conditions to strongly favor seedling survival, such as increasing light, or planting older seedlings could also avoid detrimental effects of residual pathogens.

In summary, we found two pathogens of *Pr. serotina*, *Py. intermedium* and *Py. irregulare*, present in soils surrounding live conspecific trees and stumps aged up to 1.5 years old. Their persistence after *Pr. serotina* tree death creates the potential for pathogens to continue impacting conspecific seedlings after tree death. Increased seedling biomass and the modest (but not statistically significant) improvement in *Pr. serotina* seedling survival in stump soils relative to live tree soils suggests that the effects of soil pathogens may weaken following tree death though the time frame is unclear. Taken together, pathogen persistence demonstrates the need to consider legacies of prior species on soil pathogen presence in forest gaps, though the impacts of this hidden signature of prior trees on seedling performance may mitigate over time.

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CHAPTER 4

Acer rubrum plant-soil feedbacks are positive and their legacies persistent

ABSTRACT

Mature trees can affect tree seedling performance in their vicinity through plant-soil feedbacks (PSFs). How PSFs manifest depends on both the tree species influencing the soil and seedling species responding. Legacies of conspecific PSFs, present as soil signatures after tree death or harvest, affect seedlings of a single species, *Prunus serotina*, in the crucial period of early (< 1.5 yr) post-disturbance regeneration. Given that both PSFs and PSF legacies can impact species distinctly, understanding their effects across multiple species and soil sources could be critical for refining expectations of post-disturbance regeneration. In a greenhouse experiment, we grew seedlings of *Acer rubrum* in conspecific and five heterospecific soils collected around live trees and stumps of varying ages. We evaluated PSFs as the difference between seedling performance in each species soil source and PSF legacies as the difference in stump and live tree soils within each soil source. We found positive conspecific PSFs, likely driven by beneficial effects of arbuscular mycorrhizae, and limited variation among heterospecific PSFs. We also detected multiyear (> 8 yrs) conspecific PSF legacies but were unable to resolve legacies of heterospecific PSFs. Positive conspecific PSFs and longer-lasting legacies for *A. rubrum* could reinforce the presence of *A. rubrum* in forests. The present study of *A. rubrum* supports near-decadal persistence of PSF legacy effects, which could have important consequences for forest regeneration dynamics, providing motivation for examining PSF legacies in a broader range of tree species.

INTRODUCTION

Mature trees influence nearby tree seedlings through a myriad of processes, including plant-soil feedbacks (PSFs), which in turn can influence forest succession and species composition. PSFs occur as plants modify soil biota and/or abiotic factors in ways that influence the growth and survival of co-occurring or subsequent plants (Bever et al. 1997). Importantly, mature tree effects on soils can shape tree seedling performance via PSFs and thereby determine community composition (Mangan et al. 2010; Bennett et al. 2017; Reinhart et al. 2021). Oftentimes, seedlings have poor performance under conspecific canopies due to attack by soil pathogens (Packer and Clay 2000; Bagchi et al. 2010; Liang et al. 2016). PSFs enhance forest diversity when conspecific seedlings are disfavored relative to heterospecifics near mature trees (Bever 2003; Bagchi et al. 2010; Crawford et al. 2019) and are dependent on both the tree affecting the soil and seedling species responding (Kiers et al. 2000; Gustafson and Casper 2006; Bever et al. 2012; Hersh et al. 2012).

Numerous biotic and abiotic mechanisms can contribute to PSFs, which may persist as soil signatures after tree death and extend PSFs as PSF legacies (see Chapter 2). Biotic soil components of PSFs such as bacteria, mycorrhizal fungi, and soil pathogens can enter dormancy and persist for years as spores or cysts (Martin and Loper 1999; Packer and Clay 2000; Adl and Gupta 2006; Kardol et al. 2007; Raaijmakers et al. 2009; Nguyen et al. 2012; Bennett et al. 2017). Alterations to soil nutrients associated with individual trees can remain for decades near stumps and persist through land use transition (Finzi et al. 1998a, b; Døckersmith et al. 1999; Wardle et al. 2008). These residual soil effects following tree death create the potential for PSF legacies to affect seedling regeneration. Here we evaluate PSF legacies as soil signatures present months to years after tree death, as opposed to PSFs occurring when the tree initiating the

feedback is alive. Following tree death, PSF legacies are expected to fade over time, manifesting as weakening effects on tree seedling performance relative to PSFs of live trees (see Chapter 2).

PSF legacies in forests have received limited attention, but understanding their effects could be critical when developing expectations for regeneration following tree harvest or other disturbance. A single-species study of PSF legacies demonstrated that negative PSF legacies of *Prunus serotina* Ehrh. diminish conspecific seedling survival < 1.5 years after harvest in low light (5% full sun) (see Chapter 2). Though short-lived in this case, PSF legacies influencing initial regeneration dynamics can have long-lasting effects on community composition by affecting seedling success during the critical period of post-disturbance regeneration. Thus, elucidating PSF legacy effects, particularly across multiple species soil sources, could inform the trajectory of seedling regeneration in both the context of natural disturbance and post-harvest. Quick dissipation of PSF legacies could create new opportunities for seedling recruitment (where PSFs are negative) or reduce relatively advantageous conditions (where PSFs are positive).

To examine PSFs and PSF legacies in multiple soil sources, we grew *Acer rubrum* L. (red maple) seedlings in soils from stumps and live trees of conspecific and five co-occurring heterospecific species in Michigan hardwood forests. We focused on *A. rubrum* because it is a well distributed species across forests of eastern North America (Burns and Honkala 1990). *A. rubrum* seedlings are usually disadvantaged in conspecific soils relative to heterospecific soils (negative conspecific PSF) (McCarthy-Neumann and Kobe 2010; McCarthy-Neumann and Ibáñez 2012; Bennett et al. 2017) or do not perform differently based on soil source (neutral PSF) (Nijjer et al. 2007; Reinhart et al. 2012; McCarthy-Neumann and Ibáñez 2013). Though PSFs vary depending upon the species involved (Kiers et al. 2000; Gustafson and Casper 2006; Bever et al. 2012; Hersh et al. 2012), responses to heterospecific soils may be similar; *A. rubrum*

seedlings had higher mass in both *A. saccharum* Marshall (sugar maple) and *Quercus rubra* L. (northern red oak) than conspecific soils (McCarthy-Neumann and Kobe 2010). Higher recruitment of *A. rubrum* far from conspecifics would align with expectations of the Janzen-Connell hypothesis and be reflected in patterns of negative conspecific density dependence (Janzen 1970; Connell 1971; Comita et al. 2014). *A. rubrum* abundance has risen in many deciduous forests of eastern North America during the past century (Lorimer 1984; Abrams 1998; Fei and Steiner 2007), which provides motivation for understanding PSFs and PSF legacies of *A. rubrum*. We hypothesized: (1) *A. rubrum* seedlings will have higher survival and growth (experience more positive PSFs) in heterospecific soils relative to *A. rubrum* soils. (2) *A. rubrum* seedling survivorship or growth will not differ among heterospecific soil sources derived from different tree species. To examine PSF legacies after tree death or harvest, we further hypothesized that: (3a) over time, PSF legacies in conspecific soils will weaken relative to PSFs in conspecific soils and (3b) PSF legacies in heterospecific soils will be weaker relative to heterospecific PSFs. Discerning both PSFs and PSF legacies effects in conspecific and heterospecific soils could elucidate how persistent soil signatures (i.e., the 'ghosts of trees past') may shape seedling regeneration.

METHODS

We studied *A. rubrum* seedling growth and survivorship in soils of six species from Michigan hardwood forests: *A. rubrum*, *A. saccharum*, *Prunus serotina* Ehrh. (black cherry), *Betula alleghaniensis* Britton (yellow birch), *Q. rubra*, and *Tilia americana* L. (basswood). To form a chronosequence of time since tree death, we selected live trees and stumps of each species at sites in the Northern Lower and Eastern Upper Peninsulas of Michigan (USA) utilizing

harvest records to establish time of aboveground tree death. We used single tree selection harvests, rather than larger cuts, to mimic spatial patterns of individual mortality events common in these forests. From 11 total sites, we sampled soils from stumps and live trees at 4 sites for each species, each corresponding to a distinct harvest time falling into 4 bins: < 2 years, 2–4 years, 5–6.5 years, > 7 years since harvest (hereafter referred to as harvest times) (Table A4.1). All study species typically co-occurred in the overstory of our sites in varying abundance. We maximized the number of study species sampled at each site, but it was difficult to find conditions where all species were harvested. All sites had soils ranging from sandy to loamy sand (Web Soil Survey 2020) and similar moisture and fertility regimes ranging from mesic to dry-mesic and medium to medium-poor nutrient content based on understory indicator species using a site quality index (Burger and Kotar 2003).

We selected six stumps and three live trees for soil sampling for each species per harvest time (Table A4.1). Stumps were identified by distinguishing bark and/or wood characteristics. Stumps with sprouts were generally avoided though moderate sprouting occurred infrequently in the youngest (< 2 yr since harvest) stumps. We sampled soil from live trees of each species to establish a site-specific baseline for that species' PSF and from stumps forming a chronosequence to evaluate PSF legacies. Thus, we selected 36 focal trees or stumps per species ((6 stumps + 3 live trees) × 4 harvest times) and a total of 216 focal trees or stumps (6 study species × 36 individuals/species).

We collected soils in August 2018 within 2 meters of each individual, where ~80% of the fine root biomass occurs (Meinen et al. 2009). We sampled loose soil (1–10cm depth) from 4–6 points around the bole of each tree or stump using a shovel (disinfected with 70% ethanol between individuals to prevent cross contamination). Field soil from each individual was

thoroughly homogenized by hand. To minimize potential nutrient differences between field soils and provide a more hospitable environment for seedling growth than field soil alone we used a 1:1:2 blend of field soil, autoclaved-field soil, and autoclaved potting mix (Sunshine® Mix #8, SunGro®, Agawam, MA, USA)). Autoclaved soils were treated at 121°C for 4 hours. We measured soil pH of each field soil using an electrode (MP 220, Mettler Toledo, Columbus, Ohio, USA).

Within 2–3 weeks of collection, field soils were transitioned into greenhouse pots (D40 Deepots (6.8 cm diameter x 25.4 cm deep), Stuewe and Sons, Inc. Tangent, Oregon, USA). We did not condition soils in the greenhouse by growing seedlings in them as is common in many PSF studies (Brinkman et al. 2010) to avoid altering legacy effects in stump soils. In using field-cultured soils, we aimed to mimic realistic conditions, though these soils often produce smaller effects than greenhouse-trained soils (Kulmatiski et al. 2008) and are potentially influenced by neighboring trees (Brinkman et al. 2010).

In September 2018 we planted an *A. rubrum* seed with radicle into each pot. For each *A. rubrum* soil sample (from an individual stump or live tree) 5 pots were planted (5 pots × 36 individuals = 180 pots). For each heterospecific species 72 pots were filled (2 pots × 36 individuals), with a total of 360 heterospecific pots from the other five study species (5 heterospecific species × 72 pots). Pots were placed on greenhouse benches in a complete randomized design. Prior to planting, *A. rubrum* seeds (Sheffield's Seed Co, Locke, New York, USA) were treated for 10 minutes with 0.6% NaOCl, rinsed with deionized water, cold stratified in perlite for < 30 days, then washed with 0.06% NaOCl, and rinsed with deionized water directly prior to planting. Light was reduced using shade cloth (Green-Tek®, BFG Supply Co., Burton, OH, USA) to 5% full sun, which is similar to conditions where soils were collected.

We monitored seedling survival three times a week for twenty weeks by assessing emergence, survivorship, and changes in seedling health. Each pot was watered with ~50 mL of deionized water every 4–5 days. When watering we also applied a selective larvicide, Gnatrol (Active Ingredient: *Bacillus thuringiensis* (37.4%); Nufarm Americas Inc., Alsip, IL), to control a common greenhouse pest, fungus gnats. At the study's end we harvested all surviving seedlings, separated above- and belowground tissues, and oven-dried them at 65°C for at least 48 hours prior to weighing.

Statistical Analyses

All analyses were performed in R version 3.6.2 (R Core Team 2019).

We analyzed seedling survival using a mixed effects proportional hazards survival model using the R package 'coxme' (Therneau 2020). In this model, soil source (soil collected from different tree species) and status as tree vs. stump were fixed effects and site, greenhouse bench, and individual tree or stump were random effects. Hypothesis 1 is evaluated in this model by comparing seedling survival responses in *A. rubrum* soils vs heterospecific soils and hypothesis 2 is assessed by comparing between heterospecific soils using Tukey's HSD and the 'emmeans' package in R (Lenth 2020).

To estimate the duration of conspecific PSF legacies (hypothesis 3a) we used an additional mixed effects proportional hazards survival model with only *A. rubrum* soils, tree vs. stump nested within site as a fixed effect, and bench and individual live tree or stump as random effects. To compare PSF legacies and PSFs in heterospecific soils (hypothesis 3b) we nested status as tree vs. stump within soil source as a fixed effect and used site, greenhouse bench, and individual tree or stump as random effects in a mixed effects proportional hazards survival model with only heterospecific soils.

Total dry-weight biomass of surviving seedlings was analyzed in a linear model with soil source as a fixed effect, and site and individual live tree or stump as random effects using the 'lme4' package in R (Bates et al. 2015); root mass fraction was analyzed in a model with the same structure.

Soil pH was analyzed in a linear model with soil source and tree vs. stump as fixed effects, and site as a random effect using the 'lme4' package in R (Bates et al. 2015). All pairwise comparisons were made between species soil sources using Tukey's HSD and the 'emmeans' package in R (Lenth 2020).

RESULTS

PSFs

In contrast to hypothesis 1 which predicted higher survival and growth in heterospecific soils*, A. rubrum* seedling survival was higher in *A. rubrum* soils relative to all other species' soils (Fig. 4.1). Hazard ratios greater than 1 (which demonstrate an increase in mortality hazard relative to *A. rubrum* soils) for all heterospecific soils further illustrates poor seedling survival in heterospecific soils (Table 4.1A). Final survival in *A. rubrum* soils was 22.8% higher than mean final survival in heterospecific soil. Of the seedlings surviving to the study's end, those grown in *A. rubrum* soils had greater biomass than in heterospecific soils (Fig. 4.2, Table A4.2); biomass allocation as indicated by root, stem, and leaf mass fractions did not vary with soil source (results not shown).

Because seedling performance did not differ between live tree and stump soils (HR = 1.035, p = 0.78; Table 4.1A), we used both to compare survival curves across species soils (hypothesis 1). We also tested hypothesis 1 with only live tree soils and results were similar, with

seedling survivorship in *A. rubrum* soil differing from *Q. rubra*, *B. alleghaniensis*, and *P. serotina* soils but no longer significantly different from *A. saccharum* and *T. americana* soils.

Addressing hypothesis 2, *A. rubrum* survival was similar in all heterospecific soil sources (Table 4.1B; Figure 4.1).

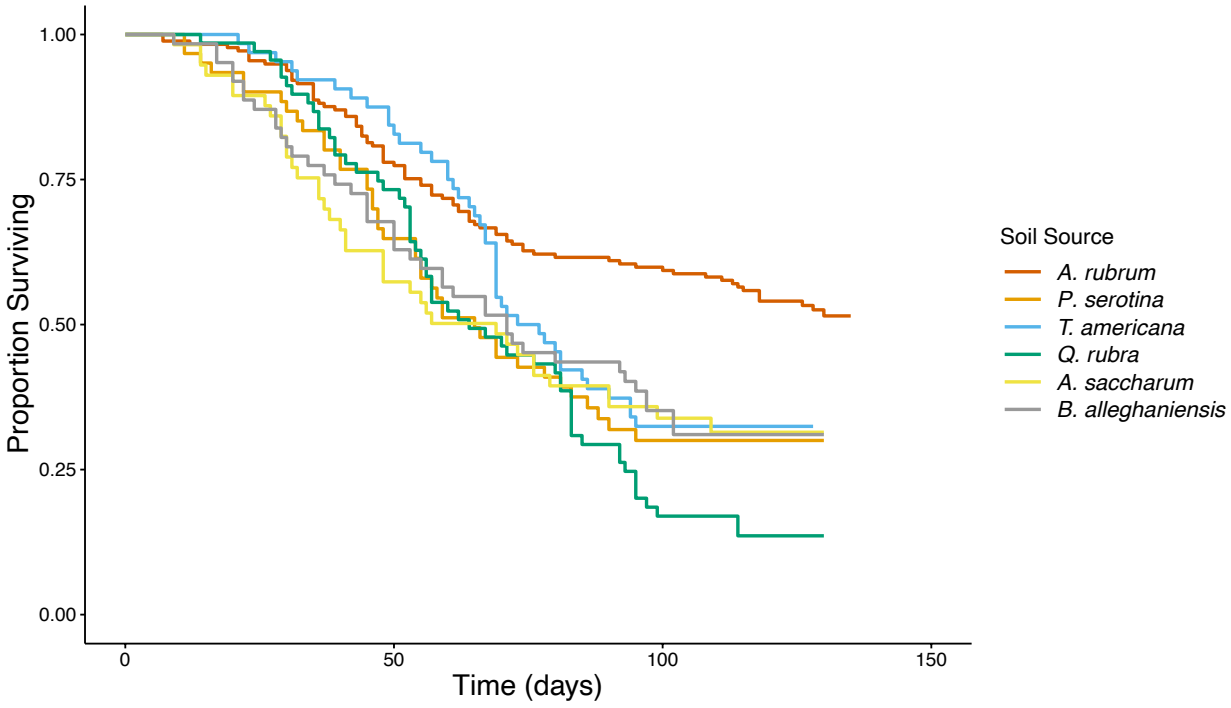

Figure 4.1 Survival curves of *A. rubrum* seedlings grown in a greenhouse in soils sourced from six tree species. Live tree and stump soils for each species are combined. Comparing curves between soil sources evaluates PSF.

Table 4.1

A) Hazard ratios from a Cox proportional hazards survival model of *A. rubrum* seedlings grown in soils from 6 tree species. All soils are referenced against *A. rubrum* soils. Model includes random effects for site, individual tree or stump, and greenhouse bench. Hazard ratios > 1 represent an increase in hazard for that parameter.

	Hazard Ratio	Std. Error	p-value
P. serotina	2.048	0.198	**<0.001**
T. americana	1.617	0.206	**0.02**
Q. rubra	2.384	0.193	**<0.001**
A. saccharum	2.094	0.202	**<0.001**
B. alleghaniensis	1.830	0.199	**0.002**
Live Tree vs. Stump	1.035	0.124	0.78

B) Pairwise comparisons of *A. rubrum* seedling survival between soils from 6 tree species. Similar to Table 4.1A, most contrasts with *A. rubrum* soil are significantly different, with the exception of *T. americana* due to correcting p-values for additional comparisons.

Contrast	Estimate	Std. Error	p-value
A. rubrum - P. serotina	-0.717	0.198	**0.004**
A. rubrum - T. americana	-0.481	0.206	0.18
A. rubrum - Q. rubra	-0.869	0.193	**0.0001**
A. rubrum - A. saccharum	-0.739	0.202	**0.003**
A. rubrum - B. alleghaniensis	-0.605	0.200	**0.03**
P. serotina - T. americana	0.236	0.234	0.92
P. serotina - Q. rubra	-0.152	0.222	0.98
P. serotina - A. saccharum	-0.023	0.227	1
P. serotina - B. alleghaniensis	0.112	0.227	1
T. americana - Q. rubra	-0.388	0.205	0.41
T. americana - A. saccharum	-0.259	0.239	0.89
T. americana - B. alleghaniensis	-0.124	0.233	0.99
Q. rubra - A. saccharum	0.129	0.227	0.99
Q. rubra - B. alleghaniensis	0.264	0.222	0.84
A. saccharum - B. alleghaniensis	0.135	0.232	0.99

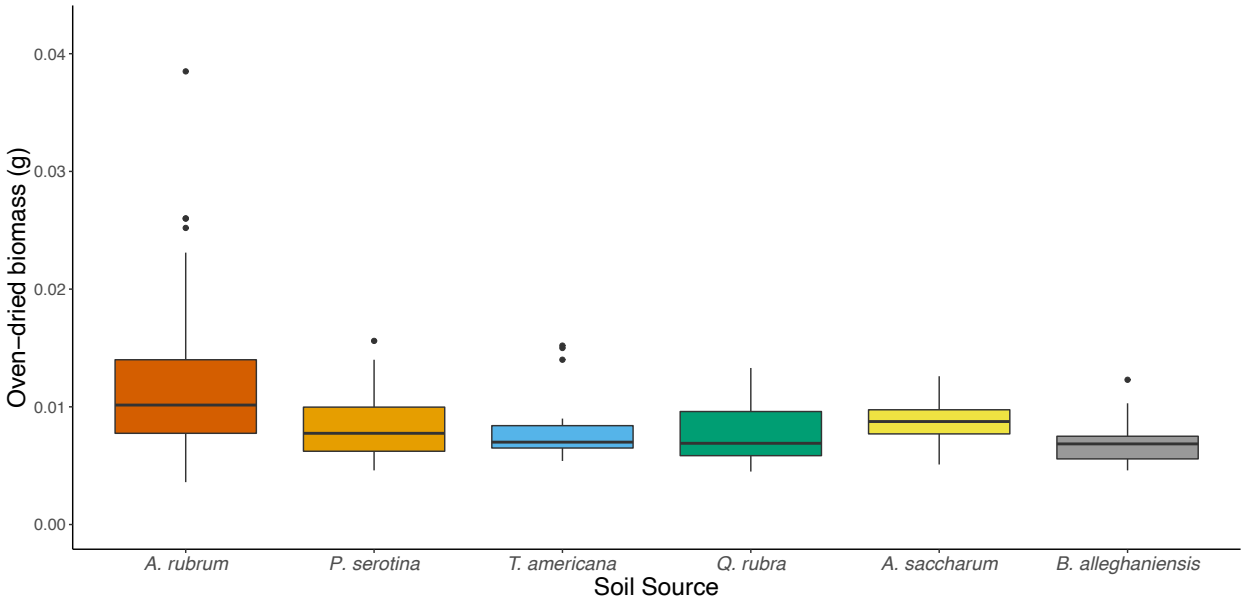

Figure 4.2 Oven-dried biomass of surviving greenhouse grown *A. rubrum* seedlings in soils from six tree species. Live tree and stump soils for each species are combined. Seedling biomass in *A. rubrum* soils is greater than all other soil sources (Table A4.2).

PSF legacies

In assessing conspecific PSF legacies across *A. rubrum* stump ages (hypothesis 3a), we found seedling survival did not differ between stump and live tree soils for all stump ages (Fig. 4.3, Table A4.4). In the oldest (8-yr-old) stump soils there was a non-significant trend of poorer survival relative to live tree soils (HR = 0.556, p = 0.26) and ~13.3% difference in final survival.

In heterospecific soils (hypothesis 3b), seedling survival was not different in stump or live tree soils for all soil sources (Fig. B4.1), as indicated by a hazard ratio near 1 (HR = 1.035, p = 0.78; Table 4.1). In a further analysis to test for species by status effects (live tree vs. stump nested within soil source (Table A4.3)), seedling survival was not different between live tree and stump soils for each species from which soil was sourced.

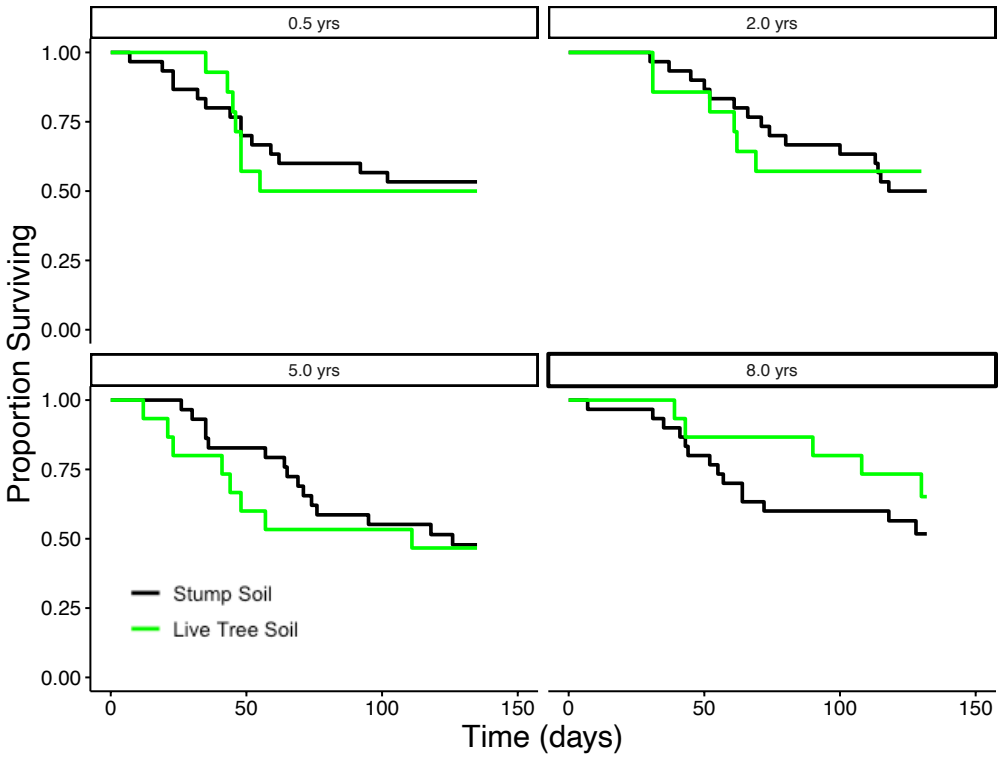

Figure 4.3 Survival curves of greenhouse grown *A. rubrum* seedlings in soils sourced from *A. rubrum* stumps and live trees. Survival curves within each panel are not different from each other (Table A4.4).

Soil pH

A. rubrum soil pH (mean = 4.7) was lower than most other soil sources in the full model comparison (Table A4.5A), but in post-hoc pairwise comparisons differed only from *T. americana* ($p = 0.001$) and differed marginally from *A. saccharum* ($p = 0.06$). *T. americana* soil also had a higher pH (mean = 5.9) than all other soils (Table A4.5B, Fig. B4.2).

DISCUSSION

In contrast to our expectations, we found positive PSFs for *A. rubrum* seedlings in conspecific soils relative to heterospecific soils; both seedling survival and growth responses

were higher in *A. rubrum* soils compared to all heterospecific soils (addressing hypothesis 1) (Fig. 4.1 & 4.2, Table 4.1A & A4.2). *A. rubrum* seedling survival did not differ significantly among heterospecific soil sources (addressing hypothesis 2) (Fig. 4.1, Table 4.1B), suggesting limited variability in heterospecific PSFs. In conspecific soils, similar survival in live tree and stump soils except for a non-significant trend of lower survival in the oldest (8-yr-old) stump soils suggests that PSF legacies are long-lived (> 8 yrs.) in *A. rubrum* soils (addressing hypothesis 3a) (Fig. 4.3, Table A4.4). *A. rubrum* seedling survival was not different between live tree and stump soils of any heterospecific species (addressing hypothesis 3b) (Fig. 4.3, Table 4.1 & A4.3), indicating that PSF legacies in heterospecific soils do not shift after tree death, which could arise because PSFs are near neutral or PSF legacies in heterospecific soils are long lasting (and not weakening). We are not able to distinguish between these two possibilities in the current study. Taken together, positive conspecific PSFs and multi-year PSF legacies in *A. rubrum* soils support that soil conditions benefiting *A. rubrum* seedlings may be present long after tree death and that such legacies should be considered in regeneration dynamics.

PSFs

Our findings suggest that the positive response of conspecific seedling growth and survival to *A. rubrum* soils relative to heterospecific soils is driven by soil mutualists and to a lesser degree by soil nutrients. Because our study lacks a soil sterilization treatment, we cannot definitively disentangle whether soil biota or abiotic factors are influencing seedling performance, but several lines of evidence point to the importance of soil mutualists.

Soil mutualists of *A. rubrum*, specifically arbuscular mycorrhizal fungi (AMF) likely contribute to positive conspecific seedling growth and survival responses because more beneficial AMF are present in *A. rubrum* soils than heterospecific soils (Lovelock and Miller

2002). *A. rubrum* inocula (in the form of chopped roots) promoted conspecific seedling growth more effectively than *Quercus falcata* Michx. (southern red oak), even though the extent of root colonization by AMF was similar (Lovelock and Miller 2002). Furthermore, soils from ectomycorrhizal species, like *Q. rubra* and *B. alleghaniensis* in the present study, could be expected to have less beneficial AMF inoculum in their soils. However, lack of variation among heterospecific soil sources (Fig. 4.1, Table 4.1B) suggests that despite variation in type of mycorrhizal association and soil pH (Fig. B4.2, Table A4.5B) the magnitude of heterospecific PSFs for *A. rubrum* are minor relative to positive PSFs in conspecific soils.

Our finding of positive PSFs in *A. rubrum* relative to heterospecific soils runs counter to our expectations and results of other studies (e.g., Nijjer et al. 2007; McCarthy-Neumann and Ibáñez 2012; Reinhart et al. 2012; Bennett et al. 2017). As an AMF tree species, *A. rubrum,* is expected to experience negative PSFs (Bennett et al. 2017) because AMF provide relatively little protection from soil pathogen damage (but see Borowicz 2001). PSF direction can be influenced by light levels, although we grew seedlings under 5% full sun which is lower than the ~8.5% full sun transition where conspecific PSFs switch from neutral to positive for *A. rubrum* (Ibáñez and McCarthy-Neumann 2016).

Study duration may be driving differences between our findings and others. In our study, seedling survival in *A. rubrum* soils only began to differentiate from other species after ~70 days (Fig. 4.1). In contrast most other experiments evaluating *A. rubrum* PSFs last 12 weeks. If we truncated our study at 10 weeks, our results would be similar to other work and suggest that neutral PSFs occur for *A. rubrum* (Nijjer et al. 2007; Reinhart et al. 2012; McCarthy-Neumann and Ibáñez 2013).The delay in appearance of positive effects on survival until ~70 days may be driven by competition between AMF and soil pathogens for root space (Borowicz 2001) and/or

slow development of seedlings and mutualisms under relatively low light conditions of 5% full sun. Lengthier studies may allow time for growth and survival effects to manifest and sufficient time in seedling development that PSFs may shift with plant life stage (Dudenhöffer et al. 2018). Notably, Bennett and colleagues (2017) grew *A. rubrum* seedlings for 6 months and found negative PSFs on biomass; however, seedling survival and light environment were not reported.

Variation in soil nutrients between soil sources is unlikely to have influenced results in this study. While *A. rubrum* soil tended to have a lower pH than soils of other species, we do not think that these slight differences in soil pH could explain differences in *A. rubrum* seedling survival. First, we blended 50% potting medium with 50% field soil, thereby diluting soil chemistry effects. Second, there was no variation in seedling survival across other species' soils, even though they varied in pH (especially *T. Americana* versus other species). Beyond pH effects, survival was lowest in *Q. rubra* soils relative to *A. rubrum* (~34.9% difference in final survival), which soil nutrients might contribute to because N mineralization rates tend to be higher in *A. rubrum* than *Q. rubra* soils (Finzi et al. 1998b). However, other study species like *A. saccharum*, are typically associated with soil nutrient concentrations similar to those under *A. rubrum* canopies (Finzi et al. 1998a, b), making it unlikely that soil nutrients are the sole driver of positive conspecific PSFs in *A. rubrum*.

PSF legacies

Conspecific PSF legacies are likely present and might be weakening with time, indicated by similar survival in live tree and stump soils of all ages except for the oldest (8-yr-old) stumps where survival was not significantly lower relative to live tree soils (Fig. 4.3, Table A4.4). A trend towards poorer survival (~13.3% lower) in the 8-yr-old stump soils suggests a weakening of PSF legacies and also reinforces the finding of positive PSFs in *A. rubrum* soils. These

positive and multiyear effects in *A. rubrum* soils could be caused by residual effects on soil chemistry (Wardle et al. 2008), mutualists such as mycorrhizae persisting as dormant spores (Nguyen et al. 2012) or perhaps residing on the roots of neighboring trees or saplings. Such long-lasting PSF legacies could then facilitate continued growth of *A. rubrum* populations even after mature trees die or are harvested.

In heterospecific soils, legacies of PSFs for *A. rubrum* were not apparent because seedling survival did not differ between live tree and stump soil sources (Table 4.1A); this relationship was consistent for all heterospecific soil sources (Table A4.3). The lack of difference in seedling performance between live tree and stump soils could be indicative of long-lived legacies or near neutral PSFs near heterospecifics that would not be expected to change after tree death. We consider the latter most likely given the consistency of seedling responses among heterospecific soil sources. A soil sterilization treatment could have provided an important reference to determine whether heterospecific soil PSFs are neutral.

Caveats

Although we diluted the effects of soil nutrients by blending 50% field soil with 50% potting medium, we cannot completely exclude the possibility that our finding of conspecific PSFs arises in part from *A. rubrum* affinities for soil microsite conditions during establishment (Binkley and Giardina 1998; Finzi et al. 1998a, b) rather than tree modifications of soil. The absence of a sterilized soil treatment also limits our ability to isolate drivers of the seedling response patterns. However, regardless of whether seedlings are responding to tree-driven alterations or pre-existing soil conditions, determining their responses is valuable for understanding seedling regeneration patterns.

Our findings are restricted to a single light level of 5% full sun and light levels can determine how PSFs and PSF legacies manifest (Ibáñez and McCarthy-Neumann 2016; see chapter 2). Although we have not evaluated *A. rubrum* seedling performance across a broad range of light environments, 5% full sun is representative of the understory or small gaps of forest types where *A. rubrum* is recruiting into the understory (Alexander et al. 2008).

Conclusions

Longer lived (> 8 years) positive PSF legacies of *A. rubrum* found here contrast with prior work finding short-lived (< 1.5 yr) negative PSF legacies for *P. serotina* (see chapter 2) and demonstrate the potential for variability in PSF legacies depending upon the species and mechanisms involved. *P. serotina* PSF legacies were likely driven by pathogenic oomycetes leading to dramatic declines in survival (see chapters 2 & 3), whereas *A. rubrum* PSF legacies were likely brought about through benefits of AMF. Thus, different PSFs mechanisms may lead to differing PSF legacy effect duration. PSF legacies, like PSFs, appear to vary depending upon the plant species involved (Kiers et al. 2000; Gustafson and Casper 2006; Bever et al. 2012; Hersh et al. 2012), motivating further study of PSF legacies across multiple species to understand the role of PSF legacies in post-disturbance regeneration.

Though the rising abundance and expansion of *A. rubrum* throughout forests of eastern North America has received substantial attention (Lorimer 1984; Abrams 1998; Fei and Steiner 2007), our findings suggest that conspecific PSFs and PSF legacies are not likely involved. If PSFs were significant drivers, we would expect better *A. rubrum* seedling performance in heterospecific than conspecific soils. Furthermore, we would expect higher *A. rubrum* performance relative to other seedling species, which were not included in this study. Growing additional seedling species in multiple soil sources could better elucidate PSFs and PSF legacies

as mechanisms of seedling regeneration patterns. Nevertheless, the positive conspecific PSFs and multiyear PSF legacies we found suggest that where *A. rubrum* is established, PSFs and their legacies will reinforce the presence of *A. rubrum* as a component of the forest.

Overall, we found unexpectedly positive conspecific PSFs for *A. rubrum* and minimal variation in heterospecific PSFs. Conspecific PSF legacies were long lasting (> 8 years) and we were unable to determine if heterospecific PSF legacies were present. These findings suggest that beneficial conditions for *A. rubrum* recruitment will persist after conspecific tree death and continue to shape seedling recruitment. The range of effects from past findings of short-lived (< 1.5 yr) PSF legacy effects for *P. serotina* (see chapter 2) to multi-year (> 8 yr) PSF legacies of *A. rubrum* in the present study support that PSF legacies vary with species and point to the potential importance of PSF legacies to forest dynamics and succession, which should be further elucidated through studies incorporating multiple species of seedlings and soil sources.

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CHAPTER 5

Restoring with forest monocultures:

a mechanistic look at exotic species supression

ABSTRACT

Restoring forests on abandoned pastureland often requires suppressing exotic grasses that negatively influence tree seedling survival and recruitment. Tree planting is one restoration tool that reduces grass growth through shading and accumulation of leaf litter, while also benefiting desired woody species by moderating microclimate. Nitrogen-fixing trees are often planted in tropical reforestation for their fast growth rates and ability to improve soils, but nitrogen (N) contributed to the soil from N_2-fixing trees can also facilitate undesired exotic grasses. The balance between grass suppression and facilitation by trees may depend upon tree density and site conditions. Using restoration forests of a native N_2-fixer, *Acacia koa* (koa), on Hawai‘i Island, we evaluated whether koa suppresses invasive grasses and if so, by what mechanisms. We used a range of tree densities and sites spanning the island. We found consistent effects of grass suppression by koa via shading and litter accumulation. Sites with lower soil moisture may better reduce grass growth, but this effect was not linked to koa density. Importantly, total grass suppression rarely occurred. Grass persistence under koa canopies may be driven by a shift in composition to more shade tolerant grass species. If complete grass suppression and/or more diverse forest are desired management goals, then further management interventions, like diverse understory plantings, could amplify the mechanisms of grass suppression we identified by introducing species that cast deeper shade and/or have litter that is slower to decompose.

INTRODUCTION

Few plant communities in the world are free from exotic species, making management of exotic species a near universal challenge in achieving many restoration goals (D'Antonio et al. 2016). Invasive, exotic plants can displace native species and thereby alter community composition and functioning (Mack et al. 2000; Ehrenfeld 2003; Levine et al. 2003). Exotic pasture grasses in particular are widespread and frequently introduced by humans for grazing livestock (D'Antonio and Vitousek 1992). When restoring forests from such pasturelands, exotic grasses can be a substantial barrier by competing with desired native woody species, inhibiting native recruitment, altering fire regimes, and exhibiting priority effects leading to continued grass dominance after pasture abandonment (D'Antonio and Vitousek 1992; Holl et al. 2000; Williams and Baruch 2000; Denslow et al. 2006; Yelenik 2017). Therefore, methods that can be used over large scales are needed to effectively control invasive pasture grasses during restoration efforts.

One such method is planting trees, which suppress grasses through a number of mechanisms (Parrotta 1992; Holl et al. 2000; Lamb et al. 2005). Shade cast by trees can reduce grass growth because grasses are primarily shade intolerant (Holl et al. 2000; McDaniel and Ostertag 2010). The physical accumulation of tree leaf litter negatively affects herbaceous vegetation, including grasses (Xiong and Nilsson 1999). As trees mature, their canopies moderate daily temperature and moisture extremes and create less stressful environments, which can benefit the recruitment of additional woody species (Nepstad et al. 1996; Holl 1999; Rehm et al. 2021). Disentangling mechanisms by which trees suppress grasses could aid in targeting management interventions that reinforce or compliment ongoing mechanisms where trees are planted.

Nitrogen-fixing (hereafter N_2-fixing) trees are common candidates for tree planting in many tropical regions in part because they grow quickly and rapidly form canopy structure (Carpenter et al. 2004; Scowcroft et al. 2004; Siddique et al. 2008; Griscom and Ashton 2011). N_2-fixing trees benefit plant growth by contributing organic matter and N to the ecosystem, primarily through decomposition of their N-rich leaf litter (Scowcroft et al. 2004; Batterman et al. 2013). N contributions to soils can be crucial in overcoming N limitation, which occurs in many younger tropical soils (Vitousek and Farrington 1997; Batterman et al. 2013). In combination, grass suppression via shading and litter accumulation, plus improved conditions for tree seedling recruitment (moderated microclimate, increased soil N and organic matter) potentially create conditions to set in motion passive restoration of forest communities following initial tree planting (Corbin and Holl 2012; Yelenik et al. *in press*).

However, planting N_2-fixing trees may not always successfully suppress pasture grasses and lead to forest succession, especially since many mechanisms that enhance woody species growth can also facilitate invasive grasses. Indeed, increased soil N driven by N_2-fixers can benefit grasses to the detriment of native species (Maron and Connors 1996; Sierra and Nygren 2006). The N-rich leaf litter of N_2-fixing trees decomposes rapidly, creating a thinner litter layer and likely less grass suppression than the litter of co-occurring non-N_2-fixing species (Scowcroft 1997; Tateno et al. 2007; Yelenik 2017). In addition, grasses may have higher foliar N from N_2-fixing tree inputs, which can further increase decomposition and ecosystem N cycling rates (Perakis et al. 2012). Thus, a mixture of mechanisms suppressing and facilitating grasses occur under N_2-fixing trees.

The balance between grass suppression and facilitation by trees may shift depending upon tree density or abiotic variables (Callaway and Walker 1997). Increasing stem density

should intensify grass suppression via shading and litterfall, but increased N inputs from additional stems could also facilitate grasses and counteract suppressive effects from additional trees (Sitters et al. 2013). Furthermore, as stem density and shading increases in transitions from savanna to forest, grass composition shifts to more shade tolerant species (Charles-Dominique et al. 2018). Thus, grass suppression by tree canopies could be undermined as shade intolerant grass species are replaced by more shade tolerant grasses (McDaniel and Ostertag 2010). Climate variables such as rainfall and temperature can affect decomposition rates (Austin 2002; Powers et al. 2009), which then alters the thickness of the litter layer and rate at which N is contributed to the soil. These interactions with climate add further complexity to the balance between competition and facilitation. Understanding mechanisms involved in tree-grass interactions can aid in understanding their outcomes, which are critical for successful forest restoration.

To examine interactions between a N_2-fixing tree and invasive grasses in a restoration effort, we studied the native Hawaiian leguminous species koa (*Acacia koa* A. Gray). Koa is commonly planted in monoculture for reforestation in Hawai‘i because it grows quickly and is easy to propagate relative to other native Hawaiian tree species (Jeffrey and Horiuchi 2003; Scowcroft et al. 2004; Scowcroft and Yeh 2013; Friday et al. 2015). However, when restoring forest from old pastureland by planting koa, invasive grasses often persist in the understory and impede recruitment of native forest species thereby stalling succession in a koa-grass state (Denslow et al. 2006; McDaniel et al. 2011; Yelenik et al. *in press*). Across the Hawaiian landscape, steep topography creates wide gradients in environmental conditions across relatively small expanses (Giambelluca et al. 2013), which may lead to disparate restoration outcomes when using koa to suppress grasses.

To examine the efficacy of grass suppression by koa monocultures, we asked the following questions: Does increasing koa density decrease grass biomass? And if so, by what mechanisms? We hypothesized (a) that grass biomass decreases with increasing densities of koa stems and this decrease is associated with less light, lower soil moisture, greater litterfall, and slow decomposition leading to a build-up of litter. Alternatively, (b) if pasture grass biomass increases with koa density, then greater soil N facilitates grasses and counteracts competitive effects and/or species composition shift to more shade tolerant grasses. To examine the generality of these effects we carried this study out at sites spanning a range of temperatures and rainfall regimes on Hawai‘i Island.

METHODS

Study System

We studied koa-grass interactions in koa-dominated forest stands on Hawai‘i Island, USA. In Hawai‘i, like many other tropical and sub-tropical areas, much of the forest was cleared and converted to pasture for grazing livestock (McDaniel and Ostertag 2010; Corbin and Holl 2012). Common exotic grasses on Hawai‘i Island and at our study sites are meadow ricegrass (*Ehrharta stipoides* (Labill.) R. Br.) and kikuyu grass (*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone) (McDaniel and Ostertag 2010; Yelenik 2017). In recent decades, ranching has become less profitable, leading to abandonment of pastureland and growing interest in reforestation to increase habitat for endangered birds and aid other ecosystem services (Scowcroft and Jeffrey 1999). Koa is a canopy dominant or codominant tree occurring on many Hawaiian islands and spanning a range of elevations, soil types, and rainfall regimes (Wagner et al. 1990). Koa is highly valued, both culturally and commercially (Elevitch et al. 2006), and

grows relatively quickly, leading to its widespread use in reforestation. In addition, koa can resprout from root suckers, making it a dominant species in passive regeneration after grazer removal when remnant koa are present (McDaniel et al. 2011; Scowcroft and Yeh 2013).

Mature koa trees rarely produce true leaves and typically have canopies composed of phyllodes (sickle-shaped modified petioles) (Baker et al. 2009). When collecting koa leaves for measures of decomposition we used only phyllodes and koa leaf litter collected from the forest floor was almost entirely phyllodes. For simplicity, we will use the term koa leaves to refer to phyllodes.

Site Descriptions

We established 10 study sites across five protected areas encompassing a wide range of climatic conditions on Hawai‘i Island (Table 5.1, Fig. 5.1). These protected areas are managed by a diverse group of stakeholders, showing the ubiquity of koa as a restoration tool across Federal, State, and Non-Profit agencies. Some sites are located within the same protected area, but are spatially separated, and owing to steep climatic gradients, experience distinct temperature and moisture regimes. Though the protected areas have varying restoration and previous land use histories (Table 5.1), this variation likely has minimal effects on the mechanisms of koa-grass interactions and incorporating variation across sites makes our findings more broadly applicable. The sites are similar in that they all contain koa-dominated stands on land occupied by exotic grasses that was previously disturbed by some combination of deforestation, burning, grazing, and/or logging. Most of the sites have similar soils and substrate ages (750-3,000 years old), except Hakalau which is significantly older (Table A5.1). To exclude feral ungulates, all sites except Hilina Pali 1 and 2 are fenced. However, maintaining intact fences is difficult and

ungulates, particularly pigs, may have influenced our sites. When selecting plots, we avoided any areas recently damaged by ungulates.

Table 5.1 Characteristics of the 10 study sites of koa-dominated restoration stands ranging across Hawai‘i Island.

Site	Approx. Elevation (m)	Avg. Annual Temp. (ºC)[1]	Avg. Annual Rainfall (mm)[2]	Restoration History
Hakalau	1845	12.1	2559.7	Koa was planted (1985-1990) in corridors extending from intact forest[3]
Kahuku 1	1105	16.5	1358.6	Fenced in 2005 with various restoration
Kahuku 2	1292	15.4	1302.5	treatments (herbicide, soil turnover, planting
Kahuku 3	1357	14.9	1522.8	and seedling) applied[4]
Hilina Pali 1	732	19.4	1796.6	Regenerated from 1950s koa plantings after
Hilina Pali 2	846	18.3	2037.3	fires in 1970s and 1980s[5]
Kona Hema 1	1728	12.3	877.3	Fenced in 2004, koa was planted and
Kona Hema 2	1456	14.2	808.8	regenerated from existing trees[6]
Pu‘u Wa‘awa‘a 1	1319	15.5	702.74	Fenced in 1985, koa regenerated from
Pu‘u Wa‘awa‘a 2	1334	14.7	709.08	existing trees[7]

1) Giambelluca et al. 2014
2) Giambelluca et al. 2013
3) Jeffrey and Horiuchi 2003
4) McDaniel et al. 2011
5) Tim Tunison, National Park Service, personal communication 2013
6) Giffin 2017
7) Giffin 2010

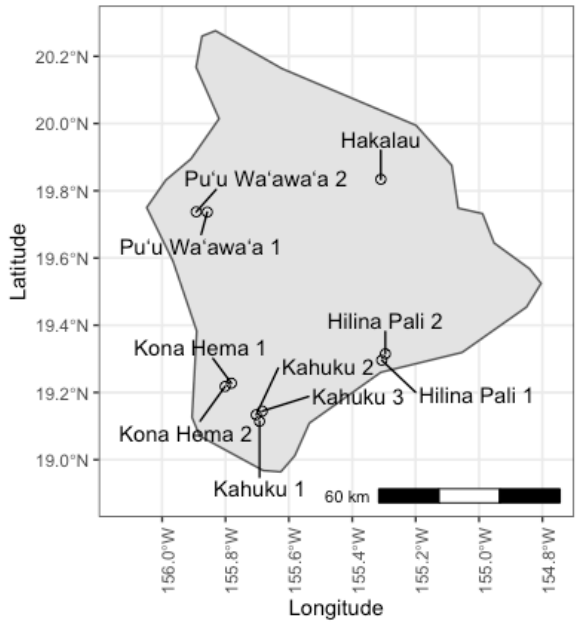

Figure 5.1 Map of site locations.

Plot Selection

At each site, we established fifteen 2.5 m radius circular plots (each plot ~20 m^2), with at least 20 cm soil depth, and located them to maximize the range of koa densities sampled at each site (Fig. B5.1). Plot edges were separated by at least 10 m, except at Kahuku 3 where the higher density plots were separated by at least 5 m because few areas of high density koa existed. Of the fifteen plots at each site, 10 plots included koa stems and grass (hereafter koa plots) and five control plots contained only grass and no koa stems or koa canopy cover. Woody species rarely occurred in the understory of these sites unless planted (pers. obs.); to avoid any confounding effects of tree species other than koa, all plots contained only herbaceous species in the understory. We measured diameter at breast height (DBH) for all koa stems exceeding 0.5 cm at breast height (1.37 m) in each plot.

To stratify sampling across a given site, plots were selected in clusters of three: two koa plots (one high density and one low density) and one control plot. Control plots were in the vicinity of koa plots in open spaces in the stand. The exception to the plot clustering method was Kahuku (1, 2, and 3); at these sites we distributed our plots across the limited space (200 × 200 m) of the restored area. High versus low density koa was relative within a site and plot cluster, and we aimed to capture a wide range (1–79 stems/plot). Thus, density range varied by site, e.g., at dry sites koa grows less densely and has a more open growth habit leading to a lower maximum plot stem density than at many wet sites. To account for the high variability of stem density, within and across sites, we used this as a continuous variable for analysis.

The following measures were made in each plot at every site, except at Pu‘u Wa‘awa‘a 1 and 2 where decomposition measures did not take place.

Light

We measured light using a light meter (MQ-301, Apogee Instruments, Inc, Logan, UT) on clear to minimally overcast days to assess light availability near the ground. At Pu‘u Wa‘awa‘a a cloudless sky was not encountered, so light measures were taken on a uniformly overcast day. We averaged five measures in every plot, one at plot center and then at four points 1 m distant from plot center. The light wand was held at ~1 m height, exceeding grass height. Measures were made when the sun was unobscured by clouds and directly or nearly directly overhead to capture shade cast only by the plot's vegetation and avoid neighboring tree shade.

To control for diurnal variation in light, we assessed light under koa canopies as a percent of light available in the open. Thus, to calculate the percent of full sun available we divided the average amount of light in a koa plot by light levels in absence of canopy (usually the nearest control plot). All measures took place within a month (June 24–July 24, 2019). Light levels were slightly greater in four koa plots than in their nearest control plot, likely due to some small clouds passing overhead when measuring the control plot; for these koa plots light levels were truncated at 100% full sun.

Soil Nitrogen

To measure soil N, we sampled soil from 0–10 cm depth using a spade from three haphazardly selected points within each plot. Samples from within each plot were combined, homogenized, refrigerated, passed through a 4.75 mm sieve, and processed within 48 hours of collection. To extract nitrate and ammonium, subsamples of field wet soil were shaken with 2M KCl for 1 hour. Extracts were analyzed colorimetrically using an ELx808 Absorbance Microplate Reader (BioTek Instruments, Inc, Winooski, VT). We measured inorganic soil N again following a ~5-week incubation to calculate N mineralization. We prepared the incubations

by placing 50 grams of each soil sample in loosely capped jars and adding deionized water to all samples to homogenize soil moisture levels to the wettest sample (3.2 g H_2O/g dry soil). Incubating samples were kept in a cooler in the Magma Lab at Hawai‘i Volcanoes National Park (average temp. = 16 °C) to modulate temperatures.

Soil Moisture

To measure soil moisture in the field, we used a soil moisture probe (Hydrosense II, Campbell Scientific, Inc, Logan, UT) inserted at three points to 12 cm depth within each plot. The probe was placed at least 0.5 meters from the bole of any tree over 10 cm DBH to avoid roots. We took these measures three times over the course of the study, each roughly 1.5 months apart to capture variation in soil moisture while transitioning into the dry season, and then averaged the measures for analysis.

Decomposition

To examine decomposition rates and determine the speed at which the litter layer turns over, we placed koa leaves in the field using a tethered leaf method (Vitousek et al. 1994). We collected fully expanded koa leaves grown in full sun at each site and placed them in a drying oven at 35°C for at least a week to dry them to a consistent state without brittleness. Additional leaves from each site were dried at 60°C to create a conversion from starting mass (dried at 35°C) to final dry mass. We weighed each leaf individually before tethering them together in strands of 5 leaves. Strands of tethered leaves were buried underneath leaf litter and grass rhizomes so that contact was made between our leaves and the soil. At each plot, we placed four strands of five leaves strung together with a washer at each end. One string of leaves was removed after each consecutive month of decomposition for a total of 4 months. After retrieval, the leaves were dried at 60°C, weighed individually, and subtracted from their initial mass

(converted to dry mass) to calculate change in mass. The percent change in mass for all leaves on a strand was then averaged and used to determine the rate of decomposition (k; $mass_t = mass_0 \times e^{-kt}$) per plot.

In the first month, a few leaves (8) gained mass, which may be due to variation in the initial drying. We truncated the mass gain of these leaves as 0.01 % mass loss. In the third and fourth months some leaves became untethered, or substantial parts broke off and could not be retrieved. Often, other leaves were retained on a strand and decomposition data from that plot could still be collected, just with fewer leaf replicates on a strand. Six whole strands of leaves (out of 480) were lost resulting in missing data for a plot for a month.

Grass and Koa Litter Biomass

We measured biomass by removing all plant material within a 0.6 m × 0.6 m quadrat in each plot and drying it at 70°C for at least 48 hours. The quadrat was placed haphazardly such that it was representative of the plot vegetation. We sorted the dried samples into grass, koa leaf litter, ʻōhiʻa (*Metrosideros polymorpha* Gaudich.) leaf litter, coarse woody debris, and other material (usually seed pods and flowers) and weighed each component.

Percent Cover/Species Composition

We estimated percent cover of all herbaceous vegetation and woody species litter in 0.6 m × 0.6 m quadrats. The quadrat was haphazardly placed three times in each plot to be representative of the vegetation. We identified all species of grass and herbaceous plants.

Leaf C and N Content

At each site, we collected koa leaves grown in full sun to assess % C and N content. Leaf samples were processed at the University of Hawaiʻi Hilo Analytical Laboratory using a Costech Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA).

Statistical Analysis

We completed all analyses using R version 3.6.2 (R Core Team 2019).

To evaluate the effects of koa density on grass biomass and the mechanisms involved, we developed a Structural Equation Model (SEM) using the piecewiseSEM package (version 2.1.0) (Lefcheck 2016). SEMs are useful for understanding direct and indirect pathways (Grace et al. 2012), making them ideal for our system due to the many ways our factors of interest could influence each other and grass biomass. Our initial SEM was based on knowledge of the system and hypothesized relationships between koa and grass, including processes like decomposition (Fig. 5.2a, Table A5.2). Each component model within the SEM used a gamma distribution and included a random effect for site. Two plots at Kahuku 3 did not include any grass and were modified to include 0.0001 g grass biomass in order to be modeled with a gamma distribution, which does not include 0. Koa basal area and stem density in our plots were not strongly correlated; thus, we ran two separate SEMs, one for each measure of koa, using the same initial SEM (Table A5.2). These SEMs include data only from koa plots; control plots are used to reference the range of variation present in the absence of koa canopies.

We evaluated indirect paths between koa density and grass biomass mediated by light, litter, soil N, litter decomposition rates, and soil moisture. We selected the SEM that best fit our data by examining model AIC and Fisher's C to determine global goodness of fit. We added a single path from koa density (or basal area) to grass biomass, which improved model fit based on tests of directed separation (Lefcheck 2016). Path coefficients were standardized by scaling the raw data prior to analysis. Data were scaled to have a standard deviation of 1 and centered on 2 to avoid negative values that would have resulted from 0-centering, as negative values cannot be modelled with a gamma distribution.

To evaluate shifts in understory composition based on percent coverage of litter and grass, we used a PERMANOVA with koa stem density and light as fixed effects and site as a random effect using the adonis in function in R's vegan package (Oksanen et al. 2019).

RESULTS

We used SEMs to incorporate direct and indirect paths between our variables of interest and present here our overall model fit prior to addressing the component parts of the SEM. The SEMs fit the data well based on Fisher's C, a global goodness of fit measure where $p > 0.05$ indicates good fit, with $p = 0.55$ and $p = 0.18$ for the models using koa density and basal area, respectively. The models using either measure of koa were similar; here we focus on koa stem density (see Table A5.3 and Fig. B5.2 for basal area). Path coefficients (r) are standardized and can be compared to assess relative effects.

Increasing koa density was associated with decreasing grass biomass through two indirect paths (Table 5.2, Fig. 5.2b). Greater koa density led to increasing koa litter biomass ($r = 0.169$, $p = 0.0013$), which then diminished grass biomass ($r = -0.138$, $p < 0.0001$) (Fig. 5.3b). More koa stems also reduced light reaching the understory ($r = -0.179$, $p < 0.0001$) and decreasing light reduced grass biomass ($r = 0.070$, $p = 0.027$) (Fig. 5.3d). Lower soil moisture decreased grass biomass ($r = 0.126$, $p = 0.013$) (Fig. 5.3c), although reduced soil moisture was not associated with koa stem density ($r = 0.007$, $p = 0.776$).

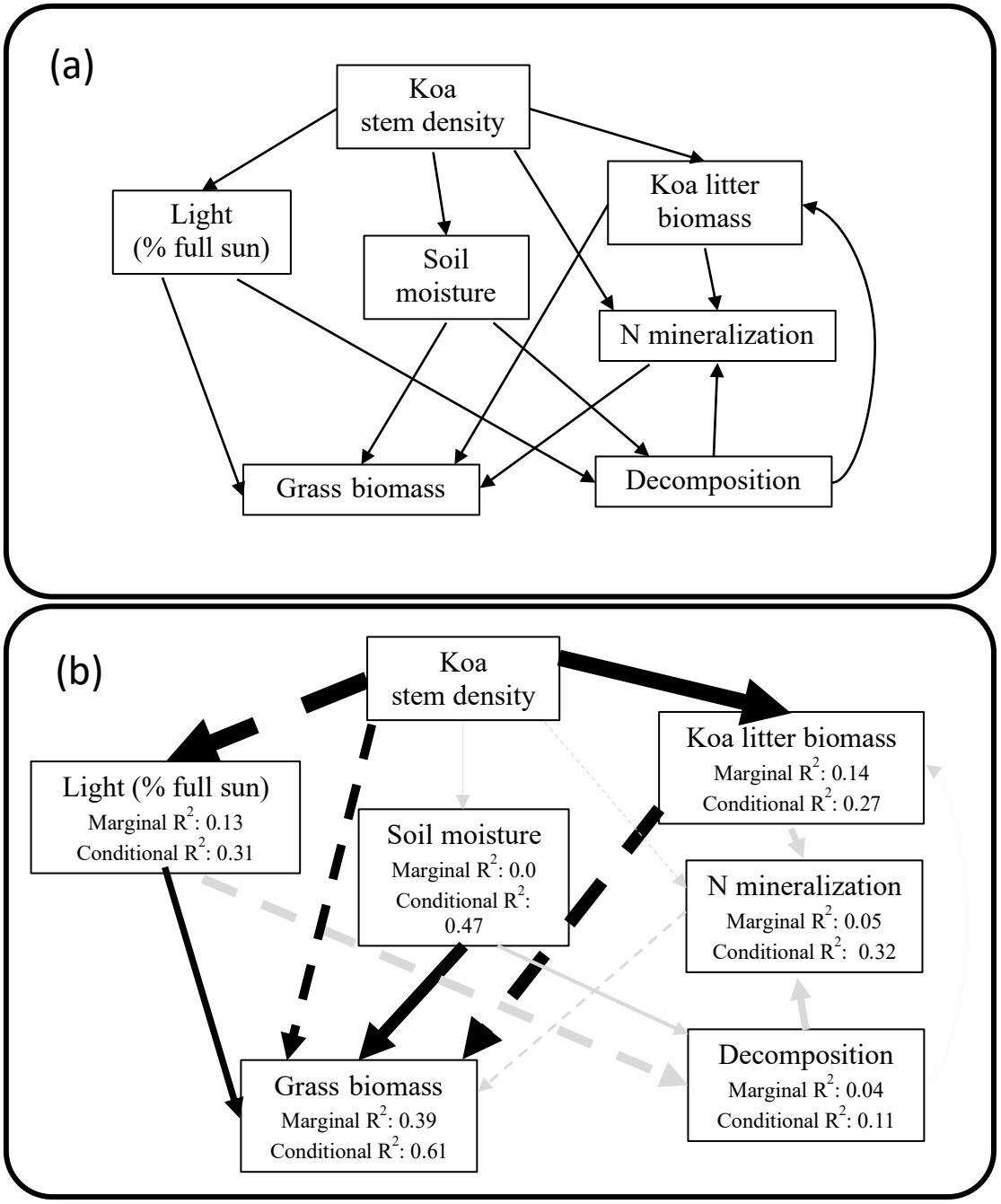

Figure 5.2 Initial (a) and final (b) SEM evaluating the effects of koa stem density on grass biomass and possible mechanisms. Black arrows signify statistically significant ($p < 0.05$) paths and gray arrows non-significant paths. Solid arrows denote positive relationships and dashed arrows negative relationships. Arrow width scales with standardized path coefficients. Marginal R^2 includes variance from fixed effects and conditional R^2 considers fixed effects and the random effect of site.

Table 5.2 Parameter estimates for each path included in the final SEM using koa stem density. Significant p-values (< 0.05) are in bold. Path coefficients are standardized.

Response	Predictor	Path Coefficient	Std. Error	DF	p-value
Grass biomass	Koa litter biomass	-0.138	0.030	97	**< 0.0001**
Grass biomass	Light	0.070	0.032	97	**0.027**
Grass biomass	Soil moisture	0.126	0.051	97	**0.013**
Grass biomass	N mineralization rate	-0.028	0.034	97	0.396
Grass biomass	Koa stem density	-0.093	0.026	97	**0.0004**
Koa litter biomass	Koa stem density	0.169	0.053	72	**0.0013**
Koa litter biomass	Decomposition	0.001	0.053	72	0.979
N mineralization rate	Koa litter biomass	0.068	0.050	71	0.174
N mineralization rate	Koa stem density	-0.012	0.048	71	0.804
N mineralization rate	Decomposition	0.076	0.052	71	0.141
Light	Koa stem density	-0.179	0.036	100	**< 0.0001**
Soil moisture	Koa stem density	0.007	0.024	100	0.776
Decomposition	Light	-0.096	0.057	74	0.093
Decomposition	Soil moisture	0.039	0.070	74	0.573

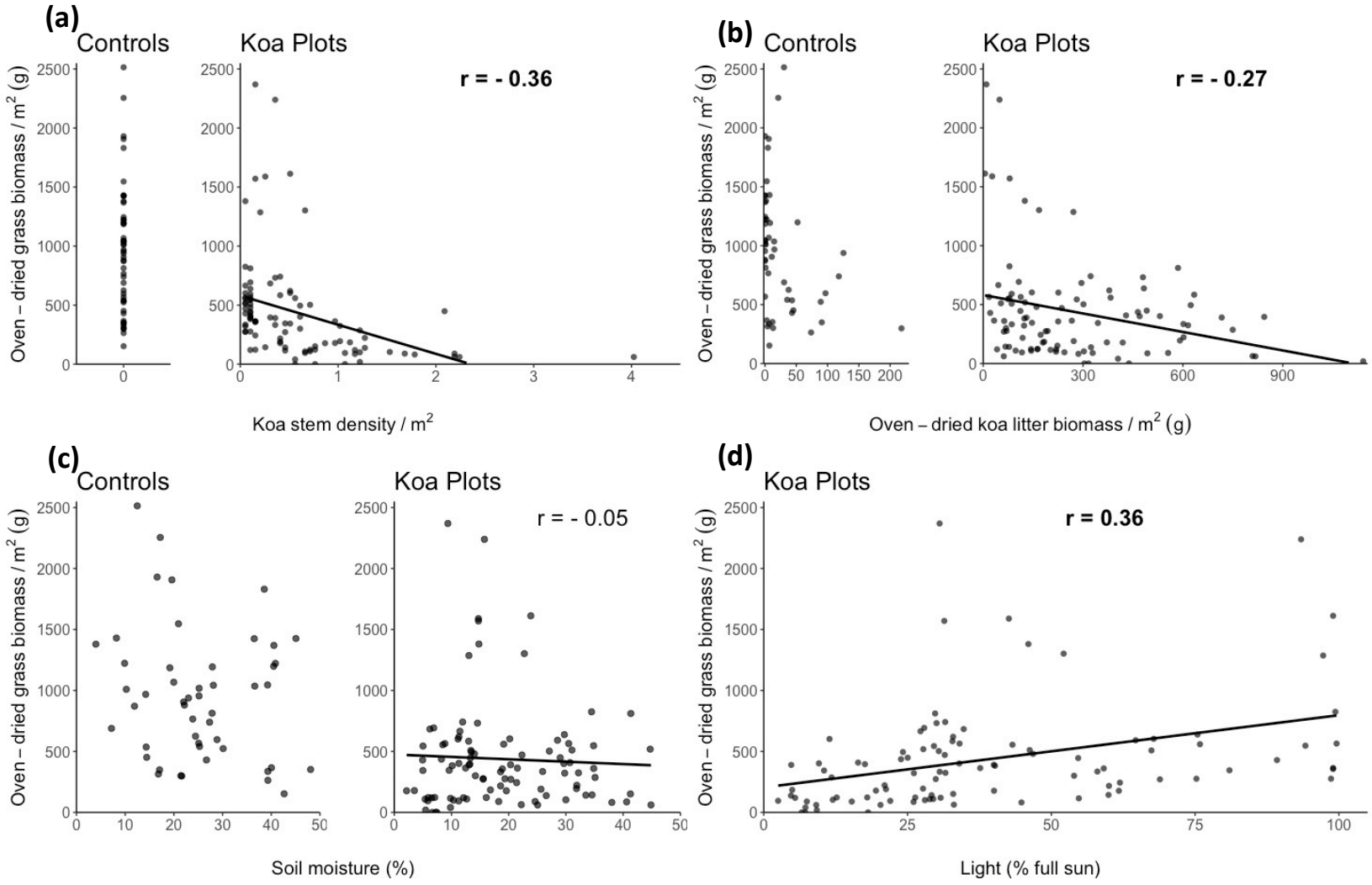

Figure 5.3 Bivariate correlations between grass biomass and (a) koa stem density, (b) koa litter, (c) soil moisture, and (d) light in koa and control (only grass) plots. Controls are plotted only for reference and were not used in analysis. Bolded r values are significant Pearson's correlations ($p < 0.05$

Differences between sites accounted for a portion of variation in the SEM, particularly for soil moisture, as shown by the increase from marginal R^2 to conditional R^2 values (Fig. 5.2b & B5.2). Conditional R^2 values account for the site random effect in addition to fixed effects, while marginal R^2 values account for fixed effects alone. Conditional R^2 values were 0.07–0.47 units greater than marginal R^2 values. Notably, 47% of soil moisture variation is explained by site. We used an additional SEM to assess whether annual rainfall and temperature might drive these site effects (Table A5.6). To ensure model fit, we also added a path from light to koa litter biomass. The data were well fit by the model based on Fisher's C ($p = 0.53$), $p > 0.05$ indicates good fit. Paths in this model were similar to those in the model without rainfall and temperature (Table A5.7). We found effects of rainfall and temperature on soil moisture ($r = 0.084$, $p < 0.0001$; $r = -0.268$, $p < 0.0001$, respectively) (Fig. B5.4) and a temperature effect on grass biomass driven primarily by Hilina Pali 1 and 2 sites where a grass species distinct from all other sites dominates (i.e., *Melinis minutiflora* P. Beauv. as opposed to *C. clandestinus* or *E. stipoides*).

To improve model fit, we added a direct path from koa density to grass biomass. This additional path indicates that there are further negative effects of koa density on grass biomass ($r = -0.093$, $p = 0.0004$) (Fig. 5.3a) not mediated through measured factors in the model.

Grass and litter cover shifted with koa stem density and the amount of light reaching the understory (Table A5.4, Fig. B5.3) to include greater koa litter cover. Cover of *C. clandestinus*, a relatively shade intolerant species (McDaniel and Ostertag 2010), decreased under koa canopies (relative to control plots) at the 8 sites where it was present and *E. stipoides* (a shade tolerant species) (McDaniel and Ostertag 2010) cover increased under koa canopies at 5 of the 6 sites where it is present (Table A5.5).

N mineralization rate was not associated with grass biomass as expected ($r = -0.028$, $p = 0.396$) and not influenced by koa stem density, koa litter biomass, or decomposition rate. Decomposition rate was not influenced by light ($r = -0.096$, $p = 0.093$) or soil moisture ($r = 0.039, 0.573$), nor did it affect koa litter biomass ($r = 0.001$, $p = 0.979$).

DISCUSSION

The net effect of koa on invasive grasses is suppression, both within and across sites (Fig. B5.5), and higher soil N with increasing koa density does not seem to facilitate grasses. Increasing koa stem density suppressed grasses via light reduction and greater koa litter biomass. Low soil moisture also suppressed grasses, but was associated with koa basal area, not stem density. Although reductions in grass biomass are significant, koa eliminated grass in only 2 out of 100 plots. More typically, substantial amounts of grass remained under koa canopies (Fig. 5.3b). A shift in composition to more shade tolerant grass species under dense koa canopies might allow grasses to persist (McDaniel and Ostertag 2010) and help maintain koa-grass states because grasses reduce recruitment of native forest species (Cabin et al. 2002a; Denslow et al. 2006). Thus, if total grass suppression and/or more diverse forests are management goals, then additional interventions (e.g., diverse understory plantings, herbicide application) are likely needed to amplify mechanisms of grass suppression and ensure conditions suitable for further recruitment of woody species after planting koa.

Multiple mechanisms contribute to grass suppression by koa. Increasing koa stem density decreased light levels, which in turn reduced grass biomass (Fig. 5.2b & 5.3c). This finding aligns with observations of reduced grass biomass with decreasing light levels on Hawai‘i Island (McDaniel and Ostertag 2010) and the importance of canopy cover in diminishing competition

with pasture grasses in reforestation work throughout the tropics (Holl et al. 2000; Zahawi et al. 2013). Light reduction to 5% full sun, which substantially reduces grass growth in this system (McDaniel and Ostertag 2010), was infrequently reached at our sites (Fig. 5.3d). Planted koa stands typically reduce light to 10–20% full sun (Scowcroft and Jeffrey 1999), which is relatively high compared to ~1.9–10% full sun in the understory of Hawaiian forests (Burton and Mueller-Dombois 1984) and other tropical forests (Canham et al. 1990; Record et al. 2016). Relatively high light transmission through koa canopies may be driven by the orientation of koa leaves. Most mature koa trees have canopies filled with koa phyllodes, not true leaves, which have chlorophyll on both sides and tend to be oriented vertically to capture light and conserve water (Baker et al. 2009). Incorporating additional tree, shrub and fern species into koa stands could create a more structurally complex canopy and might improve the ability of these restoration forests to lower light levels and thus grass biomass (Pretzsch 2014; Sapijanskas et al. 2014). Such an increase in diversity could lead to greater resource uptake and potentially invasion resistance as is often hypothesized (Naeem et al. 2000; Levine et al. 2004; Chazdon 2008). It is also important to note that while koa canopies reduce light levels and diminish grass biomass, shading is not a mechanism acting in isolation.

As leaves fall from koa canopies and accumulate into a litter layer, this layer also suppresses grasses. More leaf litter accumulates as koa density increases, which in turn reduces grass biomass (Fig. 5.2b & 5.3b). Light is substantially reduced beneath litter (Vazquez-Yanes et al. 1990). Leaf litter can physically impact understory vegetation by reducing establishment of new individuals and directly shading existing grass at the forest floor (Amatangelo et al. 2008; Barbier et al. 2008). In a temperate forest, leaf litter reduced light reaching the soil and decreased

the density of two annual grasses (Facelli and Pickett 1991). Here we find that koa leaves, both when in the canopy and after falling to the ground, decrease grass biomass.

We found no effect of decomposition rate on koa litter biomass (Table 5.2). Our decomposition measures were limited to a relatively short time span of four months during the dry season. Though substantial koa leaf decomposition can occur in a four-month span (Yelenik et al. *in review*), the rate of decomposition in the present study was likely limited by the dry season, as decomposition increases with precipitation (Austin and Vitousek 2000; Powers et al. 2009). Using leaves collected at each site (rather than a common substrate) to measure decomposition was the best approximation to actual decomposition rates, but it introduced variation because litter quality affects decomposition rates (Swift et al. 1979). Indeed, koa leaf C:N ranged from 18.7 – 30.3 across sites. This hampers our ability to say what aspects of site variation might drive changes in decomposition. Thus, although the physical effects of accumulating leaf litter are often balanced by decomposition rates (Barbier et al. 2008), we found no relationship between decomposition and koa litter biomass.

As koa litter decomposes it contributes N to the soil (Scowcroft et al. 2004). In contrast to expectations that N_2-fixing trees increase N cycling rates (Bernhard-Reversat 1988; Scowcroft et al. 2004; Yelenik et al. 2004), we found that neither the amount of litter nor density of koa affected N mineralization rates (Table 5.2). Additionally, N mineralization rates did not affect grass biomass. The lack of an effect of N on grasses runs counter to expectations of an association between N_2-fixing trees and understory vegetation facilitation (Blaser et al. 2013). Prior work suggests that high soil N under koa canopies drives the persistence of grasses, although we note the lack of variation in soil N in this study (Yelenik 2017). It may be that the presence of koa, regardless of density, speeds N cycling relative to non-fixing species in ways

that alter grass growth. Phosphorus could also become limiting in the presence of abundant N fixed by koa (Scowcroft et al. 2007, but see 2008) and other *Acacia* spp. (Ludwig et al. 2004; Sitters et al. 2013). Our study shows that suppression via litterfall and shading overwhelms any benefits for grasses of higher soil N with increasing koa density (Pearson correlation: $r = 0.23$, $p = 0.02$). Thus, when considering the balance between competition and facilitation in koa-grass interactions (Callaway and Walker 1997), the net effect of koa density on invasive grasses across our sites is negative and higher soil N does not appear to aid grasses.

Decreasing soil moisture reduced grass biomass, suggesting that competition for water is an important mechanism for grass suppression. This result is similar to studies from East Africa investigating interactions between *Acacia tortilis* and grasses in savanna (Ludwig et al. 2004). Koa basal area, but not stem density, was linked to reduced soil moisture ($r = -0.046$, $p = 0.055$; Table A5.3). However, in SEMs (with either basal area or stem density) much of the variation in soil moisture was accounted for by the random effect for site (Fig. 5.2b & B5.2). Site moisture regime may determine the effects of soil moisture. At dry sites, hydraulic lift of water by tree roots can increase soil moisture and lead to grass facilitation, while at wet sites, grasses and trees compete for soil water, as occurs in savannas (Dohn et al. 2013). Thus, though soil moisture is not clearly linked to koa stem density, competition for water may be an additional mechanism affecting grass biomass through competition or facilitation depending upon the site.

The addition of a direct path from koa density to grass biomass indicates there are further negative effects of koa density not accounted for in our measures. There may be belowground competition between koa and grass, similar to effects of savanna trees on grass at high rainfall sites (Belsky 1994). This additional path might also account for error in measures already in the model; many of our measurements represent a single snapshot in time. For example, light

filtering through the forest canopy is notoriously spatially and temporally variable (Théry 2001), making accurate measures of the light environment difficult. Soil moisture is also highly variable, being influenced by rainfall events and soil characteristics. Thus, our three measures of soil moisture throughout the study are a coarse approximation. Nonetheless, this work identifies two mechanisms by which increasing koa density reduces grass biomass, namely shading and litter accumulation, and found that reducing soil moisture may also be important.

A shift in grass composition to more shade tolerant species might act counter to mechanisms of grass suppression. We found that koa litter and grass cover changed as shade and stem density decreased (Table A5.4, Fig B5.3). Though we selected plots to exclude any woody species in the understory, herbaceous cover (primarily grasses) varied and was representative of each site. The shift in understory cover composition is likely driven in part by increasing koa litter cover but also a transition from shade intolerant grasses, like *C. clandestinus*, to shade tolerant grasses, such as *Ehrharta stipoides* Labill., just as McDaniel and Ostertag (2010) observed at Hakalau. As a bunch grass, *E. stipoides* may provide more opportunities for woody species recruitment than rhizomatous species like *C. clandestinus* and *M. minutiflora* that often form mats across the forest floor (Yelenik 2017). Thus, the mechanisms we observed might drive suppression of some grasses which are subsequently replaced by other more tolerant species, in a pattern of continual invasion (D'Antonio et al. 2017; Nsikani et al. 2019).

Our sites ranged across Hawai'i Island, encompassing varying abiotic conditions that could affect the efficacy of grass suppression by koa. For example, leaf production rate typically increases across the range of rainfall our sites spanned, potentially leading to more litter accumulation and thereby grass suppression, but this might be tempered by faster decomposition in wet conditions (Austin 2002). Thus, there might be more nuance involved in the mechanistic

patterns we identified. However, it is difficult to disentangle effects of site-level climate on grass biomass because along with rainfall and temperature, grass composition varied among sites. Given the breadth of variation our sites span in terms of climate and grass species, our findings are relatively applicable across the island and show a consistency in the mechanisms involved in grass suppression.

Although planting koa monocultures and raising koa density offer routes towards grass suppression, grass remains present in the understory of nearly all our plots (98 out of 100), much like other areas of regenerating koa (Scowcroft et al. 2008). Because grass cover is linked to negative effects on seedlings in tropical (Cabin et al. 2002a; Denslow et al. 2006) and temperate forests (Flory and Clay 2010) and little to no passive recruitment of woody species occurs among grasses under koa (Yelenik 2017; Rehm et al. 2019) additional management interventions are needed if restoration of diverse forest species assemblages is desired. A suite of management options can reinforce the mechanisms of shading, resource uptake, and litter accumulation and/or directly reduce grass biomass. Shading can be enhanced by planting other species with koa that cast deeper shade(McDaniel and Ostertag 2010). The effect of the litter layer on grass suppression can be strengthened by planting other native Hawaiian tree species, such as ʻōhiʻa, that produce litter that decomposes slower than koa litter (Scowcroft 1997; but see Yelenik et al. *in review*), thereby leaving greater standing biomass on the forest floor (Yelenik 2017). Grass biomass can be directly reduced through management techniques like applying herbicide (Cabin et al. 2002b; Elgar et al. 2014; Pinto et al. 2015). Similar to conclusions from Yelenik (2017), this study suggests that planting or facilitating more species in the understory to create a more structurally complex canopy, take up more resources, and increase litter inputs will ultimately help reduce invasive grass biomass (Naeem et al. 2000). The benefits of jumpstarting succession

by planting a diverse assemblage will likely extend beyond grass suppression and enhance ecosystem function and productivity (Chazdon 2008; Tuck et al. 2016).

In sum, we identified two mechanisms by which increasing koa density causes the net effect of suppressing grasses: shading and litter accumulation. Decreasing soil moisture may also suppress grasses, though its effects were more closely tied to site. The mechanisms of shading and litterfall were consistent across sites spanning a range of abiotic conditions and varied site history, demonstrating their ubiquity. However, total grass suppression rarely occurred, indicating that planting koa monocultures is an ineffective tool if inciting passive restoration of diverse forest communities is desired. Rather, planting additional species could be used to amplify mechanisms of grass suppression and create conditions favorable for further forest species recruitment.

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CHAPTER 6

Conclusion

Mature trees influence seedlings regenerating beneath their canopies through a myriad of pathways; some effects are direct, such as competition for resources like light or soil nutrients, while others are indirect and mediated through soil biota or interactions with other vegetation (Finzi et al. 1998a, b; Tinya and Ódor 2016; Bennett et al. 2017; Yelenik 2017). In this dissertation I examined two forms of indirect effects of trees on seedling regeneration. In chapters 2-4, I focused on plant-soil feedbacks (PSFs) and their legacies in Michigan forests focusing on two study species, *Prunus serotina* and *Acer rubrum*, and elucidated one mechanism by which *P. serotina* PSF legacies may occur. In chapter 5, I moved to a more applied context and focused on interactions between invasive grasses and a native tree species, *Acacia koa*, commonly planted for reforestation in Hawai‘i. In this system, suppression of exotic, invasive grasses is a necessary precursor to tree seedling recruitment and the restoration of native forests.

PSFs and their legacies

PSFs shape plant community dynamics via differential effects on species-specific plant performance (Klironomos 2002; Ehrenfeld et al. 2005; Mangan et al. 2010; Bauer et al. 2015). This dissertation research shows that conspecific PSFs can persist after tree death and influence seedling regeneration of two species, *P. serotina* (chapter 2) and *A. rubrum* (chapter 4).

Negative PSF legacies of *P. serotina* were short-lived, lasting < 1.5 years, restricted to low light (5% full sun) and likely driven by soil pathogens (chapter 2). Specifically, I isolated two oomycetes pathogenic to *P. serotina*, *Pythium intermedium* and *Pythium irregulare*. Both were present in soils up to 1.5 years after tree harvest, demonstrating their potential to impact

seedling regeneration (chapter 3). Despite their brief persistence, negative PSF legacies could restrict *P. serotina* seedling survivorship after conspecific tree death during a crucial post-disturbance time window for establishment of regeneration. More broadly, these findings demonstrate the potential for PSF legacy effects to impact seedling communities (under certain conditions) via the persistence of oomycetes in soil. Short persistence of PSF legacies, in this case, limits the duration of direct PSF legacy effects in community dynamics, but their overall effects on communities may be longer lasting through differential species establishment of tree seedlings immediately after disturbance.

A. rubrum PSFs were unexpectedly positive in conspecific relative to heterospecific soils, and their legacies appear to be relatively long lived (> 8 years) in conspecific soil (chapter 4). Variation between heterospecific PSFs was minimal and heterospecific PSF legacies could not be resolved. Positive *A. rubrum* PSFs and their legacies are likely mediated by a combination of distinct arbuscular mycorrhizal fungi (Lovelock and Miller 2002) and soil nutrient concentrations associated with different canopy trees (Finzi et al. 1998a, b). Multiyear PSF legacies suggest that legacy effect duration may vary depending upon the agents involved and lengthier duration signifies that soil signatures of long-gone trees may be another determinant of seedling regeneration success.

Furthermore, my results demonstrate that PSF legacies can vary widely from short-lived negative PSF legacies to long-lived positive PSF legacies depending upon the species involved (and possibly the environmental context). This wide range of variability in PSF legacy effect direction and persistence could be attributed to different mechanisms. PSFs, and therefore their legacies, can operate through many potential mechanisms, including alterations to soil nutrient cycling, allelochemicals, and soil biota (Packer and Clay 2000; Klironomos 2002; Stinson et al.

2006; Waring et al. 2015; Bennett et al. 2017) each with their own mode (e.g. spores, cysts) and potential duration of soil persistence (Martin and Loper 1999; Wardle et al. 2008; Nguyen et al. 2012). Thus, depending upon the plant species and PSF mechanisms involved, PSF legacies will likely manifest in distinct ways.

PSFs are dependent not only on the plant species and mechanisms involved, but also environmental context (Smith-Ramesh and Reynolds 2017; McCarthy-Neumann and Kobe 2019) and PSF legacies likely are as well. Conditions such as higher light levels could overwhelm effects of negative PSFs and therefore their legacies, as I found in chapter 2. Understanding whether these patterns hold true outside of the greenhouse would be important for managing forests with PSF legacies in mind. The work in this dissertation was designed to maximize the chances of detecting PSF legacies by isolating the effects of solitary tree death through selection harvests and minimizing soil disturbance. Yet oftentimes disturbance and harvest regimes occur at much larger scales (Sommerfeld et al. 2018). Thus, it is important to explore additional forms of disturbance causing tree death, which may leave a patchwork of soil legacies behind (Døckersmith et al. 1999) or create conditions that overwrite signatures of past trees through a drastic turnover in environment and plant communities.

Hawaiian forest restoration

In evaluating the balance between invasive grass suppression and facilitation by an N-fixing tree, *A. koa* (koa), I found that increasing koa density suppresses grasses through two processes, greater shading and litter accumulation, and any facilitation by higher soil N was overwhelmed by these suppressive effects (chapter 5). Decreasing soil moisture may also contribute to grass suppression but was more closely linked to site than koa density. However, koa did not consistently lead to total grass suppression and grass suppression is necessary if

further passive seedling regeneration under koa canopies is desired (Yelenik 2017; Rehm et al. 2019). Thus, when restoring Hawaiian forests, if planting koa is intended to 'jumpstart succession' and create conditions for the passive reestablishment of a diverse native forest, additional management interventions are needed to amplify mechanisms of grass suppression.

Despite expectations of soil N fertilization by N-fixers facilitating exotic invasive species (Maron and Connors 1996; Sierra and Nygren 2006) this work suggests that other characteristics lead to the persistence of grass under koa canopies. Although koa canopies shaded grasses, light transmission through koa canopies is relatively high (Scowcroft and Jeffrey 1999) and grass composition shifted to more shade tolerant species. Rather than initiating tree planting with koa monocultures, future work could include additional species to grow a more structurally complex canopy and create conditions more favorable for seedling regeneration. More broadly, this work invites consideration of species' characteristics, such as litter quality and shade tolerance, and their interactions in restoration work.

Conclusions and Synthesis

Mature trees affect seedling regeneration through innumerable direct and indirect paths. Through this dissertation I have examined how tree effects on soils feedback and impact seedlings even after trees are dead, and also identified mechanisms of grass suppression and facilitation by trees in a restoration context to better understand how to bring about conditions for successful seedling recruitment. These effects on seedling regeneration are indirect and, for PSFs and PSF legacies, mediated through soils and biota, or through competitive exclusion of woody seedlings by grasses in the case of restoring forests with koa.

Across this work, it is clear that considering the characteristics of plants involved, such as mycorrhizal association, shade tolerance, depth of shade cast, and litter quality, is important in

predicting the outcome of interactions. As I found in Hawai‘i, planting a single species was insufficient to completely suppress invasive grasses, but complementing the impacts of one species with greater diversity that reinforces mechanisms of grass suppression is a potential route forward. For PSF legacies, this dissertation demonstrated both their occurrence and variability. Though it has long been known that soil diseases or nutrient depletions can persist after plant death and affect subsequent plants in the same soils (a motivation for crop rotation in agriculture), the persistence of individual tree effects on soils as PSF legacies that are sufficient to affect seedling survival is novel and demonstrates their relevance for determining future forest composition. The variability of potential strength, direction, and duration of PSF legacies invites further research to determine the extent and magnitude of PSF legacy effects on plant communities. Studies of PSF legacies may also transfer from forest dynamics to restoration, where soil signatures of past plants may likewise shape the success of future recruits. With PSFs and their legacies, just as with koa forest restoration, considering species' characteristics, their ongoing interactions with soils and other vegetation, and legacies of these impacts on the landscape can further elucidate constraints on forest regeneration.

APPENDICES

APPENDIX A.

Tables

Table A2.1 Seedling mortality events in the greenhouse divided into before (pre-emergence) or after (post-emergence) seedlings emerged above the soil. Light levels were applied in the greenhouse using shade cloth to reduce light to 5 and 30 % full sun for low and high light respectively. Sites are Russ Forest (RF), Lux Arbor (LA), DeWitt (DW), and Rose Dell (RD). Soil sampling distance occurred near and far from each live tree or stump. Near cores were removed within 2 meters. Far cores were sampled at least 20 meters from the focal individual (live tree/stump), 5 meters from other *P. serotina* trees or stumps, and 4 meters from any trees greater than 5 cm diameter at breast height.

Light	Site	Soil Source	Distance	N	Pre-emergence	Post-emergence
High	RF	live tree	near	11	4	4
			far	12	3	3
		stump	near	20	3	12
			far	20	4	6
	LA	live tree	near	12	4	4
			far	12	7	4
		stump	near	24	8	8
			far	24	6	7
	DW	live tree	near	12	5	3
			far	12	4	6
		stump	near	20	9	7
			far	19	3	11
	RD	live tree	near	12	8	3
			far	12	4	5
		stump	near	20	7	7
			far	19	5	7
Low	RF	live tree	near	12	2	10
			far	10	0	8
		stump	near	20	4	16
			far	20	2	10
	LA	live tree	near	12	7	5
			far	12	6	6
		stump	near	24	10	11
			far	22	2	16
	DW	live tree	near	12	5	7
			far	12	3	7
		stump	near	20	9	7
			far	20	4	14
	RD	live tree	near	12	4	8
			far	12	4	8
		stump	near	20	5	12
			far	20	5	13

Table A2.2 Estimates from a linear model of dry-weight biomass (g) of surviving seedlings. Light levels, sites, and soil sampling distances are described in Table A2.1. An asterisk denotes statistical significance ($p < 0.05$).

	Estimate ± Std. Error
Light: Low vs High	-0.122 ± 0.05
Overall site effect	
Site: LA	0.018 ± 0.007
Site: DW	-0.030 ± 0.007
Site: RD	-0.018 ± 0.100
Seed Mass (g)	0.763 ± 0.190 *
Far vs Live Trees (nested w/n site)-assessing PSFs	
Site: RF	0.037 ± 0.053
Site: LA	0.011 ± 0.048
Site: DW	0.063 ± 0.051
Site: RD	-0.006 ± 0.090
Stumps vs Live Trees (nested w/n site)-assessing PSF legacies	
Site: RF: (0.5-yr-old stumps)	0.020 ± 0.063
Site: LA: (1.5-yr-old stumps)	-0.00007 ± 0.050
Site: DW: (3.5-yr-old stumps)	0.039 ± 0.054
Site: RD: (~15-yr-old stumps)	0.037 ± 0.091

Table A2.3 Dry-weight biomass (g) and sample size of surviving seedlings.

Light	Site	Soil Source	Distance	N	Mean Biomass (g) ± Std. Error
High	RF	live tree	near	3	0.176 ± 0.126
			far	6	0.228 ± 0.032
		stump	near	5	0.232 ± 0.101
			far	10	0.185 ± 0.033
	LA	live tree	near	4	0.209 ± 0.104
			far	1	0.144
		stump	near	8	0.153 ± 0.044
			far	11	0.212 ± 0.033
	DW	live tree	near	4	0.145 ± 0.031
			far	2	0.118 ± 0.049
		stump	near	4	0.188 ± 0.053
			far	5	0.231 ± 0.042
	RD	live tree	near	1	0.079
			far	3	0.164 ± 0.049
		stump	near	6	0.146 ± 0.06
			far	7	0.155 ± 0.014
Low	RF	live tree	far	2	0.09 ± 0.024
		stump	far	8	0.063 ± 0.011
	LA	stump	near	3	0.05 ± 0.009
			far	4	0.055 ± 0.02
	DW	live tree	far	2	0.022 ± 0.018
		stump	near	4	0.072 ± 0.024
			far	2	0.083 ± 0.011
	RD	stump	near	3	0.067 ± 0.023
			far	2	0.061 ± 0.024

Table A2.4 Results of a PERMANOVA examining soil nutrients (sum of base cations, phosphate, C:N ratio, and total inorganic N (ammonium + nitrate)) using Bray-Curtis dissimilarities with site as a random effect, based on 999 permutations.

	Df	Sum of Squares	R^2	P-value
Stump vs Live Tree	1	0.055	0.024	0.35
Residuals	31	2.287	0.976	
Total	32	2.342	1.000	

Table A3.1 Oven-dried biomass (g) and sample size of surviving seedlings.

Site	Soil Source	N	Mean Biomass (g) ± Std. Error
RD	live tree	33	0.067 ± 0.008
	0.5-yr-old stump	27	0.066 ± 0.007
RF	live tree	49	0.073 ± 0.007
	0.5-yr-old stump	38	0.093 ± 0.008
	1.5-yr-old stump	37	0.086 ± 0.007

Table A3.2 Results of pairwise PERMANOVAs examining soil nutrients (sum of base cations, phosphate, C:N ratio, and total inorganic N (ammonium + nitrate)) using Bray-Curtis dissimilarities with site as a random effect, based on 999 permutations.

Comparison		Df	Sum of Squares	R^2	p-value
Live Trees vs 0.5-year-old stumps		1	0.081	0.054	0.19
	Residuals	23	1.405	0.946	
	Total	24	1.485	1.000	
Live Trees vs 1.5-year-old stumps		1	0.213	0.187	0.09
	Residuals	18	0.927	0.813	
	Total	19	1.140	1.00	
0.5-year-old stumps vs 1.5-year-old stumps		1	0.409	0.393	**0.03**
	Residuals	13	0.630	0.607	
	Total	14	1.039	1.000	

Table A3.3 Odds ratios from logistic regression of oomycete species detection from both baiting experiments. P-values are in parentheses. Each column corresponds to a separate model. *Py.* = *Pythium*, *Pp.* = *Phytopythium*.

	Pp. vexans	*Py. irregulare*	*Py. intermedium*
RF:RD (sites)	0.34 (0.41)	0.93 (0.92)	0.20 (0.06)
Study	2.55 (0.28)	2.10 (0.15)	9.25 **(0.0003)**
Site RD: 0.5 yr old stump vs live	0.30 (0.35)	1.06 (0.94)	1.34 (0.72)
Site RF: 0.5 yr old stump vs live	1.91 (0.62)	1.58 (0.56)	3.00 (0.24)
Site RF: 1.5 yr old stump vs live	0.66 (0.71)	0.17 (0.13)	1.29 (0.80)

Table A3.4 Odds ratios from logistic regression of oomycete species detection based on status (symptomatic vs asymptomatic) of seedlings grown in soil cores in greenhouse. P-values are in parentheses. Each column corresponds to a separate model. *Py.* = *Pythium*, *Pp.* = *Phytopythium*.

	Pp. vexans	*Py. irregulare*	*Py. intermedium*
Status	0.15 (0.11)	1.36 (0.66)	0.67 (0.70)

Table A3.5 Odds ratios comparing effectiveness of different bait materials in detecting three species of oomycetes in loose field soils and soils surrounding seedlings grown in soil cores in the greenhouse. Comparisons were made using Tukey's HSD in a post-hoc test following analysis with a negative binomial model. P-values are in parentheses. Each column corresponds to a separate model. *Py.* = *Pythium*, *Pp.* = *Phytopythium*, *Ph.* = *Phytophthora*.

	Pp. vexans	*Py. irregulare*	*Py. intermedium*	*Ph. citricola*
A. stolonifera - Pi. japonica	0.53 **(0.004)**	2.76 (0.28)	7.3 **(0.004)**	-
A. stolonifera - S. cereale	0.44 **(0.0001)**	0.45 (0.16)	1.41 (0.52)	-
Pi. japonica - S. cereale	0.83 (0.44)	0.16 **(0.005)**	0.19 **(0.02)**	6.96 **(0.02)**

Table A3.6 Counts of isolates assigned to each species acquired by baiting from: 1) loose field soils from 30 stumps and live trees, 2) 21 pairs of symptomatic and asymptomatic seedlings grown in soil cores removed from the same locations as loose field soil samples. *Py.* = *Pythium*, *Pp.* = *Phytopythium*, *Ph.* = *Phytophthora*.

Species	**# of cultures from:**	
	Loose Field Soil	Seedling Soil Cores
Py. irregulare	30	13
Py. intermedium	42	14
Pp. vexans (group 1)	114	108[2]
	19[2]	
Pp. vexans (group 2)	81	
Ph. citricola I[1]	6	13[2]
Ph. citricola I/III[1]	5	
Py. salpingophorum/ conidiophorum	1	0

1) Subgroups based on Jung and Burgess, 2009
2) Isolates grouped directly into species based on morphology prior to discernment of two genetically distinct groupings

Table A3.7 Counts of isolates from pairs (N=21) of symptomatic and asymptomatic greenhouse-grown *Prunus serotina* seedlings in soil cores removed from the same locations as loose field soil samples broken down by seedling status (asymptomatic or symptomatic). *Py.* = *Pythium*, *Pp.* = *Phytopythium*, *Ph.* = *Phytophthora*.

	# of cultures from seedling soil cores	
Species	Asymptomatic	Symptomatic
Py. irregulare	7	6
Py. intermedium	6	8
Pp. vexans (group 1)	50	58
Pp. vexans (group 2)		
Ph. citricola I	4	9
Ph. citricola I/III		
Py. salpingophorum/ conidiophorum	0	0

Table A4.1. Harvest ages for each species and sites where soil was sampled. Sites beginning with UP and LP refer to Michigan's Upper Peninsula and Lower Peninsula respectively. Stump ages are in italics. Soil was sampled from six stumps and three live trees of each species for each harvest time.

Harvest Time	*A. saccharum*	*A. rubrum*	*P. serotina*	*B. alleghaniensis*	*T. americana*	*Q. rubra*
< 2 yrs.	*0.5 yrs.* **Site:** UP1	*0.5 yrs.* **Site:** UP1	*0.5 yrs.* **Site:** UP1	*0.5 yrs.* **Site:** UP2	*1.5 yrs.* **Site:** LP1	*1.5 yrs.* **Site:** LP1
2–4 yrs.	*2.0 yrs.* **Site:** UP3	*2.0 yrs.* **Site:** UP3	*2.0 yrs.* **Site:** UP3	*2.0 yrs.* **Site:** UP3	*3.5 yrs.* **Site:** LP2	*3.5 yrs.* **Site:** LP2
5–6.5 yrs.	*5.0 yrs.* **Site:** UP4	*5.0 yrs.* **Site:** UP4	*5.0 yrs.* **Site:** UP4	*5.0 yrs.* **Site:** UP4	*6.5 yrs.* **Site:** LP3	*6.5 yrs.* **Site:** LP3
> 7 yrs.	*7.0 yrs.* **Site:** UP5	*8.0 yrs.* **Site:** UP6	*7.0 yrs.* **Site:** UP5	*14.0 yrs.* **Site:** UP7	*8.5 yrs.* **Site:** LP4	*8.5 yrs.* **Site:** LP4

Table A4.2 Estimates from a linear model of dry-weight biomass (g) of surviving *A. rubrum* seedlings grown in soils from six tree species. All soil sources are referenced against *A. rubrum* soils.

	Estimate	**Std. Error**	**p-value**
Intercept	0.012	0.0005	**< 0.001**
P. serotina	-0.0032	0.001	**0.007**
T. americana	-0.0035	0.001	**0.002**
Q. rubra	-0.0039	0.001	**0.007**
A. saccharum	-0.0029	0.001	**0.02**
B. alleghaniensis	-0.0047	0.001	**< 0.001**

Table A4.3 Hazard ratios from a Cox proportional hazards survival model of *A. rubrum* seedlings grown in soils from 6 tree species. All soil sources are referenced against *A. rubrum* soils. Live tree versus stump soil comparisons are made for each species using a nested term. Model includes random effects for site, greenhouse bench and individual live tree or stump.

	Hazard Ratio	Std. Error	p-value
T. americana	0.897	0.299	0.72
Q. rubra	1.092	0.289	0.76
A. saccharum	1.327	0.279	0.31
B. alleghaniensis	0.869	0.288	0.62
P. serotina: Live Tree vs. Stump	1.332	0.325	0.38
T. americana: Live Tree vs. Stump	0.761	0.333	0.41
Q. rubra: Live Tree vs. Stump	1.494	0.281	0.15
A. saccharum: Live Tree vs. Stump	0.589	0.360	0.14
B. alleghaniensis: Live Tree vs. Stump	1.346	0.331	0.37

a) Hazard ratios > 1 represent an increase in hazard for that parameter.

Table A4.4 Hazard ratios from a Cox proportional hazards survival model of *A. rubrum* seedlings grown in *A. rubrum* live tree and stump soils of four ages. Model includes a random effect for greenhouse bench and individual tree or stump.

	Hazard Ratio[a]	Std. Error	p-value
2.0 yr old harvest site	0.972	0.379	0.94
5.0 yr old harvest site	1.106	0.379	0.79
8.0 yr old harvest site	1.032	0.383	0.93
0.5 yr old harvest site: live tree vs. stump	1.380	0.478	0.50
2.0 yr old harvest site: live tree vs. stump	0.882	0.493	0.80
5.0 yr old harvest site: live tree vs. stump	1.099	0.448	0.83
8.0 yr old harvest site: live tree vs. stump	0.556	0.526	0.26

a) Hazard ratios > 1 represent an increase in hazard for that parameter.

Table A4.5

A) Estimates from a linear model of soil pH from live trees and stumps of 6 tree species, all referenced against *A. rubrum* soils. Site is included as a random effect.

	Estimate	Std. Error	p-value
P. serotina	0.200	0.108	0.07
T. americana	1.155	0.201	**<0.001**
Q. rubra	0.545	0.202	**0.02**
A. saccharum	0.311	0.108	**0.005**
B. alleghaniensis	0.178	0.117	0.13
Live Tree vs. Stump	-0.032	0.061	0.60

B) Pairwise comparisons of pH from soils of 6 tree species.

Contrast	Estimate	Std. Error	p-value
A. rubrum - T. americana	-1.155	0.201	**0.001**
A. rubrum - P. serotina	-0.200	0.110	0.45
A. rubrum - Q. rubra	-0.545	0.202	0.15
A. rubrum - A. saccharum	-0.311	0.110	0.06
A. rubrum - B. alleghaniensis	-0.178	0.119	0.67
T. americana - P. serotina	0.955	0.202	**0.006**
T. americana - Q. rubra	0.610	0.101	**<0.001**
T. americana - A. saccharum	0.844	0.202	**0.01**
T. americana - B. alleghaniensis	0.977	0.204	**0.005**
P. serotina - Q. rubra	-0.345	0.203	0.56
P. serotina - A. saccharum	-0.111	0.099	0.88
P. serotina - B. alleghaniensis	0.022	0.117	1.0
Q. rubra - A. saccharum	0.234	0.203	0.85
Q. rubra - B. alleghaniensis	0.367	0.205	0.51
A. saccharum - B. alleghaniensis	0.133	0.117	0.86

Table A5.1 Substrate age and soil classification for each study site.

Site	Substrate Age[1]	Soil Classification[2]
Hakalau	11,000-64,000 yrs. Laupāhoehoe flows	Acrudoxic Hydrudands
Kahuku 1	1500-3000 yrs. Ka‘ū Basalt	Lithic Haplustands
Kahuku 2	1500-3000 yrs. Ka‘ū Basalt	Humic Haplustands
Kahuku 3	1500-3000 yrs. Ka‘ū Basalt	Humic Haplustands
Hilina Pali 1	1500-3000 yrs. Puna Basalt	Lithic Haplustands & Vitric Haplustands
Hilina Pali 2	750-1500 yrs. Puna Basalt	Lithic Haplustands & Vitric Haplustands
Kona Hema 1	1500-3000 yrs. Ka‘ū Basalt	Typic Ustifolists
Kona Hema 2	1500-3000 yrs. Ka‘ū Basalt	Typic Ustifolists
Pu‘u Wa‘awa‘a 1	1500-3000 yrs. Hualālai Volcanics	Lithic Haplustands
Pu‘u Wa‘awa‘a 2	1500-3000 yrs. Hualālai Volcanics	Lithic Ustifolists

1) Wolfe and Morris 1990
2) Web Soil Survey 2020

Table A5.2 List of model statements from SEM. Two models were run, one with koa basal area and the other with koa stem density.

Response	Predictor(s)
Grass biomass	~ Koa litter biomass + Net N mineralization + Light + Soil moisture
Koa litter biomass	~ Koa basal area/stem density + Decomposition
Net N mineralization	~ Koa litter biomass + Koa basal area/stem density + Decomposition
Decomposition	~ Soil moisture + Light
Soil moisture	~ Koa basal area/stem density
Light	~ Koa basal area/stem density

Table A5.3 Parameter estimates for each path in the SEM using koa basal area. Significant p-values (< 0.05) are in bold.

Response	Predictor	Path Coefficient	Std. Error	DF	p-value
Grass biomass	Koa litter biomass	-0.124	0.029	97	**< 0.0001**
Grass biomass	Light	0.090	0.029	97	**0.0016**
Grass biomass	Soil moisture	0.073	0.048	97	0.123
Grass biomass	N mineralization rate	-0.020	0.031	97	0.512
Grass biomass	Koa basal area	-0.116	0.022	97	**< 0.0001**
Koa litter biomass	Koa basal area	0.215	0.057	72	**0.0001**
Koa litter biomass	Decomposition	0.042	0.049	72	0.387
N mineralization rate	Koa litter biomass	0.050	0.051	71	0.328
N mineralization rate	Koa basal area	0.032	0.051	71	0.528
N mineralization rate	Decomposition	0.074	0.049	71	0.129
Light	Koa basal area	-0.088	0.036	100	**0.016**
Soil moisture	Koa basal area	-0.046	0.024	100	0.055
Decomposition	Light	-0.096	0.057	74	0.093
Decomposition	Soil moisture	0.0394	0.070	74	0.573

Table A5.4 Results of a PERMANOVA examining composition of the understory of koa plots based on percent cover using Bray-Curtis dissimilarities with site as a random effect, based on 999 permutations.

	Df	Sum of Squares	R^2	p-value
Koa stem density	1	5.74	0.07	0.001
Light	96	69.79	0.83	0.001
Residuals	202	8.13	0.10	
Total	299	83.67	1.00	

Table A5.5 Percent cover based on visual estimation of understory of koa plots by site. Each column sums to 100% cover and data for each site represents 10 koa plots each sampled with 3 quadrats.

	Hakalau	Hilina Pali 1	Hilina Pali 2	Kahuku 1	Kahuku 2	Kahuku 3	Kona Hema 1	Kona Hema 2	Pu‘u Wa‘awa‘a 1	Pu‘u Wa‘awa‘a 2
Monocots										
Commelinaceae										
Commelina diffusa	0	0	0	0.18	0	0	0	0	0	0
Cyperaceae										
Carex wahuensis	0	0	0	0	0	0	0.44	0	0	0
unknown sedge	0	0	0	0.67	0	0	0	0	0	0
Poaceae										
Andropogon sp.	0	0	0	0	0.22	0	0	0	0	0
Anthoxanthum odoratum	15.16	0	0	0	0	0	0	0	0	0
Axonopus fissifolius	0.22	0	0	0	0	0	0	0	0	0
Dactylis glomerata	0	0	0	0	0	0	0	0	11.16	0.89
Digitaria eriantha	0	0	0	25.38	0	0	0	0	0	0
Holcus lanatus	31.42	0	0	0	0	0	0	0	0.56	1.04
Cenchrus clandestinus	18.60	0	0	43.27	52.31	63.69	58.27	84.69	43.02	47.47
Ehrharta stipoides	17.29	0	0	0	3.44	0	20.69	3.16	34.82	39.16
Melinis minutiflora	0	91.73	93.16	0	0	0	0	0	0	0
Paspalum dilatatum	0	0	0	0.44	0.33	0	0	0.11	0	0
Schizachyrium condensatum	0	0	0.36	0	0	0	0	0	0	0
unknown	0	0	0	0	0	0	0	0	3.58	0.67
Dicots										
Fabaceae										
Desmodium incanum	0	0.31	0	0	0	0	0	0	0	0
Oxalidaceae										
Oxalis sp.	0	0	0	0	0	0	0	0	0.04	0.02
Polygonaceae										
Persicaria capitata	0	0	0	0	0	0	0.22	1.13	0	0
Rumex acetosella	0	0	0	0	0	0	0.07	0	0	0
Leaf Litter										
Acacia koa litter	17.31	7.96	6.49	30.07	43.38	36.31	19.49	10.91	6.82	10.76
Metrosideros polymorpha litter	0	0	0	0	0.38	0	0	0	0	0
Rock	0	0	0	0	0	0	0.82	0	0	0

Table A5.6 List of model statements from SEM with additional parameters, rainfall and temperature.

Response	Predictor(s)
Grass biomass	~ Koa litter biomass + Net N mineralization + Light + Soil moisture + Temperature + Rainfall
Koa litter biomass	~ Koa basal area/stem density + Decomposition + Temperature + Rainfall + Light
Net N mineralization	~ Koa litter biomass + Koa basal area/stem density + Decomposition + Temperature + Rainfall
Decomposition	~ Soil moisture + Light + Temperature + Rainfall
Soil moisture	~ Koa basal area/stem density + Temperature + Rainfall
Light	~ Koa basal area/stem density

Table A5.7 Parameter estimates for each path included in the SEM using koa stem density with additional paths for temperature and rainfall. Significant p-values (< 0.05) are in bold.

Response	Predictor	Path Coefficient	Std. Error	DF	p-value
Grass biomass	Koa litter biomass	-0.129	0.031	97	**< 0.0001**
Grass biomass	Light	0.064	0.032	97	**0.043**
Grass biomass	Soil moisture	0.153	0.047	97	**0.001**
Grass biomass	N mineralization rate	-0.039	0.035	97	0.262
Grass biomass	Koa stem density	-0.094	0.026	97	**0.0003**
Grass biomass	Temperature	0.262	0.074	97	**0.0004**
Grass biomass	Rainfall	0.019	0.067	97	0.776
Koa litter biomass	Koa stem density	0.110	0.051	72	**0.031**
Koa litter biomass	Decomposition	-0.020	0.049	72	0.683
Koa litter biomass	Temperature	0.070	0.084	72	0.407
Koa litter biomass	Rainfall	-0.170	0.100	72	0.089
Koa litter biomass	Light	-0.202	0.058	72	**0.0005**
N mineralization rate	Koa litter biomass	0.068	0.050	71	0.178
N mineralization rate	Koa stem density	-0.019	0.048	71	0.702
N mineralization rate	Decomposition	0.076	0.052	71	0.143
N mineralization rate	Temperature	-0.222	0.118	71	0.060
N mineralization rate	Rainfall	0.087	0.143	71	0.541
Light	Koa stem density	-0.179	0.036	100	**< 0.0001**
Soil moisture	Koa stem density	0.005	0.007	100	0.449
Soil moisture	Temperature	-0.268	0.007	100	**< 0.0001**
Soil moisture	Rainfall	0.084	0.007	100	**< 0.0001**
Decomposition	Light	-0.078	0.056	74	0.165
Decomposition	Soil moisture	-0.034	0.074	74	0.646
Decomposition	Temperature	-0.128	0.075	74	0.0866
Decomposition	Rainfall	-0.022	0.070	74	0.7543

APPENDIX B.

Figures

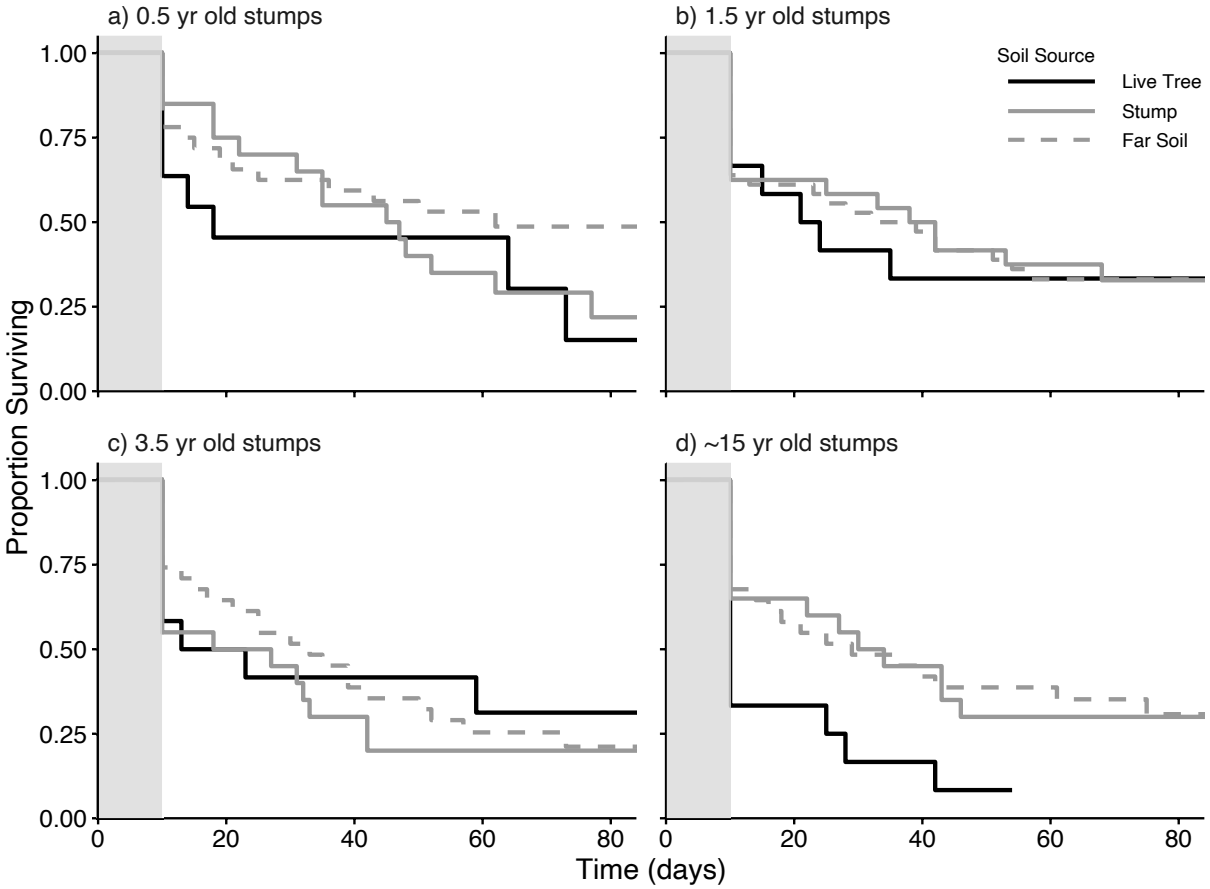

Figure B2.1 Survival curves of *P. serotina* seeds/seedlings in high light (30% full sun). Gray band (0-10 days) covers time between planting seed with radicle to aboveground emergence. Far soils represent background forest soil conditions without the influence of any individual tree. Comparing survival curves of far soils and live tree soils within a site evaluates PSFs, while comparing stump soil and live tree soil survival curves within a site assesses PSF legacy effects. Panels show different times since *P. serotina* tree removal: a) 0.5 yrs at Russ Forest (RF), b) 1.5 yrs at Lux Arbor (LA), c) 3.5 yrs at DeWitt (DW), and d) ~15 yrs at Rose Dell (RD).

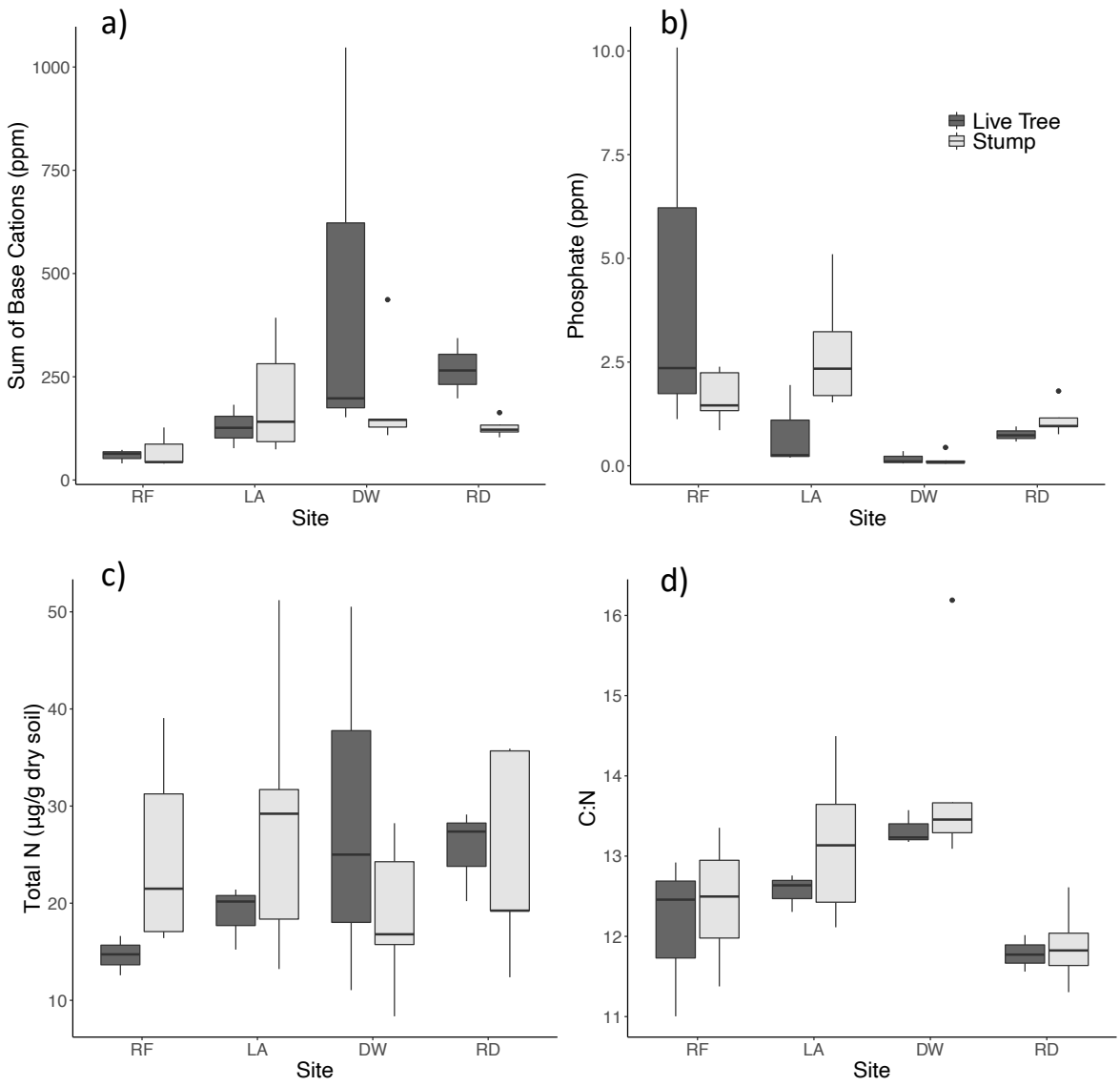

Figure B2.2 Nutrient concentrations of soil samples (1-10 cm deep) collected around each live tree and stump. Panels depict various soil nutrients: a) sum of base cations (Ca, K, & Mg) (ppm), b) phosphate (ppm), c) total inorganic N (nitrate + ammonium) (µg/g dry soil), and d) C:N ratio. Sites are Russ Forest (RF), Lux Arbor (LA), DeWitt (DW), and Rose Dell (RD).

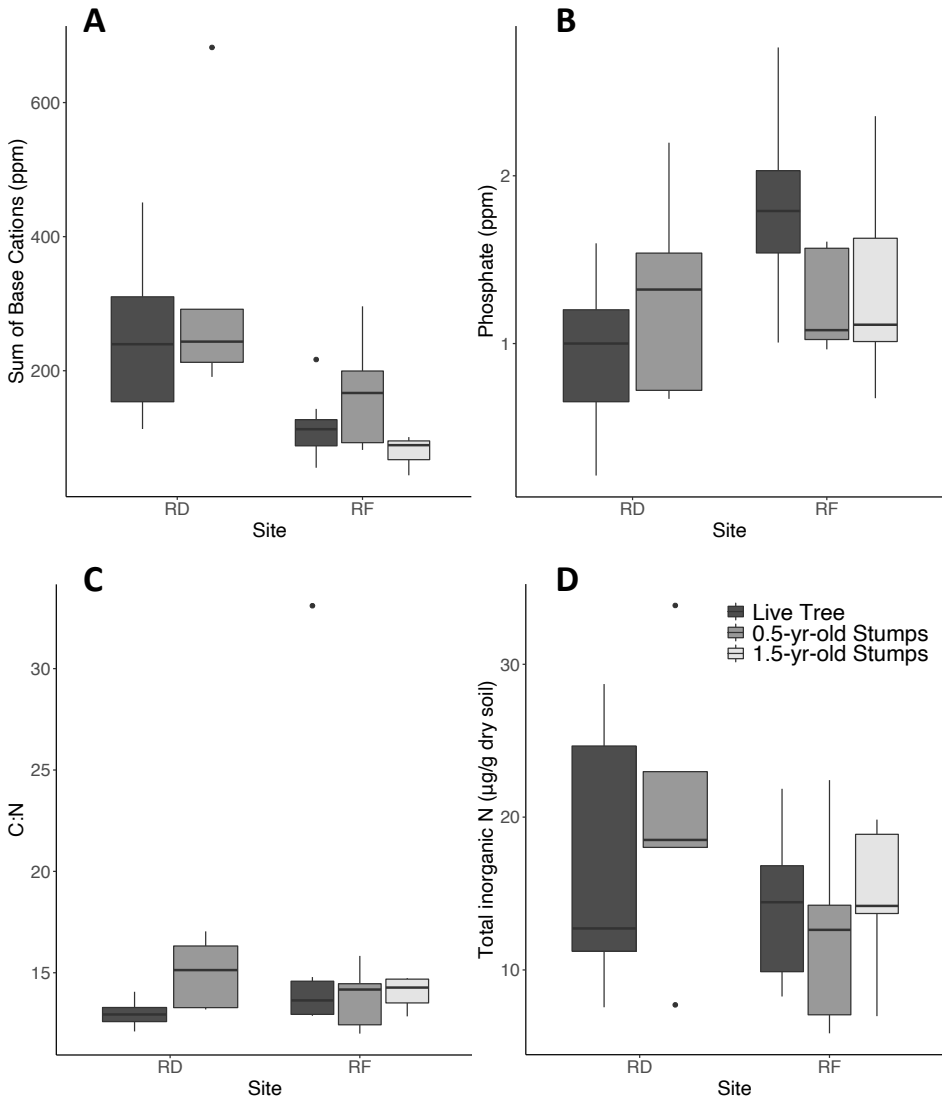

Figure B3.1 Nutrient concentrations from soils (1-10 cm deep) around each live tree and stump. Each panel shows a different soil nutrient: a) sum of base cations (Ca, K, & Mg) (ppm), b) phosphate (ppm), c) total inorganic N (nitrate + ammonium) (µg/g dry soil), and d) C:N ratio.

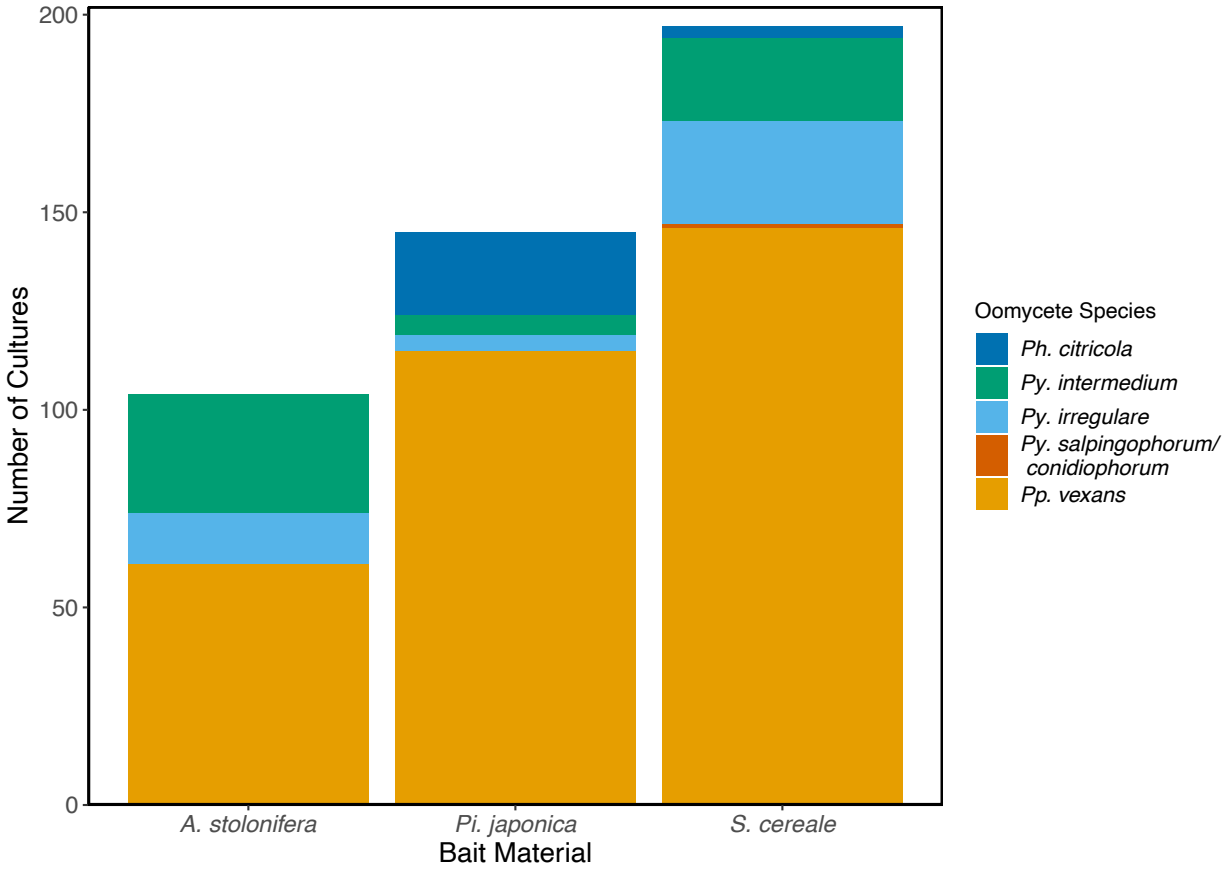

Figure B3.2 Comparison of oomycetes colonizing each bait material. Error bars depict the standard error of the mean. Order of oomycete species in legend corresponds to order of species in stacked bars. *Py.* = *Pythium*, *Pp.* = *Phytopythium*, *Ph.* = *Phytophthora*, *A.* =*Agrostis*, *Pi.* = *Pieris*, *S.* = *Secale*.

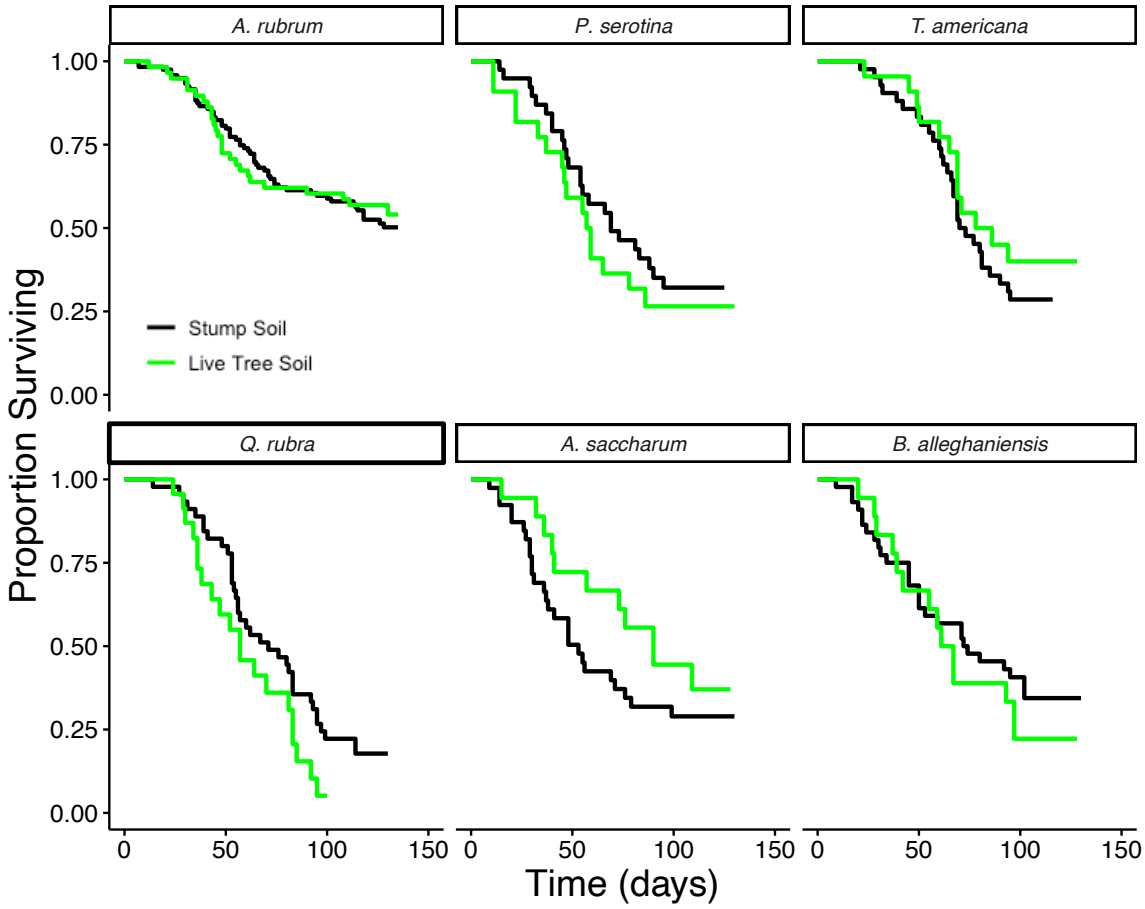

Figure B4.1. Survival curves of greenhouse grown *A. rubrum* seedlings in live tree and stump soils sourced from six tree species. Stumps ranged in age from 0.5 to 14 years since harvest and all stump ages are pooled. Comparing stump and live tree soils evaluates PSF legacies.

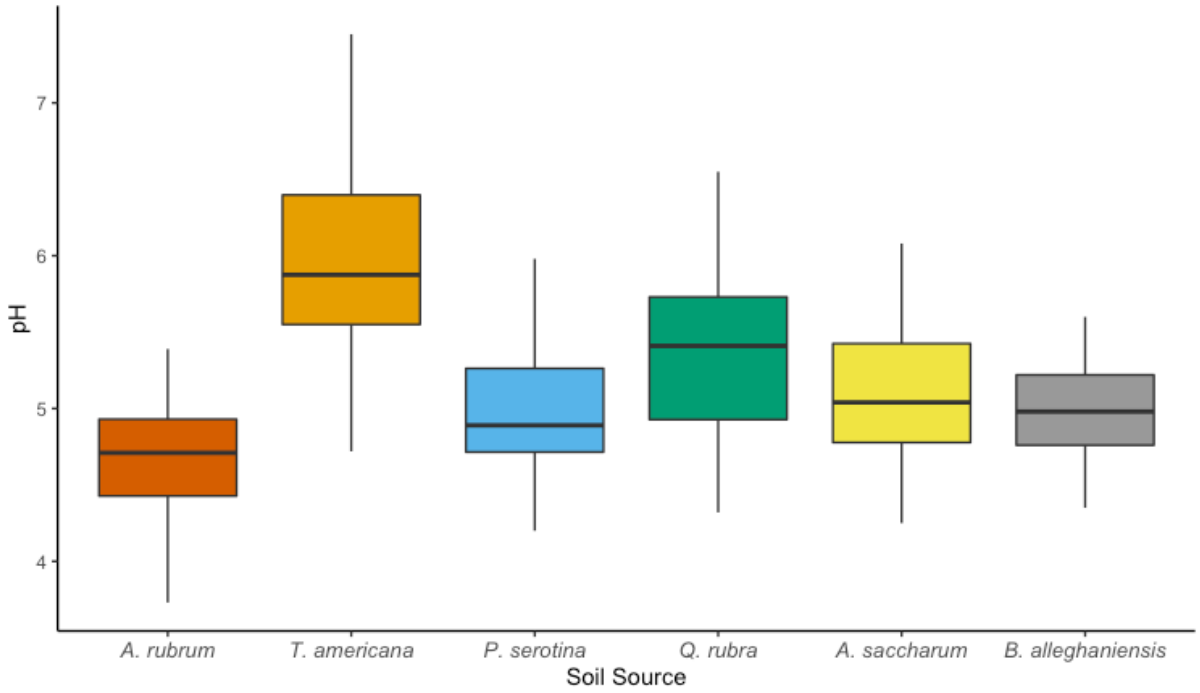

Figure B4.2 Soil pH of soils sourced from live trees and stumps of 6 tree species.

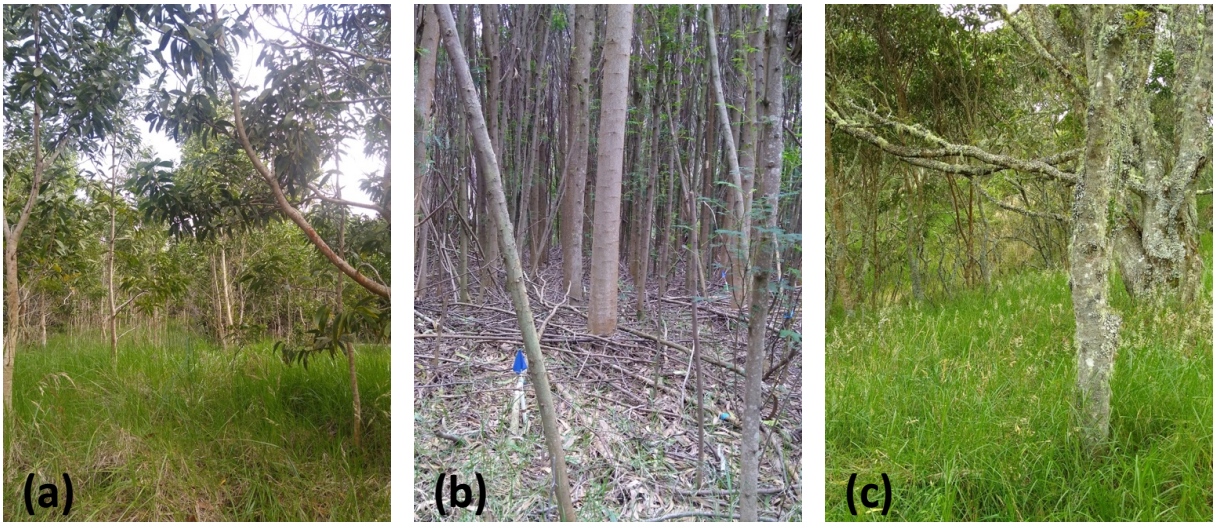

Figure B5.1 Photos of koa plots of varying densities from (a) Kona Hema, (b) Kahuku, and (c) Hakalau.

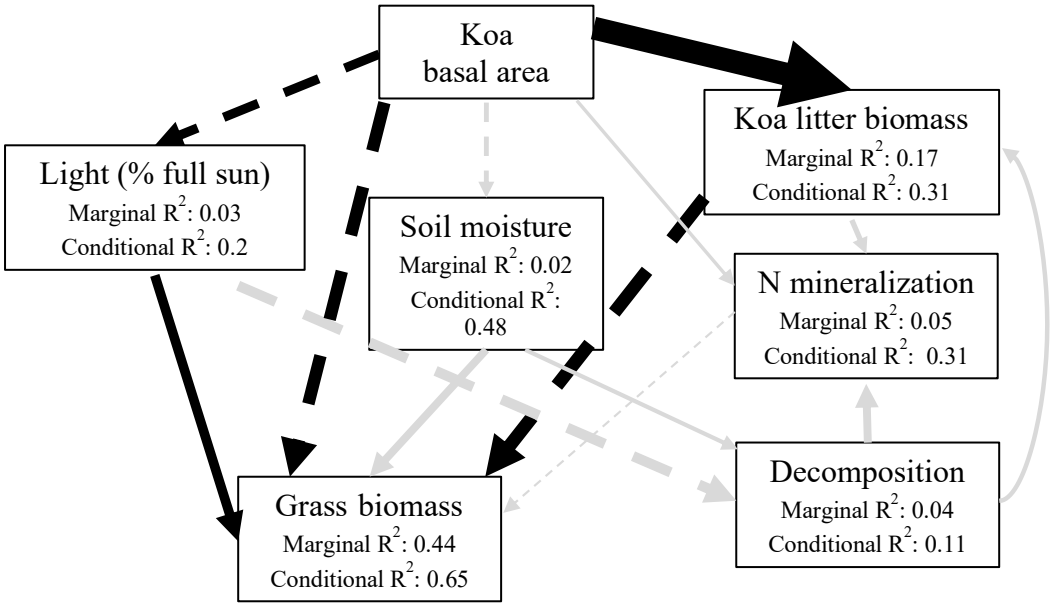

Figure B5.2 Final SEM evaluating the effects of koa basal area on grass biomass and possible mechanisms. Black arrows signify statistically significant ($p < 0.05$) paths and gray arrows non-significant paths. Solid arrows denote positive relationships and dashed arrows negative relationships. Arrow width scales with standardized path coefficients.

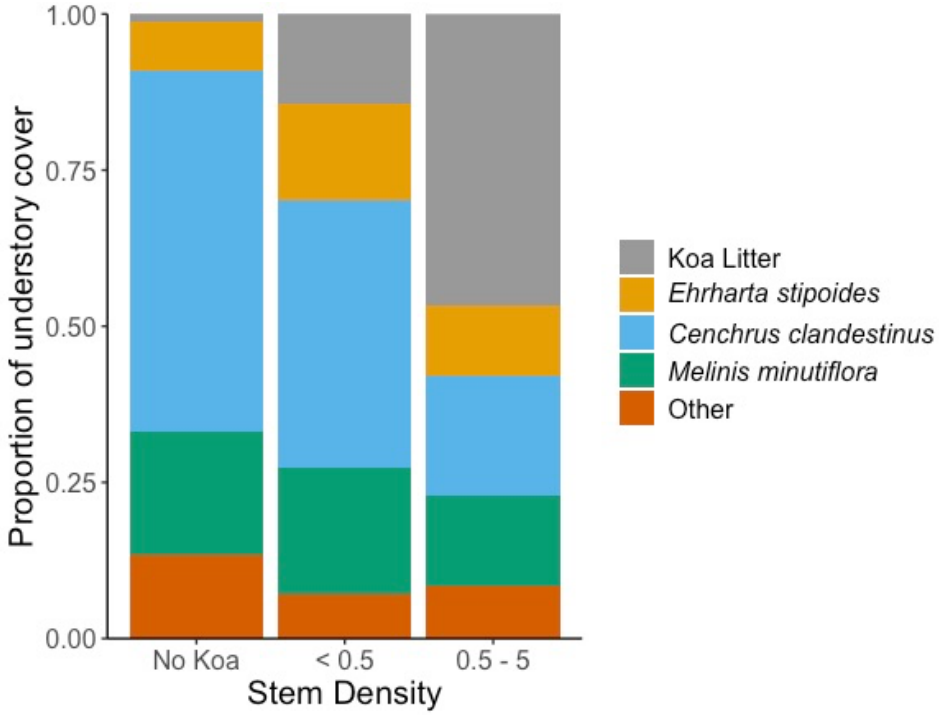

Figure B5.3 Composition of understory cover by koa stem density averaged across all sites and plots. Species with < 5% cover were condensed into the 'other' category.

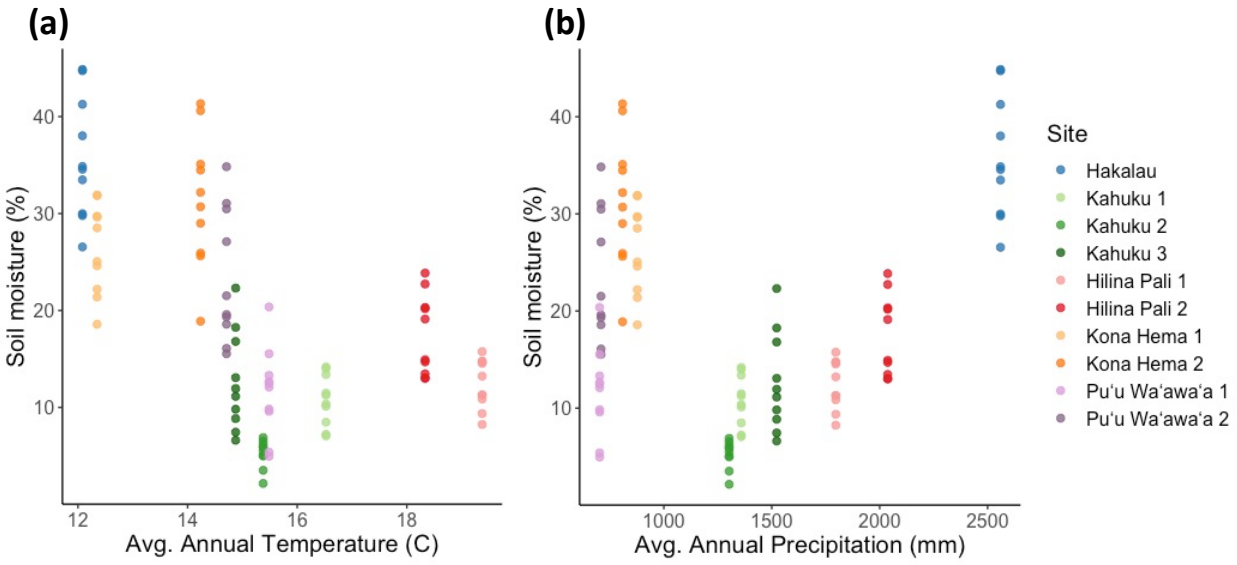

Figure B5.4 Relationships between soil moisture and site temperature (a) and precipitation (b).

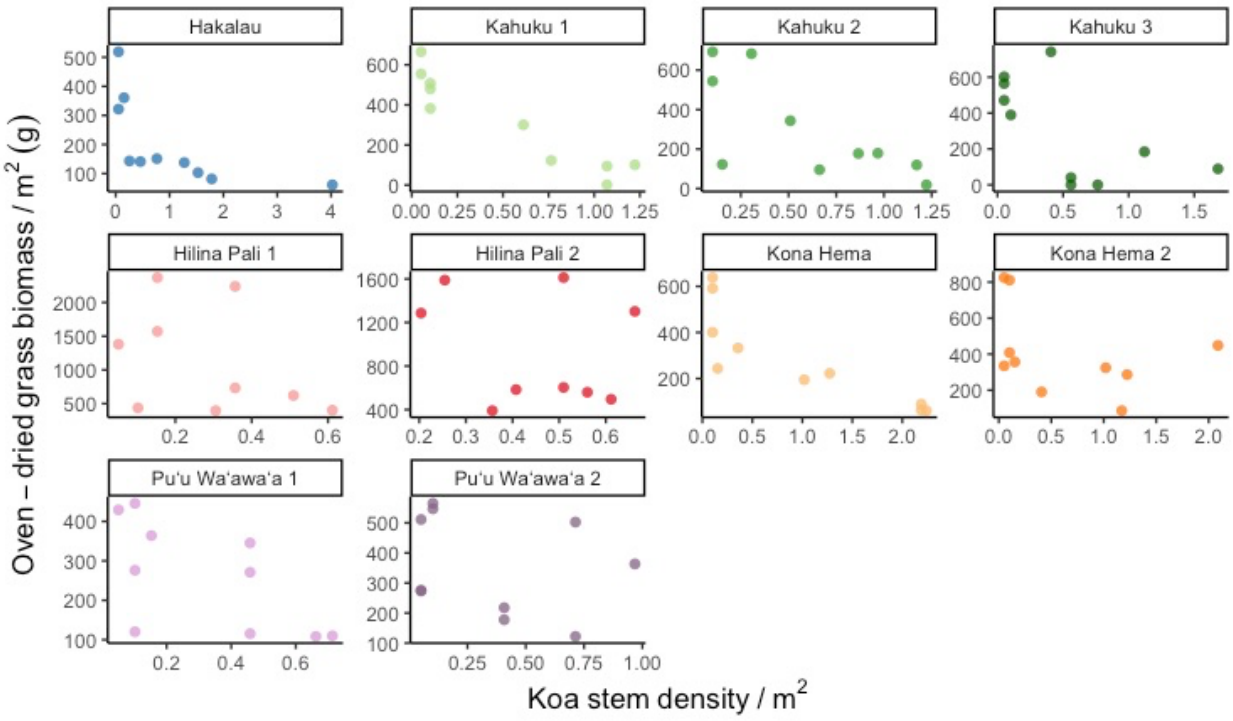

Figure B5.5 Relationships between grass biomass and koa stem density at each site.

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