TREE SEEDLING MYCORRHIZAL TYPE AND FUNCTIONAL TRAITS INTERACT WITH LIGHT AVAILABILITY TO MEDIATE PLANT-SOIL FEEDBACKS

By

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ABSTRACT

The seedling recruitment phase is a major demographic bottleneck and is critical for future forest community dynamics. Plant-soil feedbacks (PSFs) are often considered to be key drivers of seedling recruitment. PSFs are a continuous feedback loop in which adults modify properties of the soil beneath their crown, thereby influencing the ability of seedlings to grow and survive in that soil. Mechanisms underlying the strength and direction of PSFs include soil-borne microbes, seedling functional traits that confer defense against or recovery from microbes, and matching/mismatching of mycorrhizal type between juvenile and adult trees. Additionally, the strength and direction of PSFs may shift with light availability, which can modify both microbial abundance and functional traits. In this dissertation, I investigated the role each of these mechanisms and their interactions on tree seedlings PSFs.

In Chapter 2, I investigated how shade tolerance may be shaped by, not only responses to light availability, but also by species' defense and recovery functional traits, soil microbial communities, and interactions of these factors with light availability. I found that shade tolerance may be explained by interactions among soil-borne microbes, seedling functional traits, and light availability, providing a more mechanistic and trait-based explanation of shade tolerance and thus forest community dynamics.

In Chapter 3, to determine the extent to which functional traits mediate PSFs via seedling survival, I conducted a field experiment in which I planted seedlings of four temperate tree species across a gradient of light availability and into soil cores collected beneath conspecific (sterilized and live) and heterospecific adults. Results from this chapter indicate that functional trait values in seedlings as young as three weeks vary in response to both soil source and light

availability. Furthermore, traits play an important role in mediating effects of local soil sources and light on seedling survivorship, and thus plant traits could play an important role in PSFs.

In Chapter 4, to assess the role of mycorrhizal type matching on juvenile trees' defense/recovery trait response and PSFs, I carried out a greenhouse experiment where I grew seedlings of five temperate tree species under soils cultured by adults of the same species and under three light levels. I found that AM seedlings experience lower survival in soils cultured by AM adults and EM seedlings experience higher survival in soils cultured by EM adults. Additionally, as differences in mycorrhizal colonization and defense/recovery traits between conspecific and heterospecific soils decrease, PSFs are effectively neutralized, providing new insights into how mismatching of mycorrhizal type interacts with traits to influence PSFs.

In Chapter 5, to investigate the potential trade-offs between $PSF_{survival}$ at low light and $PSF_{biomass}$ at high light availability, I evaluated biomass data from the parallel factorial blocked field (Chapter 3) and greenhouse (Chapter 4) experiments. AM seedlings experienced negative $PSF_{biomass}$ that shifted to positive with increasing light availability, and EM seedlings always experienced positive $PSF_{biomass}$, irrespective of light level. In addition, I found that measuring $PSF_{survival}$ may be more important than $PSF_{biomass}$ when studying species sensitive to soil-borne microbes and that are expected to grow in low light-environments.

Together, these results provide a more mechanistic understanding to the factors underlying PSFs. Tree seedling mycorrhizal type and functional traits appear to interact with light availability to mediate PSFs, thereby influencing seedling regeneration dynamics and subsequent forest community dynamics.

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CHAPTER 1

Introduction

The seedling recruitment phase is a major demographic bottleneck and is critical for future forest community dynamics (Gurevitch et al., 2020). Plant-soil feedbacks (PSFs) are often considered to be key drivers of seedling recruitment (Crawford et al., 2019; Putten et al., 2016). PSFs are a continuous feedback loop in which adults modify properties of the soil beneath their crown, thereby influencing the ability of seedlings to grow and survive in that soil (Bever et al., 1997). Mechanisms underlying the strength and direction of PSFs include soil-borne microbes (Bever et al., 2010; Jiang et al., 2020), seedling functional traits that confer defense against or recovery from microbes (Cortois et al., 2016; Xi et al., 2021), and matching/mismatching of mycorrhizal type between juvenile and adult trees (Chen et al., 2019; Kadowaki et al., 2018). Additionally, the strength and direction of PSFs may shift with light availability, which can modify both microbial abundance and functional traits. In this dissertation, I investigated the role of each of these mechanisms and their interactions on tree seedlings PSFs.

The putative agents of PSFs are soil-borne microbes, namely pathogens and mycorrhizal fungi (Bever et al., 2010; Jiang et al., 2020). Soil-borne pathogens (including fungi, oomycetes, and bacteria) can cause high seedling mortality (Song & Corlett, 2022), especially in shade (McCarthy-Neumann & Ibáñez, 2013; McCarthy-Neumann & Kobe, 2008; O'Hanlon-Manners & Kotanen, 2004), where wetter conditions enhance microbe reproduction and dispersal (Hersh et al., 2012; Y. Liu & He, 2019; Reinhart et al., 2010). Mycorrhizal fungi are typically thought of as mutualists, providing water and nutrients in exchange for sugars (S. Smith & Read, 2008; Wipf et al., 2019). Mycorrhizal fungi are more abundant in high light availability (Bereau et al.,

2000; Koorem et al., 2017; Shi et al., 2014). However, at low light availability, where photosynthate production is more limited, they may act parasitically (Konvalinková & Jansa, 2016; McCarthy-Neumann & Ibáñez, 2013).

Interactions between soil-borne pathogens and different groups of mycorrhizal fungi can shift the strength and direction of PSFs. At low light availability, the cost of maintaining the mycorrhizal symbiosis may exacerbate the negative effects of pathogens. Mycorrhizae can also confer protection against pathogens, but the degree of protection depends upon mycorrhizal type (Bennett et al., 2017) and may depend upon resource availability (McCarthy-Neumann and Ibáñez 2013). AMF can provide indirect defense against pathogens by competing for space on plant roots (Borowicz, 2001) and EMF can provide direct defense by forming a protective physical sheath on young roots (Laliberté et al., 2015). Also, both AMF and EMF can increase their host plant's resource acquisition (Liang et al., 2015; Sikes, 2010).

Moreover, matching or mismatching of mycorrhizal type (AMF or EMF) between the seedling growing in and the adult tree culturing the soil may influence PSFs. (Here, we refer to species that typically associate with AMF and EMF as "AM species" and "EM species", respectively). Whereas AM trees typically experience negative PSFs (i.e., inhibition of seedlings around conspecific adults), EM trees more often experience positive PSFs (i.e., facilitation around conspecific adults) (Bennett et al., 2017; Kadowaki et al., 2018). In addition, AM trees have a higher abundance of plant pathogens in their soil (Eagar et al., 2022, 2023) and AM seedlings accumulate soil-borne pathogens faster when growing under AM adults (Chen et al., 2019). However, when there is mismatching of mycorrhizal type (e.g., AM seedlings growing beneath EM trees, and vice-versa), both AM and EM seedlings experience positive or neutral PSFs (Kadowaki et al., 2018).

Differences in PSFs may be partially explained by seedling defense and recovery functional traits, which are defined as measurable morphological or physiological attributes affecting plant performance (Violle et al., 2007). Plant functional traits could influence PSFs and vice-versa (P. Ke et al., 2015; Kuťáková et al., 2018; Xi et al., 2021). Functional traits that influence plant defense against and recovery from attack by soil-borne microbes include phenolics, lignin, and nonstructural carbohydrates (NSC). Phenolics and lignin can serve as chemical (Ichihara & Yamaji, 2009) and physical (Augspurger, 1990) defenses against soil-borne microbes. NSC can be mobilized to repair damaged tissues (Dietze et al., 2014).

Phenolics, lignin, and NSC are likely affected by soil source (e.g., conspecific versus heterospecific soil). Phenolics production can be induced by mycorrhizal colonization (Wallis & Galarneau, 2020) and potentially by fungal pathogens (Witzell & Martín, 2008). Therefore, phenolics production should subsequently be higher in conspecific soils, where there should be higher colonization by mycorrhizal fungi and infection by effectively-specialized soil-borne pathogens (Benítez et al., 2013; Hersh et al., 2012). It is unclear whether lignin production is influenced by conspecific soils. However, it could be driven by soil nutrient availability, which can be impacted by microbes (J. Li et al., 2020; Luo et al., 2022). NSC should be lower in conspecific soils, due to greater resource allocation to symbionts (Schiestl-Aalto et al., 2019) and recovery against pathogen infection (Martínez-Vilalta, 2014; Saffell et al., 2014).

Both PSFs and defense/recovery traits can shift across environmental gradients like light availability (McCarthy-Neumann & Ibáñez, 2013; Smith-Ramesh & Reynolds, 2017). However, most studies have not integrated abiotic factors when evaluating both traits and PSFs (Cortois et al., 2016). Shifts in light can change microbial composition and abundance (Koorem et al., 2017; Y. Liu & He, 2019), which may alter seedlings' ability to defend against or recover from disease.

AMF are more abundant in higher light (Bereau et al., 2000; Koorem et al., 2017; Shi et al., 2014); however, in low light they can act parasitically and thereby decrease seedling survival (Konvalinková & Jansa, 2016). Higher mortality from pathogens typically occurs in low light (McCarthy-Neumann & Ibáñez, 2013; McCarthy-Neumann & Kobe, 2010a), where wetter and cooler conditions enhance microbe reproduction and dispersal (Hersh et al., 2012; Y. Liu & He, 2019; Reinhart et al., 2010). Light availability can also modify trait levels, including reduced production of phenolics (Ichihara & Yamaji, 2009) and lignin (Falcioni et al., 2018; Rogers et al., 2005). Additionally, carbon limitation in shade and lower stored nonstructural carbohydrates (NSC) may constrain recovery from disease (Kobe, 1997; Kobe et al., 2010).

There is an increased need to understand the mechanisms underlying forest community dynamics, especially those that regulate the coexistence of tree species and promote species diversity, like PSFs. Negative PSFs (lower performance in conspecific versus heterospecific soils) may have positive effects on forest community diversity by increasing the likelihood that a seedling of a different species will replace an adult tree when it dies. Conversely, positive PSFs (higher performance in conspecific than heterospecific soils) may decrease community diversity by increasing the likelihood that an adult tree is replaced by a seedling of the same species. Together, negative and positive PSFs can mediate species coexistence within forests. However, how defense and recovery traits, in addition to mismatching of mycorrhizal type between juvenile and adult trees, mediate PSFs is relatively unknown. Furthermore, it is unclear how theses relationship might shift across abiotic gradients, such as light availability.

This dissertation includes four chapters focusing on different aspects of tree seedling PSFs, including separating roles of mycorrhizal fungi versus soil-borne pathogens, defense and recovery functional traits (phenolics, lignin, and NSC), and mismatching of mycorrhizal type

between juveniles and adult trees, all in the context of light availability. A short summary of each chapter follows.

Chapter 2: I investigated how shade tolerance may be shaped by, not only responses to light availability, but also by species' defense and recovery functional traits, soil microbial communities, and interactions of these factors with light availability. I conducted a greenhouse experiment, controlling for AMF and soil-borne pathogen presence/absence and light availability, and measuring defense/recovery traits, for three temperate tree species from the genus *Acer* that vary in shade tolerance. I found that persistence of tree seedlings under low light availability, which we often interpret as shade tolerance, is not due to light limitation alone, but rather is due to interactions between low light availability and soil-borne microbes. Differences in seedling survival between low and high light only occurred when microbes were present. AMF colonization, phenolics, and NSC generally increased with light availability. Measured amounts of phenolics also were higher when pathogens were present, signifying that phenolics may be an induced defense response. Furthermore, across species, microbe treatment, and light availability, survival increased as phenolics and NSC increased. These results suggest that shade tolerance may be explained by interactions among soil-borne microbes, seedling defense and recovery functional traits, and light availability, providing a more mechanistic and trait-based explanation of shade tolerance and thus forest community dynamics.

Chapter 3: To determine the extent to which defense and recovery functional traits mediate PSFs via seedling survival, I conducted a field experiment in which I planted seedlings of four temperate tree species across a gradient of light availability and into soil cores collected beneath conspecific (sterilized and live) and heterospecific adults. I monitored seedling survival twice per week over one growing season, and randomly selected subsets of seedlings to measure

mycorrhizal colonization, phenolics, lignin, and NSC levels at three weeks. Results from this study demonstrate that defense and recovery functional trait values in seedlings as young as three weeks vary in response to both soil source and light availability. In general, I found higher measured amounts of mycorrhizal colonization and defense/recovery traits in conspecific than heterospecific soils and in higher light availability. Moreover, seedling survivorship was associated with AMF colonization and phenolics for two species. These results suggest that seedling traits could have an important role in mediating the effects of local soil source and light levels on seedling survivorship, and thus plant traits could play an important role in PSFs.

Chapter 4: To assess the role of mycorrhizal type matching on juvenile trees' trait response and PSFs, I carried out a greenhouse experiment where I grew seedlings of five temperate tree species under soils cultured by adults of the same species and under three light levels. After 12 weeks, I quantified seedling survival, colonization by mycorrhizal fungi (AMF and EMF), and measured their defense and recovery traits (phenolics, lignin, and NSC). I found that negative PSFs experienced by seedlings associating with AMF almost always occurred when they were compared with heterospecific adults associating with EMF. Conversely, positive PSF experienced by EM seedlings occurred when compared to soils cultured by AM adults. For both AM and EM species, the magnitude of effect for PSFs was greatest at low light. Furthermore, soil microbes from conspecific-cultured soils reduced survival for AM species but had no effect on survival for EM species. PSFs for AM seedlings became less negative as percent AMF colonization and defense/recovery traits increased, and PSFs for EM seedlings became less positive as percent AMF colonization and lignin increased. These results suggest that functional traits and increased colonization by mycorrhizal fungi effectively neutralize both negative and

positive PSFs, providing new insights into how mismatching of mycorrhizal type interacts with traits to influence PSFs, and thus forest community dynamics.

Chapter 5: To investigate the potential trade-offs between $PSF_{survival}$ at low light and $PSF_{biomass}$ at high light availability, I evaluated biomass data from the parallel factorial blocked field (Chapter 3) and greenhouse (Chapter 4) experiments. I found that $PSF_{biomass}$ was typically negative for AM species and positive for EM species, but these results did not depend upon mycorrhizal matching/mismatching of the juvenile and adult tree. Furthermore, $PSF_{biomass}$ became more neutral (i.e., less negative) for the AM species as light availability increased. There was also a negative relationship between $PSF_{survival}$ in low light availability and $PSF_{biomass}$ at high light availability. At high light availability, all species experienced positive $PSF_{biomass}$, in contrast to low light where AM species experienced negative $PSF_{survival}$ and EM species experienced positive/neutral $PSF_{biomass}$. This research suggests that measuring $PSF_{survival}$ may be more important when studying species sensitive to soil-borne microbes and that are expected to grow in low light-environments. Conversely, measuring $PSF_{biomass}$ may be more beneficial when seedlings do not experience high mortality. Together, these results help elucidate the mechanisms underlying variation in PSF studies.

The final chapter of this dissertation is a synthesis of the findings of the four research chapters, in addition to a discussion of how these results contribute to the scientific field of forest ecology. I demonstratee how soil-borne microbes can mediate differences in shade tolerance. In addition, I show that mismatching of mycorrhizal type and defense/recovery traits may help explain forest regeneration dynamics. Together, my results provide a more mechanistic understanding of seedling recruitment patterns in the context of PSFs, mycorrhizal type, defense/recovery functional traits, and light availability.

CHAPTER 2

Tree seedling shade tolerance arises from interactions between
light availability and soil-borne microbes and is mediated by functional traits

ABSTRACT

Shade tolerance is a central concept in forest ecology and strongly influences forest community dynamics. However, the plant traits and conditions conferring shade tolerance are yet to be resolved. I propose that shade tolerance is shaped not only by responses to light but also by a species' defense and recovery functional traits, soil microbial communities, and interactions of these factors with light availability.

I conducted a greenhouse experiment for three temperate species in the genus *Acer* that vary in shade tolerance. I grew newly germinated seedlings in two light levels (2% and 30% sun) and controlled additions of microbial filtrates using a wet-sieving technique. Microbial filtrate treatments included: <20 μm, likely dominated by pathogenic microbes; 40-250 μm, containing arbuscular mycorrhizal fungi (AMF); combination, including both filtrate sizes; and sterilized combination. I monitored survival for nine weeks and measured fine root AMF colonization, hypocotyl phenolics, stem lignin, and stem+root nonstructural carbohydrates (NSC) at three-week intervals.

I found that differences in seedling survival between low and high light only occurred when microbes were present. AMF colonization, phenolics, and NSC generally increased with light. Phenolics were greater with <20 μm microbial filtrate, suggesting that soil-borne pathogens may induce phenolic production; and NSC was greater with 40-250 μm filtrate, suggesting that mycorrhizal fungi may induce NSC production. Across species, microbe

treatments, and light availability, survival increased as phenolics and NSC increased. Therefore, shade tolerance may be explained by interactions among soil-borne microbes, seedling traits, and light availability, providing a more mechanistic and trait-based explanation of shade tolerance and thus forest community dynamics.

INTRODUCTION

Tree seedling mortality responses to understory light availability are an important filter of mature tree species composition and drivers of forest community dynamics (Pacala et al., 1996). Thus, plant survival in low light or shade tolerance (Shirley, 1943) is a central concept in forest ecology. The seedling establishment phase, a major demographic bottleneck, is also critical for future community dynamics (Gurevitch et al., 2020). However, seedling responses to shade are far more complex than responses to light availability alone (Valladares et al., 2016; Valladares & Niinemets, 2008). Understanding the functional traits and biotic and abiotic conditions that convey shade tolerance are key to a more mechanistic understanding of forest community dynamics.

A plant's ability to tolerate low light conditions can be moderated by soil-borne microbes, like soil-borne pathogens and mycorrhizal fungi (Jiang et al., 2020). Soil-borne pathogens (including fungi, oomycetes, and bacteria) can cause high seedling mortality (Song & Corlett, 2022), especially in shade (McCarthy-Neumann & Ibáñez, 2013; McCarthy-Neumann & Kobe, 2008; O'Hanlon-Manners & Kotanen, 2004), where wetter conditions enhance microbe reproduction and dispersal (Hersh et al., 2012; Y. Liu & He, 2019; Reinhart et al., 2010). Arbuscular mycorrhizal fungi (AMF) can provide water and nutrients in exchange for sugars (Wipf et al., 2019). However, they may parasitize tree seedlings and increase mortality in low

light (Ibáñez & McCarthy-Neumann, 2016; Konvalinková & Jansa, 2016), despite mutualistic tendencies and greater abundance in high light (Bereau et al., 2000; Koorem et al., 2017; Shi et al., 2014).

AMF, pathogens, and light levels can interact to influence seedling mortality. In high light, AMF root colonization is greater (Ibáñez & McCarthy-Neumann, 2016; Konvalinková & Jansa, 2016; Koorem et al., 2017), which can reduce the growth of fungal pathogens, potentially by competing for root space (Borowicz, 2001). AMF may also indirectly ameliorate pathogen effects (Liang et al., 2015), by providing water and nutrients to the host plant (Graham, 2001) and inducing production of defensive traits (Pozo & Azcón-Aguilar, 2007; Zamioudis & Pieterse, 2012) that protect against pathogens (Azcón-Aguilar et al., 2002; Violle et al., 2012). Conversely, in low light, seedling mortality may increase, due to the combined carbon costs of maintaining the AMF mutualism and recovery from pathogen attack.

Seedlings could also experience higher mortality from soil-borne microbes due to shade-induced changes in defensive functional trait values, such as reduced phenolics (Ichihara & Yamaji, 2009) and lignin (Falcioni et al., 2018; Rogers et al., 2005). Additionally, carbon limitation in shade and lower stored nonstructural carbohydrates (NSC) may constrain recovery from disease (Kobe, 1997; Kobe et al., 2010). Functional trait values may not only differ within a species based on light availability, but may also vary among species in relation to shade tolerance (Imaji & Seiwa, 2010). Shade tolerant species are typically less vulnerable to mortality by soil-borne microbes than shade intolerant species (Alvarez-Clare & Kitajima, 2007; Augspurger, 1984a; McCarthy-Neumann & Kobe, 2008, 2010a) at least partly because shade tolerant species allocate more carbon to chemical and physical defenses (Coley et al., 1985;

Coley & Barone, 1996), and recovery (Kitajima, 1994; Myers & Kitajima, 2007; Poorter et al., 2010).

To examine the effects of light availability, soil-borne microbes, tree seedling functional traits, and their interactions on light-dependent seedling survival, I established an experiment to test the following hypotheses: I hypothesized that:

1) Within species, decreased survival under low versus high light only occurs in the presence of soil microbes.

2) Mycorrhizal colonization is lower in the <20 um microbial filtrate where pathogens are likely to be the dominant microbial group.

3) As defensive traits, phenolics and lignin are induced to higher levels in soils where microbes are present (non-sterilized) and in higher light availability.

4) As a recovery trait, NSC is lower in soils where microbes are present and in low light availability.

5) Across all species and when microbes are present, survival increases as phenolics, lignin, and NSC increase.

MATERIALS AND METHODS

I conducted a fully factorial blocked-design greenhouse experiment at the Michigan State University Tree Research Center in Lansing, Michigan, USA (42.7 ºN, 84.5 ºW). The experiment consisted of three species, four microbial communities (<20 µm filtrate, representing pathogenic microbes; 40-250 µm filtrate, representing AMF; combined filtrate (both <20 µm and 40-250 µm); and sterilized combined filtrate) and two light levels (2% and 30% full sun, representing shade and light gap environments). Individual pots were set up on six different benches (three

per light level), where all treatment combinations were represented. I planted 80 seedlings per treatment combination for a total of 1,920 seedlings. I monitored seedlings every three days for survival, and randomly selected subsets for trait measurements at three, six, and nine weeks.

Species selection

I selected three biogeographically widespread, co-occurring tree species within the genus *Acer: saccharum*, *rubrum*, and *negundo*. These species have similar seed sizes (Osunkoya et al., 1994), but vary in shade tolerance (Burns & Honkala, 1990a; Niinemets & Valladares, 2006a).

Light availability

I grew seedlings at two light levels (2% and 30% full sun). I created light treatments by covering six greenhouse benches (three per treatment) with an inner layer of black shade cloth and an outer layer of reflective knitted poly-aluminum shade cloth (BFG Supply, Burton, Ohio, USA). I confirmed light levels using PAR (photosynthetically active radiation) measurements at each bench with a LI-COR 250A quantum sensor (LI-COR, Lincoln, Nebraska, USA) on a uniformly overcast day.

Soil collection and preparation of soil inocula

Soils were collected from Alma College's Ecological Field Station in Vestaburg, Michigan, USA (43.4 °N, 84.9 ºW), in a 100-ha mixed-hardwood forest stand containing a 3-ha subplot with mapped and tagged trees. In August 2016, I randomly selected three adult trees per species. I selected adults that were at least two crown diameters away from other study species to reduce potential cross-culturing of soil. I collected soil (top 15 cm) within 1 m of each focal tree stem, maintained as separate replicates throughout the remainder of the experiment (as recommended by Rinella and Reinhart 2018). I prepared soil by dicing roots and sifting soil through a 1 cm mesh sieve, retaining all roots that passed through the 1 cm sieve, as they may

harbor host-specific microbial communities. Soil samples were stored at 4 □C for up to 2 months before preparation of soil inocula filtrates.

I created four microbial communities from sifted field soil using a wet-sieving method (Callaway et al., 2011; Klironomos, 2002; König et al., 2016; Liang et al., 2015; Pizano et al., 2014). For each extraction, I agitated 50 g of soil in a blender with 250 mL of deionized water at high speed for 60 sec, then passed the slurry through three analytical sieves (250-, 40-, and 20 μm) using a high-pressure water hose, for a total volume of 800 mL. To minimize contamination between treatments, I cleaned the sieves ultrasonically for 5 min between each extraction. The 250 μm sieve collected larger roots and coarser soil. I floated material retained by the 40 μm sieve on the surface of a 60% sucrose solution and centrifuged it at 688 g for 20 min. I collected material in the water and at the water-sucrose interface on 47 mm Whatman no.1 filter paper, surface sterilized it with 10% NaOCl for 10 sec, and washed it with distilled water under a filtration vacuum. I divided each filter paper into eight equal pieces. I collected the filtrate that passed through the 20 μm sieve and separated it into eight 100 mL containers. In sum, I created three microbial communities based on filtrate size classes: <20 μm, 40-250 μm, and combined (containing both <20 μm and 40-250 μm filtrates).

To test for abiotic effects of conspecific cultured soils due to nutrients or allelopathy, I combined filtrates (<20- and 40-250 μm) and sterilized by steam autoclaves (at 121 °C for two hours). I also quantified and compared AMF colonization in the sterilized and <20 μm filtrates against a control containing only filter paper and deionized water to test the effectiveness of the sterilization for reducing microbes. I found no AMF colonization in soils treated with the sterilized ($t = 2.97$, $df = 176$, $p = 0.003$) or <20 μm filtrates ($t = 3.79$, $df = 178$, $p < 0.001$),

relative to a distilled water control (Figure 2.2A). Filtrates were kept refrigerated at 4 □C for up to 48 hours, before adding them to the greenhouse pots.

In the greenhouse, I filled 1,920 (655 cm^3) deepots (Stuewe and Sons, Tangent, Oregon, USA) with sterilized commercial topsoil (Hammond Farms Landscape Supply, Lansing, Michigan, USA). To aid seedling germination, I also topped each pot with 2 cm of 85% sterilized commercial soil mix, containing peat moss, perlite, and vermiculite (Fafard 4P Mix, Sun Gro Horticulture, Agawan, Massachusetts, USA). In pilot trials of this experiment, I found over 50% seedling mortality in the first two weeks, without the addition of the commercial soil mix (personal observation). I steam sterilized the topsoil and soil mix by autoclaving twice at 121 °C for two hours, with a 48-hour incubation period between cycles.

Within 48 hours of wet sieving, I added microbe treatments to soil, keeping filtrate from each adult and species separate. To enable microbial communities to sporulate and AMF hyphae to establish, I cultured the soil with *Allium* as bait plants, before planting *Acer* seedlings (Al-Yahya'ei et al., 2011; Klironomos et al., 1999). In January 2017, each pot was planted with three germinating *Allium tuberosum* seedlings. After two months, I removed aboveground *A. tuberosum* seedling biomass.

One week after *Allium* removal, I planted *Acer* seedlings whose hypocotyls had emerged within the three previous days. I purchased seeds from Sheffield's Seed Company (Locke, New York, USA). To minimize microbes from non-experimental soil sources, I surface sterilized the seeds with 0.6% NaOCl both prior to cold stratification and germinating in perlite. I watered the seedlings three times per week with 50 mL deionized water.

Survival and functional trait measurements

I grew the *Acer* seedlings for nine weeks. I recorded emergence and survival every three days and assigned date of death as the first census with total leaf and stem tissue necrosis. I harvested a random subset of 20 seedlings for each species and each treatment at three, six, and nine weeks, to quantify AMF colonization, phenolics, lignin, and NSC. I chose these times for our sub-harvests, because in a previous greenhouse experiment, mortality curves for tree seedlings subjected to soil-borne pathogens often increased at week three and peaked between four to six weeks after germination (McCarthy-Neumann & Ibáñez, 2012).

To quantify percent AMF colonization, I stained seedling roots with a 5% Shaeffer black ink in vinegar solution (Vierheilig et al., 1998) and counted fungal structures (e.g., arbuscules, coils, vesicles, hyphae) along 100 intersections under the microscope (McGonigle et al., 1990). To quantify phenolics, I analyzed hypocotyl samples, using a microplate-adapted colorimetric total phenolics assay with Folin-Ciocalteu reagent (Ainsworth & Gillespie, 2007). To quantify lignin, I analyzed root and stem samples with an Ankom 200 fiber analyzer (ANKOM Technologies, Macedon, NY, USA), using the acid-fiber detergent fiber filter technique. To quantify NSC, I analyzed stem samples, using a standardized enzyme method for sugar and starch extraction and quantification (Landhäusser, Chow, Dickman, et al., 2018).

Statistical analyses

To test the influence of light availability and microbial community on tree seedling survival, I used an individual based counting process in a Cox survival model (Burnham & Anderson, 2002; McCarthy-Neumann & Ibáñez, 2012). Data for each seedling i and each time t, N_{it}, were coded as 0 until the seedling was found dead, $N_{it} = 1$. I used a count process to model

the number of events (mortality, N_{it}) until the experiment ended at nine weeks. I modeled the likelihood as:

$$N_{it} \sim Poisson(\lambda_{it})$$

and the process as:

$$\lambda_{it} = h_t e^{(\mu t)},$$

where parameters were estimated as a function of the hazard (h), which is the intrinsic rate of mortality due to individual age or time within the experiment), and of risk (μ), which is the extrinsic rate of mortality due to light availability and microbial community. Simulations (3 chains) were run until convergence of the parameters was ensured (25,000 iterations) and then run for another 50,000 iterations, from which the posterior parameter values (Figure A2.1, A2.2) and predicted survival (Figure A2.3) were estimated. Predicted survival values were used to assess whether there were differences in how species responded to microbe treatments and light. I then used predicted survival values and their associated uncertainty to test if there were differences in how species responded to low versus high light and in different microbe treatments. Differences that did not include zero in their 90% credible intervals were considered statistically significant (Kruschke, 2014).

I used linear mixed effects models to evaluate the effects of light availability and microbial community on seedling traits. I ran individual models for each species and trait, where light level and microbial community were treated as fixed effects, and harvest time, bench (nested within light level), and adult tree were treated as random effects.

I then used linear mixed effects models to assess the effects of traits on seedling survival in the three weeks following trait measurement (i.e., traits collected at three weeks were used to predict seedling survival from three to six weeks, and traits at six weeks were used to predict

seedling survival from six to nine weeks). I treated AMF colonization, phenolics, lignin, and NSC as fixed effects, and included light level as a covariate. Species was treated as a random effect.

I evaluated relative support for each of our linear mixed effects models using multi-modal inference with corrected Akaike's Information Criterion (Burnham & Anderson, 2002). Models with $\Delta AICc < 6$ of the best-approximating model were considered plausible (Richards, 2008).

I performed all analyses in R 3.5.1 (R Core Team, 2020). I used the *rjags* package (Plummer, 2019) to fit survival models and to run predicted survival and contrast simulations. I used the built-in "lmer" function to fit linear mixed effects models and tested significance of main effects using the "Anova" function in the *car* package (Fox & Weisberg, 2019). Model selection for linear mixed effects models was determined with the "step" function in the *lmerTest* package (Kuznetsova et al., 2017). Post-hoc Tukey pairwise comparisons of significant main effects were made using the "emmeans" and "joint_tests" functions in the *multcomp* package (Hothorn et al., 2008; Lenth, 2020).

RESULTS

Differences in seedling survival in low versus high light occurred only when microbes were present

Differences in seedling survival in low light versus high light, a measure of shade tolerance, appeared only in the presence of soil biota (Figure 2.1A). In low light, survival decreased up to 14% with the <20 μm filtrate, 12% with the 40-250 μm filtrate, and 17% with the combined filtrate. *A. negundo* experienced the largest decreases in survival (17% reduction in

the combined filtrate), whereas *A. saccharum* had the lowest (11% reduction in the combined filtrate). Furthermore, *A. saccharum* survival decreased only in the presence of combined filtrate. Microbial treatments influenced seedling survival in only three of the nine cases in high light, but in eight of nine in low light (Figure 2.1B). At low light, the presence of soil biota relative to sterilized soil decreased survival 12-26% for all species and filtrates, except for *A. saccharum* with 40-250 µm filtrate. Additionally, negative soil biota effects were more common with the <20 µm filtrate, decreasing survival for all three species, regardless of light level.

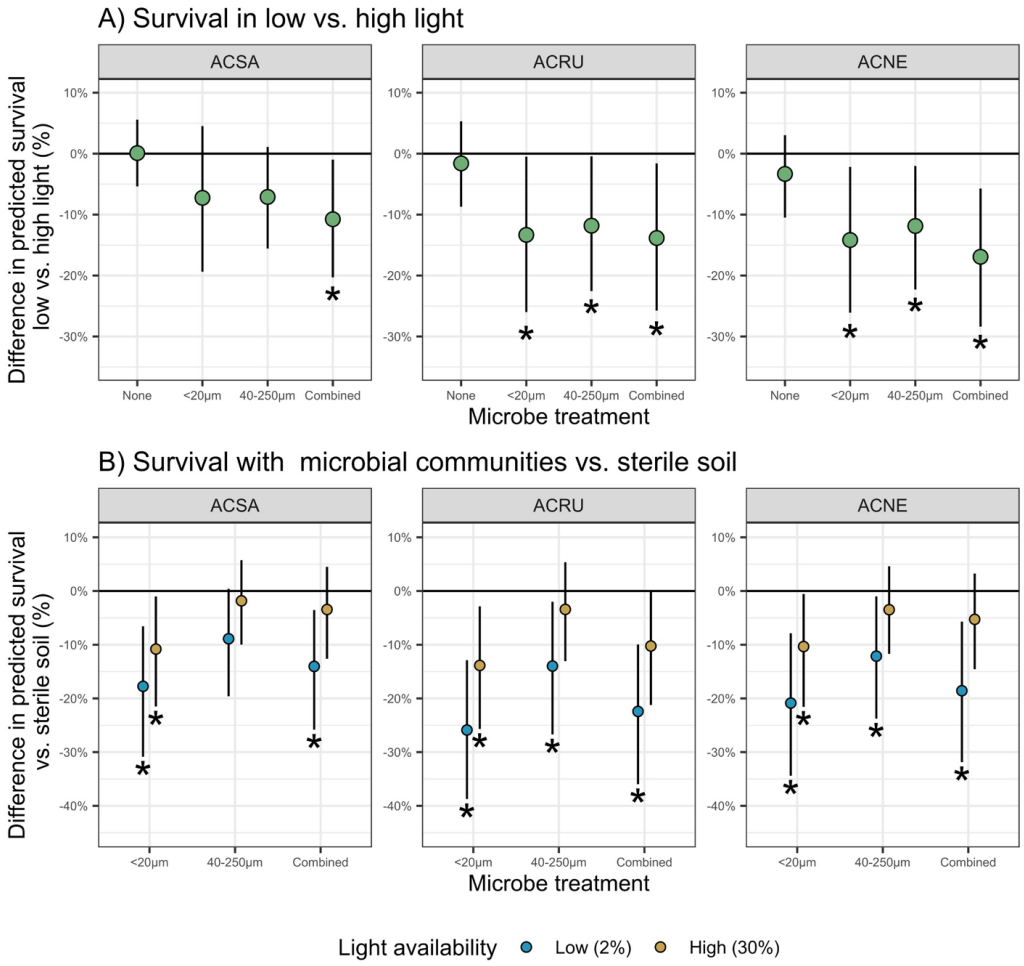


Figure 2.1 Difference in predicted survival (mean ± 90% credible interval) in **A)** low versus high light and **B)** relative to sterilized soil. For each species and microbial communities, at the end of 9-weeks. Negative values indicate decreased survival in treatment versus A) high light or B) sterilized soil. Statistically significant differences (90% CI do not overlap 0) are indicated with *.

Seedling functional trait values varied with light availability and microbial community

Across all treatments, percent root colonization by AMF was 11-18% greater in high than low light for *A. saccharum* and *A. negundo* (Figure 2.2A). There was no AMF colonization in the <20 µm filtrate, and colonization was similar in the 40-250 µm and combined filtrates. Overall, *A. negundo* (76%) had the highest AMF colonization, compared to *A. saccharum* (66%) and *A. rubrum* (45%).

Phenolic content (nmol Gallic acid equivalents per mg dry extract) increased with light availability across almost all microbe filtrate treatments (Figure 2.2B); the only exception was *A. saccharum* with the <20 µm filtrate. For *A. rubrum* and *A. negundo*, phenolic content was negligible in low light with the sterilized and combined filtrates. Overall, *A. saccharum* had the highest phenolic content (0.23 nmol/mg), compared to *A. negundo* (0.14 nmol/mg) and *A. rubrum* (0.06 nmol/mg).

Percent dry mass lignin was greater in high than low light but depended upon species and microbial filtrate (Figure 2.2C). For *A. saccharum*, lignin increased 5% across microbe filtrates, in high versus low light. For *A. negundo*, lignin increased 27% but only in the 40-250 µm filtrate. Across species, *A. saccharum* had the highest percent dry mass lignin (12%), compared to *A. rubrum* (8%) and *A. negundo* (6%).

Percent dry mass NSC was greater in high than low light for all species and was generally greatest with the 40-250 µm filtrate (Figure 2.2D). Additionally, for *A. rubrum* and *A. negundo*, NSC decreased 40-70% in the <20 µm and combined microbe filtrates, in low versus high light. Across species, *A. saccharum* had the highest dry mass NSC (12%), compared to *A. negundo* (11%) and *A. rubrum* (8%).

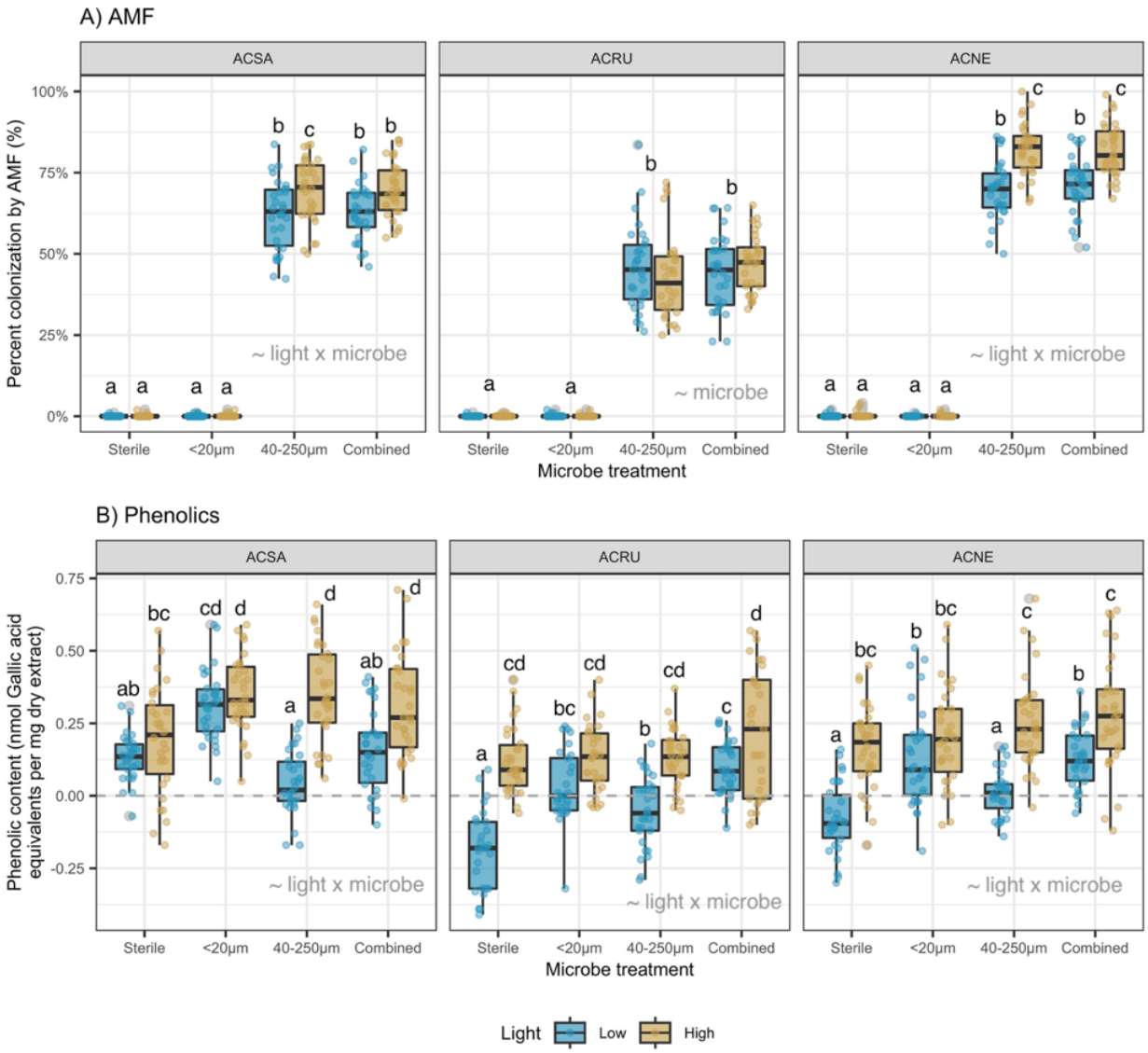
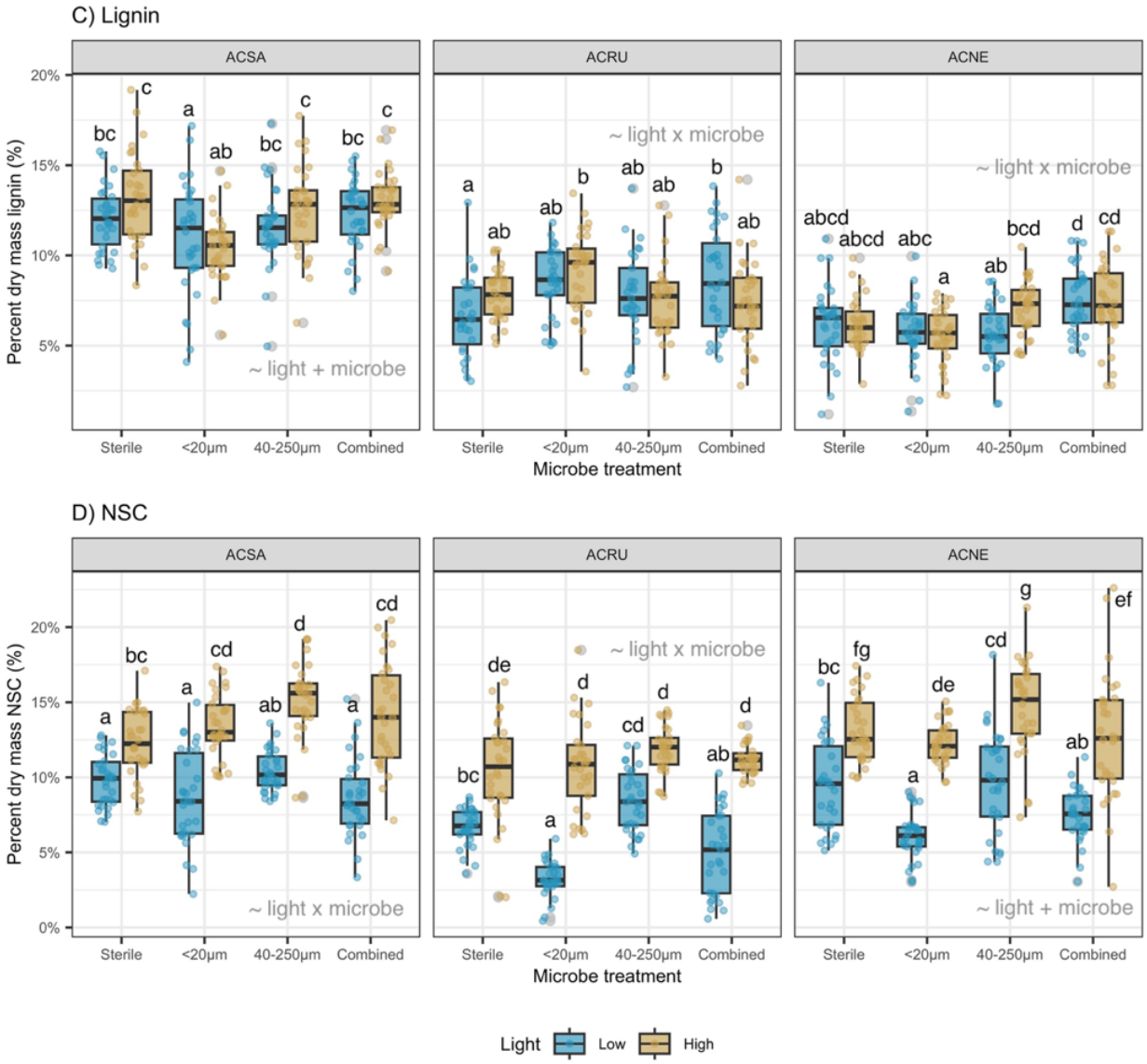


Figure 2.2 A) percent colonization AMF (%), **B)** phenolic content (nmol Gallic acid equivalents per mg dry extract), **C)** percent dry mass lignin (%), and **D)** percent dry mass NSC (%). Means within each panel not sharing a letter are statistically different by the Tukey test at alpha = 0.05. Best-fit model terms are overlayed on each panel.

Figure 2.2 (cont'd)



Seedling functional trait values are associated with survival

Survival increased with both phenolics ($\chi^2 = 5.93$, df = 1, p = 0.015; Figure 2.3A) and NSC ($\chi^2 = 7.72$, df = 1, p = 0.005; Figure 2.3B). However, there was no significant relationship between light availability and survival ($p > 0.05$), either alone or interacting with phenolics and NSC.

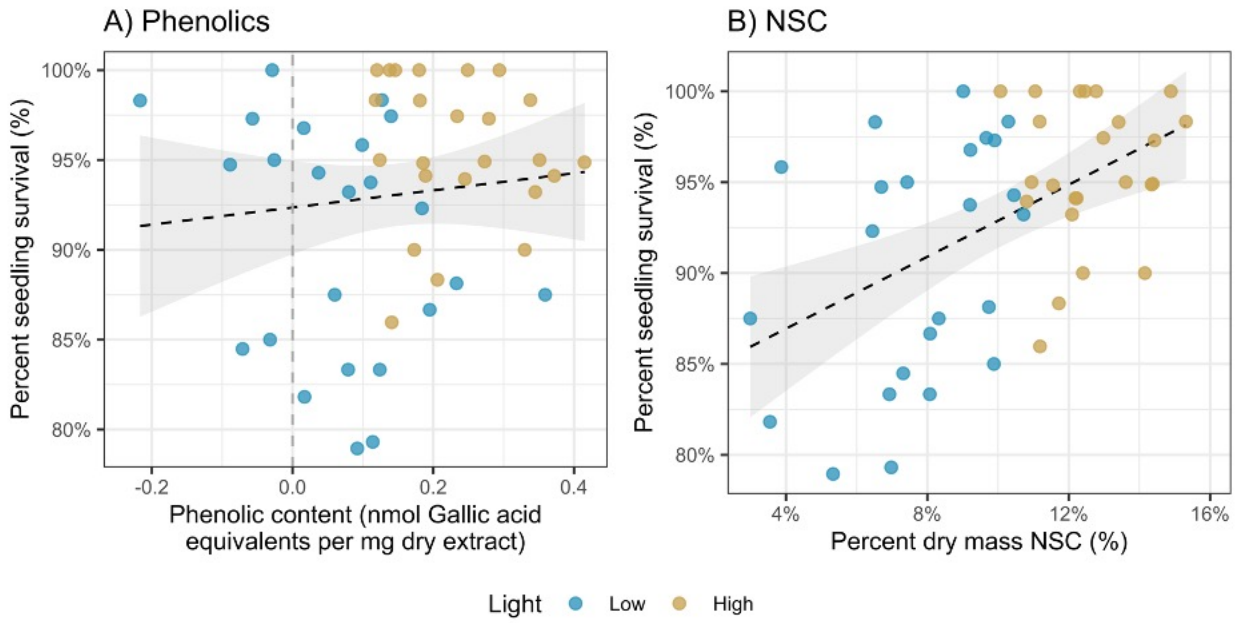


Figure 2.3 Percent seedling survival as a function of A) phenolic content (nmol Gallic acid equivalents per mg dry extract) and B) percent dry mass NSC (%). Each point represents a mean of trait values at a harvest time (3 or 6 weeks) and survival for seedlings in the 3 weeks after harvest.

DISCUSSION

My results support that survival of newly germinated tree seedlings in low versus high light, or "shade tolerance," may be due to interactions between low light and soil-borne microbes and be mediated by defense and recovery functional traits. Previous studies have demonstrated that seedling functional traits are influenced by light and microbes and that functional traits can influence growth and survival at low light (Falster et al., 2018), but have not linked resources, traits, and survival, as in this study.

Across species, overall seedling survivorship and insensitivity to shading corresponded with shade tolerance categorizations. *A. saccharum* (shade tolerant) had the highest overall survival and was least sensitive to the microbial filtrates, compared to *A. rubrum* (intermediate) and *A. negundo* (intolerant). In this study, *A. negundo* had the highest AMF colonization of the three study species (Figure 2.2A) and had the largest decreases in low-light survival with added microbial filtrates (Figure 2.1). This aligns with prior studies which have shown that shade intolerant species experience greater mortality from disease in shade (Augspurger, 1984a; Pizano et al., 2014) and higher growth when grown in high light (Xi et al., 2023). Similarly, shade tolerant species showed no significant growth responses to microbial filtrate treatments, in contrast to pioneer species that were more sensitive to the habitat from which soil microbial filtrates were collected (Pizano et al. 2017).

Soil-borne microbes explain variation in tree seedling survival responses to light

There is good support that the 20 µm filtrate in our study is primarily composed of fungi and bacteria. We found no evidence of mycorrhizal colonization in our <20 µm soil filtrate (Figure 2.2), suggesting that this treatment is mainly composed of non-mycorrhizal fungi and bacteria (also consistent with Klironomos 2002). Additionally, there is support that the 40 µm

filtrate is primarily composed of the mycorrhizal community associated with soils cultured by conspecific adults. In a prior study that investigated this methodology, Wagg et al. (2014) found that soil passing through 250 μm sieves contained ~80% of the mycorrhizal community, and an additional ~20% of the mycorrhizal community passed through the 50 μm sieves. In the Wagg et al. (2014) study, filtrate ≤25 μm in size, effectively had no nematodes, <10% mycorrhizal fungi, ~70% other fungi and ~90% bacteria of the original soil community. In addition, several other studies have used these filtrate size classes to isolate and investigate the roles of soil-borne pathogens (McCarthy-Neumann and Kobe, 2008; König et al., 2016) and AMF (Klironomos, 2002; Callaway et al., 2011; Liang et al., 2015; Pizano et al., 2017).

Differences in low versus high light survival appeared only when soil-borne microbes were present (Figure 2.1A), supporting hypothesis 1. With microbes present, survival decreased for all species, with the largest differences occurring in low light (Figure 2.2B). My results are consistent with previous research demonstrating that tree seedlings have higher mortality in shade and that the major cause of seedling death arises from disease (Augspurger, 1984a, 1984b; Vaartaja, 1962). My results are also consistent with Liang et al. (2015), who utilized the wet-sieving method and found that pathogens were associated with decreased biomass and survival, AMF were associated with increased biomass, and combined filtrate treatments canceled each other out for both biomass and survival; however, Liang et al. (2015) did not consider interactions with light availability.

I did not see increases in seedling survival when AMF were present, despite high percent colonization of seedling roots. AMF might increase seedling survival due to overall higher resource availability and also through indirect defense against pathogens via competition for root space (Borowicz, 2001; Liang et al., 2015). This result was in contrast to the study by Liang et

al. (2015), in which they found higher seedling survival with AMF. However, benefits of AMF in this study may have manifested in growth (Gehring, 2003), which I did not measure. Young seedlings still relying on maternal seed reserves and high resource availability in the greenhouse may have diminished the importance of AMF (Forero et al., 2019; Heinze et al., 2020; Kulmatiski & Kardol, 2008). Moreover, AMF may have acted indirectly by enhancing production of phenolics and NSC, which were both positively correlated with AMF colonization (Figure A2.4). I also did not see any negative relationship between AMF colonization and NSC in low light, which may suggest that AMF act parasitically when photosynthates are limited.

Amounts of functional traits varied with light availability and soil-borne microbes

For all seedling species examined, phenolic content increased when microbes were present, supporting part of hypothesis 3. Both AMF (Pozo & Azcón-Aguilar, 2007; Vierheilig, 2004; Whipps, 2004) and pathogens (Nicholson & Hammerschmidt, 1992; Witzell & Martín, 2008) can induce phenolics production. Seedling phenolics also consistently increased with light availability (Figure 2.2B), further supporting hypothesis 3 and suggesting alleviation of photosynthate constraints on chemical defense production (Ballaré, 2014).

Similarly, percent dry mass lignin increased with light availability and was highest in the 40-250 µm filtrate (Figure 2.2C), also supporting hypothesis 3. Higher lignin in the 40-250µm filtrate and lower lignin in the <20µm and combined filtrates for *A. rubrum* suggest that AMF have a positive effect while pathogens have a negative effect on lignin production.

In partial support of hypothesis 4, I found that seedling NSC decreased when pathogens were present (Figure 2.2D), consistent with NSC reserves acting as a carbon buffer after damage (Gleason & Ares, 2004; Kobe et al., 2010; McPherson & Williams, 1998; Myers & Kitajima, 2007). However, contrary to predictions, NSC increased when AMF were present, but only when

not combined with the pathogen filtrate. The positive association between AMF colonization and NSC is consistent with Y.-L. Li et al. (2022).

Functional traits were associated with greater seedling survival.

Partly supporting hypothesis 5, I found that both phenolics (chemical defense) and NSC (carbon buffer precluding recovery from damage) had positive associations with tree seedling survival. These results are consistent with previous studies that have speculated higher allocation to defensive traits increases survival of shade tolerant seedlings in low light conditions (Alvarez-Clare & Kitajima, 2007; Augspurger, 1984a; Augspurger & Kelly, 1984; Kitajima, 1994; Vaartaja, 1962).

I found no evidence of an association between lignin and survival. This was in contrast to previous studies that have posited that differences in lignin development impact seedling susceptibility to pathogens (Lee et al., 2019; Sattler & Funnell-Harris, 2013; Zhu et al., 2021). In this study, *A. saccharum*, the most shade tolerant species, had up to 50% greater lignin than the other two species (Figure 2.2D), but these differences in lignin values did not manifest in differences in survival.

Caveats and future research

There are several areas upon which future research could build on this work. I used sucrose-centrifugation to separate AMF spores from most other microbes and debris, and a bleach sterilization step to kill potential pathogens. While I was able to see high AMF colonization in the 40-250 μm and combined filtrates, and no colonization in the <20 μm or sterilized filtrates (Figure 2.2A), I cannot eliminate the possibility that I excluded some AMF in smaller filtrate sizes and included additional microbes and debris in the 40-250 μm filtrate. A more robust method would include further isolating pure AMF spores, as with Calloway (2011)

and Pizano et al. (2017). Alternatively, by adding a genetic analysis of the microbial inoculum, I could have determined more accurately which microbial groups were present, which would enhance our understanding of the results. I recommend that future research utilize the wet-sieving method in conjunction with spore isolation and/or genetic analyses.

Likewise, by culturing the pots with *Allium*, I may have inadvertently increased AMF presence, disproportionate to pathogens, or increased the relative abundance of microbes that specialize with *Allium*, rather than *Acer* species. Although often thought of as generalists, AMF can show some host specificity (Kajihara et al., 2022; H. Yang et al., 2012), which could influence post-culturing microbial communities. Furthermore, by removing the aboveground biomass and leaving the belowground root structures intact, I may inadvertently changed nutrient dynamics within the pots. Thus, I recommend that future experiments culture AMF and other microbe communities with the host species of interest (in this case, *Acer* species). In future experiments, I recommend using the host species as a bait plant in the culturing step.

While I investigated three species within a single genus to make broader generalizations, subsequent studies trying to generalize these results should include more species across additional levels of shade tolerance. Furthermore, although the three *Acer* species used in this study are similar in seed size, *A. saccharum*, the most shade tolerant species, has a larger relative seed size than *A. negundo*, the least shade tolerant species in this study. Thus, differences in seed size may have confounded effects of shade tolerance. Also, effects of light availability could be caused by changes in microclimate (e.g., soil temperature and moisture) and not directly due to irradiance. Similarly, effects of light may be mediated by photoreceptors and jasmonates, not just assimilate availability through higher photosynthetic rates (Ballaré & Austin, 2019; Pierik & Ballaré, 2021).

Additionally, I utilized shade cloth in the greenhouse to create shaded conditions, not vegetation shade. Future studies should consider teasing apart these mechanisms in the field, rather than the greenhouse, to provide more realistic seedling responses. Furthermore, although I was interested in light availability and shade tolerance, other environmental variables, such as nutrient or water availability, also could influence seedling survivorship (McCarthy-Neumann & Kobe, 2019).

Implications for forest community dynamics

This study provides a needed first step in developing a mechanistic understanding of how soil-borne microbes impact seedling shade tolerance, explained through functional traits. Although fast-growing shade intolerant species may be expected to outcompete shade tolerant species in high light (Pacala et al., 1996), shade intolerant species can be limited by the negative interactive effects of soil-borne microbes at low light (Y. Liu & He, 2019; McCarthy-Neumann & Kobe, 2010a), restricting their recruitment niche to areas with higher light and fewer soil-borne microbes. In this paper, I have demonstrated the importance of interactions between soil-borne microbes and light availability in determining tree seedling survival. Furthermore, I have related both intra- and interspecific differences in survival to functional traits, supporting a more trait-based and mechanistic approach to understanding forest community dynamics.

A modified version of this chapter has been published in Frontiers in Ecology and Evolution. The original publication is available at https://www.frontiersin.org/articles/10.3389/fevo.2023.1224540/full.

CHAPTER 3

Tree seedling functional traits mediate plant-soil feedback survival responses across a gradient of light availability

ABSTRACT

Though not often examined together, both plant-soil feedbacks (PSFs) and functional traits have important influences on plant community dynamics and could interact. For example, seedling defense and recovery traits could impact seedling survivorship responses to soils cultured by conspecific versus heterospecific adults. Furthermore, levels of defense and recovery functional traits could vary with soil culturing source. In addition, these relationships might shift with light availability, which can affect trait values, microbe abundance, and whether mycorrhizal colonization is mutualistic or parasitic to seedlings.

To determine the extent to which defense and recovery functional traits mediate PSFs via seedling survival, I conducted a field experiment. I planted seedlings of four temperate tree species across a gradient of light availability and into soil cores collected beneath conspecific (sterilized and live) and heterospecific adults. I monitored seedling survival twice per week over one growing season, and I randomly selected subsets of seedlings to measure seedling defense and recovery traits (i.e., mycorrhizal colonization and phenolics, lignin, and nonstructural carbohydrates) levels at three weeks.

Though evidence for PSFs was limited, *Acer saccharum* seedlings exhibited positive PSFs (i.e., higher survival in conspecific than heterospecific soils). In addition, soil microbes had a negative effect on *A. saccharum* and *Prunus serotina* seedling survival, with reduced survival in live versus sterilized conspecific soil. In general, I found higher trait values (measured

amounts of a given trait) in conspecific than heterospecific soils and higher light availability. Additionally, *A. saccharum* survival increased with higher levels of phenolics, which were higher in conspecific soils and high light. *Quercus alba* survival decreased with higher colonization by arbuscular mycorrhizal fungi.

I demonstrate that defense and recovery trait values (i.e., measured amounts of colonization by mycorrhizal fungi, phenolics, lignin, and NSC) in seedlings as young as three weeks vary in response to soil source and light availability. Moreover, seedling survivorship was associated with trait values for two species, despite both drought and heavy rainfall during the growing season that may have obscured survivorship-trait relationships. These results suggest that seedling traits could have an important role in mediating the effects of local soil source and light levels on seedling survivorship and thus plant traits could have an important role in PSFs.

INTRODUCTION

Though often examined separately, both plant-soil feedbacks (PSFs) and functional traits are important in plant community dynamics (Cadotte et al. 2015, Crawford et al. 2019, McGill et al. 2006, van der Putten et al. 2013, Yang et al. 2018). PSFs are a continuous feedback loop whereby plants modify properties of the soil they are growing in and influence the performance of future plants growing in that soil (Bever et al., 1997). These feedbacks subsequently affect community composition, which in turn influences soil properties, and so on. The net effect of interactions results in positive (better performance in conspecific soils), negative (better performance in heterospecific soils), or neutral PSFs.

The putative agents of PSFs are soil-borne microbes, like mycorrhizae and pathogens (Bever et al., 2010; Jiang et al., 2020). Arbuscular mycorrhizal fungi (AMF) are often mutualistic, exchanging water and nutrients for photosynthates (Wipf et al., 2019). Soil-borne

pathogens, including fungi, oomycetes, and bacteria, can cause the death of entire seedling cohorts (Mangan et al., 2010; Terborgh, 2012), and pathogens with higher effective specialization are more abundant in conspecific soils (Benítez et al., 2013; Hersh et al., 2012). Mycorrhizal colonization is frequently higher in conspecific soils and in soils cultured by adult trees of the same mycorrhizal type (Bennett et al., 2017; Chen et al., 2019; Liang et al., 2016). These soils contain mycorrhizal genotypes that are well-suited to colonizing the adult trees growing in them (Segnitz et al., 2020; J. Yang et al., 2018).

Functional traits are measurable morphological or physiological attributes affecting plant performance (Violle et al., 2007) that can translate into impacts on community dynamics. Despite the important role of plant survival in PSF (Comita et al., 2010; McCarthy-Neumann & Kobe, 2010a), traits promoting faster growth (e.g., specific leaf area, specific root length, height) have been the focus of most PSF studies (Baxendale et al., 2014; Cortois et al., 2016; Xi et al., 2021). Frequently, defensive traits are accounted for by assuming that species with fast growth rates have low investment in defense, and vice-versa (Cortois et al., 2016; Xi et al., 2021). However, tree seedling survivorship is likely to have greater effects on future community dynamics and composition than growth (Pacala et al., 1996). Thus, while little studied, functional traits that influence tree seedling survivorship in response to PSFs could be a crucial mechanism governing seedling and forest community dynamics.

Plant functional traits could influence PSFs and vice-versa (P. Ke et al., 2015; Kuťáková et al., 2018; Xi et al., 2021). Functional traits that influence plant defense against and recovery from attack by soil-borne microbes include phenolics, lignin, and nonstructural carbohydrates (NSC). Phenolics and lignin can serve as chemical (Ichihara & Yamaji, 2009) and physical (Augspurger, 1990) defenses against soil-borne microbes. NSC can be mobilized to repair

damaged tissues (Dietze et al., 2014). Additionally, percent root colonization by mycorrhizae can be treated as a trait (Maherali, 2020) conferring defense against pathogens (Bennett et al., 2017). AMF can provide indirect defense against pathogens by competing for space on plant roots (Borowicz, 2001) and EMF can provide direct defense by forming a protective physical sheath on young roots (Laliberté et al., 2015). Also, both AMF and EMF can increase their host plant's resource acquisition, which can be allocated to defensive and recovery traits (Liang et al., 2015; Sikes, 2010).

Phenolics, lignin, and NSC are likely affected by soil source (e.g., conspecific versus heterospecific soil). Phenolics production can be induced by mycorrhizal colonization (Wallis & Galarneau, 2020) and potentially by fungal pathogens (Witzell & Martín, 2008). I expect that phenolics production should subsequently be higher in conspecific soils, where there should be higher colonization by mycorrhizal fungi and infection by effectively-specialized soil-borne pathogens (Benítez et al., 2013; Hersh et al., 2012). It is unclear whether lignin production is influenced by conspecific soils. However, lignin production could be driven by soil nutrient availability, which can be impacted by microbes (J. Li et al., 2020; Luo et al., 2022). NSC should be lower in conspecific soils, due to greater resource allocation to symbionts (Schiestl-Aalto et al., 2019) and recovery against pathogen infection (Martínez-Vilalta, 2014; Saffell et al., 2014).

Both PSFs and functional traits can shift across environmental gradients like light availability (McCarthy-Neumann & Ibáñez, 2013; Smith-Ramesh & Reynolds, 2017). However, most studies have not integrated abiotic factors when evaluating both traits and PSFs (Cortois et al., 2016). Shifts in light can change microbial composition and abundance (Koorem et al., 2017; Y. Liu & He, 2019), which may alter seedlings' ability to defend against or recover from disease. AMF are more abundant in higher light (Bereau et al., 2000; Koorem et al., 2017; Shi et al.,

2014); however, in low light they can act parasitically and thereby decrease seedling survival (Konvalinková & Jansa, 2016). Higher mortality from pathogens typically occurs in low light (McCarthy-Neumann & Ibáñez, 2013; McCarthy-Neumann & Kobe, 2010a), where wetter and cooler conditions enhance microbe reproduction and dispersal (Hersh et al., 2012; Y. Liu & He, 2019; Reinhart et al., 2010). Light availability can also modify functional trait level, including reduced production of phenolics (Ichihara & Yamaji, 2009) and lignin (Falcioni et al., 2018; Rogers et al., 2005). Additionally, carbon limitation in shade and lower stored nonstructural carbohydrates (NSC) may constrain recovery from disease (Kobe, 1997; Kobe et al., 2010).

My overall conceptual framework (Figure 3.1) is that soil source and light availability influence trait levels, which in turn influence tree seedling survival. Thus, plant traits have an important role in mediating PSFs. We hypothesized that:

1) Negative PSFs are widespread across tree species and are more prevalent under low than high light. Furthermore, these differences in PSFs are only present when soil-borne microbes are present. This result would indicate that soil-borne microbes drive negative PSFs in low light availability directly through increased pathogen abundance and/or a shift from positive to negative in the plant-mycorrhizal fungi relationship, and/or indirectly through decreased levels of defensive traits.

2) Mycorrhizal colonization is greater in conspecific soils and in higher light. This result would indicate that mycorrhizal colonization is promoted by effectively-specialized microbes in conspecific soils and greater resource availability (e.g., NSC) in high light.

3) The defensive functional traits phenolics and lignin are induced to higher levels in soils cultured by conspecific adults and in high light availability. This result would indicate that defensive functional trait production is driven by the presence of effectively-specialized

parasitic microbes expected in conspecific soils and by greater carbon income expected in higher light.

4) The recovery trait NSC is lower in soils cultured by conspecific adults and in low light availability. This result would indicate that NSC is drawn down in the presence of effectively-specialized parasitic microbes expected in conspecific soils, in addition to lower carbon income relative to use expected in lower light.

5) Finally, I hypothesized that seedling survival increases as mycorrhizal colonization, phenolics, lignin, and NSC also increase. This result would indicate that PSFs can, in part, be mediated by the degree of mycorrhizal colonization and changes in functional trait values responding to variation among soil types and in light availability.

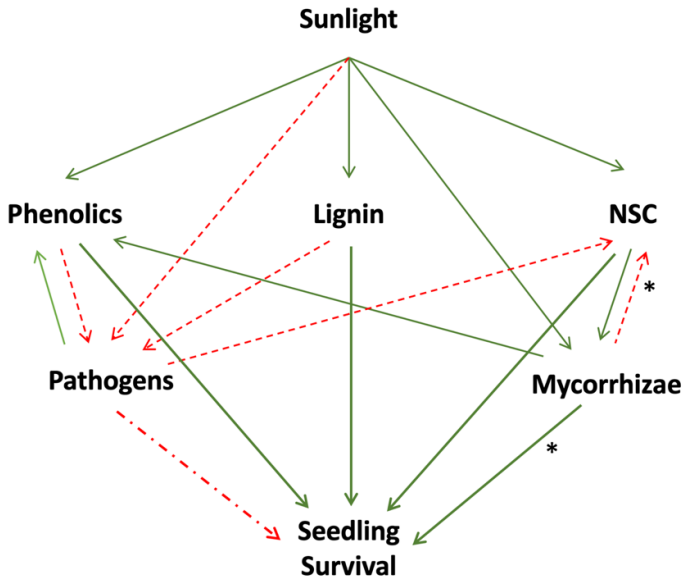


Figure 3.1 Conceptual diagram demonstrating the relationships between light availability, functional traits (phenolics, lignin, and nonstructural carbohydrates [NSC]), colonization by mycorrhizal fungi, and tree seedling survival. Green, solid lines indicate a positive relationship. Red, dashed lines indicate a negative relationship. Lines that directly influence tree seedling survival are thicker. Stars (*) next to the lines linking 'Mycorrhizae' with 'NSC' and 'Seedling Survival' indicate that this relationship is usually positive but can shift to neutral or negative.

MATERIALS AND METHODS

I conducted a factorial blocked design field experiment, consisting of four tree species, seven soil sources (sterilized conspecific, live conspecific, and five heterospecific), and a gradient of forest understory light levels (low, medium, and high), for a total of 3,024 seedlings. I monitored seedling survival twice per week over one growing season, and I randomly selected subsets of seedlings to measure mycorrhizal colonization and phenolics, lignin, and NSC measurements at three weeks. I used Cox proportional hazards survival models to evaluate survival and linear mixed effects models to test how light availability and soil source influence defense and recovery traits.

Study location

The research site is a 100 ha mixed hardwood forest stand in mid-Michigan, at Alma College's Ecological Field Station (43°23'32.0"N 84°53'41.5"W). Alma College granted permission to undertake this research and collect plant and soil materials; a formal field permit was not required. This forest has not been logged since 1897 and lies in an ecological tension zone between northern coniferous and southern deciduous forests. The dominant species in this forest is sugar maple *(Acer saccharum*), a shade-tolerant canopy tree species. Other common trees in the forest include red maple (*A. rubrum*) and big-toothed aspen (*Populus grandidentata*).

Species selection

We identified adult trees for soil collection and established field plots in a 3 ha mapped section of the forest (Table 3.1). I initially chose six tree species native to the research site: red maple (*A. rubrum*), sugar maple (*A. saccharum*), big-toothed aspen (*P. grandidentata*), black cherry (*Prunus serotina*), white oak (*Quercus alba*), and northern red oak (*Q. rubra*). *A. rubrum* and *P. grandidentata* seedlings experienced high (> 80%) mortality within two weeks of

planting, suggesting poor seed source or propagation methods. Thus, while still included as soil sources, they were not included in analyses of seedling survival, mycorrhizal colonization, or functional traits.

Table 3.1 Local adult abundance, shade tolerance, seed weight, and primary mycorrhizal association for each of our study species. [1]Local adult abundance was calculated as stems/ha at Alma College's Ecological Preserve; only adults ≥ 5cm dbh were included in this count. [2]Shade tolerance is presented as intolerant, intermediate, or tolerant and as mean ± std. dev., on a standardized scale from 1 (least tolerant) to 5 (most tolerant), calculated by Niinemets and Valladares (Niinemets & Valladares, 2006a). [3]Seed weight data was collected from Burns and Honkala (Burns & Honkala, 1990a). [4]AMF = arbuscular mycorrhizal fungi and EMF = ectomycorrhizal fungi.

Species	Local adult abundance[1]	Shade tolerance[2]	Seed weight (mg)[3]	Mycorrhizal association[4]
Acer rubrum	131	(3.44 ± 0.23)	19.7	AMF
Acer saccharum	285	(4.76 ± 0.11)	64.9	AMF
Populus grandidentata	82.33	(1.21 ± 0.27)	0.2	AMF & EMF
Prunus serotina	4.33	(2.46 ± 0.34)	94.3	AMF
Quercus alba	12.67	(2.85 ± 0.17)	6,677	AMF & EMF
Quercus rubra	71.67	(2.75 ± 0.18)	4,127	AMF & EMF

Soil sources and planting

I collected intact soil cores from May to July 2016 and April to May 2017 (Table A3.1). To minimize potential for multispecies culturing of soil, I took soil cores under trees that were at least two crown diameters away from adults of other species. Using a custom-made mechanized soil core sampler (Giddings Machine Co; Windsor, CO, USA), I removed intact soil cores (9 cm diameter and 30 cm deep for planted *A. saccharum* and *P. serotina* seedlings, or 46 cm long for *Q. alba* and *Q. rubra* seedlings) within 1 m from the bole of six mature randomly selected adults for each of the six study species (36 trees total). I maintained soil cores from each adult as separate replicates (Reinhart & Rinella, 2016; Rinella & Reinhart, 2018).

Intact soil cores with plastic liners were converted into pots by drilling two 7.5 cm diameter holes into the sides and adhering a 0.5 μm nylon mesh covering over side holes and the bottom opening. Such pots are an established method for studying common mycorrhizal networks in forests (Bingham & Simard, 2012; McGuire, 2007; Teste et al., 2006). The mesh prevents roots, fungal hyphae, oomycetes and pathogenic fungi from passing in or out, with minimal effect on water and nutrient flows (Allison et al., 2013). I did not use multi-stage greenhouse culturing (Bever et al., 2010), because in-situ natural culturing already had occurred for these long-lived trees and should more closely characterize PSFs occurring in the field.

After resting, pots were transplanted into eighteen 8.4 × 6.6 m common-garden field plots that fell within three general light groupings (low, medium, and high). Existing vegetation and leaves in each plot were removed to reduce light interception. I then took precise measurements of light availability by analyzing canopy photos with HemiView software (Delta-T Devices, Ltd., Burwell, England; Figure A3.5).

Soil samples for the sterilized conspecific soil treatment were exposed to gamma irradiation (30-70 kGy; Sterigenics International, Schaumburg, IL, USA) in July 2017 and allowed to rest for at least one month to minimize post-sterilization nutrient spikes. Gamma irradiation is highly effective at killing soil microorganisms and typically has minimal effects on soil chemical and physical properties (McNamara et al., 2003). Nevertheless, I tested the sterilized versus live soils using plant root simulator (PRS^{TM}) probes (Western Ag Innovations Inc., Saskatchewan, Canada) and found no effect of sterilization on soil nutrient availability (Tables A3.3 & A3.4; Figure A3.3).

I planted 108 seedling pots per species × soil source, evenly distributed across the 18 field plots. A single surface-sterilized seed with a newly-emerged radicle was planted into each

pot. Seeds for *Q. alba* were purchased from Sheffields Seed Co (Locke, NY, USA) and seeds for all other species were collected from mid-Michigan forests. Variation among seed source populations in survival, mycorrhizal colonization and functional traits was likely minimal (McCarthy-Neumann & Ibáñez, 2012). In June 2018, one week prior to planting, I added 1 cm of a 1:1 mixture of peat moss and fresh or sterilized soil to increase transplant success and provide fresh inoculum. In a previous trial run, I found that seedlings planted with peat moss and fresh soil had reduced transplant shock; personal observation).

To minimize disease from non-experimental soil sources, seeds were surface sterilized with 0.6% NaOCl solution prior to stratification and prior to germination. To avoid cross-contamination, all tools and surfaces that were exposed to soil were soaked in 10% bleach or surface sprayed with 70% EtOH and then rinsed with deionized water. To minimize browsing and digging-up of seedlings by vertebrates, I erected galvanized hardware cloth (6 × 6 cm openings) to 1.8 m height around each plot. I also glued hardware cloth with 0.25 cm × 0.25 cm openings to the top of each pot. Seedlings likely did not experience significant shading due to the addition of the hardware cloth and often grew above the cloth within 2 weeks of planting.

Survival and functional traits

I censused seedling survival twice per week for 16 weeks. Mortality at the first two censuses after planting were attributed to transplant shock or poor seed source; these seedlings were not used in subsequent analyses, and pots were re-planted with the same seedling species.

Three weeks after planting, I harvested six seedlings per treatment combination to measure mycorrhizal colonization, phenolics, lignin, and NSC. I chose this harvest date since, in a previous greenhouse experiment, mortality curves for tree seedlings subjected to soil-borne pathogens often increased at week three and peaked between four to six weeks after germination

(McCarthy-Neumann & Ibáñez, 2012). For measurements, I used established protocols: AMF and EMF colonization (McGonigle et al., 1990; Vierheilig et al., 1998), phenolics (Ainsworth & Gillespie, 2007; P. Waterman & Mole, 1994), lignin (ANKOM Technologies, Macedon, NY, USA), and NSC (Landhäusser, Chow, Dickman, et al., 2018). Due to the small size of three-week-old seedlings and the destructive nature of each measurement, half of the harvested seedlings were allocated to measurement of NSC (stem and root), and half of the seedlings were allocated to measurement of phenolics (hypocotyl), lignin (stem), and mycorrhizal colonization (roots).

Statistical analysis

To evaluate hypotheses 1, I analyzed seedling survival over 16 weeks with Cox proportional hazards regression (Cox & Oakes, 2017). I ran species-specific models, using soil source and light availability as fixed effects, and plot and adult tree as random effects. The best fitting models for seedling survival did not include any interactions. I compared survival in live conspecific versus heterospecific soils (Gómez-Aparicio et al., 2017; Xi et al., 2021). Greater survival in live conspecific than heterospecific soils indicated positive PSFs. I compared survival in sterilized versus live conspecific soils. Higher survival in sterilized than live conspecific soils indicated that microbes influenced PSFs.

To evaluate hypotheses 2-4, we analyzed measured amounts of mycorrhizal colonization, phenolics, lignin, and NSC with linear mixed effects models. I ran species-specific models for each trait, using soil source and light availability as fixed effects, and plot and adult tree as random effects. I used *a priori* contrasts to compare levels of measured traits in live conspecific versus heterospecific soils and to compare levels of measured traits in sterilized versus live conspecific soils.

To evaluate hypothesis 5, I analyzed seedling survival over 16 weeks with Cox proportional hazards regression (Cox & Oakes, 2017). I ran species-specific models, with mycorrhizal colonization, phenolics, lignin, and NSC as fixed effects. I imputed colonization and trait data from seedlings harvested at three weeks, for each combination of seedling species, plot, soil source, and light level. I accounted for possible collinearity between traits by calculating variance inflation factors (VIF) for each model and removing variables with VIF > 5. For *A. saccharum*, *A. rubrum*, and *Q. rubra*, NSC was removed from the final models, and for *Q. alba*, lignin was removed from the final models. NSC was highly correlated with lignin for all study species and with phenolics for *Q. rubra* (Figure A3.8).

For all models, light availability was first evaluated as a continuous variable, using Indirect Site Factor (ISF) quantified with canopy photos. ISF represents the proportion of diffuse (indirect) solar radiation reaching a given location, relative to an open site and was calculated using HemiView software (Delta-T Devices, Ltd., Burwell, England). For post-hoc comparisons and figures, I divided seedlings according to light group, splitting the range of light availability into three bins (low = 0.032-0.075 ISF, medium = 0.075-0.118 ISF, and high = 0.118-0.161 ISF). These light thresholds were determined by dividing the range of light availability across the field plots into three bins. Heterospecific soils were modeled as both pooled and unpooled/specific soils; when evaluating post-hoc comparisons, we used pooled heterospecific soils.

All analyses were performed with R version 3.5.1 (R Core Team, 2020). I used the "coxph" function in the survival package (Therneau & Grambsch, 2000) to fit Cox proportional hazards regression models. I tested the significance of main effects using a likelihood ratio test with the "Anova" function. I tested for multicollinearity variance inflation factors using the "vif" function in the car package (Fox & Weisberg, 2019). Post-hoc Tukey pairwise comparisons of

significant main effects and Bonferonni corrections for multiple comparisons were made using the "emmeans" function in the multcomp package (Hothorn et al., 2008; Lenth, 2020). I used the missForest package (Stekhoven & Buehlmann, 2012) to impute trait data for seedlings monitored for survival.

RESULTS

Negative PSFs were not widespread among tree species, nor were they more prevalent in low light availability

No species experienced negative PSFs (defined as lower survival in conspecific versus heterospecific soils). However, *A. saccharum* experienced positive PSFs with higher survival in conspecific than pooled heterospecific soil ($LR\chi^2 = 8.60$, $p < 0.01$; Figure 3.2A, Table 3.2A). Seedling survival was lower in live than sterilized conspecific soil for both *A. saccharum* ($LR\chi^2 = 61.78$, $p < 0.01$) and *P. serotina* ($LR\chi^2 = 1.52$, $p < 0.01$), suggesting an effect of soil-borne microbes. Although there was a positive effect of light on survival for *P. serotina* ($LR\chi^2 = 4.09$, $p = 0.04$) and *Q. rubra* ($LR\chi^2 = 9.02$, $p < 0.01$; Fig 2B, Table 3.2B) there was no significant interaction between soil source and light availability for any of the models with pooled heterospecific soils. When heterospecific soils were not pooled, there was a significant interaction between light and soil source, but only for *P. serotina* ($LR\chi^2 = 6.860$, $p < 0.01$). Thus, the expectation that negative PSFs are widespread among species and are more prevalent in low light availability was not supported.

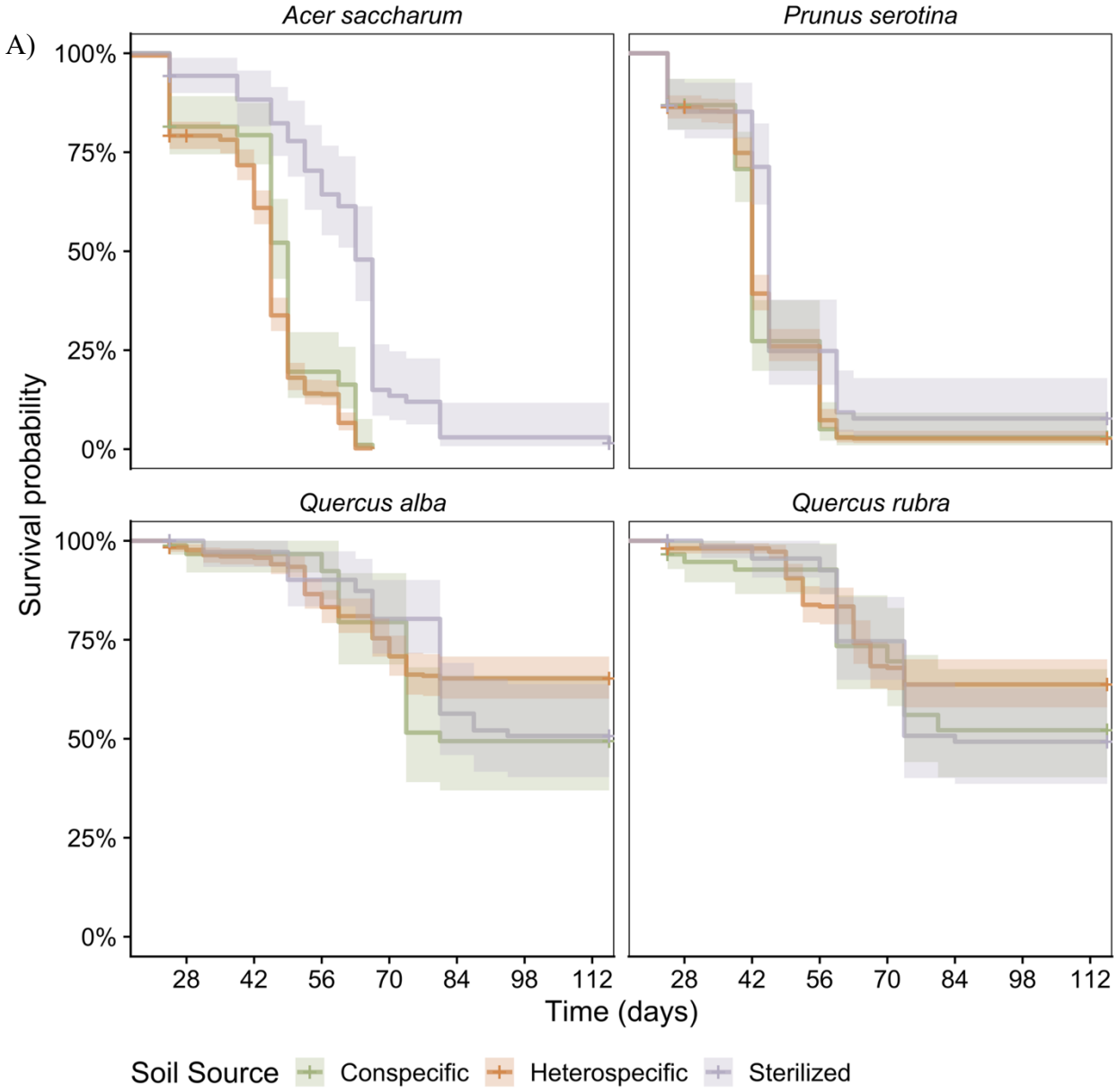


Figure 3.2 Kaplan-Meier plots evaluating the effects of **A)** soil source (conspecific, pooled heterospecific, and sterilized conspecific) on seedling survival, and **B)** light availability on seedling survival. For visualization, light availability was binned into 3 levels: Low = 0.032 - 0.075 ISF, Med = 0.075-0.118 ISF, and High = 0.118 - 0.161 ISF. Shaded regions indicate 95% confidence intervals about the mean.

Figure 3.2 (cont'd)

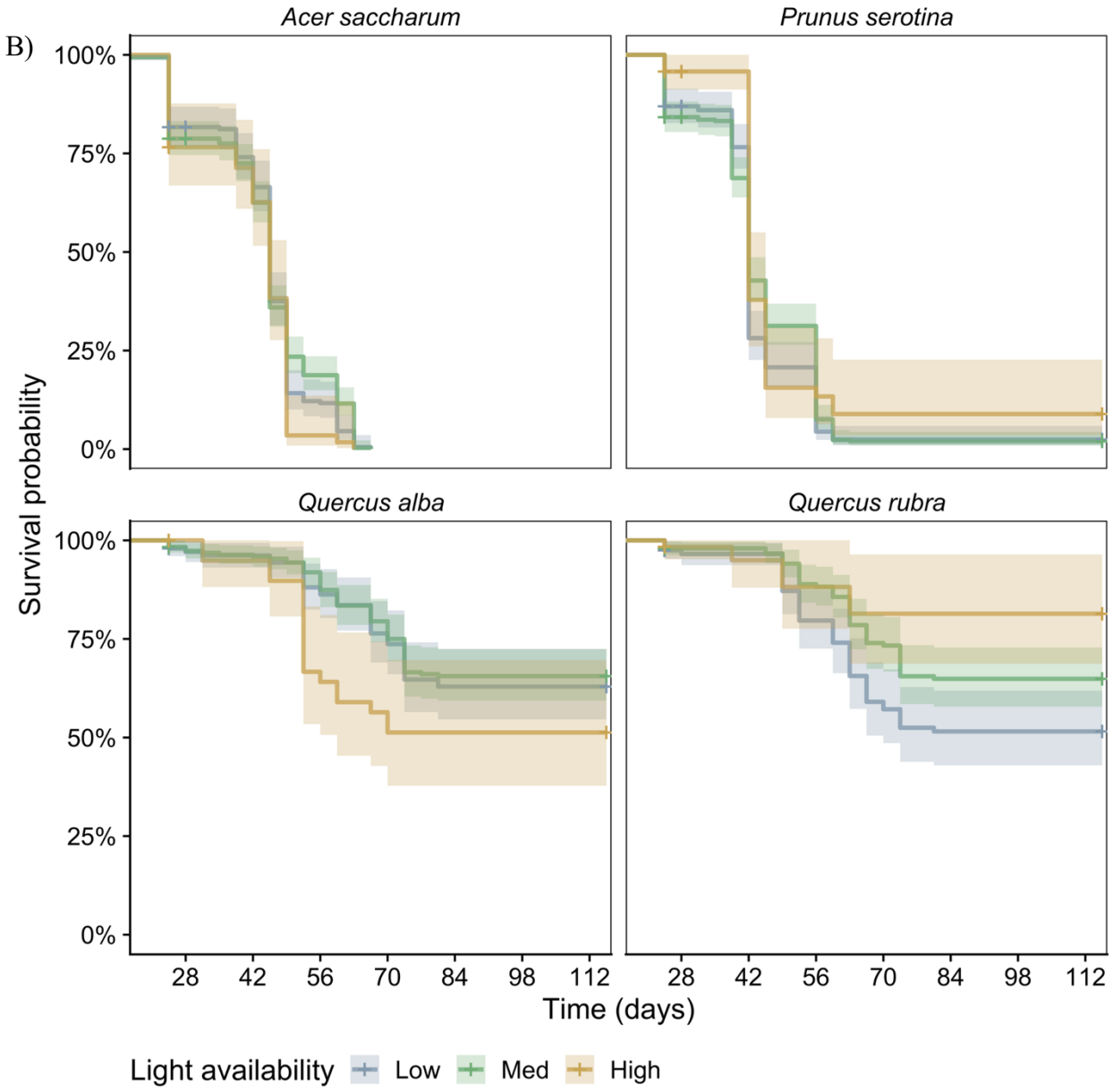


Table 3.2 Number of surviving seedlings at the end of the growing season. Data is presented for each **A)** species × soil source and **B)** species × light level as both an absolute number and percentage. Because there was not a significant interaction between soil source and light availability on seedling survival, they are presented separately, corresponding with Figures 3.2A and B. Soil sources include sterilized conspecific, live conspecific, and pooled heterospecific soils. Light availability was binned into 3 levels: Low = 0.032 - 0.075 ISF, Med = 0.075 - 0.118 ISF, and High = 0.118 - 0.161 ISF.

Species	A) Soil source			B) Light availability		
	Sterilized conspecific	Live conspecific	Hetero-specific	Low	Med	High
Acer saccharum	1 (1%)	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	0 (0%)
Prunus serotina	5 (8.9%)	3 (3.3%)	12 (2.8%)	8 (3.9%)	7 (2.3%)	5 (7.7%)
Quercus alba	36 (50.7%)	23 (50%)	199 (66.3%)	83 (60.1%)	150 (64.7%)	25 (51.1%)
Quercus rubra	33 (49.3%)	27 (54%)	152 (65%)	66 (52%)	118 (62.8%)	28 (77.8%)

Mycorrhizal colonization and seedling functional traits varied across both soil source and light availability

AMF colonization was 11% higher in conspecific than pooled heterospecific soil only for *A. saccharum* ($t_{2344} = 1.84$, marginally significant at $p = 0.07$; Figure 3.3A). For the other study species, AMF colonization was higher in pooled heterospecific than conspecific soils: 12% for *P. serotina* ($t_{2344} = 3.88$, $p < 0.01$), 16% for *Q. alba* ($t_{2344} = 2.38$, $p = 0.02$), and 12% for *Q. rubra* ($t_{2344} = 2.05$, $p = 0.04$). As predicted, AMF colonization increased with light for *P. serotina* (slope = 108% / ISF, $F_{1,2344} = 35$, $p < 0.01$) and *Q. rubra* (slope = 53% / ISF, $F_{1,2344} = 6.42$, $p < 0.01$), but not for *A. saccharum*. Contrary to our expectations, AMF colonization decreased with light for *Q. alba*, which is primarily associated with EMF (slope = -49% / ISF, $F_{1,2344} = 6.08$, $p = 0.01$).

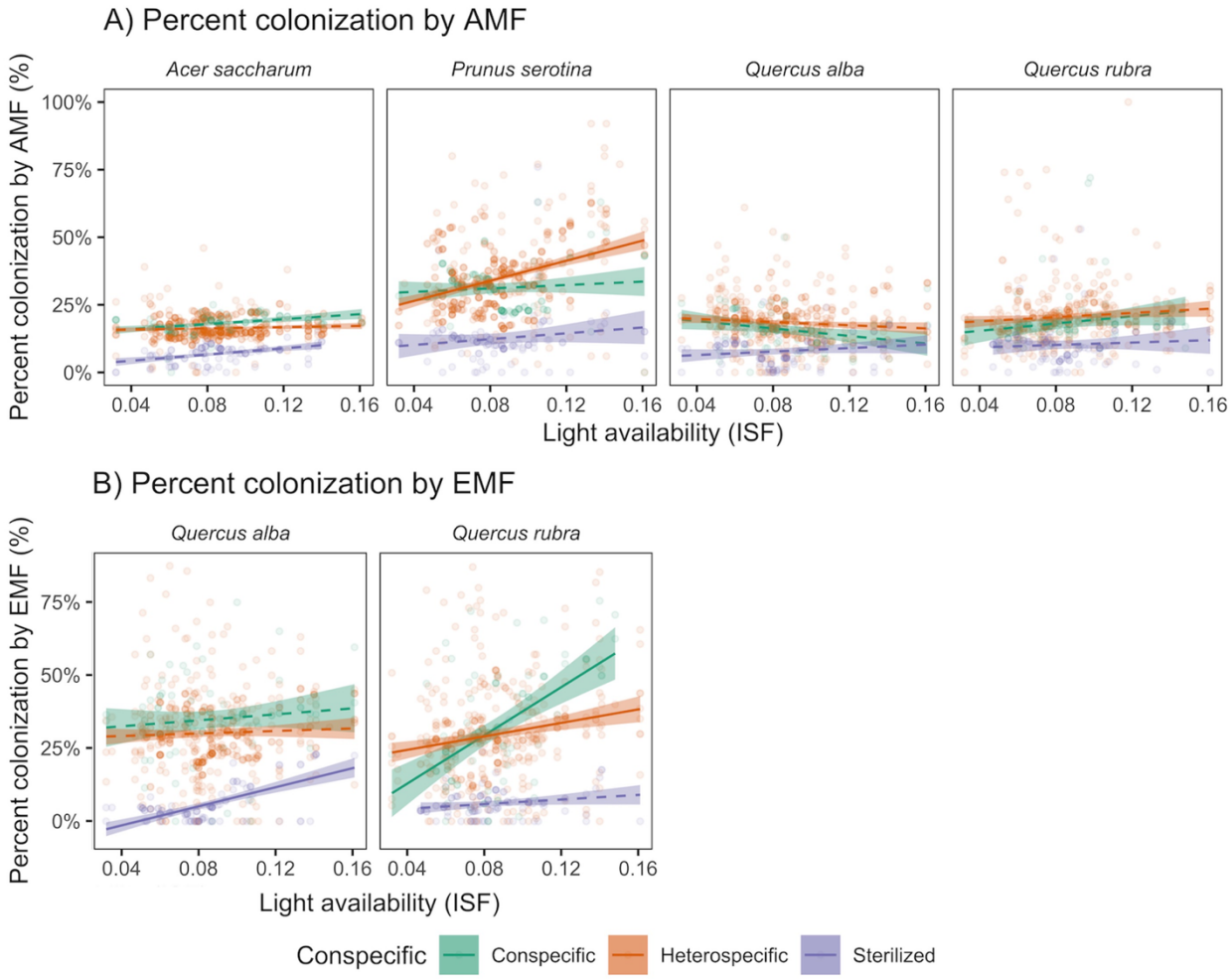


Figure 3.3 Effect of soil source and light availability on percent mycorrhizal colonization. By **A)** AMF and **B)** EMF (only *Q. alba* and *Q. rubra* are colonized by EMF). Shaded regions indicate 95% confidence intervals about the mean. Solid lines have a slope significantly different from zero ($p < 0.05$).

EMF colonization was higher in conspecific than pooled heterospecific soil by 16% for *Q. alba* across all light levels ($t_{1063} = 2.72$, $p = 0.01$; Fig 3.3B) and by 22% for *Q. rubra* in high, but not low light ($t_{1063} = 4.74$, $p < 0.01$). EMF colonization increased with light availability for *Q. rubra* (slope = 264.2% / ISF, $F_{1,1063} = 63.02$, $p < 0.01$), especially in conspecific soil (slope = 413% / ISF).

Phenolics (nmol Gallic acid equivalents per mg dry extract) were higher in live than sterilized conspecific soils for *A. saccharum* (227%, $t_{793} = 7.85$, $p < 0.001$), *P. serotina* (173%,

$t_{793} = 6.77$, $p < 0.001$), and *Q. alba* (51.7%, $t_{793} = 43.73$, $p < 0.001$). As expected, phenolics were higher in conspecific than pooled heterospecific soil for *A. saccharum* (23%, $t_{2344} = 10.56$, $p < 0.01$; Figure 3.4A) and *Q. alba* (4%, $t_{2344} = 4.44$, $p < 0.01$). Conversely, phenolics were 69% higher in pooled heterospecific soil for *P. serotina* ($t_{2344} = 6.96$, $p < 0.01$). For *Q. rubra*, phenolics were 18% higher in conspecific soil at high light ($t_{2344} = 5.89$, $p < 0.01$) and 29% higher in pooled heterospecific soil at low light ($t_{2344} = 12.77$, $p < 0.01$). Phenolics increased with light availability for all four study species (*A. saccharum*: slope = 5.70 nmol / ISF, $F_{1,2344} = 64.7$, $p < 0.01$; *P. serotina*: slope = 4.15 nmol / ISF, $F_{1,2344} = 34.48$, $p < 0.001$; *Q. alba*: slope = 10.51 nmol / ISF, $F_{1,2344} = 187.07$, $p < 0.01$; *Q. rubra*: slope = 12.73 nmol / ISF, $F_{1,2344} = 249.42$, $p < 0.01$). For *Q. rubra*, this trend appeared to be driven by conspecific soil (slope = 23.26 nmol / ISF).

Percent dry mass lignin was higher in conspecific than pooled heterospecific soil by 11% for *Q. alba* ($t_{1,2344} = 8.60$, $p < 0.01$) and 5.8% for *Q. rubra* ($t_{2344} = 5.61$, $p < 0.01$), across all light levels (Figure 3.4B). For both *A. saccharum* and *P. serotina*, lignin did not vary between conspecific and pooled heterospecific soil. Lignin increased with light availability for *A. saccharum* (slope = 38% / ISF, $F_{1,2344} = 82.34$, $p < 0.01$) and *P. serotina* (slope = 57% / ISF, $F_{1,2344} = 184.51$, $p < 0.01$). There was no effect of light on lignin for *Q. alba*. Contrary to our predictions, for *Q. rubra*, lignin decreased with light availability (slope = -22% / ISF, $F_{1,2344} = 21.42$, $p < 0.01$); this trend appeared to be driven by conspecific soil (slope = -40% / ISF). Indicating a potential effect of soil biota, lignin (percent dry mass) was higher in live than sterilized conspecific soils for all four study species: *A. saccharum* (12%, $t_{793} = 6.40$, $p < 0.001$), *P. serotina* (13%, $t_{793} = 4.18$, $p < 0.001$), *Q. alba* (12%, $t_{793} = 10.56$, $p < 0.001$), and *Q. rubra* (2.4%, $t_{793} = 2.46$, $p = 0.014$).

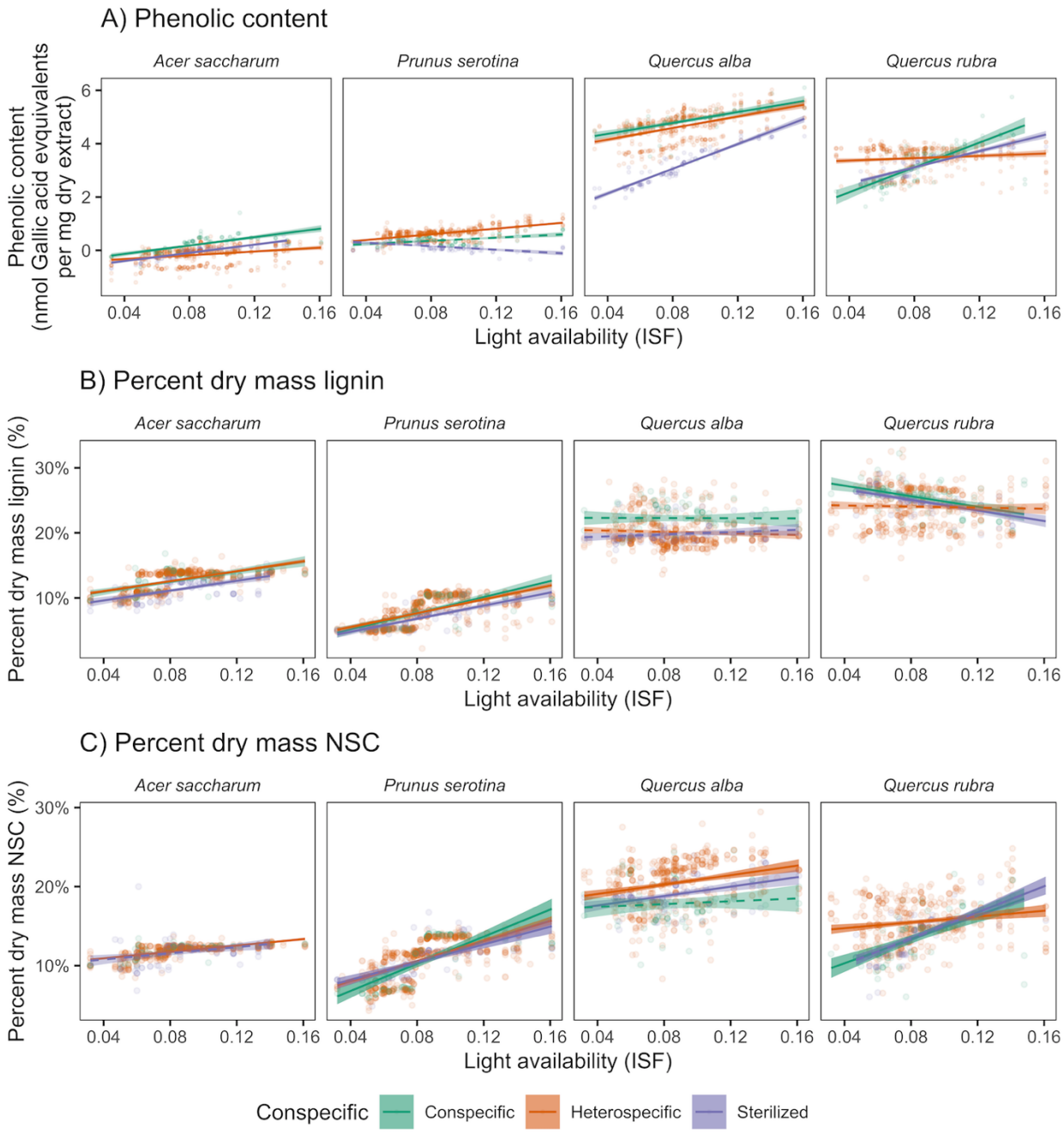


Figure 3.4 Effect of soil source and light availability on functional traits. Traits include: A) phenolics (nmol Gallic acid equivalents per mg dry mass), B) percent dry mass lignin, and C) percent dry mass NSC. Some lines are truncated, because not enough seedlings survived in that light level. Shaded regions indicate 95% confidence intervals about the mean. Solid lines have a slope significantly different from zero ($p < 0.05$).

Percent dry mass NSC was higher in pooled heterospecific than conspecific soil across all light levels for *Q. alba* (15%, $t_{2344} = 9.96$, $p < 0.01$; Figure 3.4C). For *P. serotina* and *Q. rubra*, NSC was higher in conspecific soil at low light (*P. serotina*: 1.9%, $t_{2344} = 3.41$, $p < 0.01$; *Q. rubra*: 13%, $t_{2344} = 9.34$, $p < 0.01$), but did not vary with soil source at high light. For all four study species, NSC increased with light availability (*A. saccharum*: slope = 21% / ISF, $F_{1,2344} = 22.05$, $p < 0.01$; *P. serotina*: slope = 75% / ISF, $F_{1,2344} = 287.75$, $p < 0.01$; *Q. alba*: slope = 19% / ISF, $F_{1,2344} = 16.39$, $p < 0.01$; *Q. rubra*: slope = 47% / ISF, $F_{1,2344} = 87.42$, $p < 0.01$). This trend appeared to be driven by pooled heterospecific soil for *Q. alba* (slope = 30), and conspecific soil for *Q. rubra* (slope = 76% / ISF). NSC was higher in sterilized than live conspecific soils for *Q. alba* (6.7%, $t_{793} = 4.17$, $p < 0.001$).

Mycorrhizal colonization and functional traits had limited effects on seedling survival

From the Cox survival models, I interpreted hazard ratios (HR), an integration of the hazard experienced by seedlings across the study duration. HR < 1 indicates decreased hazard relative to the baseline (i.e., increased survival); HR > 1 indicates increased hazard (i.e., decreased survival). Traits predicted survival for two species: phenolics had a positive effect on survival for *A. saccharum* (HR = 0.73, $LR\chi^2 = 4.20$, $p = 0.04$; Figure 3.5) and AMF colonization had a negative effect for *Q. alba* (HR = 1.04, $LR\chi^2 = 4.18$, $p = 0.04$).

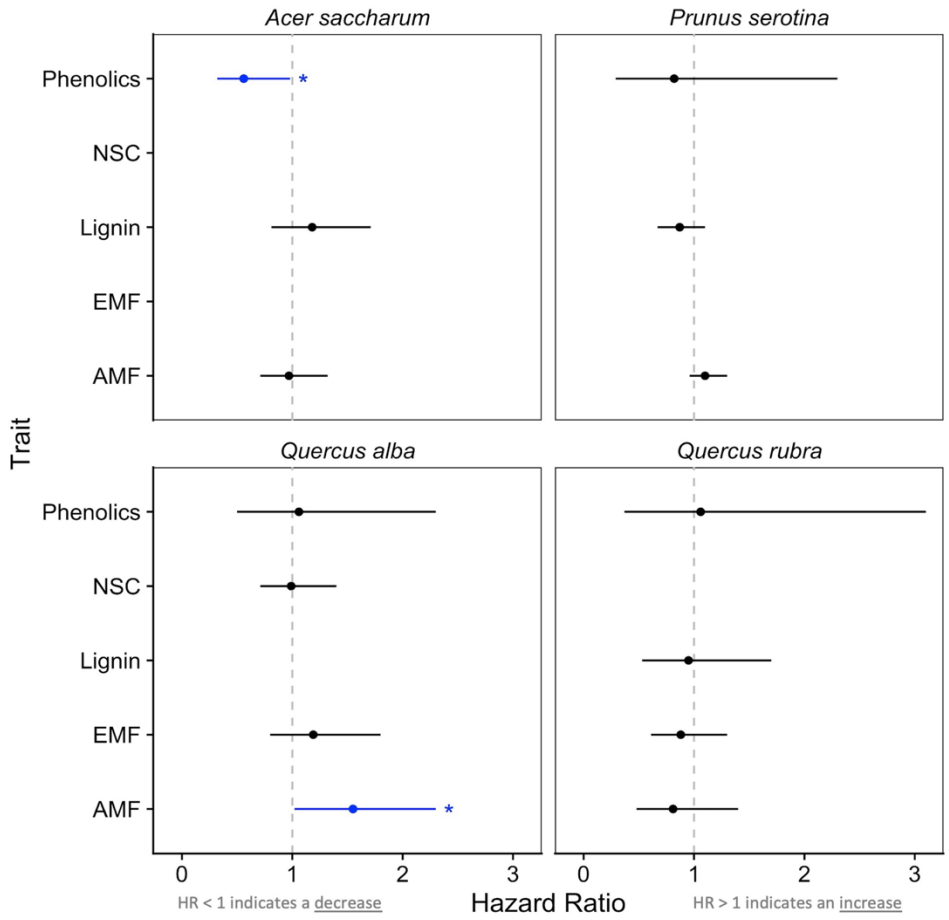


Figure 3.5 Hazard ratios (HR) demonstrating the effect of mycorrhizal colonization and functional traits on seedling survival over the growing season. HR > 1 indicates an increase in mortality and HR < 1 indicates a decrease in mortality as the trait increases. Statistically-significant effects ($p < 0.05$) are colored blue. Species x trait combinations that are blank were removed from the final models due to high collinearity (VIF > 5).

DISCUSSION

While I found limited evidence of survival-based PSFs in this field study, defense and recovery traits varied in response to soil source and light availability in seedlings as young as three weeks old. I also found limited associations between defense and recovery traits and seedling survivorship. These results support that, while seedling defense and recovery traits are very responsive to soil source and light availability, PSFs may be less prevalent in field conditions where multiple environmental factors influence seedling survivorship.

PSFs were not widespread among species, nor were they more prevalent in low light availability

Only one of four study species experienced PSFs between conspecific and heterospecific soils, and there were very few interactions between light availability and soil source on seedling survival. I expected to find stronger negative PSFs in low light conditions (McCarthy-Neumann & Ibáñez, 2013), due to both greater limitation of light availability and higher prevalence of soil-borne pathogens.

Both *A. saccharum* and *P. serotina* experienced lower survival in live than sterilized conspecific soils, indicating that soil-borne microbes have negative effects on seedling survival. For *P. serotina*, although soil-borne microbes cultured in conspecific soils may have a negative effect on survival, the net effect of PSFs (assessed as survivorship in conspecific versus pooled heterospecific soils) appeared to be neutral (Esch & Kobe, 2021; McCarthy-Neumann & Kobe, 2019; Packer & Clay, 2003).

A. saccharum seedlings experienced net positive PSFs, having greater survival in conspecific than heterospecific soils. However, they had even greater survival in sterilized than live conspecific soils, consistent with McCarthy-Neumann and Ibáñez (2013) and suggesting net

negative effects of soil-borne microbes. There are at least three mutually compatible explanations for these results: 1) mutualistic microbes may provide greater benefit in conspecific than heterospecific soils; 2) there may be a greater negative effect of harmful microbes in heterospecific soils; or, 3) there may be unmeasured, more favorable abiotic effects in soils modified by *A. saccharum* adults in comparison to heterospecific soils (McCarthy-Neumann & Ibáñez, 2012).

Comparisons of PSFs in this study are presented as differences in rates (calculated as $LR\chi^2$), rather than differences in total number of surviving seedlings at the end of the growing season (Figure 3.2, Table 3.2). Both *A. saccharum* and *P. serotina* had zero (or near-zero) survival by the end of the growing season. However, investigating the environmental conditions that influence mortality rates for these seedlings is still meaningful for understanding forest communities. When adult *A. saccharum* and *P. serotina* trees produce thousands of seeds in a single growing season (Burns & Honkala, 1990a), small differences in survival rates can scale up to meaningful impacts on community composition over longer time periods.

Mycorrhizal colonization and functional traits varied across both soil source and light availability

These results demonstrate that tree seedlings, even as young as three weeks old, express intraspecific variation in mycorrhizal colonization and functional trait values, in response to conspecific versus heterospecific soil source and light availability. AMF colonization was higher in heterospecific than conspecific soil for most of the measured species, with the largest difference being for *P. serotina* at high light availability. This was in contrast to a study evaluating PSF in temperate tree species across North America, which found that AMF colonization was equal or greater in conspecific relative to heterospecific soils (Bennett et al.,

2017). My result may be because AMF are more generalized in host associations than EMF for our study species (S. Smith & Read, 2008). AMF colonization was highest in *P. grandidentata* soil for *P. serotina* and *Q. alba* seedlings, and in *A. saccharum* soil for *P. serotina* and *Q. rubra*, suggesting that AMF from *P. grandidentata* and *A. saccharum* soils can readily colonize multiple seedling species. Surprisingly, AMF colonization increased with light availability only for *P. serotina*. Across species, *P. serotina* also had the highest total AMF colonization. I speculate that, as a shade intolerant species, *P. serotina* seedlings may regulate mycorrhizal colonization (MacLean et al., 2017; Mangan et al., 2010) by investing more resources into colonization at high light, where carbon is less limiting (Grman et al., 2012).

Consistent with expectations, EMF colonization was higher in conspecific than heterospecific soil for *Q. alba* and *Q. rubra*, especially in higher light availability (Trocha et al., 2016; Turner et al., 2009), perhaps reflecting the higher specialization of EMF than AMF (S. Smith & Read, 2008). For *Q. alba*, EMF colonization also was high in soils cultured by *P. grandidentata* and *Q. rubra*, suggesting association with multiple EMF species.

Phenolics increased with light availability for all study species, but there were no consistent effects of live soil source across all seedling species. Phenolics were higher in conspecific than heterospecific soil for *A. saccharum* across light levels, and for *Q. rubra* at high light. Additionally, phenolics were higher in live than sterilized conspecific soils, suggesting that phenolics increase in the presence of soil-borne microbes. Phenolics production can be induced in response to soil-borne microbes (Pozo & Azcón-Aguilar, 2007; Whipps, 2004), which should be more prevalent in conspecific soil. Although I did not quantify pathogen abundance, I did find that EMF colonization and phenolics were correlated for *Q. alba* and *Q. rubra*. Since EMF colonization was higher in conspecific than heterospecific soil for both species, my results

suggest that EMF colonization or the presence of host-specific pathogens induced production of phenolics, especially in conspecific soil.

For EMF-associated species, lignin was higher in conspecific than heterospecific soil, but did not vary with light. Seedling production of lignin may have already reached the upper limit in response to light availability. Seedlings may also achieve greater trait production under less stressful growing conditions, such as in conspecific soils with mutualistic EMF (Valladares et al., 2007). By improving nutrient availability, EMF can indirectly affect the allocation of seedling resources, potentially impacting lignin synthesis.

NSC increased with light availability for all study species, regardless of soil biota present. This result was consistent with previous studies (Dillaway et al., 2007; Piper et al., 2009; Zhang et al., 2013), including *Q. alba* (Dillaway et al., 2007). Contrary to expectations, for *Q. alba*, NSC was higher in heterospecific soil. For *P. serotina* and *Q. rubra*, NSC was higher in conspecific soil but only at low light. While one might speculate that seedlings may allocate more NSC to mycorrhizal mutualists or recovery from pathogens in conspecific soil or high light, I did not find strong correlations with NSC for either AMF or EMF colonization.

Mycorrhizal colonization and functional traits had limited effects on seedling survival

Species differed in which traits, if any, influenced survival. Phenolics, which provide direct chemical defense against soil-borne microbes, could increase survival for *A. saccharum* seedlings and may be the mechanism behind their positive PSFs, supported by greater production of phenolics in conspecific soils (Pozo & Azcón-Aguilar, 2007; Whipps, 2004). However, *A. saccharum* seedling survival was higher in sterilized than conspecific soil, suggesting that the positive effects of phenolics on survival did not overcome the negative effects of microbes. Furthermore, higher survival in sterilized soil confirms that microbes drove the observed positive

PSFs. For *P. serotina*, phenolics were much higher in pooled heterospecific soil and were positively correlated with AMF colonization. *P. serotina* seedlings may be more readily colonized by AMF, regardless of soil source, and thus produce more phenolics in response; this may explain why *P. serotina* are frequently found to have high mortality in conspecific soils (Esch & Kobe, 2021; Packer & Clay, 2000).

For typically EMF-associating *Q. alba* seedlings, survival decreased as AMF colonization increased. A potential explanation is that AMF can act parasitically in some environmental conditions (Ibáñez & McCarthy-Neumann, 2016; Konvalinková & Jansa, 2016) while EMF provide better direct protection against pathogens. While it is not well-understood if AMF colonization cause negative PSFs for EMF-associating tree species (Chilvers et al., 1987; Duponnois et al., 2003), previous studies (Bennett et al., 2017) found no effect of soil cultured by AMF-associating species on seedlings of EMF-associating species. Furthermore, the pot-based study design may have precluded the benefits of an EMF common mycorrhizal network (Simard & Durall, 2004), heightening the negative influence of AMF colonization. Interestingly, *Q. rubra* seedling survival was not influenced by AMF colonization, suggesting that *Q. rubra* may be less reliant upon common mycorrhizal networks or less susceptible to parasitic effects of AMF.

I expected to see traits emerge as stronger drivers of seedling survival, given large intraspecific trait variation in response to soil source and light availability. Although I found that functional traits were influenced by both soil source and light availability, I found limited instances of trait influences on seedling survival, which was likely due to abnormally high mortality experienced by seedlings during the field season. The lack of strong effects could have been driven by stressful field conditions that obscure the importance of functional traits. In

contrast to previous greenhouse studies, I did not find PSFs for *A. rubrum*, *P. serotina*, or *Q. rubra*. PSFs quantified in the highly controlled greenhouse conditions often overestimate field measured PSFs (Forero et al., 2019). In this study, I took precautions against potential competition and above-ground herbivory from rodents and deer. However, seedlings experienced high amounts of mortality, killing almost all of the *A. saccharum* and *P. serotina* seedlings, and killing half of the *Q. alba* and *Q. rubra* seedlings.

This may be in part due to the great variability in rainfall and maximum temperature experienced by seedlings across the growing season. Heavy rainfall washed away smaller seedlings and would stand in pots if preceded by dryer, warmer periods, which caused soil in the field pots to pull away from the sides of the container and harden. Evaluation of weather data (National Oceanic and Atmospheric Administration) revealed a higher amount of rainfall throughout the field season and a large rainfall event (> 7") in July. Variation in weather could have overridden the effects of traits (Putten et al., 2016) or light availability (Catford et al., 2022). PSF experiments carried out in the greenhouse may not detect such environmental effects (Beals et al., 2020) or may overestimate the strength of PSFs (Heinze et al., 2020).

There are several additional caveats to consider. I expect that there was some contamination of pots via airborne microbes, splash from rain, and falling leaf litter, which may have reduced effect sizes. However, we assume that the reported significant effects are the result of treatments, because invasions are random and microbial priority effects should be dominant, especially in whole-soil cores (P. J. Ke et al., 2021). In addition, the relationship between functional traits and seedling survival is correlative rather than causative since I did not manipulate levels of seedling functional traits. It is also difficult to disentangle some of these trait-survival relationships. For example, while I expect phenolics and lignin to be higher in

conspecific soils, and for increases in these traits to lead to higher seedling survival, I also expect higher mortality in conspecific soils where effectively-specialized pathogens are more abundant.

However, these results still elucidate the role of soil-borne microbes on tree seedling trait levels. I was unable to separate the impacts of soil-borne mutualists and pathogens on seedling trait values and subsequent survival. Also, I cannot distinguish between direct AMF colonization effects of pathogen reduction through displacement or indirect effects inducing production of phenolics, both of which can enhance seedling survival. Additionally, I was unable to tease apart the effects of colonization type (AMF versus EMF) and seed size, since the EMF-associated species used in this study were large-seeded, and vice-versa (Table 3.1). Furthermore, this study is limited to four species occurring in a single forest; future studies examining the generalizability of these results should consider additional species and habitats.

Conclusion

Linking plant traits and environmental conditions to PSFs may help us better understand the role of PSFs in community dynamics (Baxendale et al., 2014; Bennett & Klironomos, 2018; P. Ke et al., 2015). However, most studies have focused on herbaceous plants, which are predominantly colonized by AMF. A focus on tree seedling traits under different environmental conditions, especially in natural field conditions, offers both broader ecological understanding as well as potential applications for forest management. For example, selecting sites with soil and light conditions that promote higher production of defensive and recovery compounds could increase likelihood of seedling restoration success (e.g., *A. saccharum* in conspecific soil). Similarly, it may be beneficial to plant EMF-associating seedlings in soils cultured by other EMF-associating species, to increase potential for positive EMF colonization effects and limit potential negative AMF colonization effects. While environmental conditions could dilute trait

effects on seedling survival, in the absence of extreme conditions (as supported by related greenhouse studies), a sharper focus on traits promoting survival rather than growth traits will provide a more mechanistic understanding of forest regeneration dynamics.

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CHAPTER 4

Tree seedling responses to plant-soil feedbacks depend on mycorrhizal type of the adults culturing the soil

ABSTRACT

The matching or mismatching of mycorrhizal type between canopy trees and recruiting seedlings may interact with the juvenile's functional traits and thus affect their responses to plant-soil feedbacks (PSFs). Understanding these interactions will provide a more mechanistic understanding of forest community dynamics. To assess the role of mycorrhizal type matching on juvenile trees' trait response and PSFs, I carried out a greenhouse experiment where I grew seedlings of five temperate tree species under soils cultured by conspecific versus heterospecific adults. Seedlings were also grown under three levels of light availability to assess potential shifts in the PSF effects (i.e., positive at high light, and negative at low light). After 12 weeks, I quantified seedling survival, colonization by arbuscular- and ecto-mycorrhizal fungi (AMF and EMF) and measured their defense and recovery traits (phenolics, lignin, and nonstructural carbohydrates [NSC]).

I found that negative PSFs (lower survival in conspecific versus heterospecific soils) experienced by seedlings associating with AMF almost always occurred when they were compared with heterospecific adults associating with EMF. Conversely, positive PSF experienced by EM seedlings occurred when compared to soils cultured by AM adults. Although PSFs occurred regardless of light level, the magnitude of effect – both negative for AM and positive for EM seedlings – was greatest at low light. Soil microbes from conspecific-cultured soils reduced survival for AM species but had no effect on survival for EM species. Percent

mycorrhizal colonization and functional trait values were higher in high light and, except for NSC, were often higher in conspecific soils. Furthermore, PSFs for AM seedlings became less negative as percent AMF colonization and defense/recovery traits increased, and PSFs for EM seedlings became less positive as percent AMF colonization and lignin increased.

These results suggest that increased colonization by mycorrhizal fungi and increased amounts of phenolics, lignin, NSC effectively neutralize both negative and positive PSFs, providing new insights into how mismatching of mycorrhizal type interacts with defense and recovery traits to influence PSFs, and thus forest community dynamics.

INTRODUCTION

Identifying the mechanisms that maintain tree species richness is a central question in plant community ecology. The seeding-establishment phase is a critical stage for the maintenance of future community-wide species diversity, as this phase is a major demographic bottleneck for populations (Gurevitch et al., 2020). Seedling recruitment is influenced by plant-soil feedbacks (PSFs), a continuous feedback loop wherein adult trees modify the soil in which they are growing, which in turn shapes seedling community assembly (Bever et al., 1997). PSFs can be positive (better seedling performance in conspecific than heterospecific soils), negative (better seedling performance in heterospecific than conspecific soils), or neutral (no difference in seedling survival in conspecific versus heterospecific soils). The direction and strength of PSFs may be partially explained by the mycorrhizal fungal type of the host plant species, with negative feedbacks more often experienced by plant species associated with arbuscular mycorrhizal fungi (AMF) and positive feedbacks more often experienced by species associated with ectomycorrhizal fungi (EMF) (Bennett et al., 2017). In the present study, I expand this

framework by also testing the matching or mismatching of mycorrhizal type between juvenile and adult trees culturing the soil, in addition to how defense and recovery traits interact to influence PSFs.

The primary biotic agents of PSFs are soil-borne microbes, including pathogens and mycorrhizal fungi (Bever et al., 2010; Jiang et al., 2020). Soil-borne pathogens (fungi, oomycetes, and bacteria), increase seedling mortality, sometimes killing entire seedling cohorts (Mangan et al., 2010; Terborgh, 2012). While mycorrhizae typically act as mutualists, providing water and nutrients in exchange for sugars, their relationship with seedlings can shift to parasitic in low light environments (Konvalinková & Jansa, 2016; McCarthy-Neumann & Ibáñez, 2013), when photosynthates are more limited. Mycorrhizae can also confer protection against antagonists, like pathogens, but the degree of protection depends upon mycorrhizal type (Bennett et al., 2017). AMF can provide indirect defense against pathogens by competing for space on plant roots (Borowicz, 2001), whereas EMF can provide direct defense by forming a protective sheath on young roots (Laliberté et al., 2015). The net effects of PSFs may range from positive to negative, depending on interactions between soil-pathogens and mycorrhizal fungi (Laliberté et al., 2015; Reinhart & Callaway, 2006).

The type of mycorrhizal symbiont (AMF or EMF) hosted by tree seedling species can have large impacts on PSFs. (Here, I refer to species that typically associate with AMF and EMF as “AM species” and “EM species”, respectively). AM trees typically experience negative or neutral PSFs (i.e., inhibition of seedlings around conspecific adults); conversely, EM trees experience positive or neutral PSFs (i.e., facilitation around conspecific adults) (Bennett et al., 2017; Kadowaki et al., 2018). AM trees have a higher abundance of plant pathogens in the soil beneath their crowns, relative to soils beneath EM trees (Eagar et al., 2022, 2023), which may be

explained by the relatively-low amount of protection that AMF confer against these pathogens. In addition, Chen et al. (2019) found that AM seedlings accumulate soil-borne pathogens faster under AM than EM adults, providing a potential explanation for why seedlings of AM tree species experience higher root pathogen damage when grown in conspecific soil, whereas EM tree species have elevated mycorrhizal colonization in conspecific soil (Bennett et al., 2017). However, when there is mismatching of mycorrhizal types (e.g., AM seedlings growing beneath EM trees and vice-versa), both AM and EM seedlings appear to experience positive or neutral PSFs (Kadowaki et al., 2018).

Furthermore, seedling sensitivity to negative PSFs is often restricted to low light availability and increases with seedling shade intolerance (McCarthy-Neumann & Ibáñez, 2013; McCarthy-Neumann & Kobe, 2010b, 2010a). Soil moisture conditions associated with low light environments can result in greater soil pathogen density and increase fungal pathogen colonization (Hersh et al., 2012; Y. Liu & He, 2019; Reinhart et al., 2010). Colonization by mycorrhizal fungi also reduces seedling performance in low light conditions (Ibáñez & McCarthy-Neumann, 2014, 2016). In high light, AMF root colonization is greater and can compete with pathogens for carbon from plant roots (Borowicz, 2001), directly reducing pathogen infection. Mycorrhizal fungi can also indirectly ameliorate pathogen effects (Liang et al., 2015), by providing water and nutrients to the host plant (Borowicz, 2001; Graham, 2001) and inducing host plant defense traits (Pozo & Azcón-Aguilar, 2007; Zamioudis & Pieterse, 2012) that protect against pathogens (Azcón-Aguilar et al., 2002; Violle et al., 2012). Conversely, in low light, seedling mortality may be greater when seedlings encounter both mycorrhizal fungi and pathogens together (König et al., 2016), which may be due to the combined carbon costs of maintaining the mutualism and defense/recovery from pathogen attack.

Seedling functional traits – that is, measurable morphological or physical attributes that impact seedling performance (Pérez-Harguindeguy et al., 2013) – are likely influenced by soil source, mycorrhizal type, and light availability. Functional traits, such as those that confer defense against and recovery from pathogens, likely increase seedling survival in the first growing season and thus have a large influence on future community dynamics. Growth-related functional traits (e.g., specific leaf area, specific root length, height) are linked to PSFs (Baxendale et al., 2014; Cortois et al., 2016; Xi et al., 2021), but defensive, survival-related traits are rarely examined directly. When defensive traits are included, they are often inferred from an inverse relationship with growth traits (Cortois et al., 2016; Xi et al., 2021).

Traits that confer greater defense and recovery against soil-borne microbes include phenolics, lignin, and nonstructural carbohydrates (NSC). Phenolics and lignin act as chemical (Ichihara & Yamaji, 2009) and physical (Augspurger, 1990) defenses, whereas NSC can be mobilized to repair damaged tissues (Dietze et al., 2014). In previous greenhouse (Chapter 2) and field (Chapter 3) studies, I found that mycorrhizal fungi were associated with higher amounts of seedling phenolics and NSC. In those studies, I also found that increasing seedling AMF colonization, phenolics, and lignin were associated with higher survival in the presence of soil-borne pathogens.

In this study, I planted seedlings of five temperate tree species under soils cultured by adults of each of those species and in three levels of light availability in the greenhouse. My global hypothesis is that, for tree seedlings, the influence of defense and recovery traits on PSFs (calculated as the difference in survival between conspecific and heterospecific soils) is driven by mismatches between seedling mycorrhizal type and the mycorrhizal type of the adult tree culturing the soil. I predicted that:

1) AM seedlings experience negative PSFs (lower survival in conspecific versus heterospecific soils) and EM seedlings experience positive PSFs (higher survival in conspecific versus heterospecific soils) across light levels.
2) AM seedlings experience greater negative PSFs in low than high light. EM seedlings experience greater positive PSFs in high than low light.
3) Soil-borne microbes from conspecific soils have negative effects on survival for AM species and positive effects on survival for EM species, which could partly explain the PSFs found in hypotheses 1-2.
4) All study species have the greatest mycorrhizal colonization at high light availability and when grown in soils cultured by the same mycorrhizal type.
5) Phenolics, lignin, and NSC increase as light availability increases. Phenolics and lignin are higher, and NSC is lower, in conspecific soils.
6) Seedling PSFs become less negative for AM species and more positive for EM species as mycorrhizal colonization and defense/recovery traits increase. These relationships are enhanced in low light availability.

This study provides insights into how matching or mismatching of mycorrhizal type between the juvenile growing in and adult tree culturing the soil can shift the strength and direction of PSFs. Furthermore, I show that both defense/recovery traits and light availability can interact to mediate PSFs.

MATERIALS AND METHODS

I conducted a factorial blocked greenhouse experiment, consisting of five tree species, seven soil sources (sterilized conspecific, non-sterilized conspecific, and five heterospecific), across three light levels (low, medium, and high), replicated over 30 seedlings (n = 30), for a total of 3,150 seedlings. I monitored seedling survival twice per week over twelve weeks. When seedlings were three and twelve weeks old, I measured percent mycorrhizal colonization (AMF and EMF) and amounts of defense and recovery functional traits (phenolics, lignin, and NSC).

Species selection

The experiment was conducted at the Michigan State University Tree Research Center in Lansing, MI, USA (42.7 ºN, 84.5 ºW) in spring 2018. Soils were collected in a 100 ha mixed hardwood forest stand in mid-Michigan, at Alma College's Ecological Field Station (43°23'32.0"N 84°53'41.5"W) in summer 2017. This forest has not been logged since 1897 and lies in an ecological tension zone between northern coniferous and southern deciduous forests. The dominant species in this forest is sugar maple *(Acer saccharum*), a shade-tolerant canopy tree species. Other common trees in the forest include red maple (*A. rubrum*) and big-toothed aspen (*Populus grandidentata*).

I chose six tree species native to the research site: red maple (*A. rubrum*), sugar maple (*A. saccharum*), big-toothed aspen (*P. grandidentata*), black cherry (*Prunus serotina*), white oak (*Quercus alba*), and northern red oak (*Q. rubra*). The six study species vary in local adult abundance, shade tolerance, seed size, and mycorrhizal association type (Table 4.1). Due to difficulty acquiring seeds, I did not grow *P. grandidentata* seedlings. However, *P. grandidentata* soils were still included as a treatment.

Soil sources and sterilization

To minimize potential for multispecies culturing of soil, I chose adult trees for soil collection that were at least two crown diameters away from adults of other study species. In August 2017, I collected soil (top 15 cm) from within 1 m of each stem of the focal trees. I prepared soil by dicing roots and sifting through a 1 cm mesh sieve, retaining all fine roots, and maintained soil from each adult as separate replicates (e.g., Rinella and Reinhart 2018). All pots were filled with a 1:1 mixture of prepared field soil and Fafard #2 commercial soil mixture; previous trials using 100% field soil resulted in high seedling mortality in the first three weeks (personal observation).

Table 4.1 Local adult abundance, shade tolerance, seed weight, and primary mycorrhizal association for each of the study species. [1]Local adult abundance was calculated as number of individuals ≥ 5cm dbh/ha at Alma College's Ecological Preserve. [2]Shade tolerance is presented as intolerant, intermediate, or tolerant and as mean ± std. dev., on a standardized scale from 1 (least tolerant) to 5 (most tolerant), calculated by Niinemets & Valladares (2006). [3]Seed weight data was collected from Burns and Honkala (1990). [4]AMF = arbuscular mycorrhizal fungi and EMF = ectomycorrhizal fungi.

Species	Local adult abundance[1]	Shade tolerance[2]	Seed weight (mg)[3]	Mycorrhizal association[4]
Acer rubrum	131	(3.44 ± 0.23)	19.7	AMF
Acer saccharum	285	(4.76 ± 0.11)	64.9	AMF
Prunus serotina	4.33	(2.46 ± 0.34)	94.3	AMF
Populus grandidentata	82.33	(1.21 ± 0.27)	0.2	AMF, EMF
Quercus alba	12.67	(2.85 ± 0.17)	6,677	AMF, EMF
Quercus rubra	71.67	(2.75 ± 0.18)	4,127	AMF, EMF

Table from Chapter 3.

To test for plant-soil feedbacks (PSFs), I compared seedling survival in non-sterilized (live) soil collected beneath conspecific versus heterospecific adult trees. I did not use multi-stage greenhouse culturing (Bever et al., 2010), because in-situ natural culturing already occurred for these long-lived trees and should more closely characterize PSFs occurring in the field. To test for biotic components of these PSFs, I compared seedling performance in sterilized versus non-sterilized (live) soils collected beneath conspecific adults. Soil was sterilized by gamma irradiation (30-70 kGy; Sterigenics International, Schaumburg, IL, USA) and allowed to rest for at least one month to minimize post-fertilization nutrient spikes. Gamma irradiation is highly effective at killing soil microorganisms and usually has minimal effects on soil chemical and physical properties (McNamara et al., 2003). There was almost no seedling colonization by AMF or EMF in sterilized soils (mean = 0.02%, $df = 629$, $t = 84.8$, $p < 0.01$), confirming that my sterilization methods were effective.

Light availability

I grew seedlings at three light levels (~2%, 15%, and 30% full sun), which represent a typical light range experienced in Michigan forests (Schreeg et al., 2005). I created light treatments by covering greenhouse benches with an inner layer of black shade cloth and an outer layer of reflective knitted poly-aluminum shade cloth (BFG Supply, Burton, OH, USA). I confirmed light levels using PAR (photosynthetically active radiation) measurements at each bench with a LI-COR 205A quantum sensor (LI-COR, Lincoln, NE, USA) on a uniformly overcast day.

Seedling planting and measurement

Pots were set up on nine different benches in the greenhouse, where all combinations of species and soil source were represented, with three benches per light treatment. I planted 30

seedlings per species × soil source × light treatment, for a total of 3,150 seedlings. A single seed with a newly-emerged radicle was planted into each (655 cm^3) deepot (Stuewe and Sons, Tangent, Oregon, USA). To minimize disease from non-experimental soil sources, seeds were surface sterilized with 0.6% NaOCl solution prior to stratification and germination. To avoid cross-contamination, all tools and surfaces that were exposed to soil were soaked in 10% NaOCl solution or surface sprayed with 70% EtOH.

I censused seedling survival twice per week for 12 weeks. Mortality at the first two censuses after planting were attributed to transplant shock; these seedlings were not used in subsequent analysis and pots were re-planted. After three weeks of growth, I harvested six seedlings per treatment combination to measure mycorrhizal colonization and defense/recovery traits using established protocols. Half of the seedlings were allocated to measurement of mycorrhizal colonization (roots), phenolics (hypocotyl), and lignin (stem). The other half of the seedlings were allocated to measurement of NSC (stem and root).

To quantify mycorrhizal colonization, prior to drying seedlings, 5-10 root fractions per individual (1 cm sections of wet root), were retained, weighed, and stained with 5% Schaeffer black ink and vinegar solution (Vierheilig et al., 1998). Percent root colonization by AMF was quantified by inspecting 100 grid intersections for AMF structures (i.e., vesicles, arbuscules, coils, and hyphae) every 1 mm at 200x magnification (McGonigle et al., 1990). AMF fungal structures were distinguished from other fungi that can inhabit the root interior (e.g., dark septate fungi) by comparing slides to established reference images. Percent root colonization by EMF was quantified by counting the number of intact root tips with and without Hartig nets at 100x magnification every 2 mm along the root until 100 root tips were scored.

To quantify phenolics, I collected hypocotyl samples, cut into <1 mm pieces. I extracted phenolics in 5 mL methanol in the dark for 16 hours at room temperature. The methanol extracts were filtered and adjusted to 5 mL, and then I quantified total phenolics using a microplate-adapted colorimetric total phenolics assay with Folin-Ciocalteu reagent (Ainsworth & Gillespie, 2007; P. G. Waterman & Mole, 1994).

To quantify lignin, root and stem samples were lyophilized and coarsely ground at 1 mm using a Wiley Mill. We ran 0.5 g root and stem samples through a series of extractions using an ANKOM Fiber Analyzer (ANKOM Technologies, Macedon, NY, USA). I used a neutral detergent fiber extraction to wash off soluble cell contents (e.g., carbohydrates, lipids, pectin, starch, and soluble proteins). I then used an acid detergent fiber extraction with 1.00 normal sulfuric acid to wash off hemicellulose and bound proteins and an acid detergent lignin extraction with 72% sulfuric acid to wash off cellulose, leaving only lignin and recalcitrant materials. Finally, I ashed the samples to quantify dry mass lignin.

To quantify nonstructural carbohydrates (NSC), I analyzed stem samples, using a standardized enzyme method for sugar and starch extraction and quantification (Landhäusser, Chow, Turin Dickman, et al., 2018; Quentin et al., 2015). I dried seedling stems and peach leaf standard reference material (MillporeSigma-NIST1547) at 60 °C overnight to remove moisture. I then weighed out 30 mg of each for analysis and separated sugars and starches with hot ethanol extraction. I used α-amylase and amyloglucosidase to convert starch to glucose. I quantified sugars using phenol-sulfuric acid colorimetric assay and starches using a glucose-hexokinase colorimetric assay (MillporeSigma-GAK20). I calculated total NSC as the sum of soluble sugar and starch concentrations derived from the assays.

Statistical analysis

To analyze seedling survival over the 12 week growing period, I used an individual based counting process in a Cox survival model (Burnham & Anderson, 2002; McCarthy-Neumann & Ibáñez, 2012). Data for each seedling i and each time t, N_{it}, was coded as 0 until the seedling was found dead, $N_{it} = 1$. I used a count process to model the number of events (mortality, N_{it}) until the experiment ended at nine weeks. I modeled the likelihood as:

$$N_{it} \sim Poisson(\lambda_{it})$$

and the process as:

$$\lambda it = h_t e^{(\mu t)},$$

where parameters were estimated as a function of the hazard (h), which is the intrinsic rate of mortality due to individual age or time within the experiment, and of risk (μ), which is the extrinsic rate of mortality due to light availability and soil source. Risk (μ) was modeled as an interaction between species and light, plus an interaction between species and soil source, and the random effects of bench). Simulations (3 chains) were run until convergence of the parameters was ensured (25,000 iterations) and then run for another 50,000 iterations, from which the posterior distribution of parameter values and predicted survival were estimated.

Predicted survival values were used to assess whether there were differences in how species responded to soil sources and light levels. I then used predicted survival values and their associated uncertainty to test if there were differences in how species responded to low versus high light and in different soil sources. I calculated PSFs as the difference in survival between conspecific and heterospecific soil sources at each light level and for each species. I calculated the biotic effect of the soil (i.e., soil-borne microbes) as the difference in survival between live

conspecific and sterilized conspecific soils. Differences that did not include zero in their 95% credible intervals were considered statistically significant (Kruschke, 2014).

I used linear mixed effects models to investigate how light availability and soil source influenced measured mycorrhizal colonization and defense/recovery traits. Each trait (i.e., phenolics, lignin, and NSC) was evaluated in a separate model. I analyzed all traits with species, soil source, and light availability as potentially interacting fixed effects, and greenhouse bench and adult tree as random effects. I estimated marginal means for post-hoc analyses of these models.

To evaluate the effects of mycorrhizal colonization and defense/recovery traits on PSFs, I also used linear models. Since PSFs are a comparison of seedling performance between conspecific and heterospecific soils, the conspecific value (both in seedling survival, as well as traits) is a constant value per species. Thus, the trait data used in these models was from seedlings grown in soils cultured by heterospecific adults. I analyzed all models at the species level, with mean PSF for each treatment (soil source × light level) evaluated as a response of the mean amount of each type of mycorrhizal colonization or defense/recovery trait. I excluded sterilized conspecific soil controls in these analyses, since AMF and EMF colonization, in addition to amounts of phenolics for AM species, in the sterilized conspecific soils were effectively zero. Seedlings were destructively harvested for measurement of mycorrhizal colonization and traits; therefore, I could only compare means of these values to the mean PSFs experienced (survival in conspecific versus heterospecific soils at the end of the 12-week period).

All analyses were performed with R version 3.5.1 (R Core Team, 2020). I used the "rjags" package (Plummer et al., 2023) to fit survival models and to run predicted survival and contrast simulations. I used the lme4 package (D. Bates et al., 2015) to evaluate linear models. I

tested the significance of main effects using a likelihood ratio test with the "Anova" function. I tested for multicollinearity variance inflation factors using the "vif" function in the car package (Fox & Weisberg, 2019). Post-hoc Tukey pairwise comparisons of significant main effects and comparisons of estimated marginal means were made using the "emmeans" function in the multcomp package (Hothorn et al., 2008; Lenth, 2020).

RESULTS

AM seedlings experienced negative PSFs and EM seedlings experienced positive PSFs Overall, survival was relatively low for the AM seedling when grow in live conspecific soil and soils cultured by AM adults, while survival was relatively high for EM seedlings, especially when grown in conspecific soil and soils cultured by EM adults.

AM seedlings experienced negative PSFs and EM seedlings experienced positive PSFs, and PSFs occurred when there was mismatching of mycorrhizal type (H1)

PSFs differed between AM and EM host species (Figure 4.1). Across light availability, all AM seedlings (*A. rubrum*, *A. saccharum*, and *P. serotina*) experienced negative PSFs (lower survival in conspecific than in heterospecific soils). In contrast, EM seedlings (*Q. alba* and *Q. rubra*) experienced positive PSFs (higher survival in conspecific than in heterospecific soils). Although not all comparisons were statistically significant.

Negative PSFs experienced by AM seedlings almost always occurred when heterospecific soils were cultured by EM adults, while statistically significant positive PSFs in EM seedlings took place when comparing with heterospecific AM soils (Figure 4.1).

Overall, *Q. alba* and *Q. rubra* seedlings had the highest survival, followed by *A. saccharum*, *A. rubrum*, and *P. serotina* (Table A4.2).

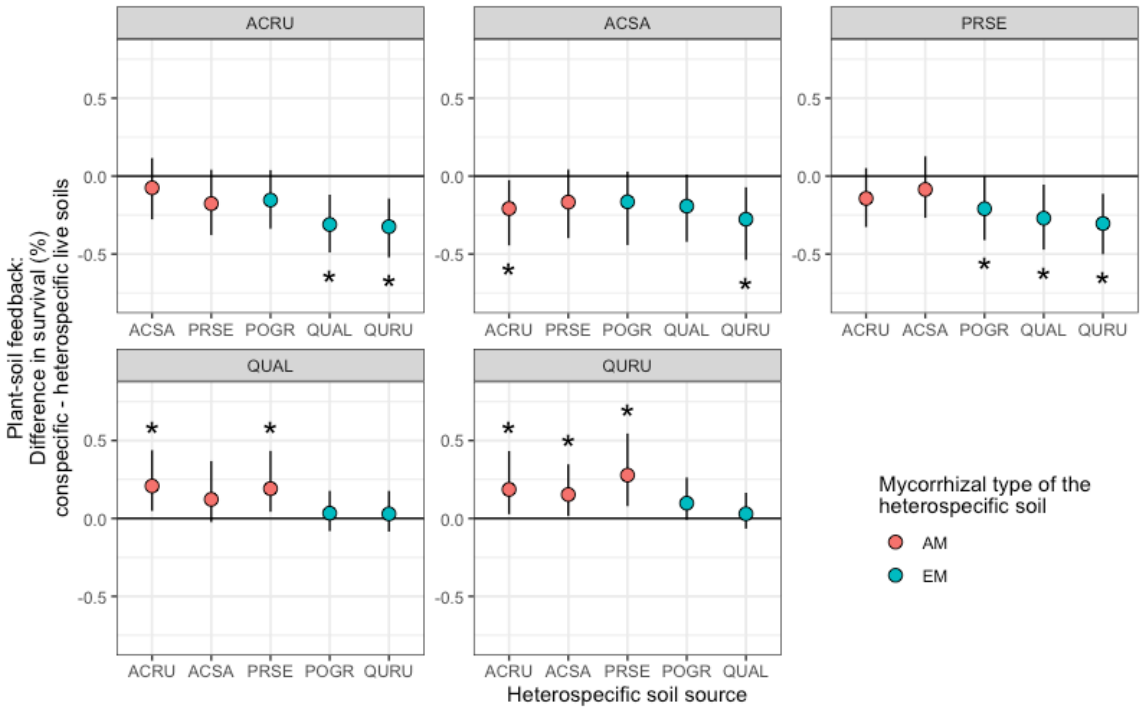

Figure 4.1 Differences in predicted seedling survival in conspecific versus heterospecific live soil when grown at low light availability. Data are means with 95% credible intervals; credible intervals that do not overlap with the zero line are statistically significant (indicated with stars, *). Differences in survival above the zero line indicate a positive PSF (higher survival in conspecific than heterospecific soils); differences in survival below the zero line indicate negative PSFs (lower survival in conspecific than heterospecific soils). As an example, *A. rubrum* experienced lower survival in soils cultured by *A. rubrum* adults than in soils cultured by *Q. alba* or *Q. rubra* adults (i.e., negative PSF).

AM seedlings experienced greater negative PSFs in low light (H2)

Negative PSFs (i.e., differences in survival between conspecific and heterospecific soils) experienced by AM species were often of greater magnitude (i.e., more negative) in low than high light availability (Figure 4.2). In contrast, positive PSFs experienced by EM species were often more positive in low than high light availability. *A. rubrum* seedlings experienced up to 32% mortality due to negative PSFs in low light versus 22% in high light. *A. saccharum* seedlings experienced up to 28% mortality due to negative PSF in low light compared to 8% in high light. *P. serotina* seedlings experienced up to 30% mortality due to negative PSFs in low light compared to 16% in high light. *Q. alba* seedlings experienced up to 21% greater survival due to positive PSFs in low light versus 10% in high light. Similarly, *Q. rubra* seedlings experienced up to 28% greater survival due to positive PSFs in low light compared to 4% in high light.

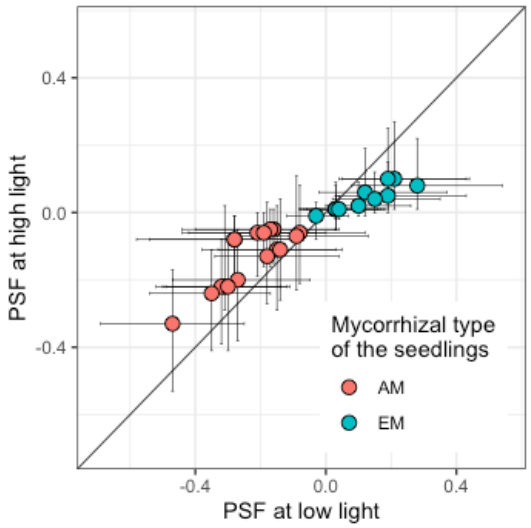

Figure 4.2 PSFs at low versus high light availability. Data are means for each species × soil combination (means ± 95% credible intervals). Sterilized conspecific soils are not included. The solid line indicates a one-to-one relationship between PSFs at low light and PSFs at high light. Points above the line demonstrate that seedlings experience more positive PSFs at low than high light; points below the line demonstrate more positive PSFs at high light.

Soil-borne microbes in conspecific soils had negative effects on AM seedling survival (H3)

Soil-borne microbes in conspecific soil reduced survival for all three AM species (*A. rubrum* = -30%, *A. saccharum* = -23% and *P. serotina* = -40% survival in live versus sterilized conspecific soils). In contrast, there was no difference in survival between live versus sterilized conspecific soils for the two EM species (Figure 4.3).

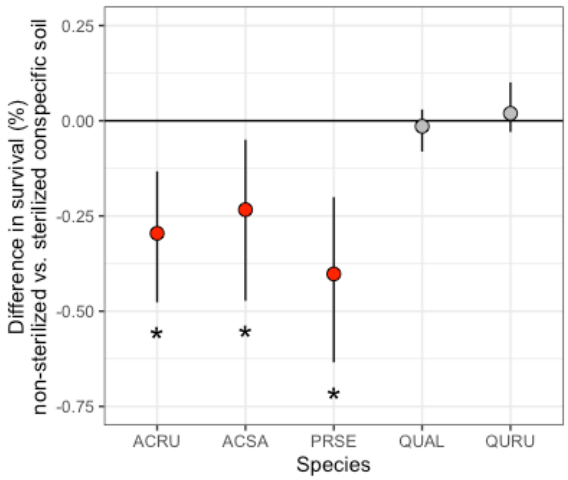

Figure 4.3 Differences in predicted seedling survival in non-sterilized versus sterilized conspecific soils and when grown at average light availability. Data are means with 95% credible intervals; credible intervals that do not overlap with the zero line are statistically significant. Differences in survival below the zero line indicate a negative effect of soil biota (lower survival in non-sterilized than sterilized conspecific soils). Here, *A. rubrum*, *A. saccharum*, and *P. serotina* experience lower survival in non-sterilized than sterilized conspecific soils, indicating a negative effect of soil-borne microbes.

AMF colonization was highest in high light availability and in soils cultured by adults of the same mycorrhizal type (H4)

Percent AMF colonization was highest when seedlings were grown in high light availability (Table A4.2). For *A. saccharum* and *P. serotina*, percent AMF colonization increased from 45% to 54% ($t = 6.9$, $df = 91$, $p < 0.01$) and from 45% to 54% ($t = 7.9$, $df = 89$, $p < 0.01$), respectively, in low versus high light. For *Q. alba*, percent AMF colonization increased from low to average light availability (13%; $t = -3.1$, $df = 91$, $p < 0.01$), and colonization in medium and high light did not significantly differ (43% at medium and 42% at high light; $p > 0.05$).

Likewise, for *Q. rubra*, percent AMF colonization increased from low to average light availability (13%; $t = 5.8$, $df = 91$, $p < 0.01$), but colonization in medium and high light did not differ (44% at medium and 47% at high light; $p > 0.05$). Also, in agreement with hypothesis 5, seedling percent AMF colonization was highest in soils cultured by conspecific adults and was higher in soils cultured by AM heterospecific adults than EM heterospecific adults (Table 4.2). For *A. rubrum*, percent AMF colonization was highest in conspecific soils (42%) and lowest in heterospecific soils cultured by EM adults (32%; $F_{2,382} = 26$, $p < 0.01$). For *A. saccharum*, AMF colonization also was highest in conspecific soils (71%) and lowest in heterospecific soils colonized by EM adults (62%; $F_{2,370} = 18.5$, $p < 0.01$). Additionally, for *P. serotina*, percent AMF colonization was highest in conspecific soils (70%) and lowest in heterospecific soils cultured by EM adults (60%; $F_{2,380} = 30.6$, $p < 0.01$). Conversely, for *Q. alba*, percent AMF colonization was highest in conspecific soils (44%) and heterospecific soils cultured by EM adults (41%; $F_{2,380} = 10.4$, $p < 0.01$). For *Q. rubra,* there was no effect of soil source on percent AMF colonization. Seedling AMF colonization increased with light availability for both AM

species (from 39 to 45%; and EM species (from 29 to 33%). Overall, AMF seedlings had higher AMF colonization than EMF seedlings (Table A4.2).

There was no effect of soil source on EMF colonization. Overall, *Q. alba* and *Q. rubra* had the highest amount of EMF colonization (Table A4.2).

Defense and recovery traits generally increased with light availability, but soil source effects varied and often depended on seedling mycorrhizal type

In agreement with hypothesis 6, seedling phenolics, lignin, and NSC generally increased with light availability for both AM and EM species (Table A4.2). Seedling phenolics (nmol Gallic acid equivalents per mg dry extract) increased in high versus low light for all five study species: *P. serotina* (100% increase; $F_{2,72} = 16.9$, $p < 0.01$), *Q. alba* (20% increase; $F_{2,72} = 69.1$, $p < 0.01$), and *Q. rubra* (20% increase; $F_{2,72} = 29.2$, $p < 0.01$). For *A. rubrum* and *A. saccharum*, phenolic values in low light were essentially zero, but increased to 0.07 nmol ($F_{2,72} = 5.2$, $p < 0.01$) and 0.06 nmol ($F_{2,74} = 9.7$, $p < 0.01$), respectively, at high light availability. Lignin (percent dry mass) increased in high versus low light: *A. rubrum* (53% increase; $F_{2,102} = 38.2$, $p < 0.01$), *A. saccharum* (20% increase; $F_{2,105} = 17.1$, $p < 0.01$), *P. serotina* (60% increase; $F_{2,102} = 46.9$, $p < 0.01$), *Q. alba* (7% increase; $F_{2,102} = 9.8$, $p < 0.01$), *Q. rubra* (6% increase; $F_{2,102} = 6.5$, $p < 0.01$). Similarly, NSC (percent dry mass) also increased in high versus low light for *A. rubrum* (47% increase; $F_{2,61} = 49.7$, $p < 0.01$), *A. saccharum* (13% increase; $F_{2,61} = 5.4$; $p < 0.01$), *P. serotina* (51% increase; $F_{2,61} = 55.1$, $p < 0.01$), *Q. alba* (14% increase; $F_{2,61} = 18$, $p < 0.01$), and *Q. rubra* (16% increase; $F_{2,61} = 14.3$, $p < 0.01$).

The effect of soil source on seedling phenolics (nmol Gallic acid equivalents) varied. For *A. rubrum*, phenolics were highest in live (0.19 nmol) and sterilized (0.06 nmol) conspecific soils and were essentially zero in heterospecific soils ($F_{5,273} = 44.2$, $p < 0.01$; Table A4.2). For *A.*

saccharum, phenolics were highest in live conspecific soils (0.19 nmol) and were essentially zero in sterilized conspecific and heterospecific soils ($F_{5,284} = 2.6$, $p = 0.02$). For *Q. rubra*, effects of soil source on seedling phenolics depended on light availability: phenolics were lowest in live conspecific soils at low light (2.5 nmol) and were highest in live conspecific soils at high light (4.3 nmol; $F_{10,505} = 6.2$, $p < 0.01$). For *P. serotina* and *Q. alba*, there was no effect of soil source on phenolics.

Lignin was highest in conspecific soils for the EM seedlings. For *Q. alba*, percent dry mass lignin was highest in live conspecific soils (23%), followed by heterospecific-EM (21%), heterospecific-AM (21%), and sterilized conspecific (21%) soils ($F_{5,320} = 4.7$, $p < 0.01$; Table 2). For *Q. rubra*, lignin was highest in conspecific soils, regardless of if they were live (25%) or sterilized (25%), followed closely by heterospecific-AM (24%) and heterospecific-EM (24%) soils ($F_{5,320} = 3.7$, $p < 0.01$). There was no effect of soil source on lignin for any of the three AM species.

NSC was lowest in conspecific soils for both EM species, with the greatest differences occurring in low light availability. For *Q. alba*, at low light, percent dry mass NSC was 18% in conspecific soils and 19% in heterospecific soils ($F_{10,510} = 2$, $p = 0.03$; Table 2). For *Q. rubra*, at low light, NSC was 11% in conspecific soils and 15% in heterospecific soils ($F_{10,510} = 3$, $p < 0.01$). There was no effect of soil source on NSC for any of the three AM species.

Overall, *Q. alba* and *Q. rubra* had higher amounts of phenolics, lignin, and NSC than *A. saccharum*, *A. rubrum,* and *P. serotina* (Table A4.2).

Table 4.2 Differences in amounts of seedling mycorrhizal colonization and defense (phenolics and lignin) and recovery (NSC) traits (% difference) in live conspecific soils compared to sterilized conspecific, heterospecific AM, and heterospecific EM soils (only significant % differences are provided). A positive % difference indicates a higher amount, and a negative % difference indicates a lower amount of the traits, in conspecific versus compared soils. Where there was a significant difference between soil sources, but this effect varied with light availability, comparisons at different light levels are also provided.

Trait	Conspecific vs. Soil Source	ACRU	ACSA	PRSE	QUAL	QURU
AMF	St. Con.	††	††	††	††	††
	Het. AM	Low: 19.4%	Low: 16.8%			
				Avg: 15.2%		
		High: 35.5%		High: 10.3%	High: 38.9%	
	Het. EM	Low: 22.0%	Low: 28.1%			
		Avg: 39.6%		Avg: 28.1%		
		High: 35.1%	High: 11.5%	High: 19.7%		
EMF	St. Con.				††	††
Phenolics	St. Con.				Low: 45.5%	
				High: +%†	High: 24.6%	High: 15.4%
	Het. AM	+%†	+%†			Low: -31.3%
						High: 24.4%
	Het. EM	+%†	+%†			Low: -26.5%
						High: 13.5%
Lignin	St. Con.				10.5%	
	Het. AM				10.1%	Low: 11.1%
	Het. EM				9.6%	Low: 10.1%
NSC	Het. AM				Avg: -17.4%	Low: -23.5%
	Het. EM				Avg: -17.7%	Low: -25.1%

† Indicates that phenolics values in one of the comparisons was essentially zero. "+%" indicates that phenolics were higher in the conspecific soil. †† Indicates that the comparison for mycorrhizal colonization is against sterilized conspecific soils, which were essentially zero.

Increasing AMF and defense/recovery traits were associated with more neutral PSFs

As amounts of AMF colonization and defense/recovery traits in heterospecific soils increased, PSFs became less negative for the AM seedling species, but, in conflict with H7, became less positive for the EM seedlings (Figure 4.4). For *A. rubrum*, PSFs became less negative as AMF colonization ($F_{1,80} = 9.66$, $p = 0.003$) in heterospecific soils increased. For *A. saccharum*, PSFs became less negative as AMF colonization ($F_{1,80} = 9.43$, $p = 0.02$), phenolics ($F_{1,80} = 8.53$, $p = 0.005$), lignin (marginally-significant at $F_{1,80} = 3.35$, $p = 0.07$), and NSC (marginally-significant at $F_{1,80} = 3.27$, $p = 0.07$) increased in heterospecific soils. For *P. serotina*, PSFs became less negative as AMF ($F_{1,80} = 13.83$, $p < 0.001$) in heterospecific soils increased. For *Q. alba*, PSFs became less positive as AMF colonization ($F_{1,80} = 4.80$, $p = 0.03$) in heterospecific soils increased. For *Q. rubra*, PSFs became less positive as AMF colonization ($F_{1,80} = 7.61$, $p = 0.007$) and lignin (marginally-significant at $F_{1,80} = 3.65$, $p = 0.07$) in heterospecific soils increased.

It is important to note that the above trend was found for increasing amounts of mycorrhizal colonization and defense/recovery traits when seedlings were grown in heterospecific soils and does not account for amounts of traits in conspecific soils. As an example, increasing percent colonization by AMF in heterospecific soils was associated with less negative PSFs (less mortality in conspecific versus heterospecific soils) for the AM species and less positive PSFs for the EM species. In general, AM seedlings had higher percent root colonization by AMF in conspecific than heterospecific soils. As amounts of colonization in heterospecific soils increased to levels comparable to the conspecific soils, differences in survival between these two soil sources (i.e., PSFs) decreased.

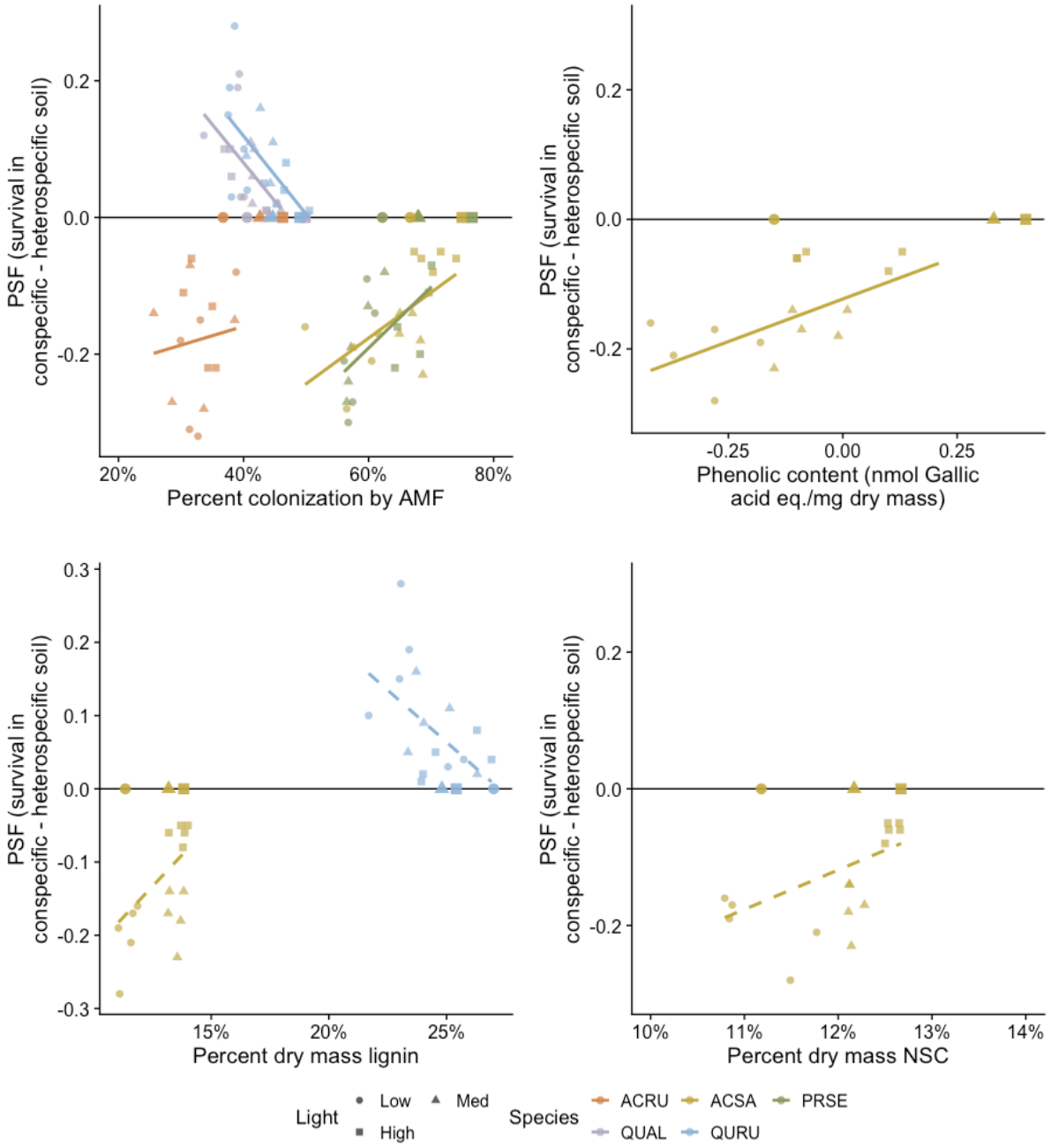

Figure 4.4 Effects of AMF colonization and defense/recovery traits (phenolics, lignin, and NSC) on seedling PSFs. Each point is a mean mycorrhizal colonization or trait value and PSF (survival in conspecific versus heterospecific soil) for a given species × soil × light combination. Trait values for conspecific soils are used for reference; they are larger in size and lie along the y = 0 axis. Species are distinguished with line color, and light level is distinguished with point shape. Regression line significance is indicated with linetype (solid = $p < 0.05$, dashed = $p < 0.1$). As an example, as percent colonization by AMF in heterospecific soils increases, PSFs for *A. rubrum*, *A. saccharum*, and *P. serotina* become less negative and PSFs for *Q. alba* and *Q. rubra* become less positive.

DISCUSSION

Disentangling the mechanisms underlying differences in AM and EM seedling PSFs is critical to predicting forest community dynamics. My results show that, not only does the mismatching of mycorrhizal type drive negative PSFs for AM seedlings and positive PSFs for EM seedlings, but higher amounts of seedling defense and recovery traits may effectively neutralize these PSFs, mediated by light availability.

Mismatching of mycorrhizal type drives negative PSFs for AM seedlings and positive PSFs for EM seedlings

In this study, the strength and direction of PSFs could be explained by mismatching of mycorrhizal type between the juvenile and adult tree. AM species experienced negative PSFs in soils cultured by AM adults and neutral PSFs in soils cultured by EM adults. In contrast, EM species experienced positive PSFs in soils cultured by EM adults and neutral PSFs in soils cultured by AM adults. Differences in AM and EM seedling responses may be due to differences in protection against pathogens conferred by AMF and EMF. Soils cultured by AM adults have a higher abundance of pathogens (Eagar et al., 2022, 2023), but seedlings growing in those soils also have higher levels of pathogen infection (Chen et al. 2019).

My results are consistent with field observations in Michigan forests, where there is an increasing abundance of maple (*Acer*) and black cherry (*Prunus*) seedlings in oak (*Quercus*) understories. Canopy oak replacement by maples may be driven by differences in species ability to take advantage of canopy gaps (Allen et al., 2018), wherein more shade tolerant maple and cherry seedlings can successfully recruit beneath oak canopies. Differences in seedling recruitment patterns may also be driven by mismatching of mycorrhizal type, as found in this study. I found that maple and black cherry experience negative PSFs when grown beneath

crowns of those same species (i.e., negative PSFs when growing beneath AM adults). However, when growing beneath oak (EM species) crowns, they experience neutral PSFs. Thus, by growing beneath oak canopies, maple and black cherry seedlings may be able to escape pathogens and/or negative effects of AMF that drive lower survival in those soils. Escape from negative soil-borne microbes likely acts in conjunction with differences in species shade tolerance (Allen et al., 2018), enabling AM species to gain a competitive advantage in EM-dominated forests.

It is important to note that, if I had grouped AM- and EM-heterospecific adults in my analyses, or only included heterospecific adults matching the seedlings' mycorrhizal type, I would have found more neutral PSFs overall, thereby limiting my interpretation of both the strength and direction of the actual PSFs. AM seedlings experienced neutral to negative PSFs, and EM seedlings experienced neutral to positive PSFs (Figure 4.1). Previous studies have also reported that AM species generally experience negative PSFs and EM species experience positive PSFs (Bennett et al., 2017; van der Putten et al., 2013). However, to disentangle the mechanisms underlying PSFs, heterospecific soils must also be evaluated by mycorrhizal type (Kadowaki et al., 2018).

PSFs became more neutral as light availability increased

PSFs became more neutral as light availability increased (Figure 4.2). For AM seedlings, PSFs were more negative at low light availability. Negative PSFs in low light could be driven by soil-borne pathogens, which are often more abundant in cooler, wetter conditions associated with shade (Hersh et al., 2012; Y. Liu & He, 2019; Reinhart et al., 2010). Moreover, AMF symbioses could also shift from mutualistic to parasitic at low light (Ibáñez & McCarthy-Neumann, 2016), thus reducing seedling photosynthates and exacerbating negative effects of pathogens on

seedling survival. In a previous experiment, I found that the presence of AMF can decrease *Acer* seedling survival, even when soil-borne pathogens are not present (Chapter 2), signifying that AMF can have detrimental impacts on seedling survival comparable to those of pathogens, when resources are limited.

Conversely, PSFs for EM seedlings in this study were more positive at low light availability and became less positive as light increased. To my knowledge, there are no instances in which EMF act parasitically (but see Ågren *et al.*, 2019). I propose that EMF were less beneficial for seedling survival at high light availability, where resources were more readily available and pathogens were likely less abundant. At low light availability, the physical protection against pathogens conferred by EMF may result in higher seedling survival, whereas, at high light availability, benefits of EMF may manifest in higher growth.

Together, these results indicate that PSFs can enhance performance of AM seedlings in higher light conditions, such as canopy gaps, but favor EM species under closed canopies. Whereas AM seedlings typically experience negative PSFs when grown under low light conditions, increasing light availability also increases survival, thereby decreasing differences between conspecific and heterospecific soils. For EM species, however, survival responses are higher under low light availability, allowing them to persist in shaded conditions, even when soil-borne pathogens are more prevalent. Differences in survival in low light availability (i.e., shade tolerance) only occur when soil-borne microbes are present (Chapter 2), which could enhance differences in niche across light gradients.

Soil-borne microbes appear to drive PSFs for AM seedlings

Soil-borne microbes appear to drive the PSFs observed in this study, but only for AM seedlings (Figure 4.3). I found that AM seedlings experienced lower survival in live than

sterilized conspecific soils, indicating that the net effect of soil-borne microbes in conspecific soils was detrimental to seedling health. Soils cultured by AM adults may encourage higher amounts of pathogens than those cultured by EM adults (Eagar et al., 2022, 2023), resulting in higher seedling mortality. For all three AM seedling species, percent AMF colonization was highest in conspecific soils and heterospecific soils cultured by AM adults (Table 4.2).

Percent colonization by AMF was higher in conspecific soils. For AM species, this result is consistent with previous studies that also found that AMF and EMF colonization is higher in soils cultured by adult trees of the same mycorrhizal type (Bennett et al., 2017; Chen et al., 2019; Liang et al., 2016). However, unexpectedly for *Q. alba*, an EM species, percent AMF colonization was also highest, not in the heterospecific soils cultured by AM adults, but in conspecific soils and heterospecific soils cultured by EM adults. Soils contain mycorrhizal genotypes that are well-suited to colonizing the adult tree species growing in them (Chen et al., 2019; Segnitz et al., 2020; J. Yang et al., 2018), which could explain the higher amounts of AMF colonization in conspecific soils. Higher amounts of AMF colonization could also be explained by differences in mycorrhizal life history strategies. The greenhouse pots would have excluded any potential common mycorrhizal networks, linking adult and juvenile trees in the forest (Bücking et al., 2016; Simard & Durall, 2004).

Contrary to expectation, I did not find similar patterns for EMF colonization; there was no effect of soil source on EMF colonization for the two EM species. I expected to see higher percent EMF colonization, especially in conspecific soils, due to the assumed higher host-specificity of EMF versus AMF (Chen et al., 2019; S. Smith & Read, 2008). Overall, these results suggest that AMF may be more specialized than expected, even when associating with EM trees species. Furthermore, soils in the greenhouse pots likely selected for more ruderal

species of mycorrhizae, which are often dominated by AMF (Gao et al., 2023; García de León et al., 2016). Together, these results support the idea that mycorrhizal communities associating with oak species shift during different stages of forest succession. AMF colonization may predominate early stages of succession, but EMF colonization (especially via common mycorrhizal networks) is more prevalent in older, established stands (Egerton-Warburton & Allen, 2001).

In contrast to the AM seedlings, EM seedling survival did not differ between live and sterilized conspecific soils, despite EM seedlings experiencing lower survival when growing near conspecific adults Jevon *et al.* (2022). I also did not find negative effects of any heterospecific soil sources on EM seedling survival. Lacking growth data, I cannot exclude the possibility that EMF may have beneficial effects on EM seedling growth but have less noticeable effects on survival.

I was unable to fully test the biotic mechanisms behind PSFs in this study because I sterilized only conspecific and not heterospecific soils. Thus, I can only draw conclusions about the role of soil-borne microbes in the conspecific soils and speculate about the role of microbes in heterospecifics soils. This is an important distinction, because PSFs (defined here as survival in conspecific versus heterospecific soils) can be driven by either soil source. Positive PSFs may be due to greater abundance of mutualists in conspecific soils or lower abundance of pathogenic microbes in heterospecific soils. For instance, Eagar et al. (2022, 2023) found higher abundance of pathogenic fungi in soils beneath AM adults. *Quercus* species growing under AM trees may also be infected by these pathogens, resulting in positive PSFs. Thus, the driving factor in this example would be due to the lack of pathogens in conspecific versus heterospecific soils, not necessarily a higher abundance of mutualists. Alternatively, PSFs could also be driven not just

by microbes, but also by abiotic factors (McCarthy-Neumann & Ibáñez, 2012). For instance, adult trees may modify nutrient availability or soil moisture, or excrete allelochemicals in the soil beneath their crown, thereby limiting seedling establishment.

As differences in percent mycorrhizal colonization and seedling defense/recovery traits between conspecific versus heterospecific soils decreased, PSFs become more neutral

Multiple studies have reported that plant traits could be important predictors of PSFs, though, the focus has been on traits promoting faster growth (Baxendale et al., 2014; Cortois et al., 2016; Xi et al., 2021). In this study, I focused on survival-based PSFs and defense/recovery traits that were expected to drive survival differences between conspecific and heterospecific soils that are likely due to soil-borne pathogens (Song & Corlett, 2022).

Here, I found that, as differences in mycorrhizal colonization and defense/recovery traits between conspecific and heterospecific soils decreased, differences in survival between these soil sources (PSFs) also decreased (i.e., became more neutral). For AM species, PSFs became less negative, and for EM species, PSFs became less positive as mycorrhizal colonization and amounts of defense/recovery traits increased. However, mycorrhizal colonization and defense/recovery traits also increased with light availability, making it difficult to disentangle the roles of these factors on PSFs.

A. saccharum illustrates the importance of traits in neutralizing PSFs. As percent colonization by AMF increased, PSFs became more neutral for all species (i.e., less negative for AM species and more positive for EM species). For the AM species, as amount of colonization in heterospecific soils increased, approaching the levels of colonization in soils cultured by conspecific adults, PSFs became less negative (i.e., seedling survival in heterospecific soils decreased to levels like those in conspecific soils). This result suggests that negative effects of

higher amounts of AMF colonization drive the differences in survival in conspecific versus heterospecific soils (i.e., PSFs).

Furthermore, as measured amounts of phenolics in heterospecific soils increased to levels present in conspecific soils, PSFs became more neutral. Phenolics production, which acts as a chemical defense against pathogens (Ichihara and Yamaji 2009), can be induced to higher amounts when soil-borne microbes, especially pathogens, are present (Chapter 2). Soil-borne pathogens are often more abundant in conspecific soils, where they are effectively-specialized for seedlings of that species; this aligns with results from this study, in which phenolics were produced in higher amounts in conspecific soils. However, although higher amounts of phenolics are associated with higher seedling survival (Chapters 2 and 3), they may not be enough to completely overcome high mortality caused by pathogens. In heterospecific soils, where there are fewer effectively-specialized pathogens, *A. saccharum* seedlings both produce fewer phenolics and have higher survival.

As amounts of lignin in heterospecific soils increased to levels similar to those in conspecific soils (i.e., the difference in lignin in conspecific versus heterospecific soils decreased), differences in survival between conspecific and heterospecific soils (i.e., PSFs) became less positive. This result suggests that lignin confers defense against soil-borne pathogens and possibly mediates PSFs for EM species. Light availability likely mediates the relationships between amounts of lignin and NSC in heterospecific soils and PSFs, at least for AM species.

Together, these results indicate that mycorrhizal colonization and defense/recovery traits can influence the strength and direction of PSFs. As differences in mycorrhizal colonization and defense/recovery traits values between soil sources decrease, so do differences in survival.

However, both mycorrhizal colonization and amounts of defense/recovery traits also increase with light availability, making it difficult to disentangle these two factors.

Caveats

The relationship between defense and recovery traits and seedling survival is correlative and thus may not reflect a causal mechanism. I also was unable to separate the impacts of soil-borne mutualists and pathogens on seedling functional traits and subsequent survival. Similarly, I cannot distinguish between direct AMF effects of pathogen reduction through displacement versus indirect effects inducing production of phenolics or NSC, both of which can enhance seedling survival. I sampled very young seedlings (3 weeks old), which resulted in some systemic measurement error when samples were smaller than typical protocols called for, especially for phenolics. The absolute amounts of phenolics in these seedlings should be interpreted with caution, but we still provide interpretation of relative amounts of phenolics between species and treatments. Also, I was unable to disentangle the effects of seed size and mycorrhizal type, since both EM seedlings were large-seeded *Quercus* species. I suggest that future studies investigate cooccurring AM and EM species with overlapping seed sizes and potentially separate out the role of mycorrhizae and soil-borne pathogens utilizing a technique, such as the wet-sieving method used in Chapter 2.

When evaluating the effects of defense and recovery traits on seedling survival in this study, it is important to note the strong relationship between light availability and those traits. Seedlings growing in higher light availability also had higher amounts of phenolics, lignin, and NSC, likely due to the higher amounts of photosynthates available. Soil sources and associated microbial communities also appear to influence defense and recovery trait production, but not as strongly as light. In this study, I was unable to disentangle the effects of light and traits on

seedling survival. However, I can infer that higher amounts of defense and recovery traits likely neutralize PSFs, and these differences are largely driven by light availability.

Implications for forest community dynamics

Understanding the mechanisms underlying PSFs is key to predicting forest community dynamics. Mismatching of mycorrhizal type between the juvenile and adult tree may shift PSFs and subsequent community dynamics. If AM species experience more negative PSFs (i.e., lower survival beneath conspecific than heterospecific adults), they are more likely to be replaced by heterospecific trees, thereby increasing diversity within that forest. For example, if AM species experience more positive PSFs (i.e., higher survival beneath conspecific than heterospecific adults), they are more likely to be replaced by conspecific trees, thereby decreasing diversity within that forest. The same could be said for EM species, as well.

These results on mycorrhizal mismatch between seedlings and cultured soils provide a possible additional explanation behind the widespread transition from oak (*Quercus*) to shade-tolerant mesophytic tree species, especially maple (*Acer*), in many eastern United States forests (Abrams, 1996; Knott et al., 2019; Nowacki & Abrams, 2008). Mesophytic trees appear to be making conditions more favorable for their own regeneration (e.g., shadier, cooler, wetter, and with less flammable woody debris) and less favorable for oak generation over time (Alexander et al., 2021; Kreve et al., 2011; Nowacki & Abrams, 2008), which has contributed to limited regeneration and seedling establishment for major oak species (Dey et al., 2008). My results suggest that mesophytic species that are associated with AMF, such as *Acer*, will have greater survival near *Quercus* adults (which are associated with EMF) due to the negative PSFs these species experience when establishing under conspecific crowns relative to establishing near

Quercus adults. Maple seedlings established in soils cultured by oak adults can escape the higher abundance of pathogens typically present beneath conspecifics.

Furthermore, maple seedlings growing in shaded oak understories can escape potentially parasitic relationships with AMF. This escape from harmful microbes, in addition to high shade tolerance, enables maple seedlings to outcompete oak seedlings and shift forest species composition. Likewise, oak establishment is reduced as the abundance of canopy adults of these AMF-associated mesophytic species increases, resulting in fewer areas for oaks to disperse that is associated with EMF where these oak seedings experience enhanced survival (i.e. positive PSF). These findings are consistent with a demographic study in a Michigan hardwood forest where newly established oak trees (>3.2 cm DBH) were less likely to establish near maple and black cherry canopy adults, but those mesophytic species were more likely to recruit near oak canopy adults (Allen et al., 2018). Although these findings were thought to be due to the mesophytic species' ability to take better advantage of the light levels under oak canopies, my work suggests that their response may be at least partially due to PSFs.

Changes in defense and recovery trait levels associated with higher light availability and conspecific soil sources can act to neutralize PSFs. While AM seedlings typically experience negative PSFs, higher amounts of defense and recovery traits can result in more neutral PSFs. Likewise, for EM seedlings, higher amounts of traits result in less positive PSFs. In this study, I demonstrated that seedlings produce greater amounts of phenolics, lignin, and NSC when grown under higher light availability. Additionally, some traits can be induced and produced in higher amounts when conspecific soil-borne microbes are present (also see Chapter 2). While there are several studies investigating the role of growth-related traits on PSFs, it is still unclear what other traits directly related to defense and recovery from soil-borne microbes can also mediate PSFs.

I suggest that future studies investigate other potential seedling functional traits and whether they are influenced by matching of mycorrhizal type. Also, studies should investigate the environmental conditions under which these relationships may shift. In addition to light availability, PSFs may shift with climate change, including increased drought, warming (Hassan et al., 2022), and wildfires (Warneke et al., 2023). Moreover, these shifts may depend on mycorrhizal type (Bennett & Klironomos, 2018). This study demonstrates that it is important to consider not only the mycorrhizal type of the seedlings, but also the mycorrhizal type of the trees that cultured the soil in which the seedlings occur (i.e., matching or mismatching mycorrhizal type).

CHAPTER 5

Mycorrhizal type and light availability explain

differences in biomass response to plant-soil feedback

ABSTRACT

Plant-soil feedbacks (PSFs) are key drivers of seedling recruitment patterns in forests and thus influence community dynamics. Recent studies have found that PSFs may be mediated by seedling mycorrhizal type (associated with arbuscular mycorrhizal fungi [AMF] or ectomycorrhizal fungi [EMF]. Furthermore, the strength and direction of PSFs may vary when measuring biomass or survival and under different light conditions. To investigate the influence of mycorrhizal type and light availability on PSFs, I conducted parallel greenhouse and field experiments, growing temperate tree species in soils collected beneath adults of those species and under three light levels ranging from shaded understory to light gap. I measured seedling survival, biomass, and colonization by mycorrhizal fungi after one growing season in the greenhouse and two growing seasons in the field.

In this study, I found that $PSF_{biomass}$ is mediated by both mycorrhizal type and light availability. Whereas AM species tend to have negative $PSF_{biomass}$ that becomes more positive as light increases, EM species experience positive $PSF_{biomass}$ regardless of light level. Also, there is a negative relationship between $PSF_{biomass}$ at high light availability and $PSF_{survival}$ at low light availability, with differences in seedling mycorrhizal type driving this relationship. Understanding the mechanisms underlying PSFs is key for understanding forest regeneration dynamics. While previous research has demonstrated that strength and direction of PSFs varies with mycorrhizal type (AM vs. EM), I show that variation within mycorrhizal types may be

attributed to differences in light availability, especially for AM seedlings. Moreover, tree seedlings express a negative relationship between $PSF_{biomass}$ at high light availability and $PSF_{survival}$ at low light availability, with relative importance of biomass and survival also mediated by mycorrhizal type. Together these results provide a more mechanistic understanding of the biotic and abiotic factors underlying PSFs and subsequent seedling regeneration dynamics.

INTRODUCTION

Plant-soil feedbacks (PSFs) are key drivers of seedling recruitment patterns in forests and subsequent community dynamics (Crawford et al., 2019; Putten et al., 2016). In forest communities, PSFs are a continuous feedback loop in which adult trees modify properties of the soil beneath their crown, thereby influencing the ability of seedlings to grow and survive in that soil (Bever et al., 1997). The strength and direction of PSFs experienced by seedlings can regulate forest community dynamics by acting as stabilizing or destabilizing mechanisms underpinning species coexistence (Chesson, 2000). PSFs are usually calculated as a comparison of plant performance in conspecific versus heterospecific soils (Bever et al., 1997; Kulmatiski et al., 2008).

Potential drivers of PSFs include soil-borne microbes, like mycorrhizae and pathogens (Bever et al., 2010; Jiang et al., 2020). Light availability could also influence microbial abundance and subsequent PSFs (Chapter 3, Chapter 4). PSFs can influence both the survival and growth of tree seedlings. Survival likely has large impacts that peak within the first few months after germination and then declines with age (McCarthy-Neumann & Ibanez 2012), whereas the influence on growth accumulates with age (Dudenhöffer et al., 2018). In addition, PSF responses differ when measured in the greenhouse versus in the field (Forero et al., 2019;

Kulmatiski & Kardol, 2008). Here, we compare seedling survival and growth responses to PSFs at both low and high light in both greenhouse and field experiments spanning 16 weeks to 2 years in duration.

PSFs are often driven by soil-borne microbes, such as pathogens and mycorrhizal fungi (Bever et al., 2010; Jiang et al., 2020), and interactions with microbes can vary with light availability (McCarthy-Neumann & Ibáñez, 2013; Chapter 2). Soil-borne pathogens, including fungi, oomycetes, and bacteria, can kill entire seedling cohorts (Mangan et al. 2010; Terborgh 2012). Furthermore, pathogens are often more abundant in low light availability, where they can proliferate in cool and damp conditions (Y. Liu & He, 2019). Another group of microbes, mycorrhizal fungi, act as mutualists, exchanging water and nutrients for sugars (S. Smith & Read, 2008). Seedlings typically have higher root colonization by mycorrhizal fungi in high light availability (Bereau et al., 2000; Koorem et al., 2017; Shi et al., 2014). However, at low light availability, where photosynthate production is more limited, they may act parasitically (Konvalinková & Jansa, 2016; McCarthy-Neumann & Ibáñez, 2013).

Interactions between soil-borne pathogens and different groups of mycorrhizal fungi can shift the strength and direction of PSFs. At low light availability, the cost of maintaining the mycorrhizal symbiosis may exacerbate the negative effects of pathogens. However, mycorrhizal fungi can also confer protection against pathogens, but the degree of protection depends upon mycorrhizal type (Bennett et al., 2017). Arbuscular mycorrhizal fungi (AMF), which form arbuscules within plant root cells, provide indirect defense against pathogens by competing for space on plant roots (Borowicz, 2001). In contrast, ectomycorrhizal fungi (EMF), which form a Hartig net around plant fine roots, can provide direct defense where the net acts as a protective sheath (Laliberté et al., 2015).

Moreover, matching or mismatching of mycorrhizal type (AMF or EMF) between the seedling growing in and the adult tree culturing the soil may influence PSFs. Hereafter, we refer to species that typically associate with AMF and EMF as "AM species" and "EM species", respectively. Whereas AM trees typically experience negative PSFs (i.e., inhibition of seedlings around conspecific adults), EM trees more often experience positive PSFs (i.e., facilitation around conspecific adults) (Bennett et al., 2017; Kadowaki et al., 2018). In addition, AM trees have a higher abundance of plant pathogens in their soil (Eagar et al., 2022, 2023) and AM seedlings accumulate soil-borne pathogens faster when growing under AM adults (Chen et al., 2019). However, when there is mismatching of mycorrhizal type (e.g., AM seedlings growing beneath EM trees, and vice-versa), both AM and EM seedlings experience positive or neutral PSFs (Kadowaki et al. 2018; Chapter 4). Together, these trends provide a potential explanation why AM seedlings experience more negative PSFs.

PSFs are typically quantified as biomass ($PSF_{biomass}$) or survival ($PSF_{survival}$). The strength and direction of $PSF_{biomass}$ and $PSF_{survival}$ may depend upon several factors, including seedling mycorrhizal type and light availability. $PSF_{survival}$ at low light may be especially important for AM species, which experience more negative PSFs (Bennett et al., 2017; Chen et al., 2019; Kadowaki et al., 2018). In contrast, $PSF_{biomass}$ may be more important for EM seedlings, which may have higher survival in low light but exhibit larger differences in growth in high light availability.

Furthermore, negative relationships between $PSF_{survival}$ at low light availability and $PSF_{biomass}$ at high light availability might provide a mechanistic basis for a growth-defense tradeoff often posited for tree species (Kobe et al., 1995). More negative $PSF_{survival}$ (reduced survival in conspecific versus heterospecific soils) is common in low light availability (Chapter

3, Chapter 4), where survival against soil-borne pathogens and resilience against parasitic mycorrhizal fungi drive seedling performance. Shade tolerant species are typically less vulnerable to mortality by soil-borne microbes than shade intolerant species (Alvarez-Clare & Kitajima, 2007; McCarthy-Neumann & Kobe, 2010a; Wood, Kobe, et al., 2023) at least partly because shade tolerant species allocate more carbon to traits that confer defense against pathogens (Wood, Kobe, et al., 2023). Similarly, slow-growing species (which are likely to invest carbon in defense, rather than growth) experience more positive PSFs, whereas fast-growing species experience more negative PSFs (Baxendale et al., 2014).

My overall conceptual framework is that $PSF_{biomass}$ is driven by seedling mycorrhizal type and light availability. Additionally, species exhibit a negative relationship between $PSF_{survival}$ at low light and $PSF_{biomass}$ at high light, with AM species experiencing negative $PSF_{survival}$ at low light, but having higher $PSF_{biomass}$ at high light, and vice-versa for EM species. I hypothesized that:

1) AM seedlings experience negative $PSF_{biomass}$ (lower biomass on conspecific versus EM heterospecific soils) and EM seedlings experience positive $PSF_{biomass}$ (higher biomass in conspecific versus AM heterospecific soils).

2) AM seedlings experience greater negative $PSF_{biomass}$ in low than high light. EM seedlings experience greater positive $PSF_{biomass}$ in high than low light.

3) Soil-borne microbes from conspecific soils have negative effects on biomass for AM species and positive effects on biomass for EM species, which could partly explain patterns of $PSF_{biomass}$ found in hypotheses 1 and 2.

4) Across species, there is a negative relationship between $PSF_{survival}$ at low light and $PSF_{biomass}$ at high light.

This study provides insight into how species mycorrhizal type can mediate PSF responses to light availability. Additionally, I present novel work, comparing biomass and survival across both a greenhouse and field study. Disentangling the impact of both seedling survival and growth will advance understanding of how PSFs can impact seedling recruitment and forest community composition.

MATERIALS AND METHODS

I conducted parallel factorial blocked field (see Chapter 3) and greenhouse (see Chapter 4) experiments to investigate the above hypotheses. In the field, I grew two temperate tree species in seven soil sources (conspecific live, conspecific sterilized, and five heterospecific), at three light levels, for two growing seasons. In the greenhouse, I grew five species in the same seven soil sources, at three light levels, for one growing season. Throughout each experiment, I monitored seedling survival. At the end of each experiment, I measured biomass and colonization by mycorrhizal fungi (AMF and EMF).

Field experiment

The field experiment (see Chapter 3) was conducted in 100 ha mixed hardwood forest stand in mid-Michigan, at Alma College's Ecological Field Station (43°23'32.0"N 84°53'41.5"W). The forest has not been logged since 1897 and lies in a transition zone between northern coniferous and southern deciduous forests. The dominant species in this forest is sugar maple (*Acer saccharum*), a shade-tolerant canopy tree species. Other common species in the forest include red maple (*A. rubrum*) and big-toothed aspen (*Populus grandidentata*).

I chose six species: red maple (*A. rubrum*), sugar maple (*A. saccharum*), big-toothed aspen (*P. grandidentata*), black cherry (*Prunus serotina*), white oak (*Quercus alba*), and

northern red oak (*Q. rubra*). The six study species vary in local adult abundance, shade tolerance, seed size, and mycorrhizal association type (Table 5.1). Due to difficulty acquiring seeds and/or poor germination, I did not grow *A. rubrum* seedlings in the field experiment. However, I still included *A. rubrum* soil as a treatment.

Table 5.1 Local adult abundance, shade tolerance, seed weight, and primary mycorrhizal association for each of the study species. [1]Local adult abundance was calculated as number of individuals ≥ 5cm dbh/ha at Alma College's Ecological Preserve. [2]Shade tolerance is presented as intolerant, intermediate, or tolerant and as mean ± std. dev., on a standardized scale from 1 (least tolerant) to 5 (most tolerant), calculated by Niinemets & Valladares (2006). [3]Seed weight data was collected from Burns and Honkala (1990). [4]AMF = arbuscular mycorrhizal fungi and EMF = ectomycorrhizal fungi.

Species	Local adult abundance[1]	Shade tolerance[2]	Seed weight (mg)[3]	Mycorrhizal association[4]
Acer rubrum	131	(3.44 ± 0.23)	19.7	AMF
Acer saccharum	285	(4.76 ± 0.11)	64.9	AMF
Prunus serotina	4.33	(2.46 ± 0.34)	94.3	AMF
Populus grandidentata	82.33	(1.21 ± 0.27)	0.2	AMF, EMF
Quercus alba	12.67	(2.85 ± 0.17)	6,677	AMF, EMF
Quercus rubra	71.67	(2.75 ± 0.18)	4,127	AMF, EMF

Table from Chapter 3.

I planted two tree species (*Q. alba* and *Q. rubra*) in seven soil sources (sterilized conspecific, live conspecific, and five heterospecific, including *A. rubrum*, *A. saccharum*, *P. grandidentata*, and *P. serotina*), and a gradient of light levels (ranging from deep shade to light gap), for a total of 1,512 seedlings. I originally planted four species (*A. saccharum*, *P. serotina*, *Q. alba*, and *Q. rubra*); however, due to low survival of *A. saccharum* and *P. serotina* seedlings in the first growing season, I was unable to evaluate biomass or traits for these species.

I collected intact soils cores from May to June 2016 and April to May 2017. To minimize potential for multispecies culturing of soil, I took soil cores under adult trees. I took soils from within 1 m of six mature randomly-selected adults for each of the six study species (36 trees

total), ensuring that each tree was at least two crown diameters away from adults of other species. I used a custom-made, mechanized soil core sampler (Giddings Machine Co; Windsor, CO, USA) to remove 9 cm × 46 cm long intact soil cores. I maintained adults as separate replicates for statistical analysis (Reinhart & Rinella, 2016; Rinella & Reinhart, 2018).

Intact soil cores with plastic liners were converted into pots by drilling two 7.5 cm diameter holes into the sides. I also adhered a 0.5 μm nylon mesh over two side holes and the bottom opening for each pot. Small nylon mesh pots are an established method for studying mycorrhizal networks in forests (McGuire 2007; Bingham and Simard 2012; Teste et al. 2017; Chapter 3). The small pores in the mesh prevent roots, fungal hyphae, oomycetes, and pathogenic fungi from passing in or out, but have minimal effects on water and nutrient flows (Allison et al., 2013).

Pots were transplanted into eighteen 8.4 m × 6.6 m common-garden field plots along a gradient of light availability (0.032 to 0.161 indirect site factor [ISF]), which I grouped into three general light levels (low, medium, high). Existing vegetation and leaves in each plot were removed to minimize potential light interception of pots. I then took precise measurements of light availability by analyzing canopy photos with HemiView software (Delta-T services, Ltd., Burwell, England; Figure A3.5).

Sterilized soil treatments were created by exposing a subset of conspecific soils in the plastic liner pots to gamma irradiation (30-70 kGY; Sterigenics International, Schaumburg, IL, USA) in July 2017. I allowed the pots to rest at least once month after irradiation, to minimize post-sterilization nutrient spikes. Gamma irradiation is highly effective at killing soil microorganisms and has minimal effects on both soil chemical and physical properties (McNamara et al., 2003). I also tested the sterilized versus live soil for difference in nutrient

availability, using plant root simulator (PRS^{TM}) probes (Western Ag Innovations Inc., Saskatchewan, Canada). I found no effect of sterilization on soil nutrient availability (Tables A3.4 and A3.5; Figure A3.3).

In the field, I planted 108 seedlings per species × soil source, evenly distributed across the eighteen field plots. I planted a single surface-sterilized seed, with a newly-emerged radicle, in each custom-made pot. I purchased seeds for *Q. alba* from Sheffields Seed Co (Locke, NY, USA). I collected seeds for all other study species from mid-Michigan forests. I expect that variation among seed source populations in survival, mycorrhizal colonization, and defense/recovery traits (e.g., phenolics, lignin and NSC) was minimal (McCarthy-Neumann & Ibáñez, 2012). Additionally, in June 2018, one week prior to planting seeds, I added 1 cm of a 1:1 mixture of peat moss and fresh or sterilized soil. This soil amendment was added to increase transplant success and provide fresh inoculum to the seedlings. In a previous trial run, I found that seedlings planted with peat moss and fresh soil experienced less transplant shock (personal observation).

To minimize disease from non-experimental sources, seeds were surface-sterilized with a 0.6% NaOCl solution prior to stratification and again prior to germination. Also, to avoid cross-contamination, all tools and surfaces that were exposed to soil were soaked in 10% bleach or surface sprayed with 70% EtOH and then rinsed with deionized water. To minimize browsing and excavation of seedlings by vertebrates, I constructed enclosures around each field plot from galvanized hardware cloth (6 cm × 6 cm) to 1.8 m. To the top of each pot, I glued 0.25 cm × 0.25 cm hardware cloth. Seedlings likely did not experience significant shading from the hardware cloth, and most seedlings grew above the cloth within 2 weeks of planting.

After planting, I censused seedling survival twice per week for 16 weeks (one growing season). I then re-censused seedlings at the end of the second growing season and quantified biomass for all surviving seedlings at the end of the experiment.

Seedling mortality in the field was exceptionally high, especially for *A. saccharum* and *P. serotina* (see Chapter 3), with almost 100% of seedlings for both species dying by the end of the growing season. Mortality for *Q. alba* and *Q. rubra* was lower (about 50% of seedlings), but was still relatively high for these species. High mortality was likely due to a dry period preceding a large rainfall event (7" in one week). Due to the seedling pots' design, drying caused soil to pull away from the edges, which then flooded during heavy rainfall.

Greenhouse experiment

I conducted a parallel greenhouse experiment (see Chapter 4) at the Michigan State University Tree Research Center in Lansing, MI, USA (42.7 ºN, 84.5 ºW). I planted five tree species (*A. rubrum*, *A. saccharum*, *P. serotina*, *Q. alba*, and *Q. rubra*). Due to difficulty acquiring seeds, I did not grow *P. grandidentata* seedlings. However, I still included *P. grandidentata* soil as treatments.

I grew seedlings in seven soil sources (sterilized, conspecific, live conspecific, and five heterospecific). I collected soils from the Alma College Ecological Field Station in August 2017 (top 15 cm from within 1 m of the adult tree bole), using the same species and adult trees as the field experiment. I prepared the soils by dicing roots and sifting them through a 1 cm mesh sieve, retaining all fine roots and maintaining soil from each adult as separate replicates (Reinhart & Rinella, 2016; Rinella & Reinhart, 2018). All pots were filled with a 1:1 mixture of prepared field soil and Fafard #2 commercial soil mixture. Previous trials using 100% field soil resulted in high seedling mortality in the first three weeks (personal observation). I did not use multi-stage

greenhouse culturing (Bever et al., 2010), since in-situ natural culturing had already occurred and likely more accurately represented PSFs experienced in the forest. Like in the field experiment, I sterilized a subset of conspecific soils by gamma irradiation (30-70 kGy; Sterigenics International, Schaumburg, IL, USA) and allowed the soil to rest for one month before planting, to minimize post-sterilization spikes in nutrient availability. There was almost zero seedling colonization by mycorrhizal fungi in sterilized soils (mean = 0.02%, df = 629, t = 84.8, $p < 0.01$), confirming that my sterilization methods were effective.

I grew seedlings at three light levels (2%, 15%, and 30% sun), representing the typical light range experienced by Michigan forests (Schreeg et al., 2005). I created light treatments in the greenhouse by covering benches with an inner layer of black shade cloth and an outer layer of reflective knitted poly-aluminum shade cloth (BFG Supply, Burton, OH, USA). I confirmed light levels using PAR (photosynthetically active radiation) methods at each bench with a LI-COR 205A quantum sensor (LI-COR, Lincoln, NE, USA) on a uniformly-overcast day.

I set up pots on nine different benches in the greenhouse, where all combinations of species and soil source were represented, with three benches per light treatment. I planted 30 seedlings per species × soil sources × light treatment, for a total of 3,150 seedlings. I planted a single seed with a newly-emerged radicle into each pot. To minimize disease from non-experimental soil sources, seeds were surface sterilized with 0.6% NaOCl solution prior to stratification and germination. To avoid cross-contamination, all tools and surfaces that were exposed to soil were soaked in 10% NaOCl solution or surface sprayed with 70% EtOH and then rinsed with deionized water.

I monitored seedling survival twice per week for the equivalent of one growing season (16 weeks). I also measured seedling biomass at the end of the experiment.

Statistical analysis

I used linear mixed effects models to investigate how soil source and light availability influence seedling biomass. Models were run for each species, with soil source and light availability as fixed effects, and greenhouse bench and adult tree as random effects. $PSF_{biomass}$ was calculated using the response-ratio of biomass in conspecific versus sterilized conspecific or heterospecific soils. I then log_{10} transformed the response-ratios to get a relative measure of PSFs, independent of differences in biomass across species. $PSF_{biomass}$ was calculated using bootstrapping (S. E. Bates et al., 2020).

To evaluate low light survival versus high light growth, I compared $PSF_{survival}$ at low light availability to $PSF_{biomass}$ at high light availability (sample sizes for each seedling species × soil source × light level in the field and greenhouse experiments are available in Table A5.1 and Table A5.2). $PSF_{survival}$ values for the field and greenhouse were previously calculated in Chapters 2 and 3, respectively. For the field experiment, I analyzed seedling survival over 16 weeks with frequentist Cox proportional hazards regression (Cox & Oakes, 2017). I ran species-specific models, using soil source and light availability as fixed effects, and plot and adult tree as random effects. The best fitting models for seedling survival did not include any interactions.

For the greenhouse experiment, I calculated $PSF_{survival}$ by using an individual based counting process in a Bayesian Cox survival model (see Chapter 4; Burnham and Anderson 2002; McCarthy-Neumann and Ibáñez 2012). I used predicted survival values to calculate differences in seedling survival between soil treatments and light levels.

All analyses were performed with R version 3.5.1 (R Core Team, 2020). For frequentist survival analysis, I used the "coxph" function in the survival package (Therneau & Grambsch, 2000) to fit Cox proportional hazards regressions models. For Bayesian survival analysis, I used

the “rjags” package to fit models and run predicted survival and contrast simulations (Plummer et al., 2023). I used the lme4 package (D. Bates et al., 2015) to evaluate linear models. I tested the significance of main effects using a likelihood ratio test with the “Anova” function. I tested for multicollinearity variance inflation factors using the “vif” function in the car package (Fox & Weisberg, 2019). I used the “emmeans” function in the multcomp package to evaluate post-hoc Tukey pairwise comparisons of significant main effects, estimated marginal means, and odds-ratios (Hothorn et al., 2008; Lenth, 2020).

RESULTS

Field seedlings $PSF_{biomass}$ was negative for *Q. alba* and positive for *Q. rubra*, regardless of soil source and light level (H1)

In the field, $PSF_{biomass}$ was negative for *Q. alba* with lower biomass in conspecific relative to all heterospecific cultured soils at all light levels (Figure 5.1, Figure A5.1, and Figure A5.2). Conversely, *Q. rubra* experienced positive $PSF_{biomass}$ with greater biomass in conspecific relative to all heterospecific soils at all light levels. Biomass values for each species × soil source × light level in the field are presented in Table A5.3.

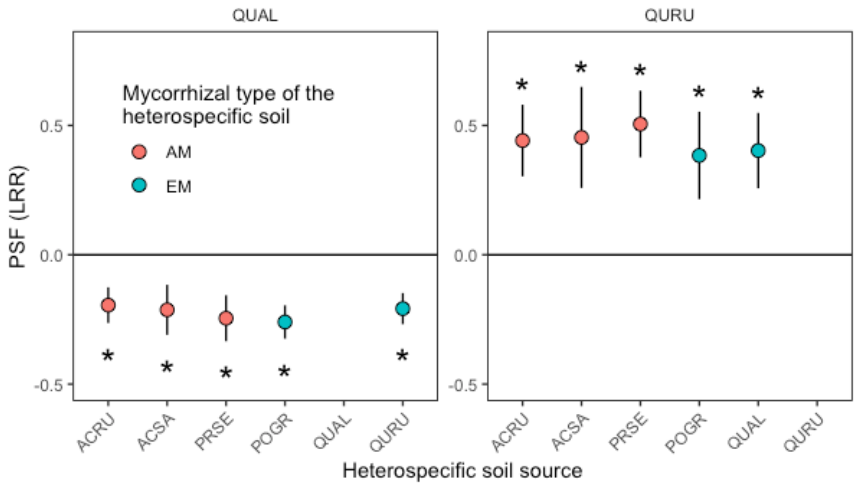

Figure 5.1 Log response ratio ± standard error seedling biomass in conspecific versus heterospecific soils at low light availability, in the field. Values < 1 indicate negative PSF and values > 0 indicate positive PSF. Values that are statistically different from 0 ($p < 0.05$) are indicated with a star *. Figures for medium and low light are available in the supporting information (Figures A5.3 and A5.4). As an example, *Q. alba* had lower biomass when growing in soils cultured by *Q. alba* adults than in soils cultured by adults of any other species (i.e., negative $PSF_{biomass}$).

Greenhouse seedlings $PSF_{biomass}$ varied with seedling mycorrhizal type and light availability (H1 & H2)

AM species experienced negative $PSF_{biomass}$ in low light availability. As light increased, $PSF_{biomass}$ became neutral, or even positive. EM species experienced positive $PSF_{biomass}$ in low and high light, and some neutral $PSF_{biomass}$ in medium light.

For AM seedlings (*A. saccharum* and *P. serotina*), $PSF_{biomass}$ varied with light availability (Figure 5.2A). At low light, seedlings experienced negative $PSF_{biomass}$, with reduced biomass in conspecific relative to all heterospecific cultured soils. *A. saccharum* also experienced negative $PSF_{biomass}$, but only in comparison to *P. grandidentata soils.* At medium light availability, *A. rubrum* $PSF_{biomass}$ was neutral in all heterospecific soils, except for a negative $PSF_{biomass}$ in *A. saccharum* soils. Additionally, *A. saccharum* seedlings experienced positive $PSF_{biomass}$ in comparison to soils cultured by EM adults (higher survival in conspecific soils compared to soils

cultured by *Q. alba* and *Q. rubra*). *P. serotina* experienced positive $PSF_{biomass}$ in all heterospecific soils. At high light, *A. rubrum* and *P. serotina* experienced neutral $PSF_{biomass}$ when compared to soils cultured by heterospecific AM adults and positive $PSF_{biomass}$ in soils cultured by heterospecific EM adults. *A. saccharum* experienced positive $PSF_{biomass}$ in all heterospecific soils.

EM seedlings (*Q. alba* and *Q. rubra*) generally experienced positive $PSF_{biomass}$, with higher biomass in conspecific relative to all heterospecific soils at low and high light (Figure 5.2B). At medium light, *Q. alba* experienced neutral $PSF_{biomass}$ compared to *A. rubrum*, *Q. alba*, and *Q. rubra* soils. Additionally, *Q. rubra* experienced negative $PSF_{biomass}$ compared to *Q. alba* soil. Furthermore, variation in the strength of $PSF_{biomass}$ varied much more in low light availability than at either medium or high light.

Biomass values for each species × soil source × light level in the greenhouse are presented in Table A5.4.

Effects of conspecific microbes on seedling biomass varied with light availability (H3)

Soil-borne microbes had a positive effect on *Q. alba* biomass in the field at low light availability (Figure 5.3A). At both medium and high light availability, microbes had a negative effect on *Q. alba* biomass and a positive effect on *Q. rubra* biomass, but sample sizes were too small to determine if these effects were statistically significant (Table A5.2).

In the greenhouse, soil-borne microbes had a negative effect on *P. serotina* biomass and a positive effect on *Q. rubra* biomass at low light availability (Figure 5.3B). However, effects of microbes shifted to neutral at high light availability for *P. serotina*. Also, at low and high, but not medium, light availability, soil-borne microbes had a positive effect on biomass *Q. rubra*.

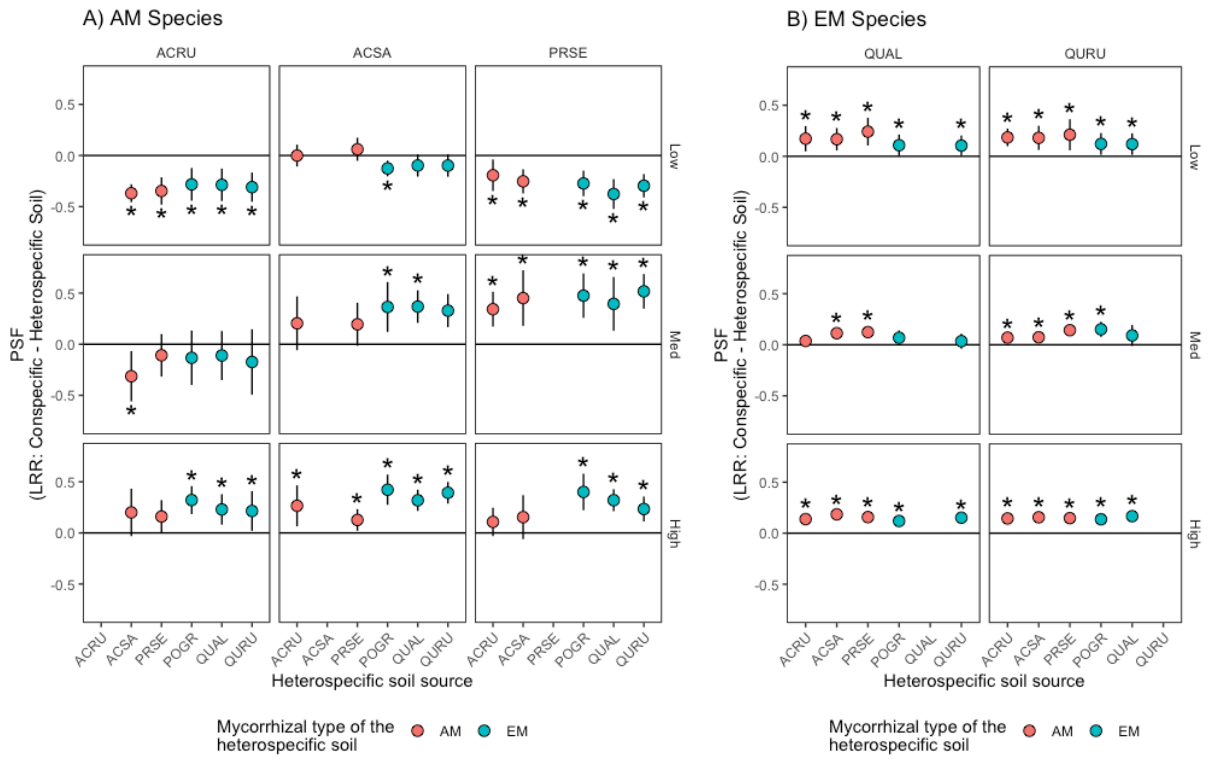

Figure 5.2 Log response ratio ± standard error of seedling biomass in conspecific versus heterospecific soils for A) AM species and B) EM species, in the greenhouse. Values > 0 indicate positive PSF. Values that are statistically different from 0 ($p < 0.05$) are indicated with a star *. As an example, at low light availability, *A. rubrum* seedlings had lower biomass in soils cultured by *A. rubrum* adults than in soils cultured by adults of any other species (i.e., negative $PSF_{biomass}$). At high light availability, *A. rubrum* experienced higher biomass in soils cultured by *A. rubrum* adults than in soils cultured by *P. grandidentata*, *Q. alba*, or *Q. rubra* adults (i.e., positive $PSF_{biomass}$).

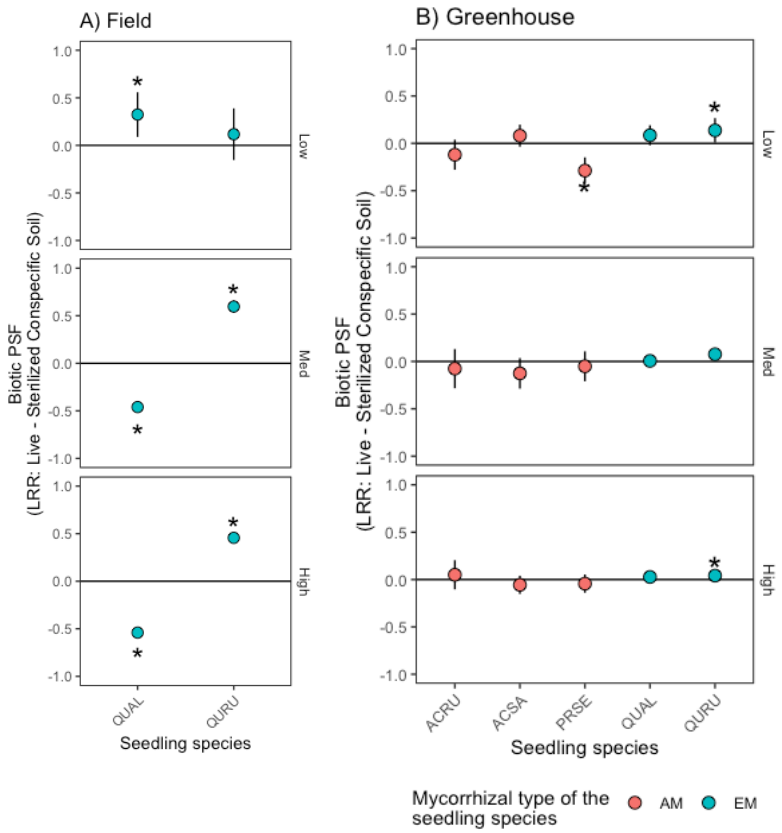

Figure 5.3 Log response ratio ± standard error of seedling biomass in live conspecific versus sterilized conspecific soils (i.e., biotic effects) at all three light levels, in the A) field and B) greenhouse. Values > 0 indicate positive PSF. Values that are statistically different from 0 ($p < 0.05$) are indicated with a star *. As an example, in the field, *Q. alba* seedlings had higher biomass in live than sterilized conspecific soils (i.e., positive $PSF_{biomass}$) and there was no difference in biomass for *Q. rubra* seedlings growing in live versus sterilized soils (i.e., neutral $PSF_{biomass}$).

There was a negative relationship between low light PSF-survival versus high light PSF-growth (H4)

There was a negative relationship between $PSF_{survival}$ in low light availability and $PSF_{biomass}$ in high light availability (Figure 5.4; $R^2 = 0.21$, $F_{6,1} = 23$, $p = 0.02$). There was no significant relationship between $PSF_{survival}$ in low light availability and $PSF_{biomass}$ in high light availability in the field experiment ($p > 0.05$; Figure A5.3).

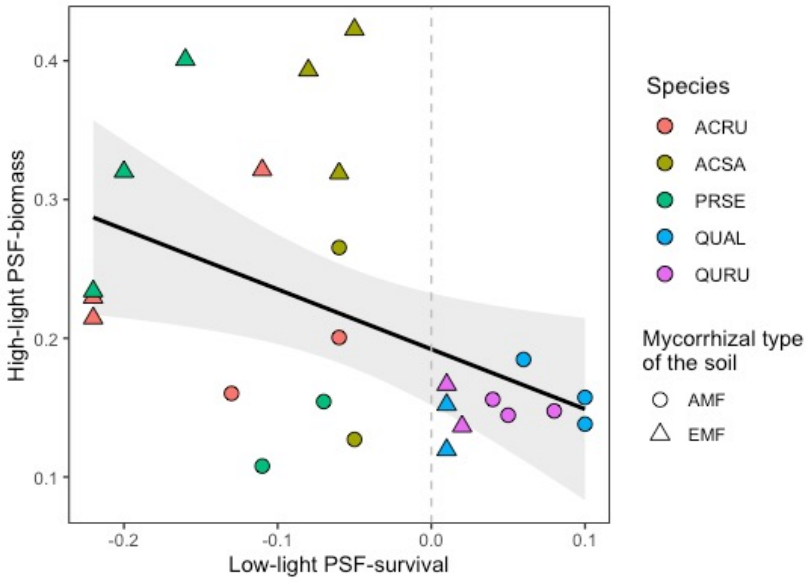

Figure 5.4 Relationship between $PSF_{survival}$ at low light availability and $PSF_{biomass}$ at high light availability in the greenhouse experiment. Each point is a mean $PSF_{survival}$ and $PSF_{biomass}$ value for a given species heterospecific soil treatment combination for the given light level, where PSF is calculated at the relative performance in conspecific versus heterospecific soil. A positive PSF indicates higher performance in conspecific than heterospecific soils, and vice-versa. The dashed vertical line at $x = 0$ is the break between negative and positive $PSF_{survival}$. Here, all AM seedlings appear to the left and all EM species appear to the right of the dashed low-light $PSF_{survival}$ line. Differences appear to be driven by both the mycorrhizal type of the seedlings and the adults culturing the soil.

DISCUSSION

In this study, I found that $PSF_{biomass}$ is mediated by both mycorrhizal type and light availability. Whereas AM species tend to have negative $PSF_{biomass}$ that becomes more positive as light increases, EM species experience positive $PSF_{biomass}$ regardless of light level. Also, I found a negative relationship between $PSF_{biomass}$ at high light availability and $PSF_{survival}$ at low light availability, with differences in seedling mycorrhizal type driving this relationship.

$PSF_{biomass}$ responses to light availability differ between AM and EM species

Environmental gradients, like light availability, can modify plant-soil interactions (Beals et al., 2020). Despite this, there are few studies investigating how light availability can influence PSFs and results are variable among those that have (McCarthy-Neumann and Ibáñez 2013; Smith and Reynolds 2015; Chapter 3; Chapter 4).

A possible explanation for variation in PSFs across light availability is mycorrhizal type. In this study, I found that AM seedlings experience negative PSFs in low light, but PSFs shifted to positive as light availability increased. Previous research has demonstrated that AM species typically experience negative or neutral PSFs and EM species experience positive or neutral PSFs (Bennett et al., 2017; Kadowaki et al., 2018). AM species may be more susceptible to soil-borne pathogens that are more prevalent in low light conditions (Hersh et al., 2012; Y. Liu & He, 2019; Reinhart et al., 2010). In addition, AMF may act parasitically in low light (Ibáñez & McCarthy-Neumann, 2016; Konvalinková & Jansa, 2016), potentially exacerbating the negative effects of pathogens on seedling performance. In a previous study, I found that AMF can have negative effects on AM seedling survival, even when pathogens are not present, suggesting that AMF can have detrimental effects on AM species performance similar to pathogens (Chapter 2).

In contrast, EM seedlings in this study, when grown in the greenhouse always experienced positive or neutral PSFs, regardless of light availability (Figure 5.1). EMF may confer greater protection from pathogens by forming a physical sheath around seedling root tips (Laliberté et al., 2015). Furthermore, EMF do not appear to act parasitically at low light availability, even when resources are limited (but see Ågren *et al.*, 2019).

Additionally, the matching or mismatching of mycorrhizal type between the juvenile and adult tree culturing the soil may also influence PSFs. For example, PSFs may shift in both strength and direction when AM seedlings are grown in EM, rather than AM soils, and vice-versa (Kadowaki et al. 2018). In a previous study (see Chapter 4), I found that negative $PSF_{survival}$ for AM species almost always occurred when heterospecific soils were cultured by EM adults; positive $PSF_{survival}$ for EM seedlings occurred when heterospecific soils were cultured by AM adults. However, $PSF_{biomass}$ in this study did not differ with mycorrhizal type, suggesting that mycorrhizal type mismatching between the juvenile and adult culturing the soil may be more important for survival than for biomass.

Effects of soil-borne microbes in conspecific soils were limited

There did not appear to be strong effects of soil-borne microbes in conspecific soils across species. In the greenhouse, soil-borne microbes had negative effects on biomass for *P. serotina* only at low light and positive effects on biomass for *Q. rubra* at low and high light (Figure 3). Similarly, in the field experiment, soil-borne microbes had a positive effect on *Q. rubra* at all light levels. AM species, like *P. serotina*, typically experience more negative PSFs due to higher abundance of specialized soil-borne pathogens in conspecific soils (Chen et al., 2019). Additionally, EM species, like *Q. rubra*, are better-defended against pathogens (Bennett et al., 2017) and likely derive more benefits from this symbiosis.

In the field experiment, soil-borne microbes in conspecific soils had a positive effect on *Q. alba* biomass in low light but a negative effect on biomass in medium and high light (Figure 5.3). This may explain the negative $PSF_{biomass}$ (lower biomass in conspecific than heterospecific soils) experienced by *Q. alba* seedlings in the field (Figure 5.1). At low light, negative $PSF_{biomass}$ is likely driven by positive effects of microbes in heterospecific soils that overwhelm the positive soil microbe effects in conspecific soils.

It is important to note that I was unable to determine if PSFs in either study were driven by soil-borne microbes in the conspecific or heterospecific soils sources, since I only compared live versus sterilized conspecific soils. It is important to distinguish which soil source drives biotic effects of PSFs. Positive PSFs may be driven by greater abundance of mutualists in conspecific soils or lower abundance of pathogenic microbes in heterospecific soils. Alternatively, negative PSFs may be driven by a higher relative abundance of pathogens in conspecific soils.

Mycorrhizal type and light availability drive differences in $PSF_{biomass}$ and $PSF_{survival}$

Whether biomass or survival is measured may also contribute to differences in PSFs across mycorrhizal type and light levels. Studies that measure survival ($PSF_{survival}$; McCarthy-Neumann and Ibáñez 2013; Chapter 4) seem to find stronger feedbacks (negative and positive) in low light availability (but see Chapter 3). Other studies measuring biomass found more negative feedbacks at high light availability that became neutral or positive as light availability decreased (L. M. Smith & Reynolds, 2015). Conversely, in this study, I found that $PSF_{biomass}$ generally became more positive as light increased, aligning with findings by Xi et al. (2023).

In the greenhouse experiment, there was a negative relationship between high-light $PSF_{biomass}$ and low-light $PSF_{survival}$. $PSF_{survival}$ may be more responsive at low light, especially for

species that are more susceptible to mortality from soil-borne pathogens, whereas $PSF_{biomass}$ may be more important at high light, especially for seedlings that do not experience strong differences in survival when grown in different soil sources. The negative relationship between $PSF_{biomass}$ at high light and $PSF_{survival}$ in low light closely aligns with a trade-off between high light growth and low light survival (Kobe et al., 1995). Shade intolerant species, which allocate more resources to growth and fewer to defense, should have better growth in high light and poor survival in low light. Shade intolerant species are also more susceptible to negative effects of soil-borne microbes in low light availability (Chapter 2).

Differences in $PSF_{survival}$ at low light and $PSF_{biomass}$ at high light appear to be driven by seedling mycorrhizal type. In this study, $PSF_{survival}$ appears to be important for AM species or species that allocate more resources to fast growth. In agreement with these results, research in grasslands has demonstrated that fast-growing, early successional species tend to experience more negative PSFs (Cortois et al., 2016; Xi et al., 2021). In a previous study, I also found that shade tolerant species, which often have slower-growth strategies and higher investment in defense traits, have lower mortality due to soil-borne microbes (Chapter 2). In contrast, $PSF_{biomass}$ may be more important for EM species or species that allocate more resources to defense/survival traits, rather than growth. I recommend future studies include both AM and EM species with differences in resource acquisition strategies to disentangle a potential growth-defense tradeoff in PSFs.

In addition to mycorrhizal type of the seedling, mycorrhizal type of the adult culturing the soil could influence the low-light $PSF_{survival}$ and high-light $PSF_{biomass}$ relationship, especially for AM species. AM seedling experienced a lot of variation in high-light $PSF_{biomass}$, with $PSF_{biomass}$ being lower in soils cultured by AM than EM adults. These differences appear to drive

the differences between low-light $PSF_{survival}$ and high-light $PSF_{biomass}$. The large amount of variation in AM seedling responses to soil sources could drive differences in seedling recruitment patterns.

Differences in $PSF_{biomass}$ at high light and $PSF_{survival}$ at low light may also be explained by seed size. In this study, I was unable to tease apart effects of mycorrhizal type and seed size. The AM species in this study were all small-seeded and were thus likely influenced more strongly by the experimental treatments. In contrast, the EM species in this study were all large-seeded and had a longer period to rely upon seed reserves and cotyledon support, thus leaving less time to respond to the treatments. Previous research has demonstrated that large-seeded species typically experience more positive PSFs (Moles, 2005). Seed-size advantage might be especially important in the first growing season, when seedlings are still reliant upon maternal seed reserves.

Additional Caveats

In this study, I was unable to directly compare the results of the field and greenhouse studies. All *A. saccharum* and *P. serotina* seedlings died by the end of the first growing season in the field (Chapter 3), and I did not harvest any *Quercus* seedlings at the end of the first growing season. However, I am still able to infer differences between field and greenhouse trends, especially between the *Quercus* seedlings that survived through both experiments. Furthermore, field experiments, regardless of whether they measure $PSF_{survival}$ (Chapter 3) or $PSF_{biomass}$, seem to result in weaker PSF responses, compared to the greenhouse. Greenhouse experiments tend to overestimate PSFs, relative to field experiments where more stochastic environment conditions may overwhelm PSF effects (Forero et al., 2019; Kulmatiski & Kardol, 2008). I suggest that future research pair both greenhouse and field experiments to better understand the underlying

mechanisms driving PSFs and also a realistic measure of the strength and direction of PSFs in the field.

Other abiotic factors, like nutrient availability, can also influence the strength and direction of PSFs. Nutrient availability might alter PSFs by increasing resources available for seedling, thus increasing their overall health and indirectly increasing their resilience against harmful microbes. I found no effect of sterilization on soil nutrient availability, nor did I find differences in soil availability in soils beneath adult trees versus soils collected for the plastic liner pots. I also did not find substantial differences in nutrient availability between adult tree species. Therefore, I do not think that nutrient availability played a strong role in mediating PSFs for either of these experiments.

Implications for forest community dynamics

Understanding the mechanisms underlying PSFs is key for understanding forest regeneration dynamics. Negative PSFs (lower performance in conspecific versus heterospecific soils) may have positive effects on forest community diversity and stabilize species coexistence by increasing the likelihood that a seedling of a different species will replace an adult tree when it dies (Bever et al., 1997; Chesson, 2000). Conversely, positive PSFs (greater performance in conspecific than heterospecific soils) may decrease community diversity by increasing the likelihood that an adult tree is replaced by a seedling of the same species.

Differences in seedling PSFs can drive forest successional dynamics and management (Jiang et al., 2020; Q. Liu & Zhao, 2023). EM species appear to derive benefit from soil-borne microbes cultured in soils by conspecific adults, regardless of light level. In contrast, AM species have reduced performance both near conspecific adults and in low light conditions, likely due to detrimental effects of pathogens or parasitic effects of mycorrhizal fungi. If seedlings are limited

by the negative effects of soil-borne microbes cultured beneath the crowns of conspecific adults (i.e., AM species), they may have more recruitment success away from conspecific adults in low light availability. Conversely, if seedlings benefit more from positive effects of soil-borne microbes cultured by conspecific adults (i.e., EM species), they may have limited recruitment success in soils farther away from conspecific adults. Together, these results help develop a deeper understanding of seedling regeneration dynamics in the context of mycorrhizal type and light availability.

CHAPTER 6

Conclusion

Disentangling the mechanisms underlying plant-soil feedbacks (PSFs) is critical to understanding forest community dynamics. In this dissertation, I examined how seedling mycorrhizal type and defense/recovery traits (e.g., phenolics, lignin and NSC) can interact with light availability to mediate PSFs. In Chapter 2, I separated the effects arbuscular mycorrhizal fungi (AMF) and pathogens on these traits and survival for three species in the genus *Acer*. In Chapter 3, I investigated variation in seedling defense and recovery traits and their effects on $PSF_{survival}$ in the field. In Chapter 4, I used a greenhouse experiment, paralleling the field experiment in Chapter 3, to study how mismatching of mycorrhizal type between juvenile and adult trees can alter these interactions. Finally, in Chapter 5, I evaluated $PSF_{biomass}$ in both the field (Chapter 3) and greenhouse (Chapter 4) experiments. I also examined the relationship between $PSF_{biomass}$ in high light and $PSF_{survival}$ in low light availability.

Seedling shade tolerance is mediated by soil-borne microbes and defense/recovery traits

In Chapter 1, I demonstrated that survival of newly germinated tree seedlings in low versus high light, or "shade tolerance," may be due to interactions between low light and soil-borne microbes and be mediated by defense and recovery traits. Previous studies have demonstrated that seedling defense and recovery traits are influenced by light and microbes and that these traits can influence growth and survival at low light (Falster et al., 2018), but have not linked resources, traits, and survival, as in this study.

This study provides a needed first step in developing a mechanistic understanding of how soil-borne microbes impact seedling shade tolerance, explained through defense and recovery

traits. Although fast-growing shade intolerant species may be expected to outcompete shade tolerant species in high light (Pacala et al., 1996), shade intolerant species can be limited by the negative interactive effects of soil-borne microbes at low light (Y. Liu & He, 2019; McCarthy-Neumann & Kobe, 2010a), restricting their recruitment niche to areas with higher light and fewer soil-borne microbes. For example, *A. negundo* seedlings may be limited to forest edges and open fields, due to high mortality in the presence of pathogens and parasitic effects of mycorrhizae in forest understories. Conversely, *A. saccharum* is able to recruit in deeply shaded environments, likely due to higher carbon allocation to defense and recovery traits, and thus lower susceptibility to negative effects of soil-borne microbes.

In this chapter, I demonstrated the importance of interactions between soil-borne microbes and light availability in determining tree seedling survival. Furthermore, I related both intra- and interspecific differences in survival to defense and recovery traits, supporting a more trait-based and mechanistic approach to understanding forest community dynamics.

Mismatching of mycorrhizal type and interactions with light availability to mediate PSFs

In Chapter 4, I found that mismatching of mycorrhizal type between seedlings and the adults culturing the soil plays a strong role in the direction of PSFs. AM seedlings generally experienced neutral PSFs when grown in heterospecific soils cultured by AM adults. However, when grown in heterospecific soils cultured by EM adults, AM seedlings experienced negative PSFs. Similarly, EM seedlings generally experienced neutral PSFs when grown in soils cultured by EM adults, but positive PSFs when grown in soils cultured by AM adults. Mismatching of mycorrhizal type can drive recruitment patterns in Michigan forests. For example, *Acer* species, which are associated with AMF, often replace *Quercus* species, which primarily associate with EMF. *Acer* species typically experience negative PSFs when grown in soils cultured by AM

adults. However, when they grow in soils cultured by adult *Quercus*, they are able to escape effectively-specialized pathogens and potentially parasitic effects of AMF.

In addition, PSFs can enhance performance of AM seedlings in higher light conditions, such as canopy gaps, but favor EM species under closed canopies. Whereas AM seedlings typically experience negative PSFs when grown under low light conditions, increasing light availability also increases survival, thereby decreasing differences between conspecific and heterospecific soils. For EM species, however, survival responses are higher under low light availability, allowing them to persist in shaded conditions, even when soil-borne pathogens are more prevalent. AM species may be able to escape soil-borne pathogens and parasitic effects of AMF when recruiting into areas with high light availability or away from conspecific adults. This lends further support to field observations that *Acer* species often replace *Quercus* in older forest stands.

Seedling traits vary in response to soil source, light availability, and mycorrhizal type

Through this dissertation, and especially in Chapter 3, I provide some of the first evidence that defense and recovery traits can vary in seedlings as young as three weeks old. Furthermore, measured amounts of these traits vary in response to both abiotic (light availability) and biotic (soil-borne microbes) factors. Despite the important role of plant survival in PSF (Comita et al., 2010; McCarthy-Neumann & Kobe, 2010a), traits promoting faster growth (e.g., specific leaf area, specific root length, height) have been the focus of most PSF studies (Baxendale et al., 2014; Cortois et al., 2016; Xi et al., 2021). Frequently, defensive traits are accounted for by assuming that species with fast growth rates have low investment in defense, and vice-versa (Cortois et al., 2016; Xi et al., 2021). However, tree seedling survivorship is

likely to have greater effects on future community dynamics and composition than growth (Pacala et al., 1996).

While little studied, defense and recovery traits that influence tree seedling survivorship in response to PSFs could be a crucial mechanism governing seedling and forest community dynamics. Seedlings growing in conditions where they produce higher amounts of defense and recovery traits (i.e., high light availability) are better able to recruit into those spaces, even when soil-borne microbes are present. For example, *Acer* seedlings growing in soils cultured by *Acer* adults would be expected to experience negative PSFs (i.e., lower survival in that conspecific soil than other heterospecific soils, especially those cultured by EM species). However, under high light availability, *Acer* seedlings may produce enough defensive and recovery traits to overcome the limitations of soil-borne microbes.

Defense and recovery traits may effectively neutralize PSFs

Results from Chapter 4 indicate that higher amounts of seedling defense and recovery traits may effectively neutralize these PSFs. Differences in seedling responses to soils cultured by AM and EM adults, and changes in PSFs with light availability, can be explained by defense and recovery traits. For AM seedlings, increasing percent colonization by AMF, phenolics, lignin, and NSC – which were influenced by light level and soil source – were associated with less negative (i.e., more neutral) PSFs. For EM seedlings, increasing colonization by AMF and higher amounts of lignin – which were influenced by light level and soil source – were associated with less positive PSFs. This study demonstrates that it is important to consider not only the mycorrhizal type of the seedlings, but also the mycorrhizal type of the trees that cultured the soil in which the seedlings occur (i.e., matching or mismatching mycorrhizal type).

There is a negative relationship between $PSF_{biomass}$ at high light and $PSF_{survival}$ at low light

While previous research has demonstrated that strength and direction of PSFs varies with mycorrhizal type (AM vs. EM), we have also shown that variation within mycorrhization types may be attributed to differences in light availability, especially for AM seedlings. Environmental gradients, like light availability, can modify plant-soil interactions (Beals et al., 2020). Despite this, there are few studies investigating how light availability can influence PSFs and results are variable among those that have (McCarthy-Neumann and Ibáñez 2013; Smith and Reynolds 2015; Wood et al. 2023b; Chapter 4). Moreover, tree seedlings may express a negative relationship between $PSF_{biomass}$ at high light availability and $PSF_{survival}$ at low light availability, with relative importance of biomass and survival also mediated by mycorrhizal type. Together, these results provide a more mechanistic understanding of the factors underlying PSFs.

The negative relationship between $PSF_{biomass}$ at high light and $PSF_{survival}$ in low light closely aligns with a trade-off between high light growth and low light survival (Kobe et al., 1995). Shade intolerant species, which allocate more resources to growth and fewer to defense, should have better growth in high light and poor survival in low light. Shade intolerant species are also more susceptible to negative effects of soil-borne microbes on survival in low light availability (Chapter 2).

Implications for forest community dynamics and management

Although PSFs are often studied in the context of individual seedlings, the impacts of these PSFs can have broader impacts on forest community dynamics. A focus on tree seedling traits under different environmental conditions, especially in natural field conditions, offers both broader ecological understanding as well as potential applications for forest management. For example, selecting sites with soil and light conditions that promote higher production of defense

and recovery compounds could increase likelihood of seedling restoration success (e.g., *A. saccharum* in conspecific soil). Similarly, it may be beneficial to plant EMF-associating seedlings in soils cultured by other EMF-associating species, to increase potential for positive EMF colonization effects and limit potential negative AMF colonization effects. While environmental conditions could dilute trait effects on seedling survival, in the absence of extreme conditions (as supported by related greenhouse studies), a sharper focus on traits promoting survival, especially under low light conditions, can provide a more mechanistic understanding of forest regeneration dynamics.

Linking mycorrhizal type, defense and recovery traits, and environmental conditions like light availability to seedling survival and biomass may help us better understand the role of PSFs in forest communities (Baxendale et al., 2014; Bennett & Klironomos, 2018; P. Ke et al., 2015). This dissertation provides some first steps in disentangling these factors and helps develop a deeper understanding of seedling regeneration dynamics.

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APPENDIX

In Chapter 2, to analyze seedling survival, the hazard was estimated for each time step, h_t, from a gamma distribution with noninformative parameter values, $h_t \sim gamma(1,1)$. This intrinsic mortality rate reflects the temporal variability in mortality that is not accounted for by the risk function μ_{it}. The risk, μ_{it}, was estimated as a function of the covariates included in the analysis, $\mu_{it} = \boldsymbol{X}_{it}\boldsymbol{B}$. $\boldsymbol{X}_{it}$ is the matrix of covariates associated with each seedling i at each time t. $\boldsymbol{B}$ is the vector of fixed effect coefficients associated with each covariate. These coefficients were estimated from normal distributions with noninformative parameter values, $\boldsymbol{B} \sim normal(0,0.0001)$. For the final model, covariates included microbe treatment and light level; in initial models, we included greenhouse bench and adult tree as random effects, but their inclusion did not improve fit of the model.

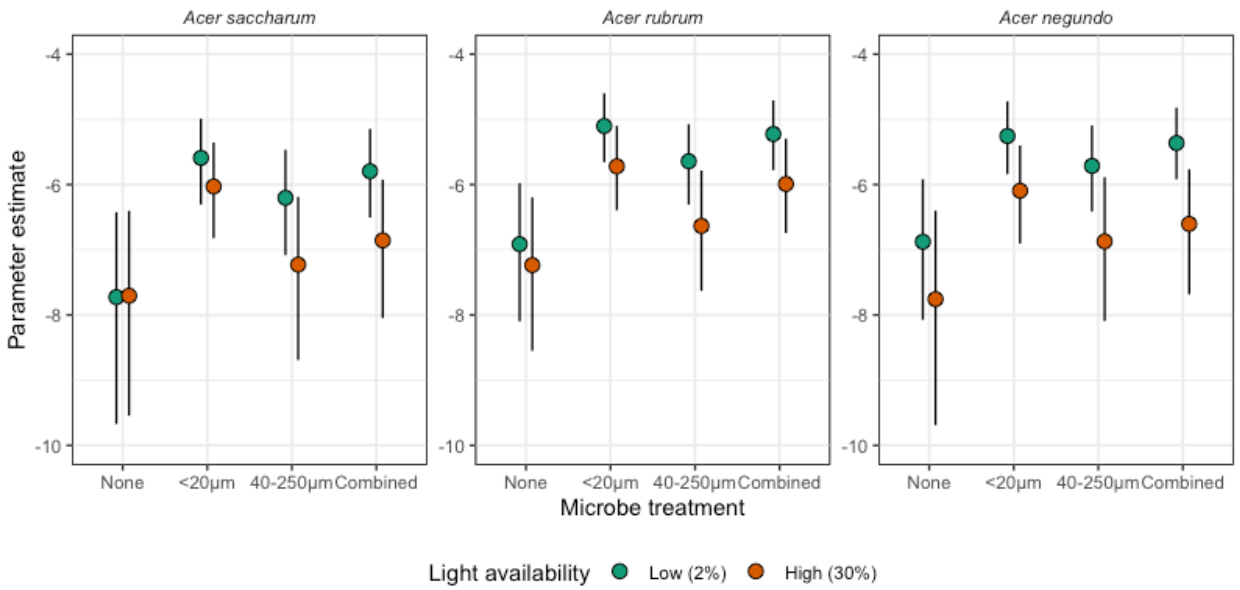


Figure A2.1 Parameter estimated (mean posterior values ± 95% credible intervals) for fixed-effect coefficients (light availability x microbe treatment; aka risk) for each species (*Acer saccharum*, *A. rubrum*, *A. negundo*).

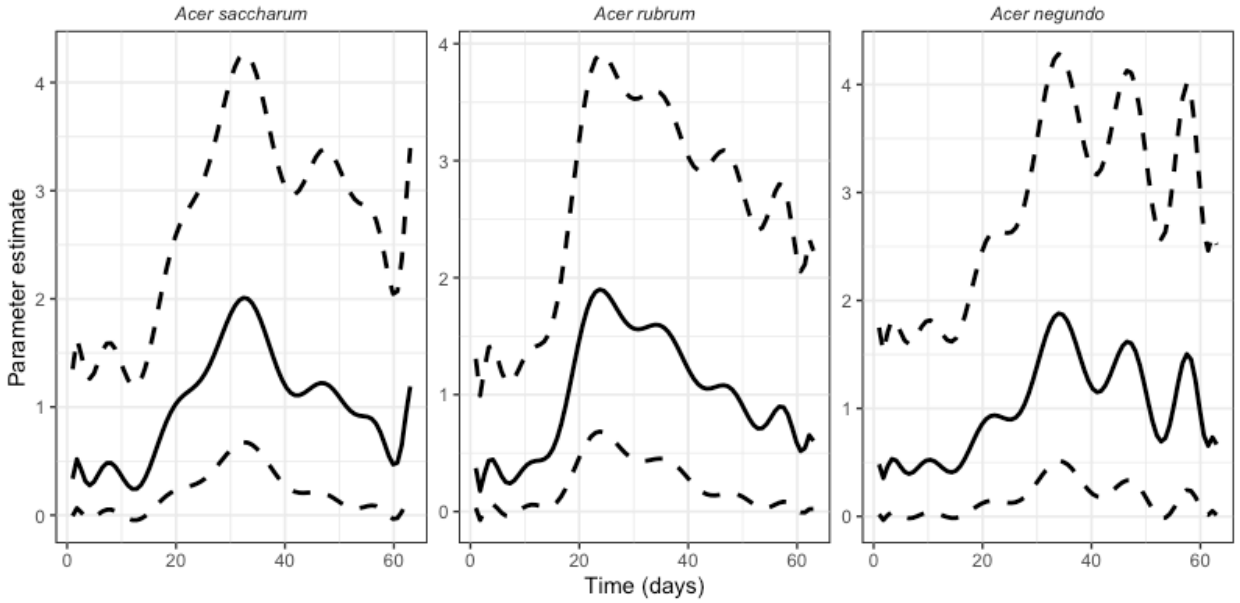


Figure A2.2 Hazard curves (mean ± 95% credible intervals) over the 9-week study period, for each species (*Acer saccharum*, *A. rubrum*, *A. negundo*).

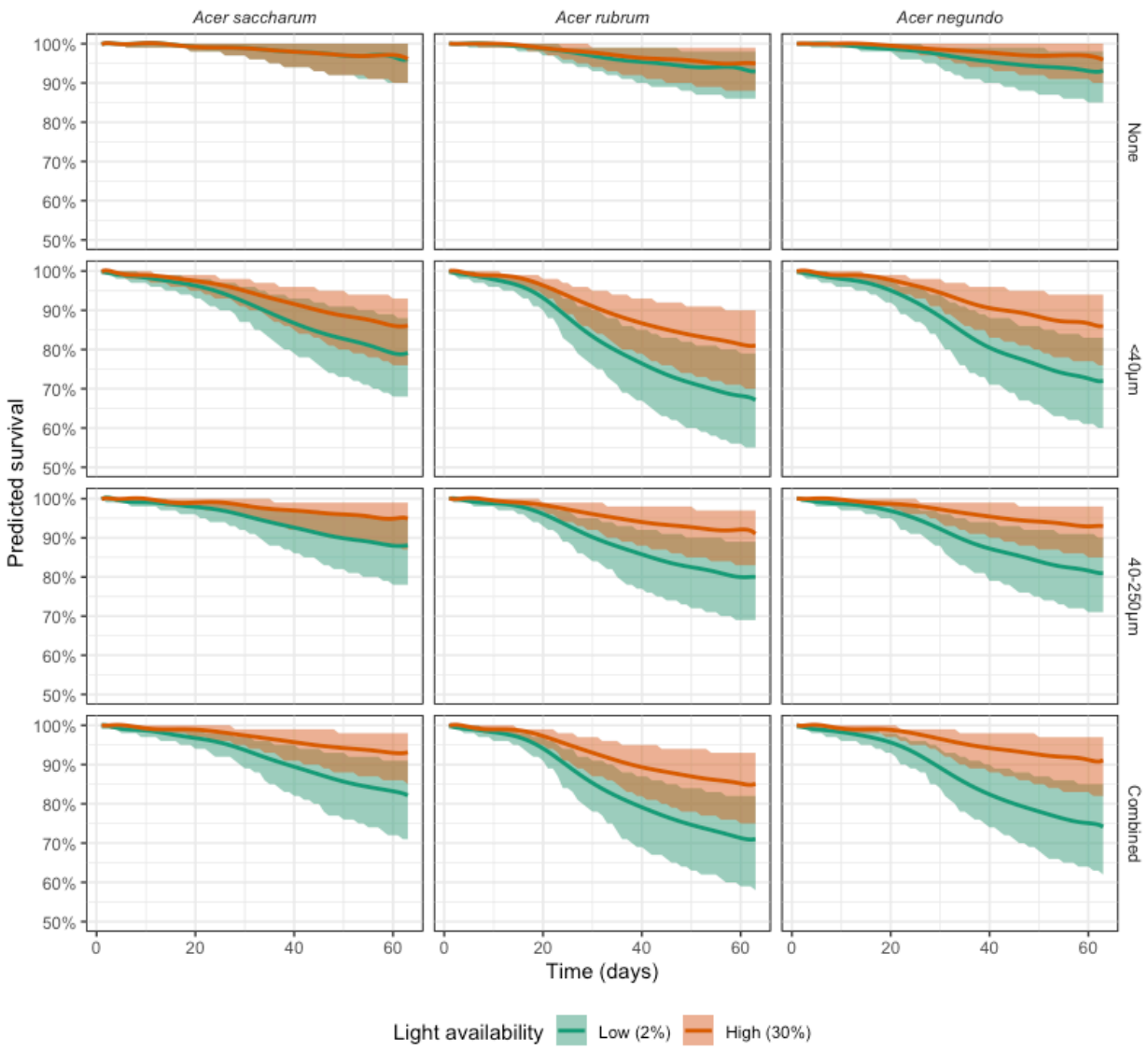


Figure A2.3 Predicted survival (mean ± 95 credible intervals for each species, light level, and microbial filtrate treatment, over the 9-week study period.

Table A2.1 Results of fixed effects linear regression model testing the effect of light availability and microbial filtrate size on seedling traits.

Species	Trait	Light	Microbe	Light x Microbe
ACSA	AMF	$\chi^2_{(1, 240)} = 0.00$, $p = 0.99$	$\chi^2_{(3, 240)} = 3305.84$, $p < 0.001$	$\chi^2_{(3, 240)} = 20.93$, $p < 0.001$
	Phenolics	$\chi^2_{(1, 240)} = 2.80$, $p = 0.09$	$\chi^2_{(3, 240)} = 53.78$, $p < 0.001$	$\chi^2_{(3, 240)} = 33.35$, $p < 0.001$
	Lignin	$\chi^2_{(1, 240)} = 4.24$, $p = 0.04$	$\chi^2_{(3, 240)} = 30.46$, $p < 0.001$	
	NSC	$\chi^2_{(1, 240)} = 14.43$, $p < 0.001$	$\chi^2_{(3, 240)} = 11.20$, $p = 0.01$	$\chi^2_{(3, 240)} = 12.97$, $p = 0.005$
ACRU	AMF		$\chi^2_{(3, 232)} = 2088.96$, $p < 0.001$	
	Phenolics	$\chi^2_{(1, 232)} = 74.21$, $p < 0.001$	$\chi^2_{(3, 232)} = 70.48$, $p < 0.001$	$\chi^2_{(3, 232)} = 20.71$, $p < 0.001$
	Lignin	$\chi^2_{(1, 232)} = 3.99$, $p = 0.046$	$\chi^2_{(3, 232)} = 13.43$, $p = 0.004$	$\chi^2_{(3, 232)} = 9.44$, $p = 0.02$
	NSC	$\chi^2_{(1, 232)} = 35.30$, $p < 0.001$	$\chi^2_{(3, 232)} = 88.27$, $p < 0.001$	$\chi^2_{(3, 232)} = 33.96$, $p < 0.001$
ACNE	AMF	$\chi^2_{(1, 240)} = 0.02$, $p = 0.90$	$\chi^2_{(3, 240)} = 4970.54$, $p < 0.001$	$\chi^2_{(3, 240)} = 68.59$, $p < 0.001$
	Phenolics	$\chi^2_{(1, 240)} = 40.91$, $p < 0.001$	$\chi^2_{(3, 240)} = 41.76$, $p < 0.001$	$\chi^2_{(3, 240)} = 14.50$, $p = 0.002$
	Lignin	$\chi^2_{(1, 240)} = 0.00$, $p = 1.00$	$\chi^2_{(3, 240)} = 24.01$, $p < 0.001$	$\chi^2_{(3, 240)} = 10.29$, $p = 0.02$
	NSC	$\chi^2_{(1, 240)} = 193.78$, $p < 0.001$	$\chi^2_{(3, 240)} = 39.85$, $p < 0.001$	

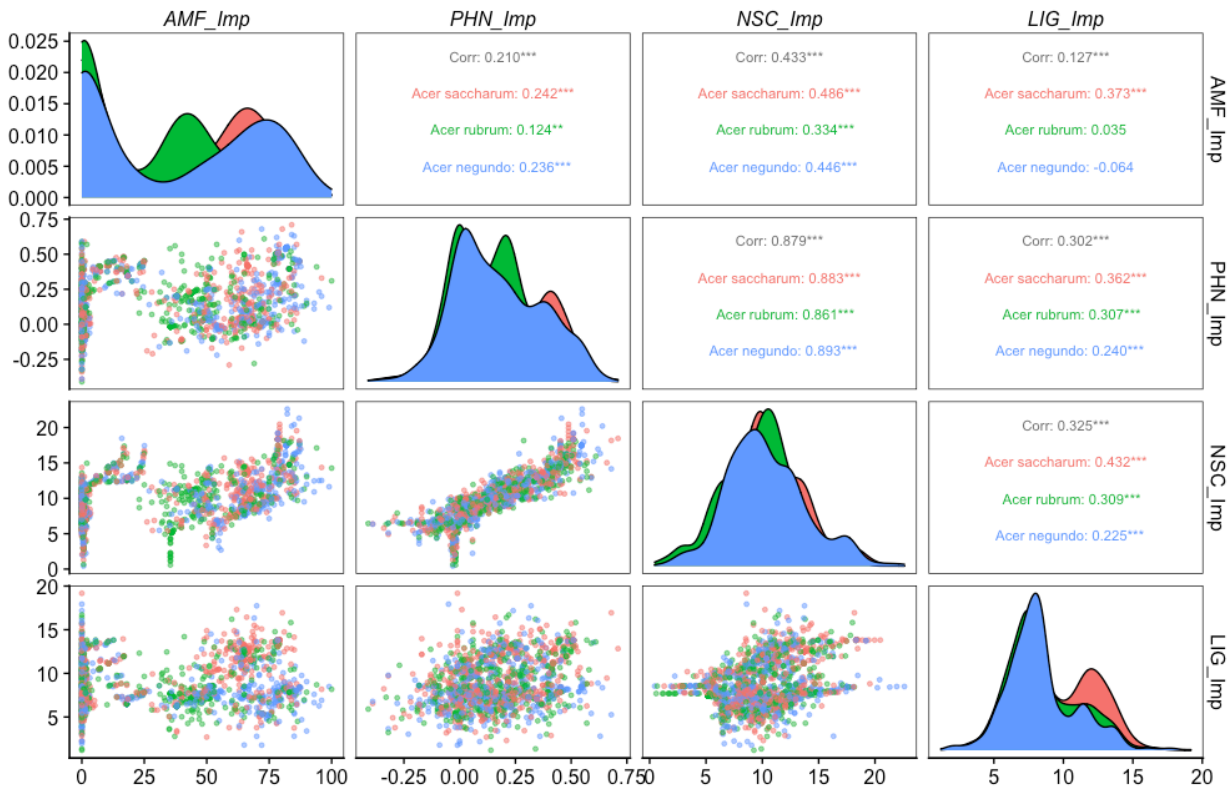


Figure A2.4 Correlation matrix for imputated seedling functional traits, pooled across species, light availability, and microbial filtrate treatments. Note that the "None" and "<40 µm" treatments were included for AMF correlations.

Table A2.2 Summary table demonstrating **A)** tree seedling survival in low versus high light in response to soil-borne microbes, **B)** functional trait responses in high versus low light in response to soil-borne microbes, and **C)** tree seedling survival responses across all light levels in response to functional traits. Arrows indicate the direction of the effect: ↑ increasing and ↓ decreasing.

A) Tree seedling survival in low versus high light in response to soil-borne microbes					
	Tolerant		Intermediate		Intolerant
Sterile	-		-		-
Mycorrhizae	-		↓		↓
Pathogens	-		↓		↓
Both[1]	↓		↓		↓
B) Functional trait responses in high versus low light in response to soil-borne microbes					
Shade Tolerant (*Acer saccharum*)					
	Overall	Sterilized	40-250µm	<20µm	Combined[1]
AMF	↑		↑		↑
Phenolics	↑	-	↑	-	↑
Lignin	↑	-	-	-	-
NSC	↑	↑	↑	↑	↑
Intermediate (*Acer rubrum*)					
	Overall	Sterilized	40-250µm	<20µm	Combined[1]
AMF	-		-		-
Phenolics	↑	↑	↑	-	-
Lignin	-	-	-	-	-
NSC	↑	↑	↑	↑	↑
Shade Intolerant (*Acer negundo*)					
	Overall	Sterilized	40-250µm	<20µm	Combined[1]
AMF	↑		↑		↑
Phenolics	↑	↑	↑	-	↑
Lignin	-	-	↑	-	-
NSC	↑	↑	↑	↑	↑

C) Tree seedling survival across all light levels in response to functional traits				
	Overall	Tolerant	Intermediate	Intolerant
AMF	-	-	-	↑
Phenolics	↑	↑	↑	↑
Lignin	-	↑	-	-
NSC[2]	↑	↑	↑	↑

In Chapter 3, we collected intact soil cores during the summers of 2016 and 2017. Before transplanting the soil cores into the common garden field plots, we stored the cores inside the research field station. Cores were stored after mesh had been glued to the bottom and 2 open sides of the enclosing pots. We covered the open top of the pot with a plastic lid specifically designed to fit our pots. This ensured that there was no potential for contamination of the pots before moving them back to the field.

Table A3.1 Percent of total soil cores collected in 2016 and 2017. For each seedling species and soil source. We collected intact soil cores during the summers of 2016 and 2017. Before transplanting the soil cores into the common garden field plots, we stored the cores inside the research field station. Cores were stored after mesh had been glued to the bottom and 2 open sides of the enclosing pots. We covered the open top of the pot with a plastic lid specifically designed to fit our pots. This ensured that there was no potential for contamination of the pots before moving them back to the field.

Soil source /	Acsa		Pogr		Prse		Qual		Quru	
collection year	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Acru	35	65	33	67	32	68	18	82	18	82
Acsa	62	38	57	43	54	46	39	61	38	62
Pogr	73	27	72	28	75	25	75	25	77	23
Prse	15	85	12	88	13	87	12	88	6	94
Qual	43	57	39	61	43	57	42	58	39	61
Quru	23	77	25	75	17	83	24	76	18	72
Con. St.	53	47	76	24	10	90	18	82	13	87

We were concerned that storing soil cores for an extended period of time would have potential negative effects on the microbial community. Specifically, we worried that the soil microbial community would be adversely affected. Preliminary analyses did not indicate any significant effect of soil collection year on seedling trait expression (AMF or EMF colonization, phenolics, lignin, NSC) or survival.

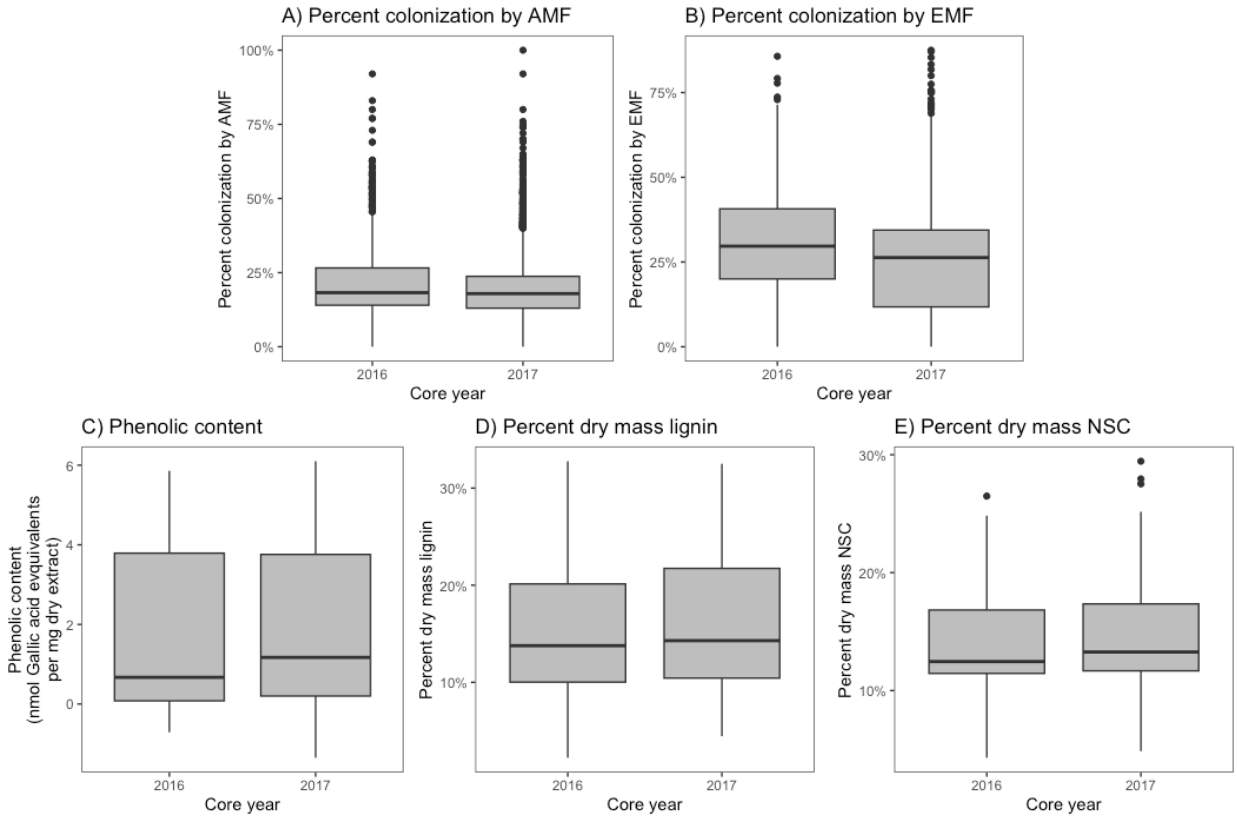


Figure A3.1 Prelimninary boxplots showing the effect of soil core year (2016, 2017) on tree seedling traits: **A)** AMF colonization, **B)** EMF colonization, **C)** Phenolics, **D)** Lignin, **E)** NSC. We were concerned that storing soil cores for an extended period of time would have potential negative effects on the microbial community. Specifically, we worried that the soil microbial community would be adversely affected. Preliminary analyses did not indicate any significant effect of soil collection year on seedling trait expression (AMF or EMF colonization, phenolics, lignin, NSC) or survival.

Nutrient availability

We tested if there were differences in soil nutrient supply rates (e.g., NH_4^+, NO_3^-, PO_4^{3-}, K^+, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, Cu^+, Zn^+, B^{3+}, Mn^{2+}, Pb^{4+}, and Cd^{2+}) by soil source treatments (between undisturbed soil under adult trees where soil was collected and in the fungal exclusion pots) and sterilization treatment (non-sterilized vs. sterilized conspecific soil in fungal exclusion pots) with plant root simulator (PRS™) probes (Western Ag Innovations Inc., Saskatoon, SK) at 0-7 cm depth. Four replicate PRS probes were installed under the adult trees we collected soil from (6 adults trees for *Acer saccharum* and *Quercus rubra* and 3 adult trees for *Acer rubrum*, *Populus granditentata*, *Prunus serotina* and *Querucs alba*). In addition, PRS probes were installed in 240 fungal exclusion pots planted with *A. saccharum* seedlings [(6 non-sterilized soil sources x adult trees (6 for *Acer saccharum* and *Quercus rubra* and 3 for the other 4 tree species) x 2 light treatments (low and high light field plots) x 4 seedling replicates) + (*Acer saccharum* sterilized soil x 6 adult trees x 2 light treatments x 4 seedlings replicates)]. PRS probes were installed 3-wks after planting and harvested 3-wks later.

"PRS probes are ion exchange resin membranes held in plastic supports that are easily inserted into soil to measure ion supply *in situ* with minimal disturbance. Anion probes have a positively-charged membrane to simultaneously attract and adsorb all negatively-charged anions... Cation probes have a negatively-charged membrane to simultaneously attract and adsorb all positive-charged cations… Prior to use, ion exchange membranes are saturated with a counter-ion that is easily desorbed, allowing ready absorption of soil ions. Anion probes are saturated with HCO_3^- and cation probes are saturated with Na^+. When buried, soil ions displace the counter-ions at a rate that depends on their activity and diffusion rate in soil solution. The quantity of soil ions adsorbed during a burial period is a function of all soil properties (physical,

chemical, and biological) controlling nutrient availability in soil."

(https://www.westernag.ca/innovations/technology/basics)

Due to systemic error in lab processing, sample sizes for some of the treatments were greatly reduced. Subsequent t-tests were conducted with pooled datasets at the nutrient or species level, rather than paired t-tests at the adult tree level.

Table A3.2 Results of t-tests comparing nutrient supply rate (micrograms / $10cm^2$ / burial length) in seedling pots versus beneath adult trees. Bolded values are significant at $P < 0.0036$, Bonferroni-corrected to $\alpha = 0.0036$, for original $\alpha = 0.05$ and n = 14 tested nutrients.

Nutrient	df	Supply rate in pots	Supply rate beneath adult trees	t	p
Al^{+}	32	15.5	14.7	0.463	0.647
B^{3+}	18	0.61	0.68	-0.454	0.655
Ca^{2+}	32	1849	2142	-1.662	0.106
Cu^{3+}	13	0.57	0.51	0.439	0.668
Fe^{2+}	24	33.0	17.1	2.509	0.019
K^{+}	19	170	267	-1.838	0.081
Mg^{2+}	28	346	371	-0.660	0.515
Mn^{2+}	29	17.7	14.5	0.797	0.432
NH_4^{+}	12	4.1	5.9	-0.869	0.402
NO_3^{-}	23	199	50	4.280	< 0.001
P^{3-}	21	4.9	8.2	-3.089	0.006
Pb^{2+}	32	2.6	2.1	0.914	0.368
S^{2+}	20	65	26	4.998	< 0.001
Zn^{2+}	24	13.7	5.1	5.033	< 0.001

Soil sterilization had some impacts on nutrient availability (Table A3.3). Sterilized soils had higher amounts of Mn^{2+}, NO_3^-, and P^{3-}, which could have potentially contributed to increased survival or biomass that would confound the effects of soil-borne microbes. However, we found limited effects of soil source, regardless of sterilization, on seedling survival and biomass, indicating that other factors (i.e., interactions between pot design and heavy rainfall events) had a larger effect than nutrient availability or soil-borne microbes.

Table A3.3 Results of t-tests comparing nutrient supply rates (micrograms / 10cm^2 / burial length) in sterilized versus live soil, in seedling pots. Alpha was Bonferroni-corrected to α = 0.0038, for original α = 0.05 and n = 13 tested nutrients. P-values marked with * are marginally significant at original α = 0.05.

Nutrient	df	Supply rate in sterilized soil	Supply rate in live soil	t	p	
Al^+	9	17	15	-1.117	0.294	
B^{3+}						
Ca^{2+}	4	1733	1844	0.478	0.656	
Cu^{3+}	5	0.43	0.53	0.885	0.420	
Fe^{2+}	9	31	32	0.174	0.866	
K^+	16	137	189	2.213	0.050	
Mg^{2+}	3	360	342	-0.368	0.736	
Mn^{2+}	3	40	13	-5.647	0.008	*
NH_4^+	2	3.0	4.4	1.24	0.370	
NO_3^-	3	381	155	-4.083	0.024	*
P^{3-}	5	6.7	4.4	-0.357	0.018	*
Pb^{2+}	10	2.5	2.5	0.071	0.945	
S^{2+}	6	44	69	2.054	0.089	
Zn^{2+}	2	21	12	-1.409	0.287	

Table A3.4 Full dataset results of t-tests comparing nutrient supply rates (micrograms / 10cm^2 / burial length) in sterilized versus live soil, in seedling pots. For many nutrients, there were not enough replicate pots for a t-test or sample size was small, so we also provide the results of a t-test using the full dataset.

Df	Supply rate in sterilized soil	Supply rate in non-sterilized soil	t	p
56	213	200	-0.160	0.874

We were concerned that the design of the seedling pots might affect soil nutrient availability. However, we found that soils in seedling pots had similar nutrient availability to undisturbed soils beneath adult trees.

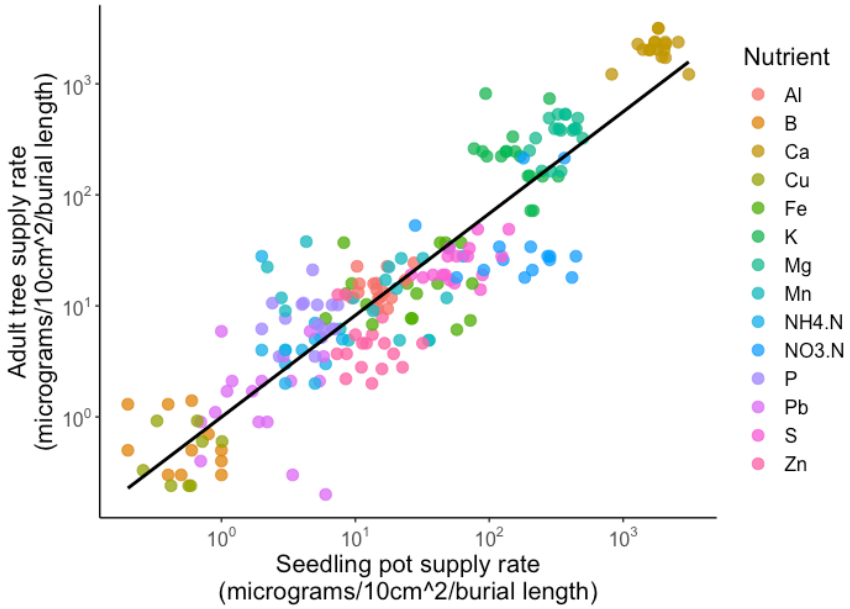


Figure A3.2 Nutrient supply rate (micrograms / 10cm^2 / burial length) in seedling pots versus beneath adult trees (Adj.-R^2 = 0.92, P = 0.03).

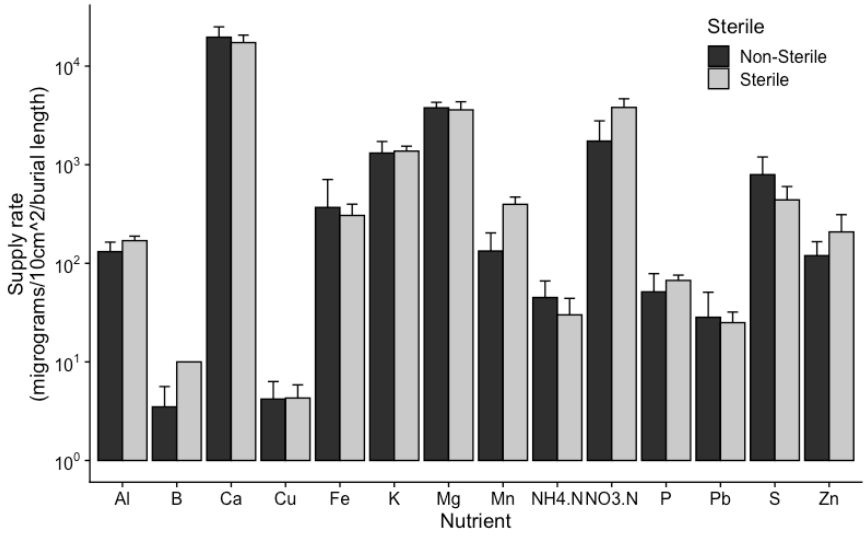


Figure A3.3 Nutrient supply rates (micrograms / 10cm^2 / burial length) in sterilized versus live soil, in seedling pots.

	Adult trees			Seedling pots		
Nutrient	df	F	p	df	F	p
Al^{+}	5	1.128	0.383			
B^{3+}	5	2.167	0.113			
Ca^{2+}	5	5.678	0.003			
Cu^{3+}	4	7.812	0.010			
Fe^{2+}	5	1.8	0.167			
K^{+}	5	2.289	0.046			
Mg^{2+}	5	9.559	< 0.001			
Mn^{2+}	5	0.781	0.577			
NH_4^{+}	4	1.487	0.263			
NO_3^{-}	5	0.647	0.667	5	0.458	0.8
P^{3-}	5	1.152	0.372			
Pb^{2+}	5	3.386	0.028			
S^{2+}	5	1.1045	0.424	5	0.806	0.571
Zn^{2+}	5	1.804	0.166	5	0.629	0.682

Table A3.5 Results of ANOVAs comparing nutrient supply rates (micrograms / $10cm^2$ / burial length) in each soil source (Acru, Acsa, Prse, Pogr, Qual, Quru) in soil beneath adult trees. Adult trees were used as a proxy for seedling pots, since preliminary analyses showed that, for most nutrients, there were no significant differences between nutrient supply rates for soil in seedling pots versus beneath adult trees. For NO_3^{-}, S^{2+}, and Zn^{2+}, there were significant differences in nutrient supply rate for soil in seedling pots versus adult trees, so we also provide results of ANOVAs for seedling pots. Bolded values are significant at $\alpha = 0.0036$, Bonferroni-corrected for original $\alpha = 0.05$ and n = 14 tested nutrients.

There were few differences in nutrient availability between soil sources (Figure A3.4). Ca^{2+} was highest in *Q. alba* soils and Mg^{2+} was higher in *A. saccharum*, then *P. grandidentata* and *P. serotina* soils.

Figure A3.4 Nutrient supply rates (micrograms / $10cm^2$ / burial length) in each soil source (Acru, Acsa, Prse, Pogr, Qual, Quru) in soil beneath adult trees. For **A)** Ca^{2+} and **B)** Mg^{2+}.

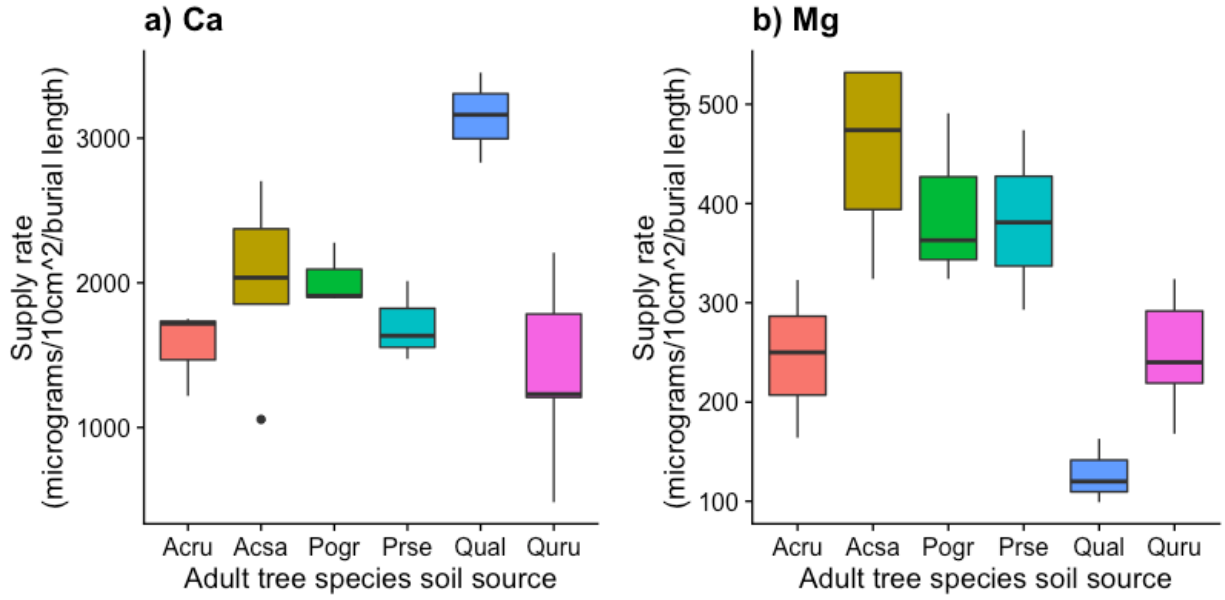
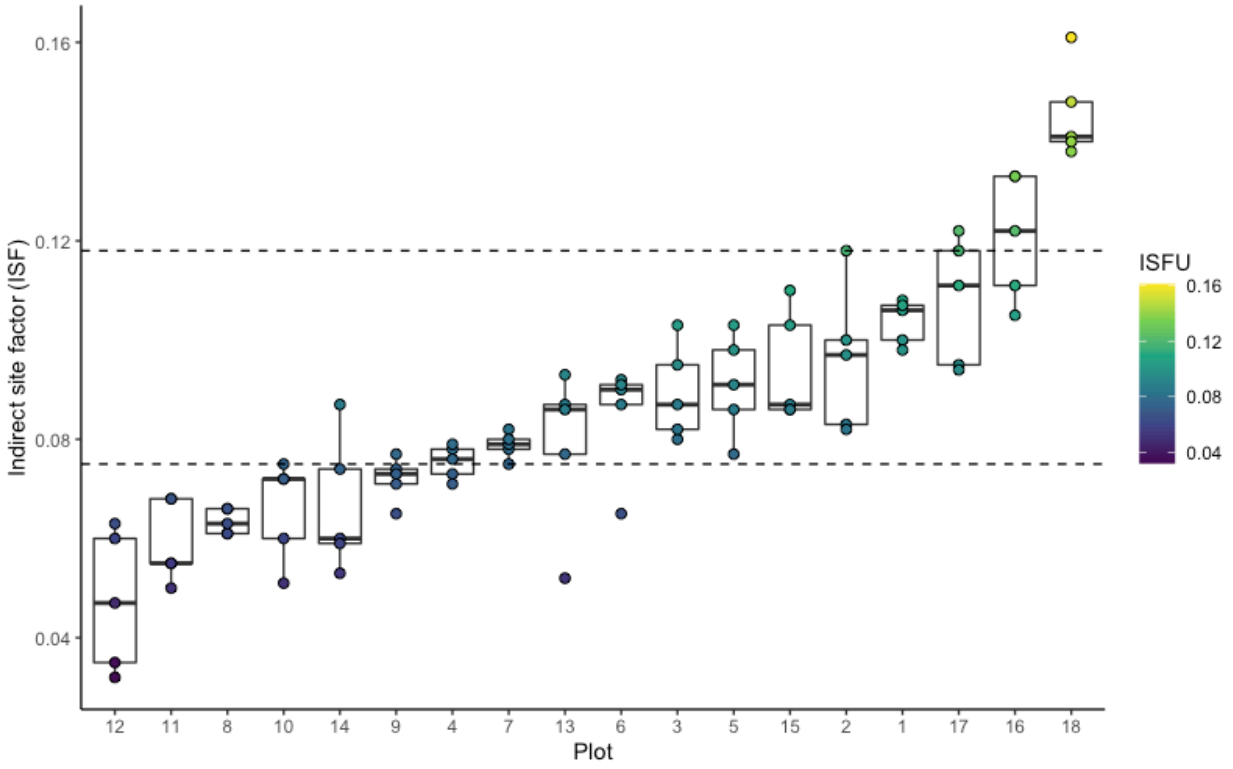


Figure A3.5 Light availability in the 18 experimental field plots. Indirect site factor (ISF, the proportion of diffuse solar radiation at a given location, relative to the amount of diffuse solar radiation in the open) in each subplot (n = 5) per common garden plot (n = 18). For analyses in which light availability was included as a categorical variable, low = 0.032-0.075 ISF, medium = 0.075-0.118 ISF, and high = 0.118 – 0.161 ISF.



Percent colonization by mycorrhizal fungi

To quantify mycorrhizal colonization, prior to drying seedlings, 5-10 root fractions per individual (1cm sections of wet root), were retained, weighed, and stained with 5% Schaeffer black in in vinegar solution (Vierheilig et al., 1998). Percent root colonization by AMF was quantified by inspecting 100 intersections between the microscope eyepiece crosshairs and roots for AMF structures (i.e., vesicles, arbuscules, coils, and hyphae) every 1mm at 200x magnification (McGonigle et al., 1990). AMF fungal structures were distinguished from other fungi that can inhabit the root interior (e.g., dark septate fungi) by comparing slides to established reference images. Percent root colonization by EMF was quantified by counting the number of intact root tips with and without Hartig nets at 100x magnification every 2mm along the root until 100 root tips were scored.

Phenolics

To quantify phenolics, we collected hypocotyl samples, cut into <1mm pieces. We extracted phenolics in 5mL methanol in the dark for 16 hours at room temperature. The methanol extracts were filtered and adjusted to 5mL, and then we quantified total phenolics using a microplate-adapted colorimetric total phenolics assay with Folin-Ciocalteu reagent (Ainsworth & Gillespie, 2007; P. Waterman & Mole, 1994).

Lignin

To quantify lignin, root and stem samples were lyophilized and coarsely ground at 1mm using a Wiley Mill. We ran 0.5g root and stem samples through a series of extractions using an ANKOM Fiber Analyzer (ANKOM Technologies, Macedon, NY, USA). We used a Neutral Detergent Fiber extraction to wash off soluble cell contents (e.g., carbohydrates, lipids, pectin, starch, and soluble proteins). We then used an Acid Detergent Fiber extraction with 1.00 normal sulfuric acid to wash off hemicellulose and bound proteins and an Acid Detergent Lignin extraction with 72% sulfuric acid to wash off cellulose, leaving only lignin and recalcitrant materials. Finally, we ashed the samples to quantify dry mass lignin.

Nonstructural carbohydrates

To quantify nonstructural carbohydrates (NSC), we analyzed stem samples, using a standardized enzyme method for sugar and starch extraction and quantification (Landhäusser, Chow, Turin Dickman, et al., 2018; Quentin et al., 2015). We dried seedling stems and peach leaf standard reference material (MillporeSigma-NIST1547) at 60□C overnight to remove moisture. We then weighed out 30mg of each for analysis and separated sugars and starches with hot ethanol extraction. We used α-amylase and amyloglucosidase to convert starch to glucose. We quantified sugars using phenol-sulfuric acid colorimetric assay and starches using a glucose-hexokinase colorimetric assay (MillporeSigma-GAK20). We calculated total NSC concentrations as the sum of soluble sugar and starch concentrations derived from the assays.

Table A3.6 Linear model evaluating the effects of sterilized versus live soil on traits. AMF colonization, EMF colonization, phenolics, lignin, and NSC. For post-hoc comparisons within species, we used joint tests of estimated marginal means.

		A. saccharum		*P. serotina*		*Q. alba*		*Q. rubra*	
Response Parameters	df	F	p	F	p	F	p	F	p
AMF col.									
Light	1, 793	6.366	0.012	4.698	0.031	0.822	0.365	4.109	0.043
Soil	1, 793	98.783	<.001	275.163	<.001	34.349	<.001	54.45	<.001
Light x Soil	1, 793	0.078	0.780	0.317	0.573	6.242	0.0127	1.069	0.302
EMF col.									
Light	1, 375	-	-	-	-	13.670	<.001	51.792	<.001
Soil	1, 375	-	-	-	-	270.962	<.001	313.466	<.001
Light x Soil	1, 375	-	-	-	-	3.742	0.0538	35.308	<.001
Phenolics									
Light	1, 793	123.267	<.001	0.064	0.780	573.880	<.001	649.5881	<.001
Soil	1, 793	52.762	<.001	64.289	<.001	1394.556	<.001	10.389	0.001
Light x Soil	1, 793	0.010	0.919	21.506	<.001	86.486	<.001	28.589	<.001
Lignin									
Light	1,793	77.208	<.001	180.293	<.001	0.959	0.328	75.411	<.001
Soil	1,793	35.188	<.001	19.296	<.001	89.096	<.001	5.171	0.023
Light x Soil	1,793	0.011	0.917	1.908	0.168	1.231	0.268	0.000	0.995
NSC									
Light	1, 793	15.844	<.001	207.577	<.001	14.497	<.001	206.174	<.001
Soil	1, 793	0.942	0.332	0.998	0.318	21.545	<.001	0.613	0.434
Light x Soil	1, 793	0.024	0.876	9.096	0.003	4.221	0.040	0.414	0.520

Bonferroni correction for species: alpha level is set to 0.0125. Bolded values are statistically significant at $p < 0.0125$. Underlined values are marginally significant at $p < 0.05$.

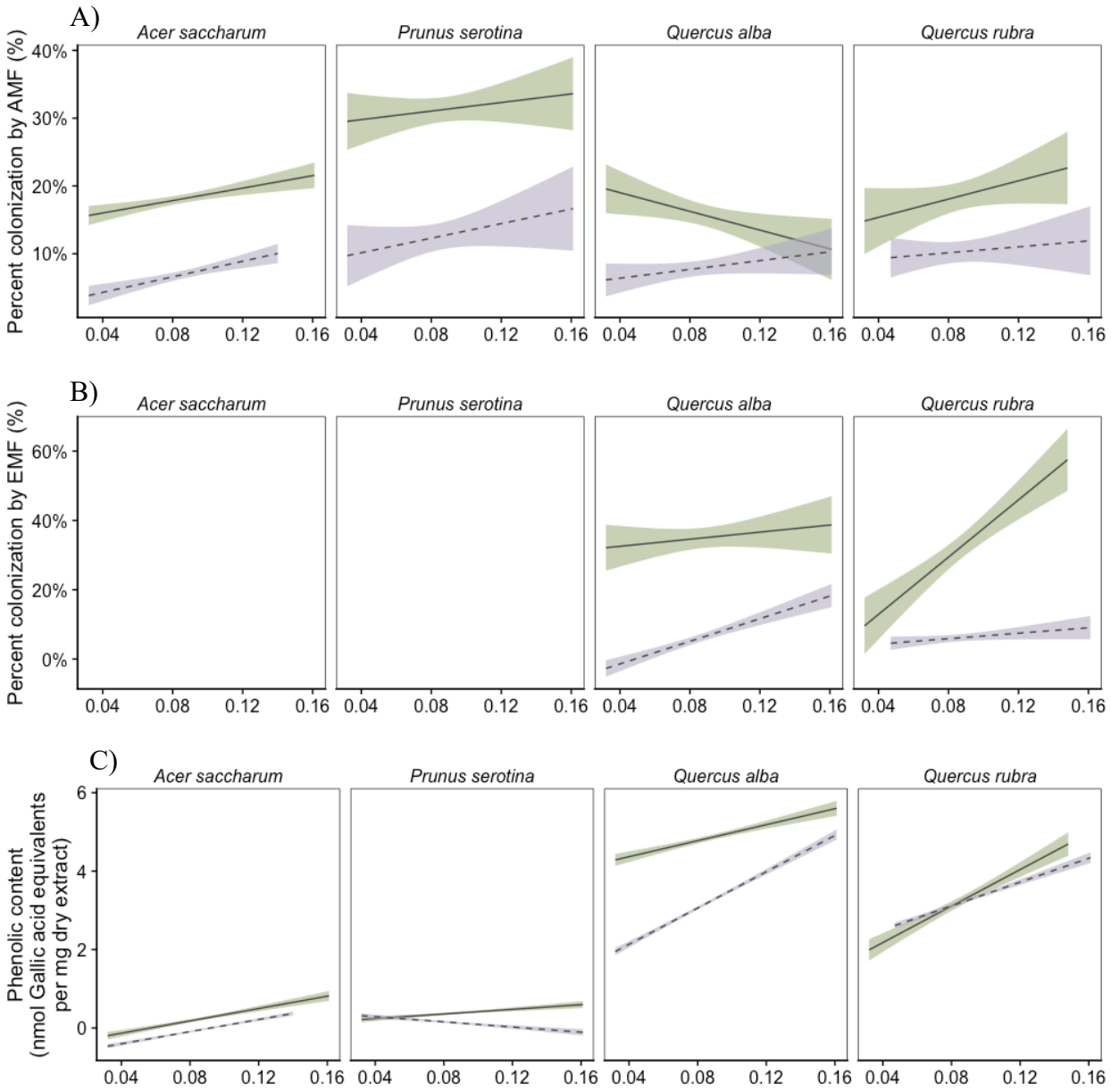


Figure A3.6 Effect of sterilized versus live soil on seedling traits. **A)** AMF colonization, **B)** EMF colonization, **C)** phenolics, **D)** lignin, **E)** NSC.

Figure A3.6 (Cont'd)

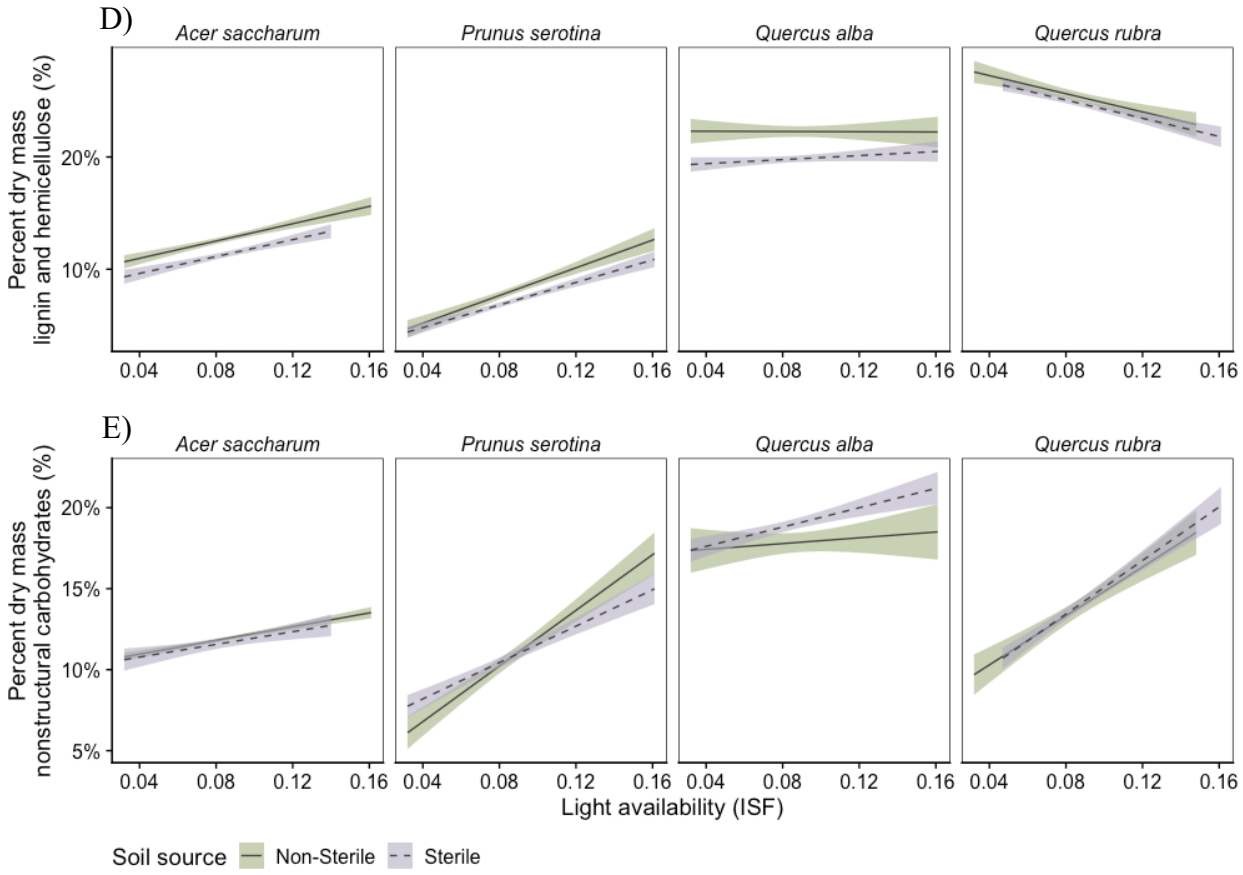


Table A3.7 Linear model evaluating the effects of light availability and soil source (conspecific versus pooled heterospecific) on traits (AMF colonization, EMF colonization, phenolics, lignin, and NSC). Sterilized soil was excluded from the model. For post-hoc comparisons within species, we used joint tests of estimated marginal means.

		A. saccharum		*P. serotina*		*Q. alba*		*Q. rubra*	
Response Parameters	df	F	p	F	p	F	p	F	p
AMF col.									
Light	1, 2344	2.421	0.1199	35.056	<.001	6.083	0.014	6.420	0.011
Soil	1, 2344	4.205	0.040	27.420	<.001	7.012	0.008	2.710	0.100
Light x Soil	1, 2344	0.889	0.346	17.640	<.001	1.002	0.317	0.471	0.492
EMF col.									
Light	1, 1063	-	-	-	-	1.313	0.252	63.024	<.001
Soil	1, 1063	-	-	-	-	7.639	0.006	8.296	0.004
Light x Soil	1, 1063	-	-	-	-	0.216	0.642	19.968	<.001
Phenolics									
Light	1, 2344	64.695	<.001	34.475	<.001	187.073	<.001	249.422	<.001
Soil	1, 2344	118.383	<.001	51.414	<.001	16.687	<.001	0.001	0.976
Light x Soil	1, 2344	9.353	0.002	2.852	0.091	0.162	0.688	170.690	<.001
Lignin									
Light	1, 2344	82.338	<.001	184.507	<.001	0.482	0.488	21.421	<.001
Soil	1, 2344	0.089	0.765	0.338	0.561	70.875	<.001	14.161	<.001
Light x Soil	1, 2344	0.001	0.975	1.041	0.308	0.328	0.567	14.176	<.001
NSC									
Light	1, 2344	22.048	<.001	287.753	<.001	16.388	<.001	87.418	<.001
Soil	1, 2344	0.128	0.721	0.018	0.893	106.576	<.001	19.305	<.001
Light x Soil	1, 2344	0.012	0.914	6.346	0.012	4.785	0.029	32.411	<.001

Bonferroni correction for species: alpha level is set to 0.0125. Bolded values are statistically significant at $p < 0.0125$. Underlined values are marginally significant at $p < 0.05$.

Table A3.8 Linear model evaluating the effects of light availability and soil source on traits (AMF colonization, EMF colonization, phenolics, lignin, and NSC). Sterilized soil was excluded from the model. For post-hoc comparisons within species, we used joint tests of estimated marginal means.

		A. saccharum		P. serotina		Q. alba		Q. rubra	
Response Parameters	df	F	p	F	p	F	p	F	p
AMF col.									
Light	1, 2312	1.733	0.188	158.201	<.001	8.746	0.003	9.374	0.002
Soil	5, 2312	12.391	<.001	68.831	<.001	16.170	<.001	14.373	<.001
Light x Soil	5, 2312	0.399	0.850	5.388	<.001	4.416	<.001	0.620	0.684
EMF col.									
Light	1, 1047	-	-	-	-	1.320	0.251	48.610	<.001
Soil	5, 1047	-	-	-	-	10.023	<.001	4.076	0.001
Light x Soil	5, 1047	-	-	-	-	1.614	0.154	5.746	<.001
Phenolics									
Light	1, 2312	146.587	<.001	196.841	<.001	830.835	<.001	261.664	<.001
Soil	5, 2312	195.305	<.001	63.129	<.001	274.609	<.001	135.613	<.001
Light x Soil	5, 2312	6.964	<.001	1.518	0.181	2.476	0.030	92.102	<.001
Lignin									
Light	1, 2312	156.314	<.001	313.426	<.001	2.374	0.124	11.809	<.001
Soil	5, 2312	4.082	0.001	4.667	<.001	30.003	<.001	36.578	<.001
Light x Soil	5, 2312	1.404	0.220	4.644	<.001	2.620	0.023	6.542	<.001
NSC									
Light	1, 2312	42.638	<.001	451.673	<.001	62.774	<.001	79.157	<.001
Soil	5, 2312	1.557	0.169	8.146	<.001	52.997	<.001	35.680	<.001
Light x Soil	5, 2312	0.527	0.756	10.567	<.001	6.235	<.001	13.492	<.001

Bonferroni correction for species: alpha level is set to 0.0125. Bolded values are statistically significant at $p < 0.0125$. Underlined values are marginally significant at $p < 0.05$.

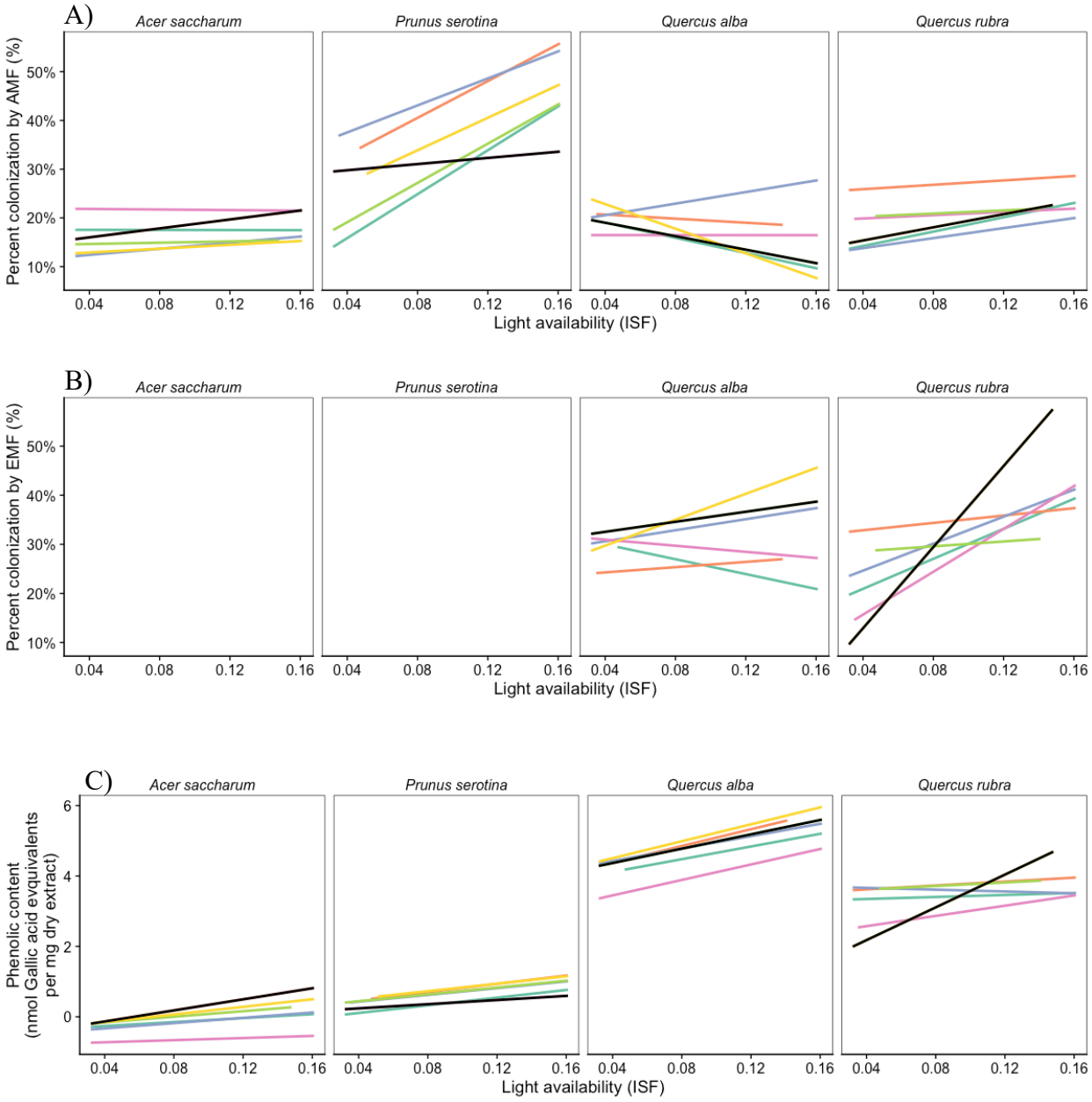
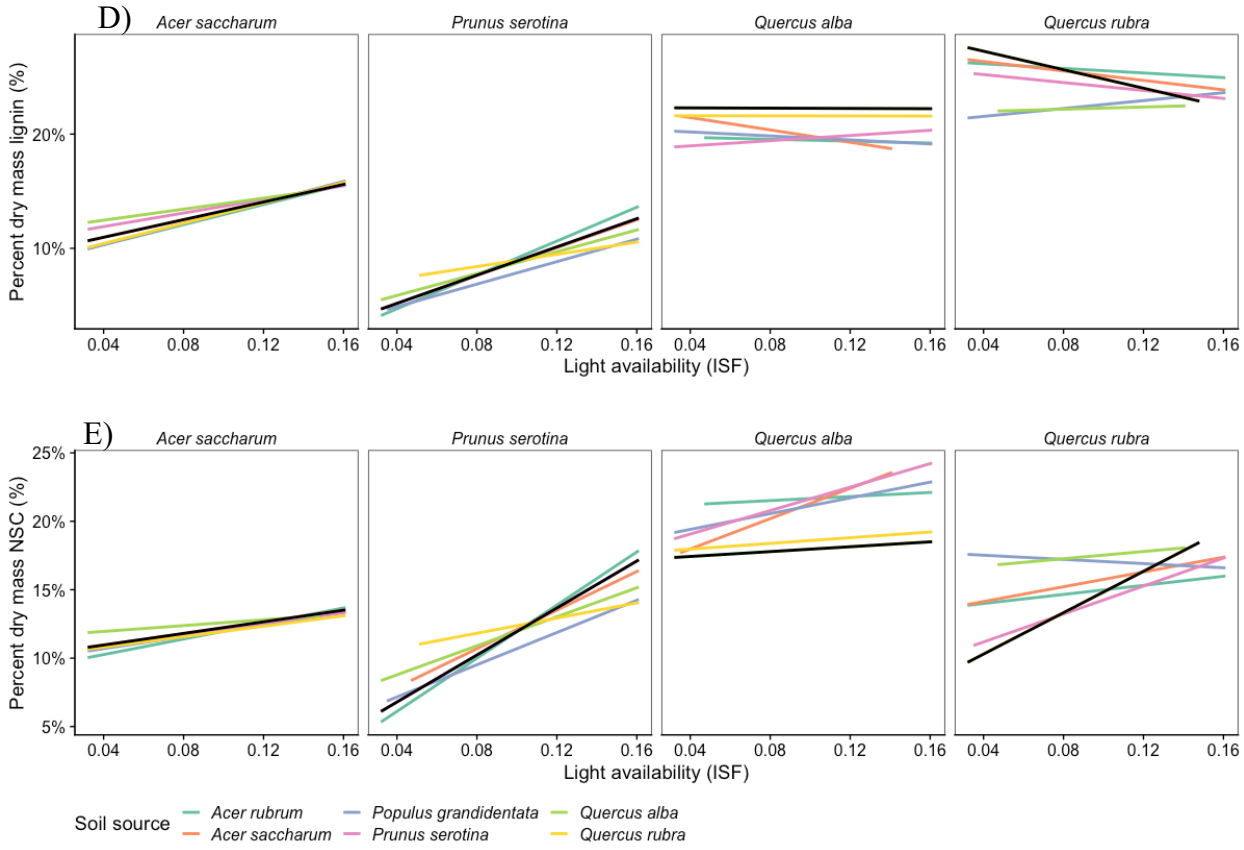


Figure A3.7 Effects of light availability and soil source on seedling traits. **A)** AMF colonization, **B)** EMF colonization, **C)** phenolics, **D)** lignin, and **E)** NSC.

Figure A3.7 (Cont'd)



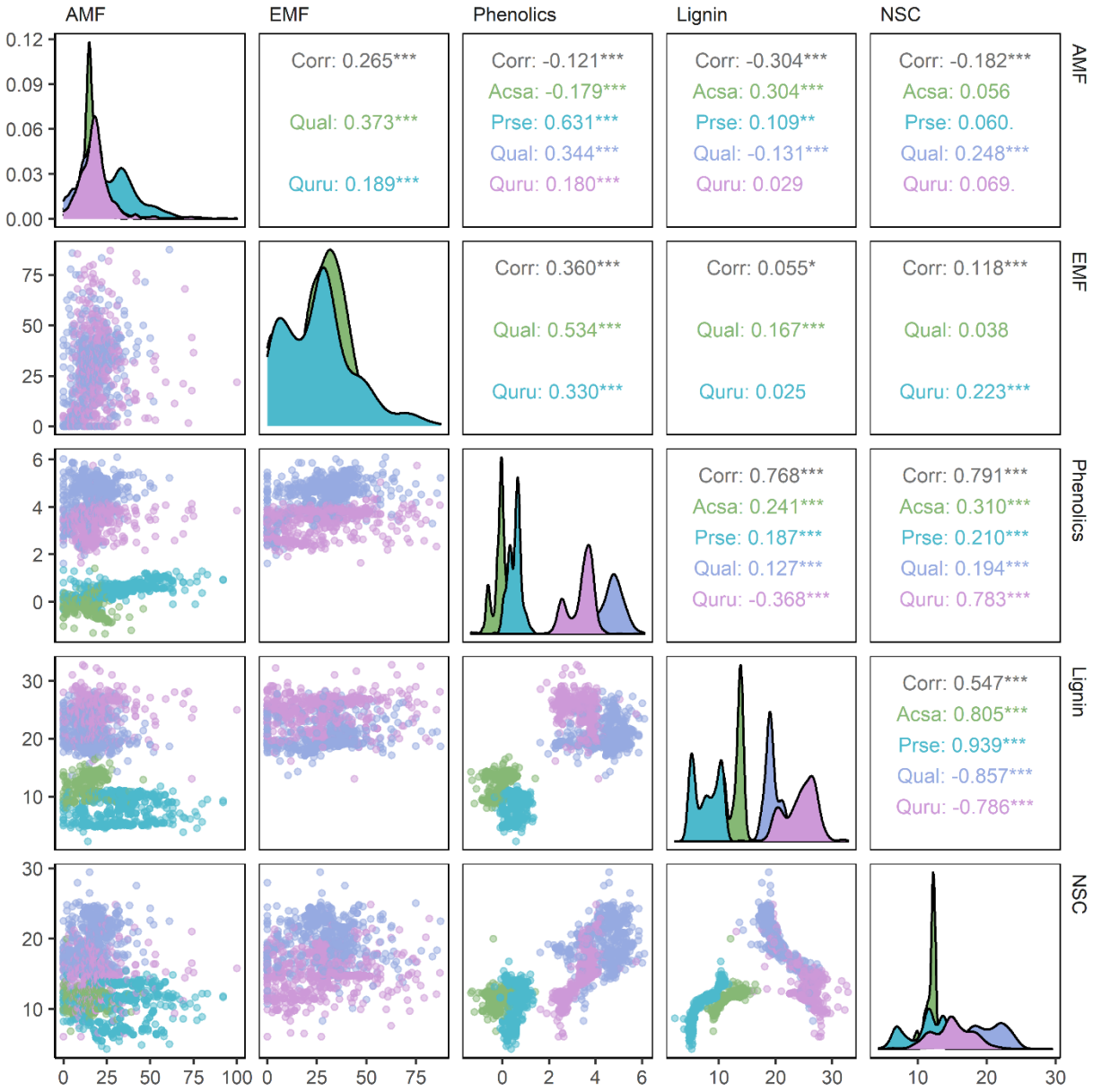


Figure A3.8 Correlations between AMF colonization, EMF colonization (for *Q. alba* and *Q. rubra*), phenolics, lignin, and non-structural carbohydrates.

Table A3.9 Cox proportional hazards survival models evaluating the effects of light availability and soil source (sterilized versus live conspecific) on seedling survival. Individual models were performed for each species.

Response Parameters	df	*A. saccharum*		*P. serotina*		*Q. alba*		*Q. rubra*	
		LRχ^2	p	LRχ^2	p	LRχ^2	p	LRχ^2	p
Light	1	0.097	0.755	1.630	0.202	3.236	0.072	6.254	0.012
Soil source	1	61.781	<.001	1.521	0.001	0.959	0.327	0.020	0.920
Light × Soil	1	0.055	0.814	2.82	0.093	0.256	0.613	0.560	0.454

Bonferroni correction for species: alpha level is set to 0.0125. Bolded values are statistically significant at $p < 0.0125$. Underlined values are marginally significant at $p < 0.05$.

Table A3.10 Cox proportional hazards survival models evaluating the effects of light availability and soil source (conspecific versus pooled heterospecific) on seedling survival. Individual models were performed for each species.

Parameters	df	*A. saccharum*		*P. serotina*		*Q. alba*		*Q. rubra*	
		LRχ^2	p	LRχ^2	p	LRχ^2	p	LRχ^2	p
Light	1	0.701	0.402	4.094	0.043	0.760	0.383	9.021	0.003
Soil source	1	8.604	0.003	1.015	0.314	1.834	0.176	2.214	0.137
Light × Soil	1	0.050	0.823	0.192	0.192	1.397	0.237	0.578	0.447

Bonferroni correction for species: alpha level is set to 0.0125. Bolded values are statistically significant at $p < 0.0125$. Underlined values are marginally significant at $p < 0.05$.

Table A3.11 Cox proportional hazards survival models evaluating the effects of light availability and soil source (conspecific versus unpooled heterospecific) on seedling survival. Individual models were performed for each species. Sterilized soil was excluded from the model.

Response Parameters	df	*A. saccharum*		*P. serotina*		*Q. alba*		*Q. rubra*	
		LRχ^2	p	LRχ^2	p	LRχ^2	p	LRχ^2	p
Light	1	0.644	0.422	4.655	0.031	0.933	0.331	9.046	0.003
Soil source	1	29.913	<.001	5.698	0.017	1.346	0.246	5.121	0.024
Light × Soil	1	0.002	0.962	6.860	0.009	0.034	0.853	0.806	0.369

Bonferroni correction for species: alpha level is set to 0.0125. Bolded values are statistically significant at $p < 0.0125$. Underlined values are marginally significant at $p < 0.05$.

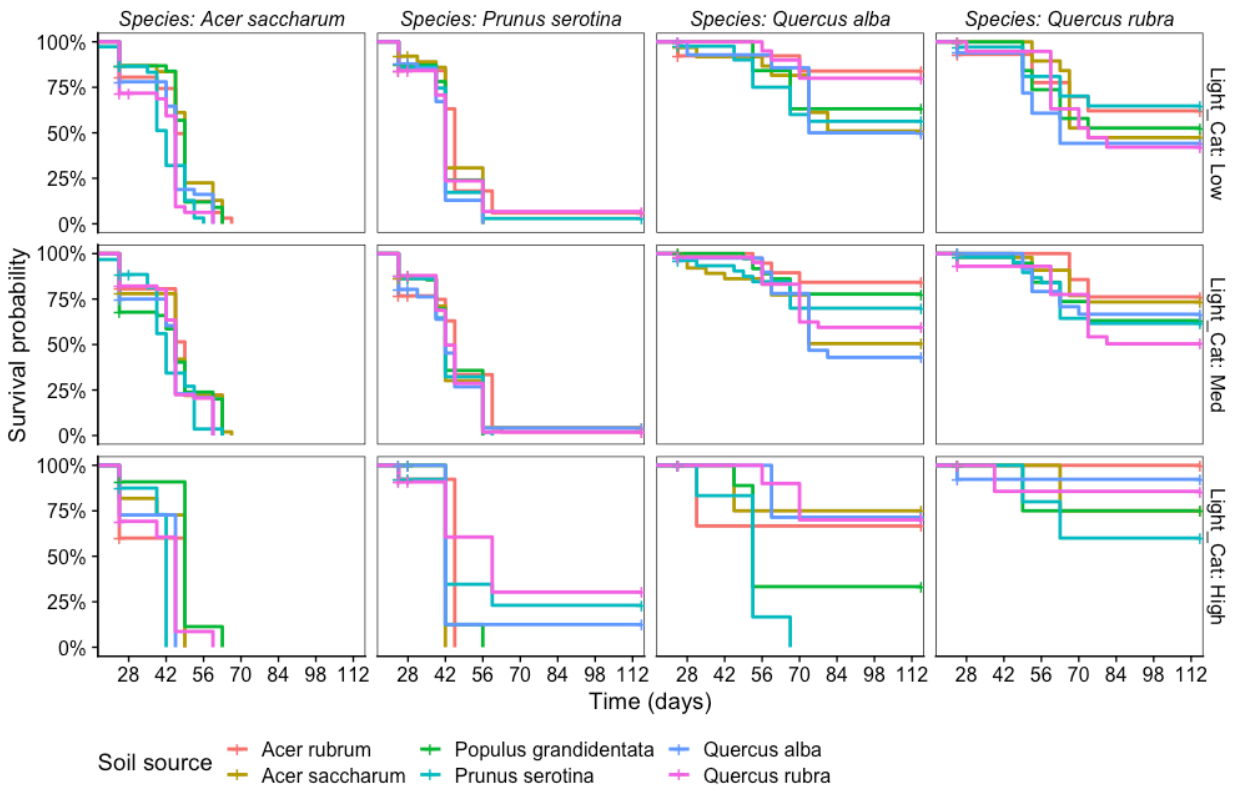


Figure A.3.9 Survival curves for each species, soil source, and light availability.

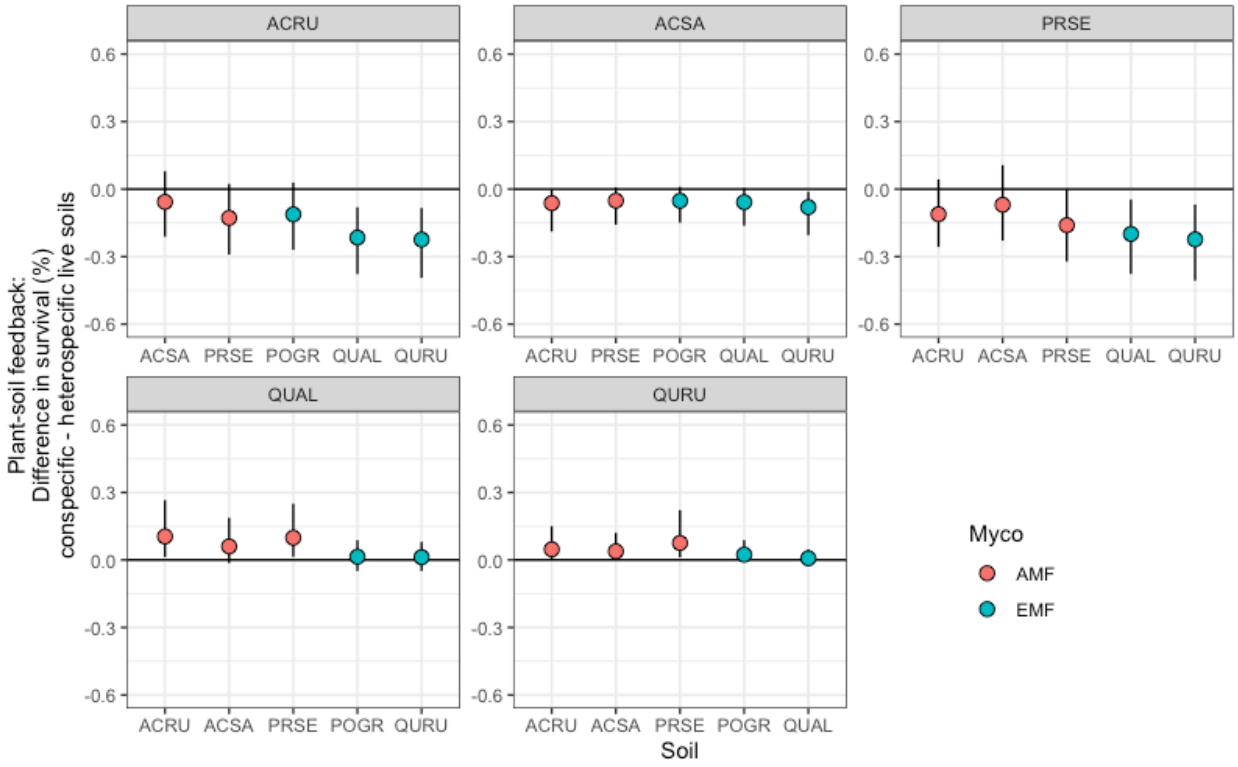


Figure A4.1 Differences in predicted seedling survival when grown at high light availability. Data are means with 95% credible intervals; credible intervals that do not overlap with the zero line are statistically significant. Differences in survival above the zero line indicate a positive PSF (higher survival in conspecific than heterospecific soils); differences in survival below the zero line indicate negative PSFs (lower survival in conspecific than heterospecific soils).

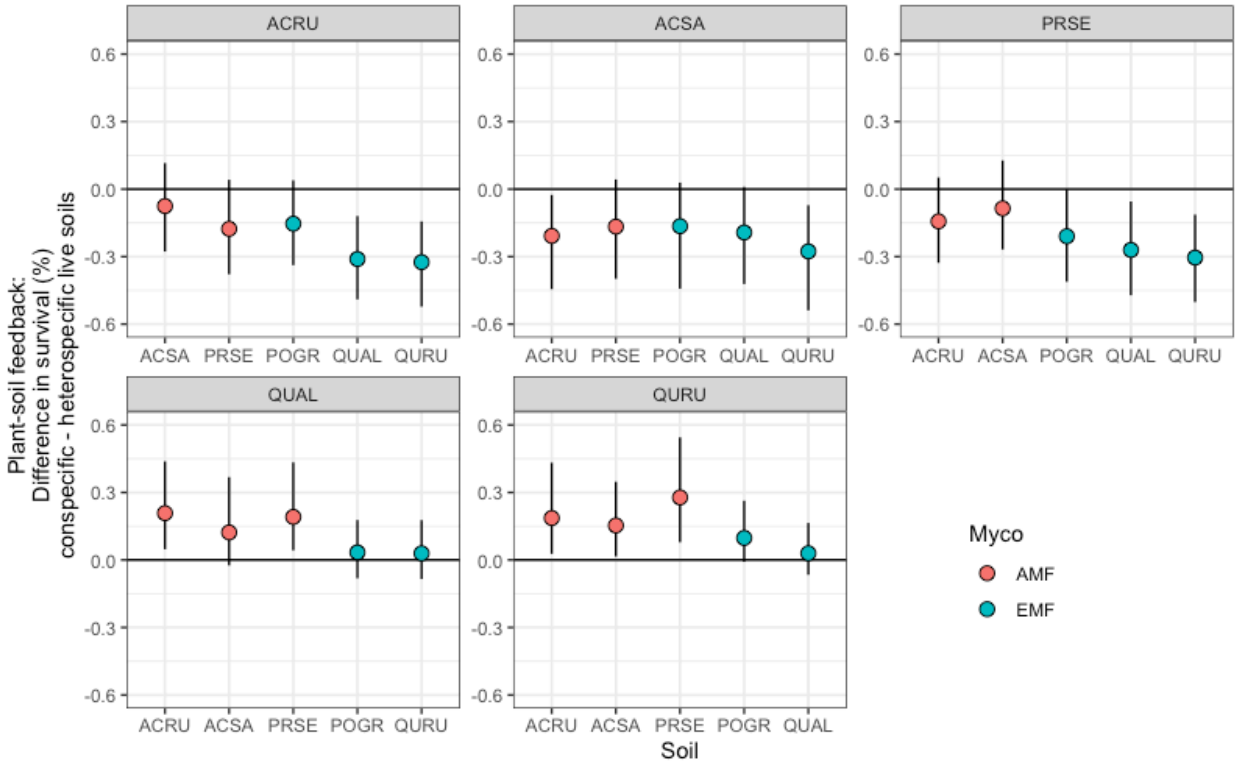


Figure A4.2 Differences in predicted seedling survival when grown at low light availability. Data are means with 95% credible intervals; credible intervals that do not overlap with the zero line are statistically significant. Differences in survival above the zero line indicate a positive PSF (higher survival in conspecific than heterospecific soils); differences in survival below the zero line indicate negative PSFs (lower survival in conspecific than heterospecific soils).

Table A4.2 Summary data (mean ± standard deviation) for percent mycorrhizal colonization by AMF and EMF, phenolics, lignin, and nonstructural carbohydrates. Data are provided for each species × soil source × light level.

Species	Soil	Light	AMF	EMF	Phenolics	Lignin	NSC
ACRU	ACRU	Low	38.82 ± 4.07		0.15 ± 0.06	5.1 ± 0.23	6.97 ± 0.69
ACRU	ACRU	Med	42.62 ± 3.64		0.3 ± 0.16	10.1 ± 0.86	12.14 ± 1.68
ACRU	ACRU	High	46.27 ± 1.9		0.34 ± 0.12	8.6 ± 1.16	12.61 ± 0.71
ACRU	ACSA	Low	36.73 ± 3.13		-0.26 ± 0.26	5.37 ± 1.15	8.96 ± 2.18
ACRU	ACSA	Med	31.45 ± 3.14		-0.16 ± 0.21	9.09 ± 1.5	11.67 ± 1.5
ACRU	ACSA	High	31.67 ± 7.34		-0.14 ± 0.31	8.8 ± 1.08	11.96 ± 1.11
ACRU	POGR	Low	33.08 ± 2.72		-0.13 ± 0.28	5.43 ± 0.73	7.84 ± 1.14
ACRU	POGR	Med	25.62 ± 3.1		-0.25 ± 0.27	8.17 ± 0.91	10.98 ± 2.4
ACRU	POGR	High	30.38 ± 6.33		0.08 ± 0.22	8.3 ± 0.77	11.85 ± 1.12
ACRU	PRSE	Low	29.92 ± 5.98		-0.35 ± 0.23	6.34 ± 1.06	8.97 ± 1.53
ACRU	PRSE	Med	38.58 ± 8.28		-0.1 ± 0.23	8.47 ± 1.56	13.56 ± 1.74
ACRU	PRSE	High	35 ± 8.26		0.06 ± 0.24	8.44 ± 1.07	12.18 ± 1.32
ACRU	QUAL	Low	31.36 ± 2.82		-0.31 ± 0.22	5.11 ± 0.92	8.51 ± 1.97
ACRU	QUAL	Med	28.57 ± 6.8		-0.06 ± 0.28	9.04 ± 1.89	12.96 ± 0.91
ACRU	QUAL	High	34.31 ± 8.02		0.07 ± 0.28	8.14 ± 1.6	12.04 ± 1.35
ACRU	QURU	Low	32.71 ± 4.95		-0.25 ± 0.18	5.6 ± 1.4	8.42 ± 1.54
ACRU	QURU	Med	33.64 ± 7.72		-0.12 ± 0.16	8.61 ± 2.02	11.24 ± 1.56
ACRU	QURU	High	35.57 ± 8.43		-0.01 ± 0.14	8.26 ± 1.41	12.57 ± 1.46
ACRU	StCon	Low	0 ± 0		-0.07 ± 0.05	5.71 ± 0.61	8.68 ± 1.29
ACRU	StCon	Med	0.15 ± 0.38		0.07 ± 0.16	7.41 ± 0.93	10.61 ± 0.89
ACRU	StCon	High	0 ± 0		0.19 ± 0.06	9.61 ± 1.18	12.64 ± 0.75
ACSA	ACRU	Low	60.5 ± 6.54		-0.37 ± 0.32	11.59 ± 1.4	11.77 ± 0.94
ACSA	ACRU	Med	68.31 ± 5.65		-0.01 ± 0.25	13.7 ± 0.57	12.11 ± 0.29
ACSA	ACRU	High	74 ± 5.51		-0.1 ± 0.39	13.87 ± 0.73	12.54 ± 0.34

Table A4.2 (Cont'd)

ACSA	ACSA	Low	66.64 ± 5.68	-0.15 ± 0.15	11.35 ± 0.95	11.18 ± 1.1
ACSA	ACSA	Med	67.83 ± 5.41	0.33 ± 0.18	13.19 ± 1.31	12.17 ± 0.42
ACSA	ACSA	High	74.93 ± 4.46	0.4 ± 0.12	13.83 ± 0.3	12.67 ± 0.14
ACSA	POGR	Low	49.85 ± 7.72	-0.42 ± 0.3	11.87 ± 1.25	10.79 ± 1.13
ACSA	POGR	Med	65 ± 3.65	0.01 ± 0.23	13.83 ± 0.53	12.12 ± 0.45
ACSA	POGR	High	67.29 ± 4.7	-0.08 ± 0.52	13.71 ± 0.5	12.53 ± 0.33
ACSA	PRSE	Low	61.54 ± 3.62	-0.28 ± 0.32	11.67 ± 1.56	10.87 ± 0.97
ACSA	PRSE	Med	67 ± 2.45	-0.11 ± 0.19	13.23 ± 0.39	12.12 ± 0.46
ACSA	PRSE	High	71.53 ± 5.64	0.13 ± 0.52	14.01 ± 0.67	12.65 ± 0.27
ACSA	QUAL	Low	57.64 ± 5.05	-0.18 ± 0.41	11.06 ± 1.75	10.84 ± 1.05
ACSA	QUAL	Med	64.93 ± 5.31	-0.09 ± 0.25	13.17 ± 0.66	12.28 ± 0.42
ACSA	QUAL	High	68.43 ± 4.11	-0.1 ± 0.46	13.2 ± 0.62	12.66 ± 0.33
ACSA	QURU	Low	56.5 ± 5.08	-0.28 ± 0.12	11.11 ± 2.78	11.49 ± 1.06
ACSA	QURU	Med	68.67 ± 5.46	-0.15 ± 0.46	13.56 ± 0.59	12.14 ± 0.35
ACSA	QURU	High	70.29 ± 5.01	0.1 ± 0.39	13.8 ± 0.76	12.5 ± 0.28
ACSA	StCon	Low	0 ± 0	-0.34 ± 0.13	9.89 ± 1.3	10.92 ± 3.22
ACSA	StCon	Med	0 ± 0	-0.02 ± 0.09	11.84 ± 1.69	12.13 ± 0.48
ACSA	StCon	High	0.07 ± 0.26	0.21 ± 0.13	13.53 ± 1.5	12.15 ± 1.46
PRSE	ACRU	Low	61 ± 3.19	0.51 ± 0.14	5.57 ± 1.23	8.87 ± 1.12
PRSE	ACRU	Med	59.91 ± 3.51	0.73 ± 0.12	9 ± 1.21	12.25 ± 1.39
PRSE	ACRU	High	69.62 ± 8.61	0.99 ± 0.08	10.22 ± 1.67	11.91 ± 1.57
PRSE	ACSA	Low	59.73 ± 5.1	0.5 ± 0.28	5.8 ± 1.14	7.98 ± 2.16
PRSE	ACSA	Med	62.55 ± 4.52	0.6 ± 0.28	10.03 ± 1.68	12.94 ± 0.71
PRSE	ACSA	High	70.08 ± 3.7	0.97 ± 0.21	9.2 ± 1.62	12.2 ± 1.22
PRSE	POGR	Low	56.08 ± 3.68	0.36 ± 0.19	6.76 ± 1.02	8.04 ± 2.09
PRSE	POGR	Med	57.18 ± 5.13	0.66 ± 0.16	8.31 ± 1.7	11.77 ± 2.12
PRSE	POGR	High	64.62 ± 5.12	1.08 ± 0.24	8.34 ± 1.92	12.75 ± 1.01

Table A4.2 (Cont'd)

PRSE	PRSE	Low	62.2 ± 4.73		0.36 ± 0.13	5.27 ± 0.08	7.03 ± 0.57
PRSE	PRSE	Med	68 ± 9.87		0.39 ± 0.1	9.9 ± 1.67	12.06 ± 1.87
PRSE	PRSE	High	76.58 ± 7.22		0.65 ± 0.11	9.84 ± 0.92	13 ± 0.79
PRSE	QUAL	Low	57.45 ± 4.89		0.49 ± 0.25	5.71 ± 1.1	9.05 ± 2.13
PRSE	QUAL	Med	56.77 ± 5.73		0.74 ± 0.19	9.37 ± 1.28	12.1 ± 1.85
PRSE	QUAL	High	68.23 ± 3.09		0.9 ± 0.21	9.3 ± 1.95	11.36 ± 1.53
PRSE	QURU	Low	56.75 ± 7.39		0.51 ± 0.1	5.5 ± 1.1	7.86 ± 2.76
PRSE	QURU	Med	56.5 ± 6.27		0.62 ± 0.16	9.57 ± 2.37	12.43 ± 1.28
PRSE	QURU	High	64.2 ± 8.41		0.94 ± 0.36	8.9 ± 1.81	12.17 ± 1.68
PRSE	StCon	Low	0 ± 0		0.27 ± 0.1	5.66 ± 0.53	8.99 ± 2.25
PRSE	StCon	Med	0 ± 0		0.05 ± 0.09	7.9 ± 1.64	11.4 ± 0.99
PRSE	StCon	High	0.21 ± 0.58		-0.06 ± 0.1	8.71 ± 0.31	12.22 ± 0.7
QUAL	ACRU	Low	39.33 ± 5.31	27.71 ± 16.85	4.16 ± 0.33	21.33 ± 2.53	18.29 ± 2.53
QUAL	ACRU	Med	41.15 ± 4.79	27.43 ± 9.91	4.8 ± 0.42	20.09 ± 2.48	21.14 ± 2.74
QUAL	ACRU	High	36.92 ± 7.89	32.43 ± 13.96	5.51 ± 0.31	21.49 ± 1.48	20.63 ± 1.6
QUAL	ACSA	Low	33.64 ± 8.63	35.86 ± 14.39	3.98 ± 0.37	21.8 ± 2.3	18.77 ± 2.49
QUAL	ACSA	Med	41.47 ± 4.17	33.71 ± 17.52	5.09 ± 0.4	21.24 ± 2.56	20.13 ± 1.7
QUAL	ACSA	High	38.07 ± 6.66	34.86 ± 12.05	5.41 ± 0.45	21.84 ± 2.57	23.21 ± 1.46
QUAL	POGR	Low	40.07 ± 5.4	34.29 ± 9.76	4.16 ± 0.63	20.76 ± 1.38	19.15 ± 2.41
QUAL	POGR	Med	41.4 ± 7.46	22.14 ± 11.75	5.04 ± 0.27	20.26 ± 1.56	21.75 ± 2.63
QUAL	POGR	High	43.73 ± 2.46	33 ± 11.22	5.42 ± 0.76	22.27 ± 1.86	20.58 ± 2.23
QUAL	PRSE	Low	39.08 ± 2.5	30.14 ± 20.32	4.73 ± 0.4	19.7 ± 2.78	20.25 ± 2.11
QUAL	PRSE	Med	41.71 ± 3.73	28.29 ± 13.41	4.69 ± 0.37	20.13 ± 1.97	22.43 ± 2.41
QUAL	PRSE	High	37.86 ± 4.62	37 ± 11.97	5.05 ± 0.56	21.91 ± 1.46	22.3 ± 3.81
QUAL	QUAL	Low	40.57 ± 2.24	35 ± 15.28	4.62 ± 0.35	22.64 ± 2.46	17.81 ± 4.22
QUAL	QUAL	Med	44.13 ± 6.32	35.29 ± 8.85	4.89 ± 0.38	22.8 ± 1.64	17.55 ± 2.4
QUAL	QUAL	High	49.87 ± 6.07	34.29 ± 7.48	5.32 ± 0.32	24.1 ± 2.49	21.29 ± 2.27

Table A4.2 (Cont'd)

QUAL	QURU	Low	39.57 ± 6.94	27.57 ± 10.97	4.52 ± 0.32	20.39 ± 1.42	19.53 ± 3.18
QUAL	QURU	Med	46 ± 3.14	30.86 ± 10.24	4.89 ± 0.42	19.8 ± 1.74	20.85 ± 1.78
QUAL	QURU	High	43.57 ± 5.29	39.86 ± 6.74	5.08 ± 0.27	23.37 ± 1.64	21.76 ± 2.76
QUAL	StCon	Low	0 ± 0	3.71 ± 1.5	2.52 ± 0.37	19.33 ± 1.42	18.95 ± 1.68
QUAL	StCon	Med	0 ± 0	6.43 ± 1.51	3.2 ± 0.28	21.04 ± 1.42	17.99 ± 2.3
QUAL	StCon	High	0 ± 0	10.29 ± 5.12	4.27 ± 0.12	22.53 ± 1.55	21.42 ± 1.88
QURU	ACRU	Low	37.77 ± 5.83	24 ± 14.11	3.22 ± 0.43	23.41 ± 3.59	14.15 ± 2.61
QURU	ACRU	Med	44.71 ± 6.28	31.57 ± 15.15	3.55 ± 0.35	25.13 ± 2.5	14.73 ± 3.67
QURU	ACRU	High	43.29 ± 5.5	26.57 ± 19.46	3.38 ± 0.45	24.53 ± 2.84	15.41 ± 1.93
QURU	ACSA	Low	37.54 ± 3.71	28.57 ± 17.31	3.26 ± 0.64	23 ± 4.39	14.97 ± 3.51
QURU	ACSA	Med	40.46 ± 4.7	35.57 ± 19.12	3.55 ± 0.38	24.03 ± 2.37	14.16 ± 1.68
QURU	ACSA	High	46.5 ± 3.57	34.14 ± 12.16	3.43 ± 0.48	26.91 ± 1.97	16.06 ± 3.5
QURU	POGR	Low	40.07 ± 4.57	37.57 ± 15.86	3.05 ± 0.36	21.69 ± 3.42	14.38 ± 2.43
QURU	POGR	Med	44.29 ± 4.94	29.43 ± 16.67	3.45 ± 0.28	23.36 ± 2.34	16 ± 1.78
QURU	POGR	High	45.27 ± 4.98	42.29 ± 18.39	3.87 ± 0.38	24 ± 1.46	15.42 ± 3.66
QURU	PRSE	Low	38.58 ± 4.89	26.86 ± 17.32	3.33 ± 0.48	23.06 ± 2.87	15.06 ± 1.84
QURU	PRSE	Med	42.69 ± 5.6	31.29 ± 9.18	3.65 ± 0.38	23.71 ± 1.76	16.19 ± 1.44
QURU	PRSE	High	46.79 ± 4.82	32.14 ± 22.65	3.52 ± 0.68	26.29 ± 2.72	17.27 ± 2.04
QURU	QUAL	Low	38.07 ± 5.91	32.14 ± 24.29	3.25 ± 0.33	25.06 ± 5.22	15.79 ± 3.75
QURU	QUAL	Med	45.43 ± 5.21	22.29 ± 23.98	3.76 ± 0.22	26.3 ± 2.44	14.68 ± 1.42
QURU	QUAL	High	50.53 ± 4.82	40 ± 20.12	3.66 ± 0.47	23.93 ± 3.68	17.64 ± 3.19
QURU	QURU	Low	40.57 ± 6.22	23 ± 18.57	2.49 ± 0.56	25.73 ± 2.15	11.27 ± 1.46
QURU	QURU	Med	44.67 ± 3.44	33.71 ± 14.35	3.25 ± 0.35	24.8 ± 2.26	14.82 ± 2.91
QURU	QURU	High	49 ± 3.95	35.43 ± 9.24	4.27 ± 1.1	25.41 ± 3.85	17.98 ± 1.49
QURU	StCon	Low	0 ± 0	4.57 ± 3.87	2.78 ± 0.33	27 ± 1.63	10.97 ± 2.26
QURU	StCon	Med	0 ± 0	6.14 ± 2.61	3.39 ± 0.25	24.4 ± 0.4	15.01 ± 0.85
QURU	StCon	High	0 ± 0	6.43 ± 2.44	3.71 ± 0.1	24.51 ± 1.42	17.7 ± 1.29

Table A4.3 Summary of predicted seedling survival (mean ± standard deviation). Data are provided for each species × soil source × light level.

Species	Soil	Light	Predicted Survival	Lower CI	Upper CI
ACRU	ACRU	Low	0.5031	0.3064	0.6853
ACRU	ACSA	Low	0.5785	0.3991	0.7636
ACRU	POGR	Low	0.6797	0.5034	0.8343
ACRU	PRSE	Low	0.6569	0.4702	0.8148
ACRU	QUAL	Low	0.8138	0.6885	0.9223
ACRU	QURU	Low	0.8277	0.6738	0.9338
ACRU	ST-CON	Low	0.8524	0.728	0.9485
ACSA	ACRU	Low	0.8133	0.5723	0.9544
ACSA	ACSA	Low	0.6054	0.2195	0.8554
ACSA	POGR	Low	0.7701	0.4524	0.9361
ACSA	PRSE	Low	0.7722	0.5012	0.9391
ACSA	QUAL	Low	0.798	0.5444	0.9524
ACSA	QURU	Low	0.8821	0.7043	0.9797
ACSA	ST-CON	Low	0.8836	0.7173	0.9813
PRSE	ACRU	Low	0.5498	0.3024	0.7467
PRSE	ACSA	Low	0.4924	0.2442	0.7295
PRSE	POGR	Low	0.6162	0.3637	0.8171
PRSE	PRSE	Low	0.4066	0.175	0.6547
PRSE	QUAL	Low	0.677	0.4599	0.8457
PRSE	QURU	Low	0.711	0.4922	0.8706
PRSE	ST-CON	Low	0.8804	0.755	0.9678
QUAL	ACRU	Low	0.7333	0.4688	0.9049
QUAL	ACSA	Low	0.819	0.5706	0.9547
QUAL	POGR	Low	0.9076	0.7576	0.9843
QUAL	PRSE	Low	0.7501	0.512	0.9076
QUAL	QUAL	Low	0.9413	0.8362	0.9952
QUAL	QURU	Low	0.912	0.7621	0.9867
QUAL	ST-CON	Low	0.97	0.8811	1.005
QURU	ACRU	Low	0.7816	0.5278	0.9432
QURU	ACSA	Low	0.8146	0.6023	0.956
QURU	POGR	Low	0.87	0.6735	0.977
QURU	PRSE	Low	0.6901	0.3815	0.8943
QURU	QUAL	Low	0.9381	0.7987	0.9947
QURU	QURU	Low	0.9679	0.8681	0.9996

Table A4.3 (Cont'd)

QURU	ST-CON	Low	0.9311	0.7742	0.9954
ACRU	ACRU	Med	0.589	0.4141	0.7643
ACRU	ACSA	Med	0.6562	0.484	0.8129
ACRU	POGR	Med	0.7435	0.589	0.8703
ACRU	PRSE	Med	0.7246	0.5714	0.854
ACRU	QUAL	Med	0.854	0.741	0.9422
ACRU	QURU	Med	0.8652	0.7505	0.9478
ACRU	ST-CON	Med	0.8844	0.7816	0.9619
ACSA	ACRU	Med	0.8541	0.6744	0.9671
ACSA	ACSA	Med	0.6765	0.3997	0.9036
ACSA	POGR	Med	0.8179	0.5965	0.9497
ACSA	PRSE	Med	0.8195	0.6144	0.9548
ACSA	QUAL	Med	0.8428	0.644	0.9631
ACSA	QURU	Med	0.9102	0.7699	0.9834
ACSA	ST-CON	Med	0.9099	0.7808	0.9821
PRSE	ACRU	Med	0.639	0.4335	0.8109
PRSE	ACSA	Med	0.5873	0.3739	0.7849
PRSE	POGR	Med	0.6961	0.5154	0.8479
PRSE	PRSE	Med	0.5071	0.2514	0.7412
PRSE	QUAL	Med	0.7466	0.556	0.8824
PRSE	QURU	Med	0.7732	0.5906	0.9053
PRSE	ST-CON	Med	0.9092	0.7996	0.9744
QUAL	ACRU	Med	0.8637	0.6491	0.9678
QUAL	ACSA	Med	0.9111	0.7567	0.9827
QUAL	POGR	Med	0.9558	0.8531	0.9946
QUAL	PRSE	Med	0.8713	0.688	0.9738
QUAL	QUAL	Med	0.971	0.8936	1.003
QUAL	QURU	Med	0.959	0.8819	0.9969
QUAL	ST-CON	Med	0.9855	0.9433	1.01
QURU	ACRU	Med	0.8751	0.6908	0.973
QURU	ACSA	Med	0.8972	0.7646	0.98
QURU	POGR	Med	0.9295	0.8143	0.9871
QURU	PRSE	Med	0.8188	0.6228	0.952
QURU	QUAL	Med	0.9666	0.8878	0.9965
QURU	QURU	Med	0.9837	0.9366	1.009
QURU	ST-CON	Med	0.9643	0.883	1
ACRU	ACRU	High	0.6741	0.4819	0.8144
ACRU	ACSA	High	0.731	0.5597	0.8621

Table A4.3 (Cont'd)

ACRU	POGR	High	0.802	0.6585	0.9128
ACRU	PRSE	High	0.7864	0.6258	0.8952
ACRU	QUAL	High	0.8894	0.7913	0.9588
ACRU	QURU	High	0.8983	0.7898	0.9627
ACRU	ST-CON	High	0.9119	0.8093	0.973
ACSA	ACRU	High	0.9558	0.8782	0.9954
ACSA	ACSA	High	0.8935	0.7485	0.977
ACSA	POGR	High	0.9454	0.8426	0.9887
ACSA	PRSE	High	0.9449	0.8438	0.9901
ACSA	QUAL	High	0.9515	0.8415	0.9932
ACSA	QURU	High	0.9737	0.9149	1.002
ACSA	ST-CON	High	0.9735	0.91	1
PRSE	ACRU	High	0.7149	0.5046	0.8564
PRSE	ACSA	High	0.673	0.4607	0.8316
PRSE	POGR	High	0.7637	0.5746	0.8816
PRSE	PRSE	High	0.6033	0.3763	0.7754
PRSE	QUAL	High	0.8026	0.6157	0.9171
PRSE	QURU	High	0.8266	0.6629	0.9285
PRSE	ST-CON	High	0.931	0.8377	0.9825
QUAL	ACRU	High	0.8668	0.6889	0.9641
QUAL	ACSA	High	0.9112	0.7775	0.9826
QUAL	POGR	High	0.9568	0.8898	0.9961
QUAL	PRSE	High	0.8725	0.7131	0.9697
QUAL	QUAL	High	0.9712	0.908	1.004
QUAL	QURU	High	0.9589	0.8654	0.997
QUAL	ST-CON	High	0.9862	0.9416	1.008
QURU	ACRU	High	0.9455	0.8366	0.9915
QURU	ACSA	High	0.9543	0.8597	0.9942
QURU	POGR	High	0.9691	0.8986	0.999
QURU	PRSE	High	0.9171	0.7608	0.9864
QURU	QUAL	High	0.9853	0.9393	1.007
QURU	QURU	High	0.9928	0.9645	1.009
QURU	ST-CON	High	0.9844	0.9433	1.007

Table A5.1 Sample size (n) for each seedling species × soil source × light level in the greenhouse experiment.

	Seedling species				
	ACRU	ACSA	PRSE	QUAL	QURU
CON-ST					
Low	14	14	14	15	15
Med	13	14	14	16	15
High	15	16	15	15	16
ACRU					
Low	8	13	8	12	12
Med	9	13	10	13	14
High	10	15	11	13	15
ACSA					
Low	8	10	7	13	13
Med	10	10	9	15	14
High	12	15	11	14	15
POGR					
Low	10	12	10	14	14
Med	12	13	10	15	14
High	12	15	12	16	16
PRSE					
Low	10	13	5	11	11
Med	11	12	9	14	13
High	13	15	10	14	14
QUAL					
Low	12	12	11	15	15
Med	14	14	11	15	15
High	14	15	12	16	16
QURU					
Low	13	14	11	15	15
Med	14	15	13	15	16
High	14	15	12	15	15

Table A5.2 Sample size (n) for each seedling species × soil source × light level in the field experiment. † Indicates a sample size that below 5.

	Seedling species	
	QUAL	QURU
CON-ST		
Low	13	11
Med	18	18
High	5	4†
ACRU		
Low	10	8
Med	32	16
High	2†	5
ACSA		
Low	10	9
Med	17	21
High	3†	3†
POGR		
Low	12	10
Med	28	12
High	3†	3†
PRSE		
Low	15	12
Med	28	22
High	0	3†
QUAL		
Low	7	8
Med	11	16
High	5	4†
QURU		
Low	16	8
Med	20	13
High	7	6

Table A5.3 Summary of seedling biomass in the field experiment. Data is presented for each species × soil source × light level.

Species	Soil	Light level	Biomass
QUAL	ACRU	High	3666.01 ± 12.51
QUAL	ACRU	Low	1689.22 ± 231.1
QUAL	ACRU	Med	
QUAL	ACSA	High	3575.54 ± 33.04
QUAL	ACSA	Low	1712.08 ± 146.55
QUAL	ACSA	Med	4042.85 ± 184.55
QUAL	CON-ST	High	5027.56 ± 115.16
QUAL	CON-ST	Low	1087.85 ± 437.61
QUAL	CON-ST	Med	3617.4 ± 269.16
QUAL	POGR	High	3520.27 ± 123.15
QUAL	POGR	Low	1792.78 ± 124.88
QUAL	POGR	Med	4000.65 ± 253.16
QUAL	PRSE	High	
QUAL	PRSE	Low	1769.46 ± 146.5
QUAL	PRSE	Med	4012.82 ± 263.91
QUAL	QUAL	High	2928.33 ± 55.41
QUAL	QUAL	Low	1383.87 ± 116.58
QUAL	QUAL	Med	2281 ± 131.29
QUAL	QURU	High	3626.45 ± 413.01
QUAL	QURU	Low	1704.09 ± 133.76
QUAL	QURU	Med	3965.82 ± 210.26
QURU	ACRU	High	3468.59 ± 126.44
QURU	ACRU	Low	990.08 ± 92.34
QURU	ACRU	Med	3467.45 ± 217.89
QURU	ACSA	High	3639.76 ± 73.42
QURU	ACSA	Low	988.03 ± 162.09
QURU	ACSA	Med	3497.36 ± 184.74
QURU	CON-ST	High	3417.24 ± 62.63
QURU	CON-ST	Low	1475.39 ± 552.04
QURU	CON-ST	Med	2225.94 ± 319.93

Table A5.3 (Cont'd)

QURU	POGR	High	3542.57 ± 126.24
QURU	POGR	Low	1051.93 ± 124.16
QURU	POGR	Med	3426.28 ± 258.21
QURU	PRSE	High	3691.07 ± 57.85
QURU	PRSE	Low	932.17 ± 98.61
QURU	PRSE	Med	3501.95 ± 223
QURU	QUAL	High	3571.43 ± 148.14
QURU	QUAL	Low	1030.77 ± 107.57
QURU	QUAL	Med	3439.6 ± 189
QURU	QURU	High	5399.9 ± 324.45
QURU	QURU	Low	1556.8 ± 276.23
QURU	QURU	Med	4008.48 ± 188.21

Table A5.4 Summary of seedling biomass in the greenhouse experiment. Data is presented for each species × soil source × light level.

Species	Soil	Light level	Biomass
ACRU	ACRU	High	435.72 ± 80.16
ACRU	ACRU	Low	10.16 ± 1.66
ACRU	ACRU	Med	140.22 ± 29.78
ACRU	ACSA	High	371.78 ± 120.84
ACRU	ACSA	Low	14.66 ± 2.25
ACRU	ACSA	Med	193.12 ± 49.32
ACRU	CON-ST	High	414.16 ± 73.51
ACRU	CON-ST	Low	11.52 ± 2.27
ACRU	CON-ST	Med	160.34 ± 56.57
ACRU	POGR	High	320.91 ± 77.12
ACRU	POGR	Low	13.42 ± 1.77
ACRU	POGR	Med	167.96 ± 54.84
ACRU	PRSE	High	373.73 ± 80.1
ACRU	PRSE	Low	14.31 ± 1.84
ACRU	PRSE	Med	156.03 ± 32.25
ACRU	QUAL	High	346.43 ± 58.94
ACRU	QUAL	Low	13.58 ± 2.3
ACRU	QUAL	Med	162.43 ± 53.13
ACRU	QURU	High	359.05 ± 95.87
ACRU	QURU	Low	13.72 ± 1
ACRU	QURU	Med	175.69 ± 62.14
ACSA	ACRU	High	710.14 ± 203.54
ACSA	ACRU	Low	80.72 ± 11.93
ACSA	ACRU	Med	313.55 ± 76.56
ACSA	ACSA	High	883.42 ± 102.86
ACSA	ACSA	Low	80.15 ± 8.16
ACSA	ACSA	Med	383.22 ± 85.6
ACSA	CON-ST	High	934.28 ± 119.87
ACSA	CON-ST	Low	74.21 ± 10.55
ACSA	CON-ST	Med	425.59 ± 52.74
ACSA	POGR	High	594.84 ± 159.14
ACSA	POGR	Low	91.07 ± 9.46
ACSA	POGR	Med	270.06 ± 74.27
ACSA	PRSE	High	782.61 ± 133.41
ACSA	PRSE	Low	76.41 ± 14.07
ACSA	PRSE	Med	319.43 ± 96.5

Table A5.4 (Cont'd)

ACSA	QUAL	High	646.41 ± 100.28
ACSA	QUAL	Low	89.19 ± 14.5
ACSA	QUAL	Med	265.99 ± 69.72
ACSA	QURU	High	605.47 ± 127.97
ACSA	QURU	Low	89.22 ± 14.72
ACSA	QURU	Med	274.01 ± 55
PRSE	ACRU	High	741.15 ± 184.93
PRSE	ACRU	Low	74.14 ± 13.59
PRSE	ACRU	Med	301.04 ± 55.39
PRSE	ACSA	High	715.52 ± 213.05
PRSE	ACSA	Low	78.11 ± 10.83
PRSE	ACSA	Med	278.63 ± 93.25
PRSE	CON-ST	High	841.51 ± 95.01
PRSE	CON-ST	Low	81.09 ± 12.17
PRSE	CON-ST	Med	442.48 ± 55.8
PRSE	POGR	High	554.95 ± 142.7
PRSE	POGR	Low	80.48 ± 16.93
PRSE	POGR	Med	271.53 ± 82.5
PRSE	PRSE	High	808.4 ± 116.55
PRSE	PRSE	Low	60.54 ± 7.66
PRSE	PRSE	Med	423.63 ± 77.77
PRSE	QUAL	High	587.39 ± 86.63
PRSE	QUAL	Low	88.4 ± 11.68
PRSE	QUAL	Med	291.2 ± 76.29
PRSE	QURU	High	641.48 ± 102.49
PRSE	QURU	Low	82.06 ± 13.79
PRSE	QURU	Med	259.74 ± 76.08
QUAL	ACRU	High	3386.46 ± 229.5
QUAL	ACRU	Low	1055.42 ± 129.87
QUAL	ACRU	Med	2529.42 ± 297.94
QUAL	ACSA	High	3232.49 ± 200.79
QUAL	ACSA	Low	1060.3 ± 116.89
QUAL	ACSA	Med	2352.63 ± 344
QUAL	CON-ST	High	3781.74 ± 229.71
QUAL	CON-ST	Low	1165.79 ± 221.2
QUAL	CON-ST	Med	2604.63 ± 265.12
QUAL	POGR	High	3448.65 ± 188.59
QUAL	POGR	Low	1124.04 ± 124.86

Table A5.4 (Cont'd)

QUAL	POGR	Med	2463.69 ± 364.43
QUAL	PRSE	High	3321.23 ± 217.76
QUAL	PRSE	Low	990.15 ± 158.15
QUAL	PRSE	Med	2327.46 ± 342.79
QUAL	QUAL	High	3883.71 ± 158.21
QUAL	QUAL	Low	1266.15 ± 227.99
QUAL	QUAL	Med	2615.77 ± 211.74
QUAL	QURU	High	3336.68 ± 157.96
QUAL	QURU	Low	1124.6 ± 93.69
QUAL	QURU	Med	2532.21 ± 254.17
QURU	ACRU	High	3360.74 ± 184.55
QURU	ACRU	Low	1046.38 ± 97.72
QURU	ACRU	Med	2500.16 ± 242.63
QURU	ACSA	High	3321.63 ± 155.43
QURU	ACSA	Low	1057.27 ± 149.69
QURU	ACSA	Med	2496.75 ± 276.45
QURU	CON-ST	High	3724.07 ± 162.16
QURU	CON-ST	Low	1109.21 ± 198.79
QURU	CON-ST	Med	2483.38 ± 223.65
QURU	POGR	High	3393.5 ± 278.17
QURU	POGR	Low	1114.29 ± 102.92
QURU	POGR	Med	2319.26 ± 347.3
QURU	PRSE	High	3348.89 ± 156.41
QURU	PRSE	Low	1022.26 ± 131.25
QURU	PRSE	Med	2329.37 ± 260.71
QURU	QUAL	High	3291.16 ± 250.07
QURU	QUAL	Low	1116.29 ± 101.97
QURU	QUAL	Med	2453.07 ± 283.18
QURU	QURU	High	3884.31 ± 239.72
QURU	QURU	Low	1275.73 ± 247.61
QURU	QURU	Med	2681.42 ± 260.84

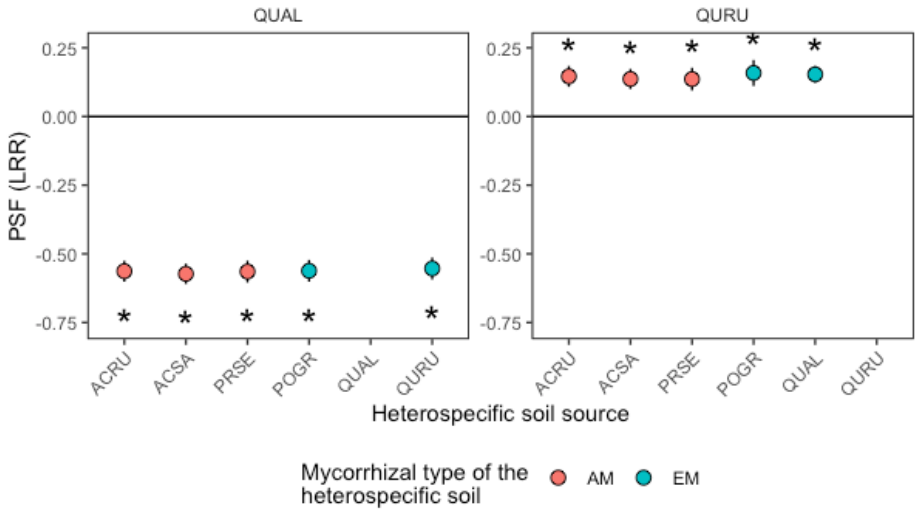


Figure A5.1. Log response ratio ± standard error of seedling biomass in conspecific versus heterospecific soils at medium light availability in the field experiment. Values < 0 indicate negative PSF and values > 0 indicate positive PSF and values < 1 indicate negative PSF. Values that are statistically different from 0 ($p < 0.05$) are indicated with a star *.

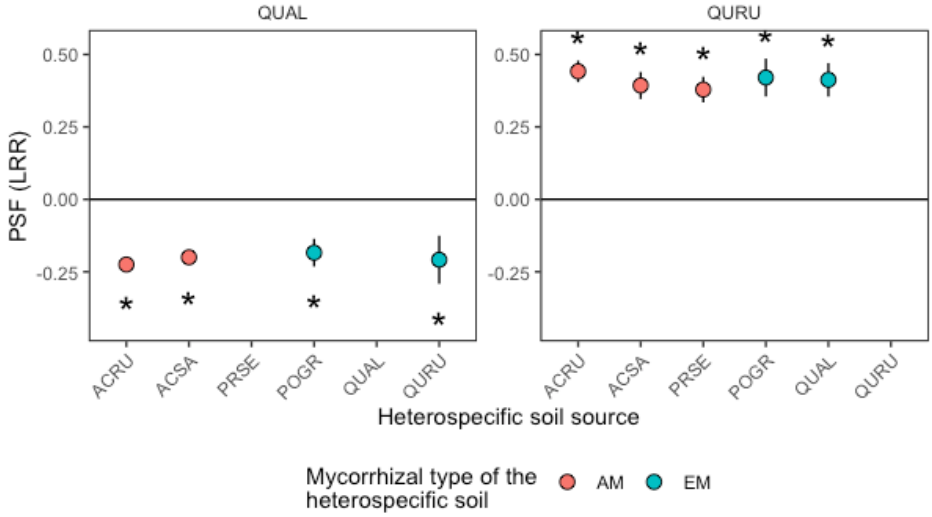


Figure A5.2. Log response ratio ± standard error of seedling biomass in conspecific versus heterospecific soils at high light availability in the field experiment. Values < 0 indicate negative PSF and values > 0 indicate positive PSF and values < 1 indicate negative PSF. Values that are statistically different from 0 ($p < 0.05$) are indicated with a star *.