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PLANT-SOIL FEEDBACKS IN TEMPERATE AND TROPICAL FORESTS

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PLANT-SOIL FEEDBACKS IN TEMPERATE AND TROPICAL FORESTS

By

Sarah McCarthy Neumann

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ABSTRACT

PLANT-SOIL FEEDBACKS IN TEMPERATE AND TROPICAL FORESTS

By

Sarah McCarthy Neumann

The Janzen-Connell (J-C) Model proposes that host-specific natural enemies maintain high tropical tree diversity by reducing seed and/or seedling survivorship near conspecific adults and/or at high conspecific densities. Such non-competitive distance or density-dependent (NCDD) mortality would favor establishment of heterospecific individuals, thus promoting species coexistence. Negative plant-soil feedback, whereby individual plants "culture" the soil community in which they grow to the detriment of themselves and other conspecific individuals, may be an important mechanism that could create NCDD mortality and/or reduced growth. I used a wet-sieving method to filter out biotic and water extractable chemical elements from soil that had been cultured by conspecific and heterospecific adults and seedlings. These soil extracts were used in greenhouse experiments with temperate and tropical tree species to examine 1) advantages to heterospecific and disadvantages to conspecific recruitment, 2) soil mechanisms underlying NCDD, 3) differences between common and rare species in sensitivity to J-C processes, 4) the strength of J-C processes in tropical versus temperate forests, 5) and the interactions of J-C processes with light availability. I found that

susceptibility to microbial extract cultured by conspecific individuals was negatively correlated with seedling shade tolerance not a species' local abundance, thereby exaggerating apparent shade tolerance differences among species and likely contributing to species coexistence through heightening niche differentiation. When comparing effects of con- vs. hetero-specific cultured soils, I found that species-specific feedbacks between adult trees (not seedlings) and soil influenced seedling performance for all temperate and tropical species. Con- and hetero-specific effects had similar prevalence and magnitude of influence for temperate species whereas three of the six tropical species had decreased performance when grown with extract cultured by con- vs. all hetero-specific adults and an additional two species had decreased performance in con- vs. two or more hetero-specific cultured extracts. In addition, in temperate forests, soils cultured by a particular species do not necessarily improve heterospecific seedling performance relative to conspecific seedlings which may impede the ability of these plant-soil feedbacks to enhance species coexistence. However, in tropical forests, heterospecific seedlings are favored relative to conspecific seedlings in soils cultured by a given species. Thus, J-C processes appear stronger in tropical vs. temperate forests, at least those mediated by plant-soil feedbacks. Surprisingly, chemical factors in the soil not micro-organisms seem to be primarily responsible for these feedbacks. Thus, my dissertation identifies a novel mechanism (feedback between adult trees and soil abiotic factors) that creates NCDD seedling mortality and/or reduced growth and moves the J-C Model beyond solely focusing on natural enemies.

DEDICATED

To my husband David and my entire family

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CHAPTER ONE

INTRODUCTION

Identifying the mechanisms that maintain species richness is a central question in plant community ecology. Under the competitive exclusion principle, competitively superior species exclude inferior species if competition for resources remains unchecked (Gause 1934). Although niche partitioning is often invoked as an explanation for species coexistence, most plants require the same resources. How then are there so many species rich forests, many in the tropics containing more than one hundred species per hectare? Out of the vast array of hypotheses that have been proposed (Palmer 1994), one of the most influential was put forth independently by Janzen (1970) and Connell (1971) (hereafter referred to as the J-C Model). They proposed that host-specific natural enemies could maintain high tree diversity of tropical forests by reducing seed and/or seedling survivorship near conspecific adults and/or at high conspecific densities. Such non-competitive distance or density-dependent (NCDD) mortality would favor establishment of heterospecific individuals, thus promoting species coexistence.

Although the Janzen-Connell Model has produced a vast body of literature of both empirical and theoretical studies, there is still contention over the importance of NCDD mortality in tropical forest community dynamics (detractors: Hubbell 1979 & 1980,

Connell et al. 1984, Hubbell and Foster 1986, Hubbell et al. 1990, Welden et al. 1991, Condit et al. 1992, He et al. 1997, Hyatt et al. 2003; supporters: Wills et al. 1997, Webb and Peart 1999, Wills and Condit 1999, Harms et al. 2000, Wright 2002, Peters 2003). In addition, the focus on explaining the maintenance of tropical diversity has detracted from testing whether similar processes are operating in temperate forests. Among the few studies conducted in temperate forests, there is evidence of distance and density-dependent tree seedling mortality (Packer and Clay 2000, Hille Ris Lambers et al. 2002), but it is not yet clear how tropical and temperate forests may differ in this regard.

Most studies on distance and density-dependent tree seedling survivorship are structured to test whether spatial patterns of tree seedling recruitment are consistent with the J-C Model. Conflicting results among the above studies could be due to the simple fact that patterns of seedling recruitment arise from several different mechanisms, which may preclude straightforward interpretations of spatial patterns. For example, in a comparative review of seed and seedling performance at near and far distances from conspecific adults, only 2 of 27 studies involving a vertebrate herbivore but 15 of 19 studies involving an insect herbivore showed negative effects of being close to a conspecific adult (Hammond & Brown 1998).

My aim in this chapter is to outline the major assumptions of the Janzen-Connell Model and to demonstrate how the J-C Model can be placed within the larger context of negative feedbacks between plants and the soil in which they grow. Specifically my dissertation research was designed to examine: 1) the mechanism underlying non-competitive distance and density-dependent mortality and/or reduced growth; 2) advantages to heterospecific and disadvantages to conspecific recruitment, 3) differences

between common and rare species in sensitivity to J-C processes, 4) the strength of J-C processes in tropical versus temperate forests, 5) and the interactions of J-C processes with light availability.

Plant-Soil Feedbacks - Individual plants not only use resources in the environment for their survival and growth but they interact and can change the environment in which they live. In particular, there is growing appreciation for the potential interactions that can occur between plants and their soil environment. Plant-soil feedbacks could be considered a two-step process: 1) the plant or population changes the soil community, and 2) the soil community affects plant survival and/or growth. Feedbacks can be either positive or negative but negative feedbacks can allow for plant species coexistence by reducing establishment, growth or reproduction of individuals under their parent plants, thereby allowing for heterospecific establishment to occur.

Although soil pathogens (e.g. fungi, bacteria and/or nematodes) have a long history of study in forestry and horticulture, their potential role as a mechanism of NCDD mortality in the dynamics of natural plant communities has only recently been recognized (Gilbert and Hubbell 1996, Packer and Clay 2000, 2003, Hood et al. 2004, Bell et al. 2006). For instance, many of these pathogens show strong host specialization, short generation times, high fecundity, long persistence in soil, and more limited dispersal than their hosts (Agrios 1997, Gilbert 2002). These characteristics could underpin the mechanisms that create negative feedbacks when individual plants "culture" the soil microbial community in which they grow to the detriment of themselves and other conspecific individuals (van der Putten et al. 1993, Bever 1994, Mills & Bever 1998, Klironomos 2002).

There are other important plant-soil feedbacks that are not mediated by soil pathogens (Ehrenfeld et al. 2005). For example, the presence of a particular plant species could be associated with the formation of mycorrhizal networks (Booth 2004), production of allelochemicals (Stinson et al 2006), alterations to soil physical properties (Rillig et al. 2002) and nutrient availability (Finzi et al. 1998a-b). All of these feedbacks could impact seedling performance in a species-specific manner and result in distance and density-dependent mortality and/or reduced growth. That is, a particular species could modify the soil to the detriment of heterospecific vs. conspecific seedlings. However, soil pathogens can be host-specific and are more likely to specialize on common species, two attributes that make them ideal for creating NCDD processes.

Distance and Density-Dependent Processes - Traditionally in studies of NCDD processes, all heterospecific individuals have been lumped into a single heterospecific category (e.g. "far" distance) (Augspurger 1983a-b, Packer and Clay 2000 and 2003; Hood et al 2004). However, tree species vary in many characteristics (e.g., resource allocation to defense vs. growth) and soil-mediated effects of mature individuals on seedlings could be species-specific as well. In this dissertation I investigate species-specific effects of conspecific vs. heterospecific individuals rather than 'near' vs. 'far' categories.

I will be defining density-dependent mortality, in this dissertation, as a feedback operating between a natural enemy and the density of seedlings within a specific area. Some studies consider juvenile mortality as a function of adult conspecific density (Connell et al 1984, Welden et al. 1991, Webb and Peart 1999). Choosing between seedling vs. adult abundance as the predictive variable for seedling mortality should

reflect the spatial scale that is most relevant for natural enemy-tree interactions. In addition, tree and soil pathogen interactions occur at small spatial scales so seedling density likely is a more relevant metric than adult density when soil pathogens are the agent of mortality.

Although distance and density-dependent mortality is at the core of the J-C Model, determining the relative importance of these processes is difficult because seed density is inversely related to distance from adult in many tree species. Most studies have not distinguished between distance and density effects (Table 1). This may be due to both mechanisms operating simultaneously or because they may operate on different life history stages. One way to investigate these differences is to compare how seedlings respond to soil micro-organisms cultured by adults vs. seedlings since each life history stage may culture unique enemies or may impact the abundance or virulence of the same enemies in a different way (Gilbert 2002). For instance, adult trees can act as a reservoir for host-specific soil pathogens that can kill seedlings. Seedlings likely culture soil pathogens that have long-lived resting spores and/or are also saprophytic since seedlings have patchy spatial distributions and relatively short life span. There is some evidence that seedlings themselves can have an impact on the biota of the soil community. Packer and Clay (2003) found that after ~4 months of *Prunus serotina* seedlings interacting with forest soil at low plant density the feedback between seedlings and the soil microbial community changed from positive to negative.

It is important to distinguish between effects of adults vs. seedlings on disease population dynamics for a few reasons. First, the increase in mortality often associated with high seedling density may have less to do with natural enemies than simple seedling

intra-specific competition. Pathogens may interact with high density simply through 'self-thinning' (intraspecific competition resulting in 'stressed' seedlings that are more susceptible to infection). Alternatively, high density may increase the negative feedback between seedling and soil fungi through increasing fungal populations and disease transmission. Also, not all species have the majority of their progeny dispersed underneath their crown. Seeds that are dispersed by birds or other mammals can have high density far from the parent tree, so it is important to determine how effective NCDD processes are on these species. Temperate species are often wind dispersed but many tropical species are bird/mammal dispersed and may be more susceptible to areas "far" from conspecific adult but with high conspecific seedling density (Clark et al. 1999). By separating out the impact that both adult and seedlings have on natural enemy populations a more mechanistic perspective of the J-C Model can emerge.

Host Specificity – Another assumption of the model is that the natural enemies causing NCDD are host-specific (Janzen 1970). If all species were equally vulnerable to natural enemies then there would be a reduction in successful recruitment for all species and coexistence would not be maintained. The more generalized the natural enemy, the weaker these processes are in maintaining species diversity. Likewise, pathogen dispersal distance must be more restricted than its host distribution (Gilbert 2002). If a species does have a natural enemy that is highly host-specific but dispersal for both the host plants and the pathogen overlap then host plant recruitment will be constrained across the host's range and may result in local extinction.

However, there is limited knowledge of both host-specificity and dispersal for most soil pathogens in forests. An analysis of the polypore (*Aphyllophorales*) community

in a tropical forest in Panama revealed that the most common species were generalists (Gilbert et al. unpublished data cited in Gilbert & Hubbell 1996). However, Augspurger and Wilkinson (2007) have demonstrated that *Pythium,* a common soil pathogen of tree seedlings, varies in pathogenicity among seedlings of different tropical tree species but does not show strict host-specificity. Thus, this intermediate level of specificity suggests that *Pythium* spp. have the potential to have some effect on forest community structure and diversity. There also appears to be localized adaptations between natural enemies (insect herbivores at least) and hosts in tropical forests where specialization occurs at the level of individual reproductive adults resulting in only non-progeny seedlings surviving under the crown of these adults (Langenheim & Stubblebine 1983, Sanchez-Hidalgo et al. 1999). There is preliminary evidence that the pathogen that causes NCDD mortality in black cherry (*Prunus serotina*) may be host-specific (Packer and Clay 2000). This is the only study currently published in the J-C literature where the mechanism of distance and density-dependent mortality patterns has been determined and the natural enemy exhibited host-specificity (Table 1).

Lack of widespread documentation for host-specificity in research of the J-C Model reflects a gap in our investigations rather than proof that host-specificity is rare in natural communities. Strict host-specialization of natural enemies, along with their more limited dispersal, would likely result in the most effective J-C process leading to species coexistence. There is a clear need to begin conducting research on the host-specificity and dispersal range of natural enemies in order to link J-C patterns in a particular species to species coexistence.

Species' Abundance - Maintaining species diversity via NCDD responses requires that these processes are more prevalent in species that are common versus those that are rare, thereby constraining the abundance of common species. Rare species may "escape" NCDD mortality because they have fewer specialist enemies due to their low abundance and unpredictable distributions in time and space (comparable to 'apparency' theory for herbivory; Feeny 1976 and Rhoades & Cates 1976). Conversely, common species, more available as hosts, could be disproportionately targeted by enemies, leading to the community compensatory trend posited by Connell (1971 & 1978). Thus, rare species would have lower pathogen loads than common species regardless of distance or density from conspecifics. Alternatively, rarity could be an advantage, regardless of the strength of NCDD processes, simply because rare species are less likely to encounter areas "cultured" by conspecifics than common species. Both scenarios would operate to promote species coexistence, but their distinction has not been widely recognized. It is also possible that rare species experience stronger NCDD mortality due to pathogens than common species, as supported in grasslands (Klironomos 2002), providing an explanation for species rarity.

In forests, it remains to be elucidated whether natural enemies target common species and keep their populations in check or keep rare species rare. Few studies have explicitly compared the incidence and strength of density-dependent mortality patterns between common and rare species. There is conflicting evidence on the relationship between species abundance and strength of NCCD among the few studies that have done this type of comparison. Negative effects of distance or density on survivorship and/or growth sometimes are reported to be more severe in common vs. rare species (Wills et al.

1997, Webb & Peart 1999, Wills et al. 2006), sometimes the converse (He et al. 1997, Hubbell et al. 2001, Ahumada et al. 2004), and sometimes are pervasive with no relationship to local species abundance (Harms et al. 2000, Peters 2003). It is important to note that most studies have investigated only common species because of inherently low sample sizes in rare species (Wills & Condit 1999, Wills et al. 2004). These conflicting observational results, occasionally from the same study site and investigators, provide strong motivation for experimentally testing specific mechanisms of NCDD and their relationship to species abundance.

It is important to note that there are two very different approaches to testing the relationship between NCDD processes and species' abundance within forest community ecology. First, many studies compare the relationship between the average population level mortality of individuals and a species' abundance within that local community (e.g. Connell et al. 1984, He et al. 1997, Welden et al. 1991, Webb and Peart 1999, Peters 2003). There are two problems with this approach: 1) these studies do not test the actual mechanism for mortality (i.e. vertebrate predator, insect herbivore or pathogens), and 2) all of these studies investigate the relationship between species abundance and NCDD using current species abundance which is a static metric. A preferable approach would be to link trajectories of tree species abundance in a community to disease pressure through time (i.e. do common species become less common due to increased pressure and vice versa). The second approach is to investigate performance for single species among plots characterized by the density of the focal species (e.g. Hubbell and Foster 1986, Condit et al. 1994, Gilbert et al. 1994, Silva Matos et al. 1999, Harms 2000). The problem with this approach is that determining negative density-dependent performance for an individual

species does not necessarily mean that species coexistence can be maintained at the community level. Common species (based upon abundance at the community level) still need to be at a disadvantage in comparison to rarer species for species diversity to be maintained. This disadvantage could be due to NCDD processes only occurring in common species or if NCDD processes occur regardless of species abundance because common species are more likely to encounter areas near or at high density of conspecific individuals.

Temperate vs. Tropical Forests – It is often assumed, but rarely tested, that mechanisms underlying forest dynamics in tropical vs. temperate forests are different. For instance, Janzen (1970) proposed that distance and density-dependent mortality would be greater in tropical vs. temperate forests because warm temperatures, greater rainfall and aseasonality in tropical forests would result in both a higher abundance of natural enemies and a greater proportion of specialist to generalist natural enemies. Givnish (1988) expanded on this idea, and proposed that increases in rainfall and soil fertility as well as decreased seasonality would not only favor herbivores and pathogens but should decrease plant investment in defenses against these natural enemies. However, Gilbert (1995 and 2002) has proposed that fungal specificity may actually decrease in tropical systems because as host diversity increases the selective pressure for specialization may diminish. For instance, Gilbert et al. (2002) found an inverse relationship between host-specificity of wood-decay fungi and tree species diversity when comparing different tropical forests. This relationship between species diversity and fungal host-specificity might be due to a decreased probability of successful colonization of pathogens as their hosts become rare.

For insect herbivores, Basset (1994) showed in feeding trials that tropical insects have greater host-specialization than temperate species. In addition, tropical leaves appear to experience more damage from herbivores than their temperate counterparts, even though tropical leaves tend to be more heavily defended (Coley and Aide 1991). I am unaware of a direct comparison of the degree of soil pathogen host-specificity and the effect of these pathogens on tree species between tropical and temperate forests. However, there is growing evidence that both individual temperate (Packer and Clay 2000) and tropical (Augspurger 1983a-b, 1984, Augspurger and Kelly 1984, Hood et al. 2004, Bell et al. 2006) tree species have higher mortality in the presence of conspecifics due to soil pathogens. Whether these pathogens exhibit host-specificity is unknown, although Packer and Clay (2000) suggest that this is the case for *Prunus serotina*. Hille Ris Lambers et al. (2002) also found density-dependent mortality patterns in six of seven species in a North Carolina temperate forest and when comparing their results to studies conducted in tropical forests (BCI and Pasoh) concluded that density-dependent mortality is prevalent in both systems.

Currently there is no clear consensus on whether the J-C Model operates similarly in temperate and tropical forests even beyond our limited knowledge of host-specificity of natural enemies in both biomes. Investigating the strength and importance of the J-C Model for maintaining species coexistence in both temperate and tropical forests also requires testing the following predictions in both biomes: 1) non-competitive distance and density-dependent processes decrease seedling performance of common species more than rare species, 2) through NCDD processes, the spatial distribution of species will become less clumped through time, 3) there should be greater recruitment of

heterospecific individuals due to NCDD effects on seedlings near conspecific adults, and 4) over time, species diversity should be greater than expected with NCDD processes compared with random survival of seeds and seedlings.

Light and Disease Interaction - Disease in the early life history stages may play an important role in maintaining species coexistence through distance and density-dependent mortality and through heightening light gradient partitioning. These two theories often have been investigated separately or as competing mechanisms for species coexistence (Itoh et al. 1997, Kobe 1999), but this dichotomy is likely more conceptual than biological.

Light availability may mediate disease induced NCDD processes. Seeds (Dalling 2004, O'Hanlon-Manners and Kotanen 2004) and seedlings (Augspurger 1983b, Augspurger 1984, Augspurger & Kelly 1984, Kitajima and Augspurger 1989, Hood et al. 2004) of some species experience higher disease related mortality at low than high light in both shade house and field environments. Increased light availability could interact with pathogen infection in at least three ways, as summarized by Augspurger (1990): 1) unfavorable conditions (e.g. increased temperature, decreased soil moisture and/or an absence of conspecific adult acting as a reservoir) that lower pathogen abundance, 2) seedlings accumulating mass more rapidly to compensate for tissue lost to disease, whereas the same tissue lost in shade might result in death, 3) seedlings reducing exposure to disease through faster lignification rates and/or growth to an invulnerable size (Niinemets & Kull 1998, Seiwa 1998), and 4) seedlings are protected from disease due to increased AMF mycorrhizal colonization in high light habitats (Lovelock & Miller

2002, Gehring 2003, Gamage et al. 2004, Gehring 2004) which in turn suppresses disease (Newsham et al. 1995, Borowicz 2001).

Interspecific differences between growth and/or survival in varying light environments result in species segregating into dominance at different light levels (Hubbell and Foster 1992, Kitajima 1994, Kobe et al. 1995, Pacala et al. 1996, Kobe 1999) which can result in species coexistence. Historically, light gradient partitioning has been viewed primarily based on plant carbon balance due solely to photosynthetic gain vs. respiration cost (Baltzer and Thomas 2007). However, the interaction between light and pathogens, as mentioned previously, may have a large impact on survival and growth that needs to be considered. An inverse relationship between high light growth and low light survivorship has been documented for many temperate and tropical species that correspond to shade tolerance classifications (Kitajima 1994, Pacala et al. 1994, Kobe et al. 1995, Kobe 1999, Walters and Reich 2000). Species susceptibility and/or response to disease may contribute to this trade-off. For instance, shade intolerant species tend to invest in traits that maximize growth (Herms & Mattson 1992, Reich et al. 1998, Walters & Reich 1999) while shade tolerant species invest more in functions that enhance survivorship, such as defense against natural enemies (Coley et al. 1985, Coley & Barone 1996) and carbohydrate storage (Kobe 1997, Myers and Kitajima 2007). Additionally, disease susceptibility has been reported to correlate negatively to shade tolerance classifications (Augspurger and Kelly 1984). Thus, classification of some tree species as shade tolerant or intolerant may have less to do with photosynthesis and respiration in low light than with characteristics that regulate the loss of tissue to all agents, including pathogens (Walters and Reich 1999).

Some of the pathways discussed previously for reduced disease pressure in high light environments may preferentially benefit shade intolerant species in high light. Shade intolerant species can increase photosynthesis more effectively in high light than shade tolerant species and thus they may be able to accumulate mass more rapidly to compensate for disease (Walters et al. 1993, Kitajima 1994). In addition, data from tropical rain forests suggest that early successional (i.e. shade intolerant) species exhibit higher colonization rates and degree of positive growth responses to mycorrhizal infection than late successional (i.e. shade tolerant) species (Siqueira et al. 1998, Zangaro et al. 2003). Since AMF mycorrhizal colonization seems to increase in high light habitats (Lovelock & Miller 2002, Gehring 2003, Gamage et al. 2004, Gehring 2004) then shade intolerant species would likely benefit more through AMF protection from diseases than shade tolerant species.

The interactions between light availability and disease need to be explored further for a better understanding of the J-C Model and its role in species coexistence. For instance, shade intolerant species may have greater susceptibility to pathogens than shade tolerant species which would allow for greater species coexistence through increased niche differentiation (i.e. exaggerating shade tolerance differences among species). In addition, shade intolerant species could be more susceptible to pathogen induced NCDD processes than shade tolerant species. How this would impact the strength of Janzen-Connell processes, since shade intolerant species are a smaller proportion of the total community than shade tolerant species, on species coexistence should be investigated.

Conclusion – Many purported tests for the J-C Model have only looked at the pattern of juvenile mortality but have not determined the mechanism causing that pattern

(Table 1). Also, the majority of studies that have looked at the mechanism causing NCDD have focused on single, common species, and thus do not test that species coexistence can be maintained in the forest community through these processes. Studies that have incorporated both common and rare species have often lacked any information on the mechanism causing J-C patterns and thus any information on host-specificity. It is critical that we progress from simply determining that distance and density-dependent mortality exists to investigating whether these patterns originate from host-specific natural enemies that constrain the abundance of common species but not rare species. Although finding J-C processes in temperate forests does not negate the importance of this mechanism for maintaining species coexistence it is still important to compare between tropical and temperate forests as it increases our knowledge of how communities are formed and structured. In a global context of deforestation and conversion of forests to agriculture and plantations, it is not a merely academic exercise to test soil pathogens as a mechanism for maintaining tree species diversity. For example, plant diseases often are viewed negatively in conservation reserves or managed forests even though they may play a role in the population dynamics of their hosts and the structure and diversity of the communities that they inhabit. Also, without adequate knowledge of plant- pathogen interactions in 'natural' systems, it is more difficult to combat the spread of exotic, invasive pathogens. Disturbance had long been viewed in ecology as a nuisance to understanding equilibrium characteristics of ecosystems. Although disturbance is now viewed as a key mechanism in most ecosystems, disease still is often disregarded. With further studies, background levels of disease may turn out to be as important a mechanism as tree fall disturbances.

Dissertation outline - My research investigated plant-soil feedbacks caused by both biotic (*Fusarium*) and abiotic factors (possibly species-induced differences in base cation availability) in soils that had been cultured by either conspecific or heterospecific adults and seedlings and the effect on survivorship and growth of focal conspecific seedlings. Through a series of shadehouse and greenhouse experiments with both tropical and temperate species, I tested the major assumptions of the Janzen-Connell Model. Chapter 2 focuses on the prevalence and effect of pathogens, derived from soils cultured by conspecific adults and seedlings, on a broad survey of 21 tropical tree species. I found that 9 of the tree species negatively responded to microbial extracts, while 2 species positively responded, and an additional 2 species experienced opposing reductions and increases in seedling life span and/or growth. In addition, species' seedling shade tolerance not their local abundance co-varies with susceptibility to these micro-organisms. This chapter is in press in *Ecology*. In chapter 3 I test whether 4 temperate tree species experience NCDD mortality and/or reduced growth, and if these processes are mediated by host-specific soil microbes and influenced by light availability. I found that species-specific feedbacks between adult trees (not seedlings) and soil (mediated through chemical mechanisms not soil microbes) influenced life span and/or growth for all temperate species. Contrary to the J-C hypothesis, however, heterospecific and conspecific effects had similar prevalence and magnitude of influence on seedling performance. In addition, soil microbes decreased seedling performance for 3 of the 4 species and this negative effect occurred regardless of light availability for some species and for others only in high light. This chapter is in review for publication in *Ecology*. Chapter 4 describes a parallel experiment to the one conducted in chapter 3 but with 6

tropical tree species. Once again I found that species-specific feedbacks between adult trees and soil (mediated through chemical mechanisms not soil microbes) influenced life span and/or growth for all species. Feedbacks from conspecific adults reduced seedling performance relative to all heterospecific adult effects for three out of six tropical tree species, and an additional two species had decreased performance in conspecific vs. two or more heterospecific cultured extracts, supporting the J-C hypothesis. In addition, conspecific seedlings were more likely to be disadvantaged versus heterospecific seedling in soils influenced by a given species. Differences in NCDD processes between temperate and tropical forests are discussed. This chapter will be revised in preparation for review by *Ecology*. Finally, in chapter 5, I discuss the implications of this research for both temperate and tropical forest community dynamics and suggest avenues of research that may answer questions raised by this study.

Table 1.1. Review of relevant published studies on Janzen-Connell processes in tropical and temperate tree species and forest communities. (+) = evidence consistent with hypothesis, (-) = evidence inconsistent with hypothesis, * = mechanism of mortality explicitly tested in study. CCT = community compensatory trend.

Species (Habitat)	Life History State	Hypothesized Mortality Patterns: Density	Distance	CCT	Mechanism of Mortality	Host-Specificity	Source
Platypodium elegans (moist tropical forest, Panama)	Seed		+		Vertebrate	Not examined	Augspurger 1983a
	Seedling		+		Pathogen (damping-off)		
Platypodium elegans (moist tropical forest, Panama)	Seedling	+	+		* Pathogen (damping-off)	Not examined	Augspurger 1983b
Platypodium elegans (moist tropical forest, Panama)	Seedling	+	+		Pathogen (damping-off)	Not examined	Augspurger 1984a
18 Wind-dispersed species (moist tropical forest, Panama)	Seedling	+ 16/18 spp			* Pathogen (damping-off)	No	Augspurger & Kelly 1984
9 Wind-dispersed species (moist tropical forest, Panama)	Seedling	+ 2/9 spp	+ 3/9 spp		Pathogen (damping-off)	Not examined	Augspurger 1984b
Borassus aethiopum (humid savanna, Cote d'Ivoire)	Seedling	-	+		Unknown (assumed resource competition with conspecific adult)	No	Barot et al. 1999

Table 1.1. (Ctd.)

Species (Habitat)	Life History State	Hypothesized Mortality Patterns: Density	Distance	CCT	Mechanism of Mortality	Host-Specificity	Source
Sebastiana longicuspis (moist tropical forest, Belize)	Seedling	+			* Pathogen (damping-off)	Not examined	Bell et al 2006
4 *Shorea* species (tropical rain forest, Indonesia)	Sapling	+ 2/4 spp (weaker effect)	+ 2/4 spp		* Herbivore (did not look at mortality; rather herbivory rates and foliar condition)	Not examined	Blundell & Peart 1998
Philendoptera sutherlandii (coastal scarp forest, South Africa)	Seedling	-	-		Herbivore	Not examined	Boudreau & Lawes 2008
Astrocaryum murumuru	Seed Seedling	+ -	- (1/2 yrs) +		* Invertebrate (bruchid beetle)	Not examined	Cintra 1997
Dipteryx micrantha (moist tropical forest, Peru)	Seed Seedling	+ -	+ -		+ Vertebrate		
Dipterex panamensis (tropical wet forest, Panama)	Seedling	+	+		Unknown (possible herbivore)	Not examined	Clark & Clark 1984

Table 1.1. (Ctd.)

Species (Habitat)	Life History State	Hypothesized Mortality Patterns: Density	Distance	CCT	Mechanism of Mortality	Host-Specificity	Source
Faramea occidentalis	Adult (recruitment)	+	-		Not examined	Not examined	Condit et al 1994
	Adult (survival)	+	+				
Desmopsis panamensis (moist tropical forest, Panama)	Adult (recruitment)	+	+				
	Adult (survival)	-	-				
Dipteryx panamensis (moist tropical forest, Panama)	Seedling		+		Unknown	Not examined	De Steven & Putz 1984
3 shade-tolerant tree species (moist tropical forest, Panama)	Seed	+ 1/3 spp			Unknown	Not examined	De Steven & Wright 2002
Maximiliana paripa (wet tropical forest, Brazil)	Seed	- (low plant mortality at high density when at far distance)	+		* Invertebrate (bruchid beetles)	Not examined	Fragoso 1997
	Seedling & Sapling		+		* Vertebrates (peccary)		

Table 1.1. (Ctd.)

Species (Habitat)	Life History State	Hypothesized Mortality Patterns: Density	Distance	CCT	Mechanism of Mortality	Host-Specificity	Source
Ocotea whitei (moist tropical forest, Panama)	Sapling	+	+		* Pathogen (stem canker)	No similar canker on most other lauraceous species)	Gilbert et al. 1994
Ocotea whitei (moist tropical forest, Panama)	Seedling	+			Unknown (assumed pathogen)	Not examined	Gilbert et al. 2001
53 woody species (moist tropical forest, Panama)	Seed/ Seedling	+			Not examined	Not examined	Harms et al. 2000
7 woody species (temperate forest, USA)	Seed Seedling	+ 5/7 spp + 1/7 spp	+ 3/7 spp + 1/7 spp		Not examined	Not examined	Hille Ris Lambers et al. 2002
10 woody species (temperate forest, USA)	Seed Seedling	+ 4/10 spp - 1/10 spp			Not examined (distribution of seed/seedlings not mortality investigated)	Not examined	Hille Ris Lambers & Clark 2003
Milicia regia (moist, semi-deciduous forest, Ghana)	Seedling		+		* Pathogen (damping-off)	Not examined	Hood et al. 2004
Virola surinamensis (moist tropical forest, Panama)	Seed		+		Vertebrate (rodents) Invertebrate (weevils)	Not examined	Howe et al. 1985

Table 1.1. (Ctd)

Species (Habitat)	Life History State	Hypothesized Mortality Patterns			Mechanism of Mortality	Host-Specificity	Source
		Density	Distance	CCT			
Cornus florida (temperate forest, USA)	Seed	- (dependson *Cornus* and *Ilix* seed density)			Not examined	No	Kwit et al. 2004
Swida controversa (temperate forest, Japan)	Seedling	+	+		Pathogen (damping-off)	Not examined	Masaki and Nakashizuka 2002
Milicia exelsa (dry semi-deciduous forest, Ghana)	Seedling		-		Invertebrate (*Phytolyma* spp.)	Not examined	Nichols et al. 1999
Swietenia macrophylla (tropical forest, Brazil)	Seedling	+ (basal area of adults)			Specialist moth (*Steniscadia poliopliaea*)	Not examined	Norghauer et al. 2006
Prunus serotina (temperate forest, USA)	Seedling	+	+ (weaker effect)		* Pathogen (Pythium spp.)	Yes (limited evidence)	Packer & Clay 2000
14 most common tree species (wet tropical forest, Australia)	> 30 cm GBH	1/14 + 1/14 -	2/14 + 3/14 -	-	Unknown (distribution of juveniles not mortality investigated)	Not examined	Penfold & Lamb 1999

Table 1.1. (Ctd.)

Species (Habitat)	Life History State	Hypothesized Mortality Patterns: Density	Distance	CCT	Mechanism of Mortality	Host-Specificity	Source
544 woody species (tropical forest, Malay)	Adult	+ 255/544 spp			Not examined	Pattern suggests yes (not explicitly tested)	Peters 2003
188 woody species (moist tropical forest, Panama)	Adult	+ 89/188 spp					
Acer pseuoplatnus (temperate forest. Britain)	Seedling	+	+		Invertebrate (slug)	Not examined	Pigot & Leather 2008
Faramea occidentalis (moist tropical forest, Panama)	Seed		+		Vertebrate	Not examined	Schupp 1988
	Seedling		-		Desiccation Vertebrate		
4 species of *Carpinus* (temperate forest, Japan)	Seed	+ 3/4 spp	– 4/4 spp		Not examined Pathogen	Not examined	Shibata & Nakashizuka 1995
	Seedling	+ 1/4 spp	+ 2/4 spp		(damping-off)		
Euterpe edulis (semi-deciduous tropical forest, Brazil)	Seedling	+	+		Not examined	Not examined	Silva Matos et al. 1999
Tabebuia ochracea (dry tropical forest, Costa Rica)	Sapling	+			Invertebrate (*Cromarcha stroudagnesia*)	Not examined	Sullivan 2003

Table 1.1. (Ctd.)

Species (Habitat)	Life History State	Hypothesized Mortality Patterns: Density	Distance	CCT	Mechanism of Mortality	Host-Specificity	Source
Fagus crenata (temperate forest, Japan)	Seed	+	+		Vertebrate Pathogen	Not examined	Tomita et al. 1999
Euterpe edulis (wet tropical forest, Brazil)	Seed		-		Vertebrate (rodent) Invertebrate (scolytidae beetle)	Not examined	von Allmen et al. 2004
149 woody species (seasonally dry forest, Indonesia)	Seedling	+		+	Unknown	Not examined	Webb & Peart 1999
84 woody species (moist tropical forest, Panama)	≥ 1cm DBH	+ 76/84 spp		+	Unknown	Not examined	Wills et al. 1997
200 woody species (moist forest, Panama & Malasia)	≥ 1cm DBH			+	Unknown	Not examined	Wills & Condit 1999
Scheelea zonensis (moist tropical forest, Panama)	Seed		+		Invertebrate (bruchid beetle)	Not examined	Wright 1983
Iriartea deltoidea	Seed		+		Invertebrate (bruchid beetle)		
Astrocaryum murumuru (moist tropical forest, Peru)	Seed		+		Vertebrate (peccary) & Invertebrate (bruchid beetle)	Not examined	Wyatt & Silman 2004

CHAPTER TWO

TOLERANCE OF SOIL PATHOGENS CO-VARIES WITH SHADE TOLERANCE ACROSS SPECIES OF TROPICAL TREE SEEDLINGS

Abstract

A negative feedback between local abundance and natural enemies could contribute to maintaining tree species diversity by constraining population growth of common species. Soil pathogens could be an important mechanism of such noncompetitive distance and density-dependent (NCDD) mortality, but susceptibility to local pathogens may be ameliorated by a life history strategy that favors survivorship. In a shade-house experiment (1% full sun), we tested seedling life span, growth, and mass allocation responses to microbial extract filtered from conspecific-cultured soil in 21 tree species that varied in abundance and shade tolerance in a wet tropical forest (La Selva Biological Station, Costa Rica). Forty-three percent of the species had significant reductions and 10% of the species significant increases in life span, growth, root length, or root surface area when inoculated with microbial extract; 10% of the species experienced opposing reductions and increases in these characteristics. Contrary to expectation, species' local abundance was not related to species-specific responses to microbial extracts from cultured soils. Across species, seedling shade tolerance (survival at 1% full sun) was negatively correlated with susceptibility to the microbial treatment for both survival and

total mass accumulation, thereby exaggerating shade tolerance differences among species. Thus, soil pathogens may contribute to species coexistence through heightening niche differentiation rather than through negative density dependence in common species.

Key words: common vs. rare species; density dependence; Janzen-Connell hypothesis; plant–soil feedback; shade tolerance; soil pathogens; species coexistence; tropical forests.

Introduction

Identifying the mechanisms that maintain tree species richness is a central question in plant community ecology. Under the competitive-exclusion principle, competitively superior species exclude inferior species in the absence of niche partitioning (Gause 1934). Although there has been a vast array of hypotheses proposed for how competitive exclusion can be precluded (Palmer 1994), one of the most influential was put forth independently by Janzen (1970) and Connell (1971). They proposed that host-specific natural enemies could maintain high tree diversity of tropical forests by reducing seed and/or seedling survivorship near conspecific adults and/or at high conspecific densities. Such noncompetitive distance- or density-dependent (NCDD) mortality would favor establishment of heterospecific individuals, thus promoting species coexistence.

Soil pathogens (e.g., fungi, bacteria, and/or nematodes) could be an important mechanism of NCDD seedling mortality and/or reduced growth because many of these pathogens show strong host specialization, short generation times, high fecundity, long

persistence in soil, and more limited dispersal than their hosts (Agrios 1997, Gilbert 2002). These characteristics could underpin the mechanisms that create negative feedback when individual plants "culture" the soil microbial community in which they grow to the detriment of themselves and other conspecific individuals (van der Putten et al. 1993, Bever 1994, Mills and Bever 1998, Klironomos 2002). Although soil pathogens have a long history of study in forestry and horticulture, their potential role as a mechanism of NCDD mortality in the dynamics of natural plant communities has only recently been more widely recognized (Gilbert and Hubbell 1996, Packer and Clay 2000, 2003, Hood et al. 2004, Bell et al. 2006).

Maintaining species diversity via NCDD responses requires that these processes are more prevalent in species that are common vs. those that are rare, thereby constraining the abundance of common species. There are at least two distinct mechanisms through which species abundance could influence NCDD responses. First, rare species may "escape" NCDD mortality because they have fewer specialist enemies due to their low abundance and unpredictable distributions in time and space (comparable to "apparency" theory for herbivory [Feeny 1976, Rhoades and Cates 1976]). From this same view, common species, more available as hosts, could be targeted disproportionately by enemies, leading to the community compensatory trend posited by Connell (1971, 1978). Thus common species would be expected to experience greater impact from enemies than rare species, regardless of distance or density from conspecifics. Under the second mechanism, rarity could be an advantage, even if rare and common species experience similar NCDD processes, simply because rare species are less likely to encounter areas "cultured" by conspecifics than common species. Both

mechanisms would operate to promote species coexistence, but their distinction has not been recognized. It is also possible that rare species experience stronger NCDD mortality due to pathogens than common species, as supported in grasslands (Klironomos 2002), providing an explanation for species rarity.

Among tropical trees, there is conflicting evidence on the relationship between species abundance and strength of NCDD. Negative effects of distance or density on survivorship and/or growth sometimes are reported to be more severe in common vs. rare species (Wills et al. 1997, 2006, Webb and Peart 1999), sometimes the converse (He et al. 1997, Hubbell et al. 2001, Ahumada et al. 2004), and sometimes are pervasive with no relationship to local species abundance (Harms et al. 2000, Peters 2003). It is important to note that most studies have investigated only more common species because of inherently low samples sizes in rare species (Wills and Condit 1999, Wills et al. 2004). Nevertheless, these conflicting observational results, occasionally from the same study site and investigators, provide strong motivation for experimentally testing specific mechanisms of NCDD and its relationship to species abundance.

Species-specific traits may also influence susceptibility to and/or impact of disease. For instance, shade-intolerant species tend to invest in traits that maximize growth (Herms and Mattson 1992, Reich et al. 1998, Walters and Reich 1999) while shade-tolerant species invest more in functions that enhance survivorship, such as defense against natural enemies (Coley et al. 1985, Coley and Barone 1996) and carbohydrate storage (Kobe 1997, Myers and Kitajima 2007). Thus, the expectation is that shade-intolerant species could be more susceptible to disease than shade-tolerant species (Augspurger and Kelly 1984). Similarly, species with larger seed mass may offset

disease-related losses during germination and establishment (Foster 1986, Armstrong and Westoby 1993).

The spatial scale of NCDD processes depends upon the biology of the particular natural enemy and host plant. Because soil-borne pathogens have limited dispersal (Agrios 1997, Gilbert 2002), we expect that soil "culturing" operates at a spatial scale commensurate with the area occupied by an individual canopy tree. From the vantage point of understanding species coexistence, density-dependence must extend beyond the scale of a single tree and is most relevant at the local community level.

The purpose of this study was to determine the prevalence and effect of pathogens, derived from soils cultured by conspecific adults and seedlings, through a broad survey of tropical tree species. This survey served as the basis for selecting species that were included in a subsequent experiment focused on the effects of soils cultured by conspecific vs. heterospecific individuals (McCarthy-Neumann and Kobe, *in review*; Chapter 3). In the present study, we tested the following hypotheses: (1) Seedling survival and growth decrease with soil microbial extract cultured by conspecific adults and seedlings vs. sterilized extract. (2) A species' vulnerability to soil pathogens increases with its abundance. (3) Among species, vulnerability to soil pathogens declines with increasing shade tolerance.

Materials and Methods

Field site.—This research took place at La Selva Biological Station (Sarapiquí Region, Costa Rica) operated by the Organization for Tropical Studies. La Selva is a 1510-ha reserve of diverse (400+ tree species), wet, tropical forest receiving ~ 4000 mm

of rain annually with a mean annual temperature of 25.88C (Hartshorn and Hammell 1994). Per distributions of focal species, we primarily collected residual soils of volcanic origin, which are the most common at La Selva (Sollins et al. 1994) and which are representative of other tropical areas. We collected alluvial soil for the study species (*Castilla*, *Luehea*, and *Neea*) that occur only under these conditions.

Species.—In 10-week-long experiments, undertaken from April 2004 to July 2005, we assessed survivorship and growth responses of seedlings of 21 tree species (Table 2.1) to soil pathogens in a shade-house experiment. Henceforth, we refer to species by genus name. We randomly selected species from those encountered in a 5.5-year field study of natural seedling dynamics (R. K. Kobe and C. F. Vriesendorp, unpublished manuscript), with species selection stratified across local abundance (based upon adult and seedling density), local dominance (basal area), seedling shade tolerance, and dry seed mass (determined from ~ 20 randomly selected seeds with emergent radicles for each species). Adult abundance was assessed as the density of $\geq$ 5 cm dbh individuals (number/ha) within three 41 x 240 m mapped stands and seedling abundance as mean standing seedling density over 5.5 years within a 1 x 200 m belt transect located in the middle of each stand (R. K. Kobe and C. F. Vriesendorp, unpublished manuscript). We characterized species shade tolerance as the probability of seedling survival at 1% full sun and zero conspecific seedling density, calculated from mortality models that were calibrated from survival time data of naturally established seedlings in 1-m2 quadrats that were censused every six weeks as part of the same 5.5-year field study (C. F. Vriesendorp and R. K. Kobe, unpublished manuscript). Light availability was assessed as percent canopy openness estimated from hemispherical canopy photos for each quadrat measured

twice during this period) and density was expressed as the mean conspecific density experienced by a seedling over its lifetime. By evaluating the mortality models at zero density, we are removing NCDD effects on seedlings from the estimates of shade tolerance. Sample sizes for model calibration for the species of interest ranged from 13 to 6051 seedlings (median N = 119 seedlings). Survival analysis and maximum likelihood techniques were used to estimate the parameters for the survival models, generally following methods in Kobe (1999). Due to low sample sizes, survival models have not been developed for four study species. For these species, shade tolerance was interpolated to our scale based on published low-light mortality (*Luehea* [R. K. Kobe, unpublished data], *Miconia* [Pearson et al. 2003], *Stryphnodendron* [Guariguata 2000], and *Trophis* [Kobe 1999]).

Soil and seed collection.—We derived microbial extract from areas predicted to have the strongest negative effect, i.e., near adults and at high seedling density. For each species, we removed a 10 cm diameter by 30 cm deep soil core from within 1 m of the bole of four randomly selected conspecific adults, with a dbh at $\geq$ 75th percentile for that species. For further culturing, each soil core was planted with four conspecific seedlings at 1% full sun for eight weeks or until all seedlings had died, whichever occurred first. Soil was stored at 4°C until seeds were available for planting. Seeds were collected within 10 m of trails throughout La Selva. Seeds were surface sterilized (0.6% NaOCl for three minutes), rinsed with deionized water and germinated in either ziplock bags with peat moss or petri dishes with filter paper in partial sun. Prior to planting, seeds were surface sterilized with NaOCl for 30 s, rinsed with deionized water, air-dried for 15 min, and weighed. Because seeds were unavailable, we used recently germinated *Coussarea*

and *Vochysia* field-collected seedlings, which were sterilized for 30 s with 0.6% NaOCl and rinsed with deionized water.

Microbial extraction and planting.—Soil microorganisms (excluding arbuscular mycorrhizal fungi, AMF [Sylvia 1994]) were extracted from cultured soil using a wet-sieving method adapted from Klironomos (2002), which cull particles < 20 μm. For each extraction, 30 g of soil was blended with 200 ml of tap water for 30 s. The liquid suspension was washed through 250-, 45-, and 20- μm analytical sieves with tap water, but keeping the extract to ≤ 450 ml. Sieves were cleaned ultrasonically between each extraction.

On the same day, seeds with newly emerged radicles were planted in a 1:4 mixture of sterilized field soil and commercial peat moss (Nutripeat, Sun Gro Horticulture Canada Ltd., Vancouver, British Columbia, Canada). Field soil was collected from a common pit in a residual, secondary forest at La Selva and was autoclaved for 1 h at 121°C followed by a 2-day incubation and a second autoclaving. Each seedling was randomly assigned to an extract from one of four soil cores (each core collected near a different conspecific adult and kept separate to test for effect of individual tree) and received either 100 ml nonsterilized extract (microbial treatment) or 100 ml extract that was autoclaved for 20 min at 121°C (control). We did not add AMF spores (collected on the 45-μm sieve) because they enhanced seedling mortality under similar conditions in a previous experiment (S. McCarthy-Neumann and R. K. Kobe, unpublished data).

Experimental treatments and seedling measurements.—To summarize, experimental treatments consisted of 21 tree species, four or two conspecific adults where

soil was collected, and two soil extract treatments (nonsterile vs. sterile). Soil for *Dussia*, *Quararibea*, and *Virola* were collected from only two conspecific adults due to limited seed availability; all other species had four conspecific adult locations. The average number of seedling replicates was 7.5 replicates per extract treatment per adult because half were replicated eight times and half were replicated seven times. The 1170 seedlings were randomly assigned to each of the six benches with the criterion that each bench had five replicates per extract treatment per species. To mimic understory irradiance (Chazdon and Fetcher 1984), potted seedlings were placed in two shade houses at ~1% full sun. We confirmed light levels with paired PAR (photosynthetically active radiation) measurements in the open and at each shade house bench with a LI-COR 250A quantum sensor (LI-COR, Lincoln, Nebraska, USA) on a uniformly overcast day. Emergence and survival were censused three times each week, height was measured weekly, and seedlings were watered (~50 ml of deionized water) by hand twice weekly throughout the experiment. We assigned date of death as the first census with total leaf and/or stem tissue necrosis. To determine mass and mass allocation, we harvested seedlings surviving to the end of the experiment, washed soil from roots, and divided seedlings into root, stem, and leaf fractions. Necrotic tissue was not included. Roots (except *Colubrina*, *Quararibea*, and *Virola*) were scanned (Epson Perfection 1260; Epson America, Long Beach, California, USA) at high resolution (400 dpi), colored black in Photoshop Plus (Adobe Systems, San Jose, California, USA) for enhanced image contrast and analyzed using WinRHIZO system version 5.0 (Regent Instruments, Blain, Quebec, Canada) for length and surface area. Tissue was oven-dried at 70°C to constant mass and weighed.

To test for unintended nutrient differences between treatment and control, we measured nitrogen concentrations (as the percentage of oven-dried leaf mass) with a CHN Analyzer (Carlo Erba Instruments, Milan, Italy) for 12 of the 21 species (N = 10 seedlings for each species–treatment group).

Statistical analysis.—Results for all analyses were considered significant at $P < 0.10$ because the study's short duration and low light conditions limited the threshold for treatment effects that could be detected, especially in growth and allocation.

Emergence time and life span, the latter of which includes mortality during both pre- and post-aboveground emergence stages (preemergence mortality was estimated as the mean emergence date for seedlings of each species), were compared between microbial and sterile treatments using the Breslow χ^2 test of homogeneity in a Kaplan-Meier survival analysis (SPSS version 14.0; SPSS, Chicago, Illinois, USA). The Breslow χ^2 was adjusted for the effect of adult in soil collection and/or bench when these terms had a P value ≤ 0.25.

We tested for main treatment (extract and adult) effects and their interactions on growth, allocation and root morphology with ANCOVA using bench as a blocking factor (SPSS) and seed mass as a covariate in the growth and root morphology analyses and total plant mass as a covariate in allocation analysis. Root mass also was used as a covariate in root morphology analysis. We ran full models (extract, adult, bench, covariate and their interactions) for each dependent variable and species and determined that the covariate effects were independent of treatment effects ($P > 0.05$). Thus, main treatments X covariate interaction terms were removed. If adult, bench, covariate or extract X adult terms were insignificant beyond the threshold suggested for pooling

variances $P > 0.25$ (Bancroft 1964), then the highest order term with the highest P value was removed and the analysis was run with the reduced model. This process was repeated until all terms with $P > 0.25$ were removed. Adjusted means of treatments were compared under ANCOVA and raw means under ANOVA. We used a 2-tailed independent t test for each species (SPSS) to test for unintended treatment differences in foliar N concentrations.

We used fixed effects meta-analysis (MetaWin, version 1.0; Sinauer Associates, Sunderland, Massachusetts, USA) to test effects of seedling shade tolerance, adult and seedling abundance, species basal area, and seed size on species sensitivity to the soil microbial extracts. Each species was placed into one of three categories for each of these characteristics, and we tested whether effect size (mean difference between treatment and control means, weighted by each group's sample size) differed among categories using a between-class homogeneity statistic (Q_b; Gurevitch and Hedges 1993) for life span, total mass, organ mass, mass allocation, and height and root morphology. In parallel, with multiple stepwise linear regression we tested for relationships between species characteristics (Table 2.1) and changes in seedling performance ([(mean total mass) X (mean life span)] / [number of days of experiment] in the microbial treatment relative to the control).

A few seedlings (2.5%) were not used for some analyses due to accidental loss of seedling tissue prior to weighing (15 seedlings), failure to scan roots (14 seedlings), or other factors (two seedlings were uprooted during watering; one *Pentaclethra* seedling had a 40% larger seed size than the second largest seed for that species).

Results

Emergence and Survival.—Life span in the microbial treatment was significantly lower for seedlings of *Luehea*, *Coussarea* and *Prestoea* (23%, 13%, and 10%, respectively) and was higher for *Iriartea* and *Welfia* (14% and 5%, respectively) compared to the sterile treatment (Table 2.2 and Appendix A). Soil microorganisms did not influence emergence time for any species.

Plant Mass and Allocation.—Microbial treatments reduced total mass for *Apeiba* (15%), *Castilla* (8%) and *Pentaclethra* (18%) seedlings ($P < 0.10$; Table 2.2 and Appendix B) and also impacted mass of individual organs in other species, which may influence future performance. Three species (*Guatteria*, *Trophis*, and *Iriartea*) had lower root mass, two species (*Coussarea* and *Prestoea*) lower stem mass, five species (*Apeiba*, *Castilla*, *Prestoea*, *Quararibea*, and *Virola*) lower leaf mass and one species (*Castilla*) lower root length and surface area in response to the microbial treatment. Only two species increased organ mass (*Dussia* for stem and *Prestoea* for cotyledon) in the microbial treatment (Table 2.2). Distribution of mass among organs was impacted by microbial extract for eight species but, there was considerable variation among species in which organ mass was impacted and whether the response was positive or negative (Table 2.2). *Castilla* and *Euterpe* seedlings decreased specific root length and *Castilla* reduced specific root surface area in the microbial vs. sterile treatment (Table 2.2). Growth and mass allocation were affected by which adult cultured the soil for 17 and 16 of the study species, respectively (Appendices B-C). Likewise, initial seed mass was a significant covariate in most growth responses for all species except *Quararibea* and

Miconia (Appendix B). Mean foliar N concentrations did not differ between treatment and control for any species (results not shown).

Meta-analysis: Functional Group Comparisons.—Contrary to expectation, common species (whether measured as adult density, seedling density, or species basal area) were not more responsive to the microbial treatments than relatively rare species with respect to emergence time (results not shown), life span (Figure 2.1A) or total plant mass (Figure 2.1B). However, species shade tolerance was associated with seedling response to the microbial treatment, which lengthened seedling life span for shade-tolerant species but not intermediate and intolerant species (d = 0.33 vs. -0.10 and -0.14; Q_b = 13.33, $P < 0.001$; Figure 2.1A). The microbial treatment also reduced total mass in shade-intolerant species and their response differed from that of tolerant species (d = -0.32 versus 0.15; Q_b = 8.31, P = 0.02; Figure 2.1B). Species with large seed mass also had a longer life span in the microbial treatment compared to medium- or small-seeded species (d = 0.27 versus -0.05 and -0.11; Q_b = 7.46, P = 0.02; Figure 2.1A).

Leaf mass (d = -0.21), leaf mass fraction (d = -0.16), and stem height (d = -0.15) were reduced in the microbial vs. sterile treatment overall but there were no differences in responses among any of the meta-analysis groupings (Appendices E and F). In addition, groupings did not differ in organ mass, mass allocation or root morphology responses to the microbial treatment (Appendices E-G).

Species shade tolerance was the only tested characteristic that was related to species sensitivity to the microbial treatment (assessed as percentage change in seedling performance in the treatment relative to the sterile control) (F = 15.79, df = 1,19, P = 0.001, R^2 = 0.45; Figure 2.2). Shade tolerance was correlated with seed size for these

species ($F = 5.15$, $df = 1,19$, $P = 0.04$, $R^2 = 0.21$), but seed size was not related to sensitivity to the microbial treatment even when shade tolerance was not included in the regression.

Discussion

Species susceptibility to soil microorganisms in low light was inversely correlated with seedling shade tolerance (Figures. 2.1 and 2.2), supporting our third hypothesis. Shade-intolerant species generally had reduced total mass in the microbial treatment (Figure 2.1B) whereas shade-tolerant and large seeded species had increased life span (Figure 2.1A). Thus, NCDD via soil microorganisms exaggerated differences in seedling shade tolerance, leading to enhanced potential for tree species coexistence through light gradient partitioning in the presence of soil microorganisms. Similarly, plant-soil feedbacks could be partially responsible for the segregation of tropical tree seedlings along light gradients found in recent experimental field studies (Kobe 1999, Montgomery and Chazdon 2002). However, because our experiment took place solely under low light, our inferences are limited to how soil microorganisms influence species' shade tolerance, but not performance at high light.

Heightened vulnerability of shade-intolerant species to the microbial treatment could reflect lower investment in defense (e.g., weak leaves, fewer secondary metabolites, and reduced carbohydrate storage [Coley et al. 1985, Coley and Barone 1996, Myers and Kitajima 2007]). These characteristics, often found in shade-tolerant species, have been documented to defend against insect herbivores but they may also help protect seedlings from disease. Soil fungal pathogens are likely the agent causing the

negative response in shade-intolerant species to the microbial treatment. These seedlings often had symptoms characteristic of damping-off (i.e., necrotic roots or stem tissue at root collar and/or leaf discoloration and wilting) and we have isolated several fungal pathogens (including species of *Fusarium* and *Rhizoctonia*) from a subsequent experiment using a subset of these species and the same extraction methodology.

Our results suggest that the negative effect of the microbial treatment outweighed any positive microbial influences for the shade-intolerant species. In contrast, in shade-tolerant species, the positive impacts of the soil microbial community (absence of AM fungi) appear to outweigh their negative impacts, for a positive net effect. We investigated whether the positive response in shade-tolerant and large seeded species to the microbial treatment could be due to microorganisms involved in nutrient cycling by measuring foliar nitrogen (N). However, N concentration did not differ between treatment and control for any species investigated and were generally high (mean = 1.7%-7.7%), suggesting that N was not limiting. Other nutrients or microorganisms could differ between the treatment and control such that the elimination of the soil microbial community led to decreased performance in these species. However, a particular factor has yet to be identified.

The covariance between shade tolerance and disease resistance documented here is consistent with the correlation between wood density (as a proxy for shade tolerance) and disease resistance in 18 tropical tree species (Augspurger and Kelly 1984). The soil used in their study was from a single common soil pit suggesting either that generalist pathogens were causing mortality or that host-specific pathogens were ubiquitous. In contrast, the present study used soil that was cultured by conspecific trees in order to

assess the potential for neighborhood-scale negative feedbacks between soil pathogens and tree seedlings. It is unknown, however, whether the study tree species vary in susceptibility and/or response to a few soil-borne pathogen species or whether there are many host-specific pathogen species, each with a unique feedback with a tree species. The former scenario is probably more likely considering that Augspurger and Wilkinson (2007) demonstrated that *Pythium,* a common soil pathogen, varies in pathogenicity among seedlings of different tropical tree species but does not show strict host specificity. A similar result was found with 4 temperate tree species and five *Fusarium* morphotypes (McCarthy-Neumann and Kobe, *in review*; Chapter 3).

We acknowledge the possibility that our estimates of shade tolerance could include some contribution from pathogens, but only to the extent that the pathogens are independent of conspecific density (since we removed density effects from the model estimates of low-light survivorship). However, it is highly unlikely that our estimates of shade tolerance arise from species differences in susceptibility to density-independent pathogens, which in turn led to a relationship between apparent shade tolerance and sensitivity to pathogens. First, many soil pathogens are passively spread and thus their transmission is density dependent. Second, species differences in shade tolerance arise from variation in morphological and physiological traits, including leaf-level gas exchange, whole-plant mass allocation to organs, and allocation to carbohydrate storage (Reich et al. 1998, Poorter and Rose 2005, Myers and Kitajima 2007). Given this well-founded body of knowledge, it is very unlikely that density-independent pathogens are the major cause of species differences in shade tolerance. Nevertheless, interactions between shade tolerance and species susceptibility to soil pathogens likely influences

species distributions across light gradients, especially since soil-borne diseases on tree seedlings appear to be higher in the shaded understory than in canopy gaps (Augspurger and Kelly 1984, Augspurger 1984). This potential spatial covariance between shade and soil-borne diseases together with the co-varying species traits of susceptibilities to shade and pathogens would act in concert to exaggerate differences among species in habitat preferences.

Species sensitivity to the microbial treatment, as assessed with seedling performance, was not related to local species abundance. These results are similar to findings in other tropical forests in seed to seedling transitions (Harms et al. 2000) and adult tree mortality (Peters 2003). In our study, both rare and common species are equally attacked by natural enemies in conspecific influenced neighborhoods, which runs counter to our second hypothesis and suggest that plant-soil microbial feedbacks are not consistent with the Janzen–Connell hypothesis. However, we have not eliminated the possibility that rare species could have an advantage over common species because their seedlings are less likely to encounter soils with host-specific pathogens due to the low density of conspecifics "culturing" these pathogens. If this community level dynamic is occurring then plant-soil microbial feedbacks could still facilitate tree species coexistence through Janzen-Connell processes. Most studies, including our own, investigate the relationship between species abundance and NCDD using current species abundance which is a static metric. A preferable approach would be to link trajectories of tree species abundance in a community to disease pressure through time (i.e., do common species become less common due to increased pressure and vice versa).

The relationship between species abundance and susceptibility to soil microorganisms could be dependent on the spatial scale at which abundance is determined. The appropriate scale for determining abundance for our study was the community since our focus was on species coexistence. Additionally, the spatial scale of interactions between trees and natural enemies depends strongly on the mobility of the enemies. Interactions between plants and the soil community appear to be quite local given that there was significant variation in seedling response to soil cultured by different trees of the same species. However, the effect of these feedbacks should be manifested at the community level per the Janzen-Connell model. Although it is difficult to place spatial boundaries on a community, 1-ha plots are likely an adequate, albeit arbitrary, representation. Even if we had quantified abundance from smaller plots, relative species rankings in abundance would not have changed (e.g., *Pentaclethra* and *Welfia* both would still be common with *Pentaclethra* having intermediate susceptibility among species and *Welfia* having a positive response to soil microorganisms). Regardless of scale, it is unlikely that soil pathogens target common species preferentially since susceptibility was not related to abundance.

There was considerable variation among the 21 species in the way that they responded to the microbial treatment. Eight of these species responded to soil microorganisms cultured by conspecific adults and seedlings through changes in their life span or total mass. An additional five species responded solely through changes in individual organ mass or root morphology. Although about half of the study species exhibited some form of negative feedback with the soil microbial community cultured by

conspecific individuals, our first hypothesis was only weakly supported since there were also a few species that exhibited positive feedbacks.

Our results support the accumulating evidence in field and greenhouse studies that negative feedbacks between plants and soil microorganisms can be an important mechanism impacting community dynamics in both tropical (Hood et al. 2004, Bell et. al 2006) and temperate (Packer and Clay 2000, 2003 and 2004, Reinhart et al. 2005, McCarthy-Neumann and Kobe, in review; Chapter 3) forests as well as grassland (Bever 1994, Mills and Bever 1998, Klironomos 2002) and dune (van der Putten et al. 1993) ecosystems. In addition, our results suggest that considerable spatial and temporal heterogeneity in plant-soil interactions likely exist in tropical forests since the seedling response to conspecific cultured soil was influenced by variability among individual adults for a majority of species. Moreover, our multi-species study determined that life history characteristics of the plant species (e.g., shade tolerance and to a lesser extent seed size) are important factors in how the plant responds to the feedback and thus, links plant-soil feedbacks with light gradient partitioning.

Our results are likely conservative due to limitations of our experimental design. Seedling growth was highly constrained by low light and the experiment's short duration, thus constricting differentiation between treatments. However, the low-light condition may have enhanced the mortality response of seedlings to the microbial treatment since low light has been shown to increase the negative effects of pathogen infection (Augspurger and Kelly 1984, Augspurger 1984). In addition, soil storage at 4°C prior to microbial extract filtration may have decreased the abundance and/or influenced the composition of the soil microbial community. We also did not consider seed density as a

metric for species abundance even though soil pathogens can cause high mortality at the preemergence seed state (Forget et al. 2004). Similarly, we focused on soil pathogens as the natural enemy and the strength of numerous other density-dependent processes (e.g., foliar pathogens, insect herbivores, and vertebrate predators) could still covary with species abundance and create NCDD patterns in forest communities.

Conclusions.—Among species, there were widespread negative feedback responses for seedlings inoculated with soil microorganisms cultured by conspecifics, but some species also responded positively. Contrary to expectations, however, local species abundance did not impact soil microbial susceptibility. The impact of soil microorganisms, however, was most strongly related to shade tolerance, which critically influences the dynamics of forest communities (Kobe et al. 1995, Pacala et al. 1996). Thus, rather than soil microorganisms causing negative feedbacks that constrain the abundance of common species, our results suggest that soil microorganisms may exaggerate seedling shade tolerance differences among species which in turn may influence species coexistence through enhancing light gradient partitioning.

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Table 2.1. Local community characteristics of the 21 tropical tree species used to assess the prevalence and patterns of soil-pathogen induced mortality.

Species	Shade tolerance	Adult density†	Seedling density†	Adult basal area†	Seed mass†
Luehea seemannii§	Intolerant (42.7)	Common (18.3, NA)	Rare (0.0, NA)	Common (1.26, NA)	Small (1, 1)
Neea psychotroides	Intolerant (24.0)	Common (41.7, NA)	Common (273.5, NA)	Intermediate (0.19, NA)	Small (24, 4)
Apeiba membranacea	Intolerant (38.9)	Intermediate (3.4, 0.6)	Intermediate (42.3, 67.7)	Intermediate (0.17, 0.13)	Small (8, 4)
Colubrina spinosa	Intolerant (35.3)	Intermediate (5.8, 7.5)	Common (1893, 3276)	Rare (0.05, 0.07)	Medium (25, 8)
Guatteria diospyroides	Intolerant (20.4)	Intermediate (5.1, 3.0)	Intermediate (51.3, 35.6)	Rare (0.03, 0.02)	Medium (77, 7)
Psychotria panamensis	Intolerant (24.5)	Intermediate (6.8, 0.6)	Intermediate (74.8, 97.8)	Rare (0.04, 0.00)	Medium (46, 10)
Castilla elastica	Intolerant (29.2)	Rare (2.0, NA)	Rare (0.0, NA)	Intermediate (0.09, NA)	Medium (159, 27)
Coussarea hondensis	Intermediate (15.3)	Common (24.7, 11.8)	Common (1233, 1147)	Intermediate (0.18, 0.09)	Medium (128, 35)
Pentaclethra macroloba	Intermediate (18.1)	Common (69.4, 9.9)	Common (761.0, 197.3)	Common (10.13, 2.3)	Large (3697, 913)
Prestoea decurrens	Intermediate (13.4)	Common (17.9, 18.1)	Intermediate (142.5, 154.2)	Rare (0.05, 0.05)	Medium (167, 22)
Quararibea bracteolosa‡	Intermediate (15.0)	Rare (2.7, 2.1)	Rare (4.3, 7.5)	Rare (0.05, 0.05)	Large (494, 97)
Trophis racemosa§	Intermediate (12.0)	Rare (0.3, 0.6)	Rare (0.8, 1.4)	Rare (0.00, 0.00)	Medium (58, 19)
Vochysia ferruginea	Intermediate (15.4)	Rare (2.0, 0.0)	Common (383.3, 598.9)	Intermediate (0.19, 0.30)	Small (32, 4)

Table 2.1 (Ctd)

Capparis pittieri	Tolerant (5.8)	Common (21.0, 10.2)	Common (277.7, 250.9)	Intermediate (0.12, 0.07)	Large (721, 107)
Euterpe precatoria	Tolerant (10.3)	Common (20.3, 2.7)	Intermediate (50.8, 36.6)	Intermediate (0.13, 0.27)	Large (370, 42)
Iriartea deltoidea	Tolerant (3.0)	Common (68.7, 30.3)	Intermediate (119.8, 31.7)	Common (1.08, 0.37)	Large (3007, 450)
Welfia regia	Tolerant (7.0)	Common (44.4, 8.5)	Common (1803, 1592)	Common (0.94, 0.22)	Large (1729, 313)
Virola koschnyi	Tolerant (5.2)	Intermediate (7.8, 0.6)	Intermediate (95.3, 16.8)	Common (0.36, 0.20)	Large (1766, 261)
Dussia macrophyllata	Tolerant (5.6)	Rare (2.7, 2.3)	Intermediate (48.3, 83.3)	Intermediate (0.24, 0.34)	Large (3500, 851)
Miconia affinia§	Tolerant (10.0)	Rare (2.7, 0.6)	Rare (15.5, 26.8)	Rare (0.02, 0.02)	Small (0, 0)
Stryphnodendron microstachyum§	Tolerant (10.0)	Rare (2.4, 1.6)	Rare (3.8, 6.2)	Common (0.38, 0.44)	Medium (62, 21)

Notes: Species are sorted by seedling shade tolerance classification and then adult abundance. Values in parentheses are mean ± SD. Shade tolerance is the percentage mortality of seedlings at 1% full-sun and with zero conspecific seedling density: shade tolerant, <10.5%; shade intermediate, 10.5-20%; and shade intolerant, >20%. Adult density is the number of individuals (with dbh ≥ 5 cm) per hectare; rare, < 3 individuals/ha; intermediate, 3-10 individuals/ha; common, >10 individuals/ha. Mean standing seedling density is the number of seedlings (defined as ≤ 5 yrs old) per hectare per year: rare, <20 seedlings/ha/yr; intermediate, 20-200 seedlings/ha/yr; common, >200 seedlings/ha/yr. Adult basal area is total area (m^2/ha): rare, < 0.06 m^2/ha; intermediate, 0.06 – 0.25 m^2/ha; common, > 0.25 m^2/ha. Seed mass is dry seed mass (mg): small, <0.03 mg; medium, 0.03 – 0.30 mg; large, > 0.30 mg. NA indicates these values came from the only mapped stand that contained these species, so no SD can be calculated. † Data presented are means and standard deviations from mapped stands. ‡ Percentage mortality at 1% full sun; without the seedling density parameter due to small sample size. § Data sets used to indirectly determine shade tolerance for species whose mortality models were not reported due to small sample sizes: *Luehea* (R. K. Kobe, unpublished data), *Trophis* (Kobe 1999), *Miconia* (Pearson et al. 2003), *Stryphnodendron* (Guariguata 2000).

Table 2.2. List of significant seedling responses to microbial vs. sterilized microbial extract for all study species (condensed from Appendices A-D).

Species	Variables with Significant Response
Luehea seemannii	life span (-23% *), root surface area fraction (-47% †)
Neea psychotroides	root length fraction (+21% *)
Apeiba membranacea	total mass (-15% †), leaf mass (-23% *), root mass fraction (+21% †)
Colubrina spinosa	no significant response
Guatteria diospyroides	root mass (-12% †)
Psychotria panamensis	no significant response
Castilla elastica	total mass (-8% †), leaf mass (-12% *), root length (-15% †), root surface area (-11% **), root mass fraction (+9% †), stem mass fraction (+7% *), leaf mass fraction (-4% **), specific root length (-12% **), specific root surface area (-8% *)
Coussarea hondensis	life span (-13% †), stem mass (-12% †), leaf mass fraction (+13% †)
Pentaclethra macroloba	total mass (-18% †)
Prestoea decurrens	life span (-10% †), stem mass (-18% **), leaf mass (-21% *), cotyledon mass (+31% *), stem mass fraction (-20% *), leaf mass fraction (-12% †), cotyledon mass fraction (+11% *)
Quararibea bracteolosa	leaf mass (-29% †)
Trophis racemosa	root mass (-13% *)
Vochysia ferruginea	no significant response
Capparis pittieri	no significant response
Euterpe precatoria	specific root length (-9% †)
Iriartea deltoidea	life span (+14% *), root mass (-23% *) (-16% *), root mass fraction (-18% *), stem mass fraction (-12% †), cotyledon mass fraction (+1% *), root length fraction (-16% †), root surface area fraction (-15% *)
Welfia regia	life span (+5% †)
Virola koschnyi	leaf mass (-15% †), stem mass fraction (+10% *), leaf mass fraction (-11% †)
Dussia macrophyllata	stem mass (+45% *)
Miconia affinis	no significant response
Stryphnodendron microstachyum	no significant response

Note: Values given are the negative (-) or positive (+) percent difference in seedling response between the microbial and control treatment. Significance is shown as: † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

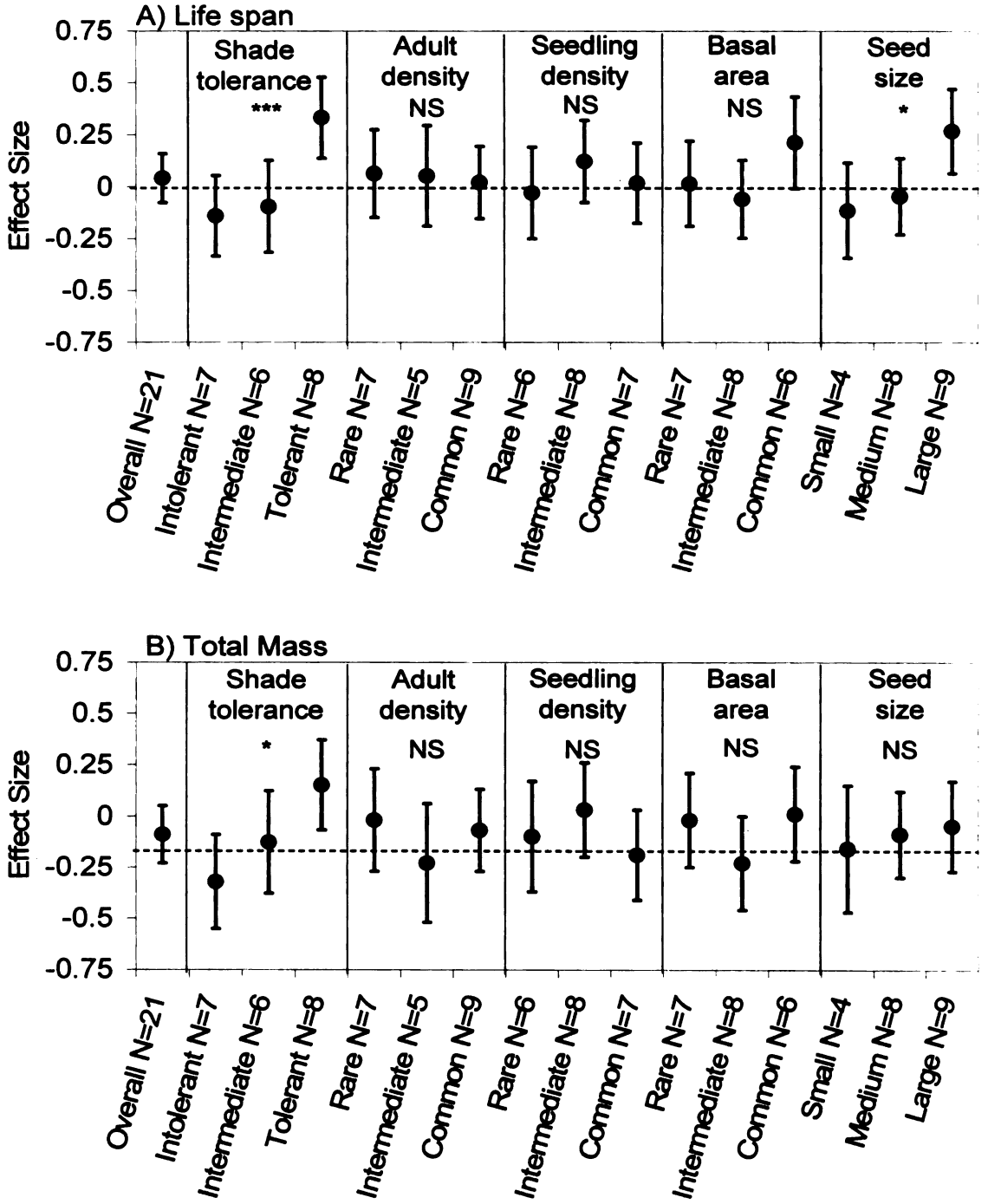

Figure 2.1. Effect size of soil pathogens on (A) life span and (B) total mass (magnitude and sign of the effect of the microbial vs. control treatment) compared to categories of local community characteristics for 21 tropical tree species. Data points show means and bars show 95% CI ranges for all studies (Overall) as well as each study category. Sample sizes and categories are indicated on the x-axis; the dotted line shows Hedges' d, indicating the absence of an effect. Significance is shown as: NS, not significant; * $P \leq 0.05$; *** $P \leq 0.001$.

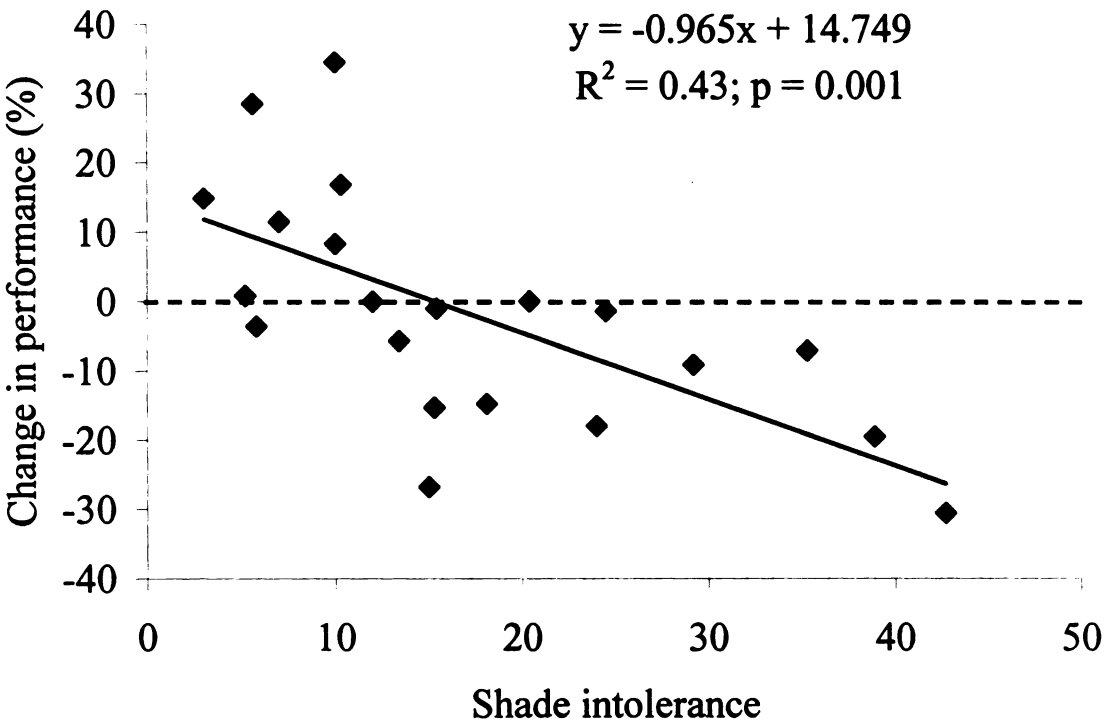

Figure 2.2. Relationship between seedling shade intolerance and change in performance between seedlings in the microbial vs. control treatments for 21 tropical tree species. Shade intolerance is measured as percent mortality at 1% full sun and zero conspecific seedling density. Change in performance is measured as ([mean total mass]X[mean life span])/(number of days in experiment). The significance value is from multiple, stepwise regression.

CHAPTER THREE

CONSPECIFIC AND HETEROSPECIFIC TREE-SOIL FEEDBACKS INFLUENCE SURVIVORSHIP AND GROWTH OF TEMPERATE TREE SEEDLINGS

Abstract

The Janzen-Connell (J-C) hypothesis proposes that host-specific enemies could maintain high tree species diversity by reducing seedling survivorship near conspecific adults and/or at high conspecific seedling densities. Negative feedback between plant and soil communities could be an important mechanism of such non-competitive distance and density-dependent (NCDD) mortality. In a greenhouse experiment, we assessed: 1) life span and growth responses of *Acer rubrum, Acer saccharum, Fraxinus americana* and *Quercus rubra* seedlings to extracts taken from soils that had been cultured by adults of each of these species; 2) whether these relationships were influenced by additional culturing of soil by different species and density of seedlings; 3) soil microbes as the mechanism creating these plant-soil feedbacks; and 4) whether low light availability increased species vulnerability to pathogens. Species-specific feedbacks between adult trees (but not seedlings) and soil influenced life span and/or growth for all species. Conspecific and heterospecific feedbacks had similar prevalence and magnitude of influence. In addition, heterospecific seedlings were not necessarily favored by these feedbacks. Chemical factors in the soil mediated these plant-soil feedbacks, whereas

microbial factors (primarily *Fusarium*) simply reduced seedling performance regardless of which tree species cultured the soil. Five *Fusarium* morphotypes were the primary infectious agents responsible for killing seedlings in the non-sterile extract treatment. Disease reduced seedling performance for some species regardless of light availability and for others only in high light. Species-specific adult-soil feedbacks impacted seedling performance, and thus have the potential to influence forest community dynamics. However, the idiosyncratic nature of these interactions likely diminishes their ability to enhance species diversity via J-C processes.

Key words: Community structure; density-dependence; distance-dependence; Fusarium; irradiance; Janzen-Connell; plant-soil feedback; soil microbes; species coexistence.

Introduction

Identifying the mechanisms that maintain tree species richness is a central problem in plant community ecology because competitively dominant species are expected to exclude inferior species (Gause 1934). Janzen (1970) and Connell (1971) hypothesized that competitive exclusion could be precluded by host-specific enemies that reduce seed and/or seedling survivorship near conspecific adults and/or at high conspecific densities. Such non-competitive distance or density-dependent (NCDD) mortality would favor establishment of heterospecific individuals, thus promoting species coexistence. Although, NCDD was proposed as a mechanism operating in tropical

forests, there is some evidence of NCDD occurring in temperate forests as well (Packer and Clay 2000, Hille Ris Lambers et al. 2002, Packer and Clay 2003).

Natural enemies such as soil pathogens are likely effective agents of NCDD mortality and/or reduced growth because many of them show host specialization, short generation times, high fecundity, long persistence in soil, and more limited dispersal than their hosts (Gilbert 2002). These characteristics could enhance the potential for "culturing" the local soil microbial community by the resident plant species, leading to a potential negative feedback for the plant species that cultured the microbes (van der Putten et al. 1993, Mills and Bever 1998, Klironomos 2002, Bezemer et al 2006, Casper and Castelli 2007, Kardol et al 2007).

Distance and density-dependent mortality is at the core of the Janzen-Connell (J-C) Model, but determining the relative importance of these processes is difficult because seed density often is inversely related to distance from adult. One way to investigate these differences is to compare how seedlings respond to soil micro-organisms cultured by adults vs. seedlings since each life history stage may culture unique enemies or may impact abundance or virulence of the same enemies in a different way (Gilbert 2002).

Irradiance may also mediate disease-induced NCDD processes. Seedlings of many tropical species (Augspurger and Kelly 1984, Kitajima and Augspurger 1989, Hood et al. 2004) experience higher disease related mortality at low than high light. Four hypotheses have been proposed for mitigated influence of disease in high light: 1) compensation for tissue lost to disease by accumulating biomass more rapidly 2) reduced exposure to disease through faster lignification, 3) unfavorable conditions (e.g. higher temperature or decreased moisture) that lower pathogen abundance and 4) increased

AMF colonization that suppresses disease (Augspurger 1990, Borowicz 2001, Gehring 2003).

Although the primary focus in this paper is on soil pathogens in NCDD processes, there are other important plant-soil feedbacks (Ehrenfeld et al. 2005). The presence of a particular plant species could be associated with formation of mycorrhizal networks (Booth 2004), production of allelochemicals (Stinson et al 2006), alterations to soil physical properties (Rillig et al. 2002) and nutrient availability (Finzi et al. 1998a-b). All of these feedbacks could impact seedling performance in a species-specific manner, i.e., a particular species could modify soil to the detriment or benefit of conspecific or heterospecific seedlings (Bezemer et al 2006). The potential for complex relationships among plant species mediated through soil feedbacks challenges lumping all non-conspecific species into a single heterospecific category (e.g. Augspurger and Kelly 1984, Packer and Clay 2000 and 2003, Hood et al 2004).

The purpose of this study was to examine mortality and growth responses of seedlings of four temperate tree species to: species of adult culturing soil in the field ("source" effects), species and density of seedlings further culturing field soil in a greenhouse, presence of microbial pathogens, and light level. Specifically, we tested the following hypotheses: H1 & H2) Soil cultured by conspecific adults (H1) or seedlings (H2) reduces seedling survival and/or growth more than soil cultured by heterospecific adults (H1) or seedlings (H2). H3) High seedling density during soil culturing increases the magnitude of seedling responses. H4) Sterilization of soil extracts enhances seedling survival and growth due to the elimination of soil pathogens. H5) Higher irradiance reduces negative effects of pathogen infection.

Materials and Methods

Soil culturing by conspecific versus heterospecific adults (H1)– To test the effect on seedling performance of soils cultured by conspecific vs. heterospecific mature trees (H1), we collected soil beneath each of the study species (*Acer rubrum, Acer saccharum, Fraxinus americana* and *Quercus rubra*) in November 2004. For each species used in the seedling response experiment, two soil cores (7.5 cm diameter x 25-cm depth) were taken within 1 m from the bole of three trees of each of the 4 species for a total of 96 soil cores (4 species of seedling x 4 species of adult culturing x 3 trees x 2 samples/ tree). Sampled trees were randomly selected from adults with a diameter at breast height at $\geq 75^{th}$ percentile for that species located in 4 mapped stands on moraines in the Manistee National Forest, MI. To minimize the potential for multi-species culturing of soil, we took soil under trees that were at least 2 crown diameters away from adults of the other species. Sampling locations for culturing by heterospecific adults had the additional criterion that no conspecific adults were closer than 20 m, except that we used a 10 m distance for *A. saccharum* due to its high local abundance. Soil was stored for ~ 2 wks at 4°C until seeds were available for planting in intact soil cores for the seedling culturing step.

Soil culturing by conspecific vs. heterospecific seedlings (H2) and seedling density (H3)– To test for effects of seedling soil culturing in addition to previous culturing by adults in the field, we planted conspecific vs. heterospecific seeds at high density in intact soil cores collected from each heterospecific adult (H2). To test effects of seedling density, paired soil cores taken from each conspecific and heterospecific adult

location were planted with one versus four conspecific seeds (H3). *A. rubrum* and *F. americana* seeds were from Sheffield's Seed Co (Locke, NY, USA) and *A. saccharum* and *Q. rubra* seeds from the Wisconsin Department of Natural Resources (Hayward, WI, USA). Seeds were surface sterilized (0.6% NaOCl solution), rinsed with DI water and weighed prior to planting. Seedlings cultured soil for 14 weeks in a greenhouse at 2% full sun; additional seedlings were planted as needed to maintain desired density for the entire culturing period. Before soil extraction, roots were cut and mixed with the soil and aboveground seedling portions were discarded. All culturing treatments were kept separate through the soil extraction process. Extracting soils that had been cultured at two seedling densities avoided potential confounding of seedling competition with NCDD effects.

Effect of sterilization on pathogen infection (H4)– Soil micro-organisms < 20 μm were extracted from cultured soil using a wet-sieving method adapted from Klironomos (2002). For each extraction, 50 g of soil was blended with 250 ml of tap water for 30 seconds. The liquid suspension was washed through 250, 45 and 20-μm analytical sieves with tap water, keeping the extract to ≤ 1 L. Sieves were cleaned ultrasonically for 5 min between each extraction, which at least minimized if not eliminated contamination between treatments. To test for microbial effects on seedling performance (H4), planted seedling pots were amended with autoclaved (30 min at 121 °C) versus unsterilized soil extract.

Planting methods and effect of irradiance on pathogen infection (H5)– Seeds with newly emerged radicles were planted in a 1:4 mixture of sterilized field soil and commercial peat moss (Fafard Mix #2, Conrad Fafard Inc., Agawam, MA USA). Field

soil was collected from a common pit in a mixed *Fagus-Acer* stand at Michigan State University's Tree Research Center and was autoclaved for 1 h at 121°C followed by 2 d incubation and a second autoclaving. Lethal temperatures (≥ 121°C) were confirmed at the center of each soil bag. Each seedling received 100 ml of non-sterilized or sterilized extract. We did not add arbuscular mycorrhizal spores (collected on the 45 μm sieve) because they enhanced seedling mortality under similar conditions in a previous experiment (McCarthy-Neumann and Kobe, unpublished data). To test for irradiance effects (H5), seedlings were grown at two light levels (2% vs. 22% full sun).

Experimental Treatments, Seedling Measurements and Harvesting– To summarize, experimental treatments consisted of species of adult, species of seedling and density of seedlings culturing soil, sterilization, and irradiance level. Density and species of seedling were tested only in low light. The 1,668 seedlings were randomly assigned to 8 benches (6 for low and 2 for high light) and were allocated among treatments per Appendix H.

Emergence and survival were censused thrice weekly and seedlings were watered (~50 ml of DI) by hand twice weekly for 12 weeks, from March to June 2005. We assigned date of death as the first census with total leaf and/or stem tissue necrosis, at which time dead seedlings were harvested for pathogen isolation. To determine live mass, we harvested seedlings surviving to the end of the experiment, washed soil from roots, divided seedlings into organ fractions, and oven-dried living tissue at 70°C to constant mass. Stem length was also measured for aboveground height.

Chemical analysis of soil extracts– To test for potential chemical differences, we measured exchangeable base cations (Ca, K, and Mg), total organic C, total N, C:N ratios

and protein-precipitable phenolics in extracts for each soil source (species of adult culturing). Extract samples were stored at 4°C for two years prior to analysis since testing for chemical effects was not part of our original research plan. Soil extracts were filtered with Whatman # 2 papers and exchangeable base cations (Ca, K, and Mg) were measured using a Perkin-Elmer Optima 2100 DV Optical Emission Spectrometer (Perkin Elmer, Norwalk, Connecticut, USA). For the total organic C, total N and C:N analysis, soil extracts were filtered through pre-rinsed Whatman GF/F papers prior to being analyzed with a TOC-V CPN Total Organic Carbon Analyzer equipped with a total nitrogen measuring unit (Shimadzu Co., Kyoto, Japan). We measured protein-precipitable phenolics with a spectrophotometer, following Makkar et al. (1988).

Pathogen Isolation and Host-Specificity of Fusarium Morphotypes - Upon seedling death, roots were rinsed of soil in DI water, surface sterilized for 1 min with 0.6% NaOCl and rinsed with sterilized water. Cross-sections from the leading edge of the disease lesion were plated on water agar amended with Ampicillin. Isolates were subcultured and maintained on water agar and potato dextrose agar, both amended with Ampicillin. Isolates were identified to genus using morphological characteristics; over 75% of isolates were in the genus *Fusarium* and were classified into five morphotypes

To determine host-specificity, seeds with emerged radicles were inoculated with each of the five morphotypes and a control of pure water agar (*A. saccharum* seeds were unavailable for inclusion in this experiment). Inoculum was obtained from pure cultures of the *Fusarium* morphotypes stored on silica beads. Individual silica beads were placed on multiple water agar plates amended with Ampicillin for conidia propagation and germination. The treatment consisted of five,10 mm disks of water agar containing

Fusarium isolates that were placed directly into the soil (a 1:5 mixture of sterilized field soil and commercial peat moss), and the control consisted of an equal number of pure water agar disks. Inoculated seedlings were assessed for original symptoms. This experiment consisted of 360 seedlings (3 species x (5 *Fusarium* morphotypes + control) x 20 replicates) all grown in low (~2% full sun) light for 8 wks from April-June 2006. Seed cleaning, soil sterilization and seedling measurements were identical to methods used in the main experiment.

Statistical Analysis– We analyzed seedling species and density separately from other treatments since they were carried out at low light only. In the absence of significant differences, data were pooled across seedling species and density treatments.

Life span was analyzed with survival analysis (SPSS v. 14.0, SPSS Inc, Chicago, IL) and includes both pre- and post- aboveground emergence stages (pre-emergence mortality date was estimated as the mean emergence date for seedlings of that species). The Breslow χ^2 test of homogeneity in a Kaplan-Meier analysis tested effects of seedling species and density on mean life span. Cox proportional hazards regression (Cox and Oakes 1984) tested relative effects of soil source, sterilization, light availability, and initial seed mass on mortality.

For growth responses, we tested for main treatment effects and their interactions with ANCOVA (treatments = species and density of seedling culturing the soil) and with a split-plot ANCOVA, split for light (treatments = soil source, sterilization and irradiance level), using bench as a blocking factor (SPSS). Because seed mass can influence seedling size, estimated dried embryo mass (based on regressions of dry embryo mass to fresh seed mass developed from ~30 randomly selected seeds for each species) was a

covariate. Data were natural-log transformed when errors were not normal. We ran full models (main treatments, bench, covariate and interactions) for each dependent variable and species to test the assumption that covariate effects were independent of treatment effects; interaction terms were removed when $P > 0.05$. If either terms for bench, covariate or the interaction between main treatments had $P > 0.25$ (Bancroft 1964), then the highest order term with the highest P value was removed and the analysis was run with a reduced model. This process was repeated until all terms with $P > 0.25$ were removed. Adjusted means were compared when the covariate was retained and raw means when the model reduced to ANOVA. When the main effect of soil source was significant (analyses were considered significant at $P < 0.10$ because of experiments short duration) a Holm adjustment was used to compare the conspecific to each of the 3 heterospecific adult soil sources for each species. Differences between treatment means were assessed and P values calculated through degree of overlap in 95% confidence intervals (Austin and Hux 2002).

To summarize reciprocal effects, we compared percent difference in integrated seedling performance [(mean total mass x mean life span) / (days of experiment)] for each species pair in soil extract (combined sterile and non-sterile treatments) relative to tap water in high and low light. To determine the relative effects of microbial versus chemical factors, we also calculated percent difference in integrated seedling performance for each species pair as: adult culturing through chemical effects alone [= seedling performance in sterile extract / seedling performance in tap water] and adult culturing through soil micro-organisms alone [= (seedling performance in non-sterile extract – seedling performance in sterile extract) / seedling performance in tap water]. We

estimated 95% confidence intervals for integrated seedling performance metrics by bootstrapping 3000 data sets for each study species (sampling with replacement), and analyzing each data set as described above using R (R Development Core Team 2008).

We used similar statistical methods to analyze seedling responses to the five Fusarium morphotypes.

Results

Conspecific and heterospecific culturing have similar influence on seedling performance (H1)– Species of adult culturing the soil (i.e., soil source) affected survivorship of *F. americana* seedlings, with reduced life span in soil extract cultured by *Q. rubra* vs. *F. americana* adults (Figure 3.1a, Table 3.1). Soil source did not affect life span for any other study species (Appendix I).

Seedling total mass for all species except *F. americana* were influenced by soil source (Figure 3.2, Table 3.1, see Appendix J-M for growth responses for all species), but conspecific soil did not disproportionately affect mass relative to heterospecific soil. Total seedling mass for *A. rubrum* was lower with soil extract cultured by conspecific vs. *A. saccharum* and *Q. rubra* adults. In contrast, seedlings of *Q. rubra* had greater mass in conspecific vs. *A. rubrum* and *F. americana* soil sources. For *A. saccharum* seedlings, the influence of soil source was mediated by irradiance. In low light, seedling mass was lower in conspecific vs. *F. americana* soil source, whereas in high light, mean seedling mass was greater in conspecific vs. *F. americana* and *Q. rubra* soil sources. Sterilization of the soil extract had no effect on whether soil source influenced life span and/or total mass for any species.

Soil culturing by conspecific and heterospecific seedlings (H2) and seedling density (H3) had negligible effects– In general, seedling culturing did not alter effects of soil source on seeding responses (results not shown). In the few significant responses effects were minor, with increased stem height for *A. rubrum* ($F = 2.76$, $df = 1,118$, $P \leq 0.10$, mean = 57.9 vs. 54.5 cm) and life span for *Q. rubra* seedlings ($\chi^2 = 3.11$, $df = 1,134$, $P < 0.10$, mean 82.5 vs. 77.4 days) in conspecific vs. heterospecific culturing by seedlings. The density of seedlings culturing the soil did not affect life span or growth for any of the species (results not shown).

Sterilization (H4) broadly influences seedling performance; irradiance (H5) interacts with sterilization– Sterilization of soil extracts increased life span and/or growth for all species, except *A. saccharum* (Table 3.1; Appendices I & J-M). *F. americana* seedlings had significantly reduced life span in the non-sterile extract across all soil sources (Figure 3.1b; Table 3.1). The non-sterile extract also reduced total seedling mass for *Q. rubra* regardless of soil source or irradiance level; *A. rubrum* and *F. americana* seedling mass was reduced across all soil sources but only in high light (Figure 3.2 and Table 3.1). Under higher irradiance, total mass was greater for all species (Figure 3.2; Table 3.1; Appendices J-M), but life span did not vary (Table 3.1 and Appendix I). Sterilization of soil extracts and efforts to minimize cross contamination were effective; 70% of dying seedlings in non-sterile treatments were infected versus 6% of dying seedlings in sterile treatments. 75% of infected seedlings harbored at least one of five *Fusarium* morphotypes.

Individual organ mass and stem height responses to the microbial extract were generally consistent with total mass responses for all of the species (Appendices J-M).

For all species, mass and height increased with initial seed mass (except for *A. saccharum* height). Random assignment of seedlings to certain low-light benches affected growth responses for all species; this effect was eliminated except for *A. saccharum* when a bench closest to the greenhouse fan was excluded from the analysis. The exclusion of this bench did not change the significance or percent differences for any growth response so all data were used in the analysis.

Integrated seedling performance– Plant-soil feedbacks can influence performance of a given species in conspecific vs. heterospecific cultured soils, (within rows of Table 3.2; significant comparisons highlighted in Table 3.1), as well as which species of seedling is most affected by soil cultured by a given species (within columns of Table 3.2). Taking the conventional approach of comparing performance in con- vs. hetero-specific soils, heterospecific soils were as likely to be detrimental as conspecific soils. For instance, *Q. rubra* culturing least affected *Q. rubra* seedlings compared to extracts cultured by the three heterospecific species (significant at low light), and under high light *A. saccharum* culturing affected conspecific seedlings less than *F. americana* soil source. However, under low light, *A. saccharum* culturing most affected conspecific seedlings compared to extracts cultured by *F. americana.* In addition, conspecific culturing tended to be the most detrimental for *A. rubrum* seedlings compared to culturing by other species (significant difference between *A. rubrum* and *Q. rubra* culturing at low light).

From an alternative perspective of interspecific comparisons of performance in soils cultured by a given study species, conspecific seedlings were not disadvantaged relative to heterospecific seedlings (Table 3.2). For instance, conspecific seedlings were less affected than at least some heterospecific seedlings for both *A. saccharum* (*F.*

americana, $P \leq 0.05$) and *Q. rubra* cultured soils (*A. saccharum*, $P \leq 0.05$ and *F. americana*, $P \leq 0.001$). In addition, *A. rubrum* seedlings tended to experience less severe feedbacks than seedlings of other species in heterospecific cultured soils (except for *F. americana* cultured soil at low light). Only *F. americana* cultured soil was more detrimental to performance of conspecific than heterospecific seedlings under high light (*A. rubrum*, $P \leq 0.05$ and *Q. rubra*, $P \leq 0.05$).

Both microbial and chemical factors in the soil extracts influenced seedling performance (Figure 3.3). Under high light, the response of *Q. rubra* seedlings to all soil extracts arose from both chemical and microbial factors; under low light, the response to con- and hetero-specific culturing was dominated by microbial and chemical factors, respectively, with stronger chemical than microbial effects. For *A. saccharum* seedlings, chemical factors from most soil sources were detrimental, except for *F. americana* source soils under low light; but conspecific vs. *F. americana* cultured soil had less (14%, $P = 0.02$) and greater (18%, $P = 0.06$) negative chemical factors in high and low light respectively. Under high light, *A. rubrum* responded negatively to biotic factors. Under low light, the negative effect of conspecific soil source on *A. rubrum* seedlings arose from both microbial and chemical factors; however, both microbial and chemical factors in *Q. rubra* cultured extract were neutral for *A. rubrum* seedlings. Soil extracts decreased *F. americana's* performance (which was similar regardless of soil source) through combined detrimental microbial and chemical factors (significant in high light only).

Chemical analysis of soil extracts– Soil extracts cultured by *Q. rubra* adults had lower base cation availability (combined Ca, K, and Mg) than extracts cultured by *A. saccharum* and *F. americana* adults, but had greater total C and C:N ratios than sources

from the other 3 species (Appendix N). *A. rubrum* source soils also had lower base cations and greater C:N ratios than *A. saccharum* and *F. americana* source soils (Appendix N). Total N (Appendix N) and protein-precipitable phenolics ($F = 0.42$, $df = 3,19$, $P = 0.74$) did not differ among soil sources.

Host-Specificity of Fusarium Morphotypes - *Fusarium* morphotypes reduced seedling life span for *A. rubrum* ($\chi = 17.99$, $df = 5,120$, $P \leq 0.01$) but not for the other species (Appendix O). *Fusarium* morphotypes also influenced total mass for *A. rubrum* ($F = 2.7$, $df = 5,91$, $P \leq 0.05$), *F. americana* ($F = 2.5$, $df = 5,49$, $P \leq 0.05$) and *Q. rubra* seedlings ($F = 2.2$, $df = 5,107$, $P \leq 0.10$) (Appendices P-R). Reductions in seedling mass were due to proportionate decreases in stem and leaf mass for all species and root mass for *A. rubrum* seedlings (Appendices P-R). Total seedling mass was positively related to initial seed mass for *F. americana* and *Q. rubra* seedlings; for all species, the random bench assignment affected total seedling mass due to a *Thysanoptera* outbreak that occurred on the second bench (Appendices P-R). The exclusion of this bench did not change the significance or percent differences for any growth response so all data were used in the analysis.

We also evaluated effects of *Fusarium* morphotype in terms of integrated seedling performance relative to the sterile treatment (Figure 3.4). *Fusarium* morphotypes 3-5 reduced *A. rubrum* seedling performance relative to the control and morphotype 2. *Fusarium* morphotype 3 reduced seedling performance relative to the control primarily through decreased total mass (22%; $P \leq 0.05$, Holm adjusted P = NS), *Fusarium* 4 through decreased life span (19%; $P \leq 0.01$, Holm adjusted $P \leq 0.10$) and *Fusarium* 5 through decreased total mass (32%; $P \leq 0.01$, Holm adjusted $P \leq 0.05$). Despite

significant F-statistics in the overall growth models for each species, seedling total mass did not differ in the control versus the various *Fusarium* morphotypes for *F. americana* and *Q. rubra* after adjusting for multiple comparisons. The following results should be viewed as suggestive trends. For *F. Americana*, seedling performance was reduced (due to a decrease in total mass) by morphotypes 1 (19%; P = NS) and 3 (17%; P = NS) relative to the control and morphotype 2, 4 and 5. For *Q. rubra, Fusarium* morphotype 1 had the lowest seedling performance (due to lower total mass) relative to morphotype 5 and to a lesser extent the control (15%; $P \leq 0.10$, Holm adjusted P = NS) and morphotype 2. In addition, *Q. rubra* seedlings had lower seedling performance (due to lower total mass) when grown with morphotype 3 and 4 than morphotype 5.

Discussion

Soil-mediated species – specific feedbacks between tree adults and seedlings were ubiquitous in this study. However, feedbacks were largely idiosyncratic and were not consistent with the Janzen-Connell Model. Most importantly, conspecific and heterospecific feedbacks occurred at similar strength across the study species. Furthermore, conspecific seedlings were not disadvantaged by these feedbacks, thus weakening the potential for these feedbacks to constrain populations and thereby contribute to species coexistence. Lastly, species-specific feedbacks tended to be chemical rather than biotic; microbial factors reduced seedling performance regardless of which tree species cultured the soil.

Conspecific and heterospecific culturing have similar influence on seedling performance (H1)- A. rubrum was the only species for which conspecific soil source

more negatively affected seedling performance than heterospecific soil sources in both high and low light; *A. saccharum* had a similar response but only in low light. For *F. americana* and *Q. rubra* at both light levels and *A. saccharum* at high light, at least one or more heterospecific soil sources were more detrimental than conspecific sources. Thus, for 3 of 4 species under high light and 2 of 4 species under low light, conspecific soil sources had the lowest adverse effect among all soil sources, however not all of these ranks were statistically significant. Similarly, the relative effect of con- versus hetero-specific cultured soils in grasslands depends upon particular species pair-wise interactions, with heterospecific cultured soils sometimes having more negative impacts than conspecific cultured soils (Bezemer et al 2006). Furthermore, none of the soil sources here were uniformly beneficial or detrimental to seedlings of all species. Likewise, the relative influences of biotic and chemical factors from a given soil source could change depending upon the species of responding seedlings. As a whole, these results support fairly specific interactions between pairs of species that are mediated through both chemical and biotic factors.

Although there are limited data to test whether similar species pair-wise interactions are operating in the field, the one result that we could test with field data was supported. We used seedling demography data spanning 6 years from northwest lower Michigan (Kobe, unpublished data) to test if *F. americana* seedlings had a shorter life span with *Q. rubra* vs. conspecific source soils. Average one year survival of newly germinated *F. americana* seedlings was 15% lower when both *Q. rubra* and *F. americana* adults were within 10 m of focal seedlings versus when only conspecific adults were present. The 15% reduction in survivorship was consistent with the present

study's results (Figure 1, 10% reduction at 77 days). Lower *F. americana* survivorship in the presence of *Q. rubra* is unlikely to arise from species differences in canopy light transmission since both tree species transmit similar irradiance (Canham et al. 1994).

Support for the Janzen-Connell Model often comes from decreased seedling performance for one or more focal species at near versus far distances from conspecific adults (Augspurger 1984, Augspurger and Kelly 1984, Hood et al. 2004, Bell et al. 2006). The implicit assumption of these studies is that a given species, whose seedlings are disadvantaged in the presence of con- versus hetero-specific adults, would more likely have its adults replaced by hetero- than con-specific seedlings. However, this assumption may not be valid if heterospecific seedlings are affected more than conspecific seedlings in a given soil source, even if the given species' seedlings perform relatively poorly in con- versus hetero- specific soil. In the present study, for example, even though *A. rubrum* seedlings were most negatively affected in conspecific source soils, both *F. americana* and *A. saccharum* seedling performance was more strongly negatively affected (under high light); *Q. rubra* seedlings were non-significantly less affected than *A rubrum* seedlings. Under low light, seedling performance of all four species was similarly affected (-17 to -20%) in *A. rubrum* source soils. Based on these feedbacks and not taking into account other aspects of species life history, the expectation is that *Q. rubra* and *A. rubrum* seedlings would be least affected by *A. rubrum* source soils and thus the most likely species to replace an *A. rubrum* canopy tree when it dies and creates a gap (with higher irradiance). Thus, knowledge of the relative benefits or costs of 'escape' from conspecific adults on seedling recruitment does not translate into knowledge of which species' seedlings are favored for recruitment near a particular tree species. But in

combination these two approaches provide a better understanding of how plant-soil feedbacks (or other mechanisms producing NCDD) contribute to tree species coexistence.

In general, *A. rubrum* seedlings were less affected by soil culturing than other species' seedlings. By absolute rank, *A. rubrum* was least affected in 5 of 8 soil source by light combinations (Table 2). Together with other factors (deer browse, fire exclusion), these feedbacks may be contributing to the documented increase in *A. rubrum* abundance throughout the eastern United States (Fie and Steiner 2007).

Contrary to expectation, the effect of adult culturing on seedling life span and/or growth occurred in both non-sterile and sterile soil extracts. If effects of adult culturing had been due only to biotic agents, then we would have expected negligible differences among soil sources for sterilized extracts. Thus, adult culturing reflects both chemical and biotic processes. Chemical analyses of the extracts suggest that base cation availability may be partially responsible for the species-specific feedbacks between tree adults and seedlings. Extracts derived from soil cultured by *Q. rubra* had significantly lower base cation availability than extract cultured by *F. americana* and *A. saccharum*, but greater total organic C and C:N ratios than extract cultured by any of the other study species. In addition, extracts derived from soil cultured by *A. rubrum* had significantly lower base cation availability and greater C:N ratios than extract cultured by *F. americana* and *A. saccharum*. Exchangeable cation availability differs under canopies of the same species (Finzi et al. 1998b) and manipulation of exchangeable cations can affect seedling and sapling performance (Kobe et al. 2002, Bigelow and Canham 2007). In this study, however, base cation availability was not significantly related to total mass for any

species, but only ~50% of the seedlings could be used for this analysis since only a sub-sample of the extracts were analyzed for cations. Variation in C:N ratios among soil sources (which was largely a function of differences in total organic C) is a less likely mechanism for observed plant-soil feedbacks since N immobilization is unlikely under the observed ratios of < 25:1. Chemical analysis of soil extracts were post-hoc and were conducted after two years of cold storage. Thus we consider these results to be suggestive. Experiments linking soil chemical factors with species-specific feedbacks between tree adults and seedlings are still needed to fully ascertain which chemical factors are responsible for creating these feedbacks.

Our soil source results would not have been interpretable or significant for *F. americana* lifespan if we had lumped all soils cultured by heterospecific adults into one treatment (e.g. "far" treatments in Augspurger 1984, Packer and Clay 2000 and 2003, Hood et al 2004). For other species, the general relationship between soil source and seedling life span or growth is consistent whether or not heterospecific species are aggregated. However, which heterospecific species influences performance relative to a conspecific adult would be unknown.

Soil culturing by conspecific vs. heterospecific seedlings (H2) and seedling density (H3) had negligible effects– Soil culturing by seedlings did not alter the effect that soil culturing by adult trees had on seedling responses, except for *Q. rubra*. Contrary to hypothesis 2, *Q. rubra* seedlings had increased life span under conspecific versus heterospecific seedling culturing. Contrary to hypothesis 3, density of seedlings during the culturing step did not affect subsequent seedling performance, presumably because higher seedling density did not change the abundance or composition of soil microbes.

Seedlings cultured soil for 14 weeks, a similar duration as other studies where seedling culturing did result in negative feedbacks (Packer and Clay 2004, Stinson et al. 2006). Similar to our results, *Milicia regia* seedling survival did not differ in soil from female versus male conspecific adults, the latter of which should have lower seedling densities (Hood et al. 2004). However, seedling density appears to mediate the impact of disease on seedling performance in studies that simultaneously vary experimental seedling density and pathogen presence (Packer and Clay 2000, Bell et al. 2006). Different methodologies could lead to conflicting results among studies, especially if density enhances disease transmission through close proximity of roots, which was eliminated in our extraction-based study.

Sterilization broadly influences seedling performance (H4) – Three of four species responded negatively to non-sterile extract, the majority of which were infected by *Fusarium*. *A. saccharum* seedlings were not affected by the non-sterile treatment, consistent with a lack of pathogen effect on the germination and viability of *A. saccharum* seeds (O'Hanlon-Manners and Kotanen 2006). *A. saccharum's* shade tolerance, the highest among the study species, also could convey tolerance to soil pathogens, as shade and pathogen tolerances are positively correlated across tropical species (Augspurger and Kelly 1984, McCarthy-Neumann and Kobe, *in press*; Chapter 2).

Five *Fusarium* morphotypes were the primary seedling mortality agents in the non-sterile extracts, and these morphotypes are likely widespread since we were able to culture the majority of them at least once from seedlings grown with soil extracts cultured by each tree species (results not shown). However, when directly inoculated with these

five Fusarium morphotypes they differentially affected *A. rubrum*, *F. americana* and *Q. rubra* seedling survival and/or growth. Similarly, Augspurger and Wilkinson (2007) demonstrated that *Pythium,* another common soil pathogen, varies in pathogenicity among species of tropical tree seedlings but was not strictly host-specific.

Irradiance interacts with sterilization (H5)– Contrary to expectation, increased irradiance did not ameliorate the negative effect of soil microbes on seedling performance. Indeed *F. americana* life span and *Q. rubra* growth were reduced by soil microbes without regard to irradiance level and growth of *A. rubrum* and *F. americana* seedlings were reduced by soil microbes only at high light availability. Seedlings have been hypothesized to be less affected by disease in high light environments through four mechanisms: tissue compensation, faster lignification, unfavorable conditions for soil micro-organisms, and through increased AMF colonization (Augspurger 1990, Borowicz 2001, Gehring 2003). The first two mechanisms likely are not operating here since they would have led to lower disease effects at high light with our methodology. However, since we minimized abiotic differences between light treatments and excluded AMF spores from microbial extracts, this study may not have adequately tested the third or fourth potential mechanisms, which may explain contradictory results with studies that allow abiotic factors (e.g. soil moisture and temperature) and/or AMF colonization to vary between irradiance levels (Augspurger 1984, Augspurger and Kelly 1984, Hood et al. 2004). Additionally, growth for some species (e.g., *A. rubrum* and *F. americana* here) may have been so severely constrained in shade that effects of soil microbes were not manifested. Similarly, interspecific competition for other resources also can influence the

magnitude of seedling responses in plant-soil feedbacks (Casper and Castelli 2007, Kardol et al 2007).

Although tree-soil chemical and microbial feedbacks occur, spatial heterogeneity in light availability has greater potential to affect seedling performance. The two strongest soil source effects were a 43% reduction in *A. saccharum* performance in *F. americana* soil under high light and a 42% reduction in *F. americana* performance in *Q. rubra* soil. In contrast, mean seedling mass increased 90-3000% across species from 2 to 22% full sun, which encompasses the typical range of variation of understory to large tree fall gap light levels in Michigan deciduous forests (Schreeg et al. 2001). However, the potential influence of spatial heterogeneity in light levels rarely is realized. Along 200 m belt transects at 4 moraine sites where adult cultured soils were originally sampled, irradiance in $1m^2$ quadrats averaged 1.5% canopy openness, ranging from 0.5 to 6% (Kobe, unpublished data). In contrast, spatial heterogeneity in soil culturing likely is ubiquitous. Thus, effects of irradiance and soil culturing on seedling performance in the field may be more similar than suggested by potential irradiance effects

Caveats– Excluding the filtrate component > 20 μm in soil extracts may have underestimated the role of soil microbes in plant-soil feedbacks, by eliminating some pathogens that could be present in field soil. Nevertheless, sterilization of extracts likely benefited seedling growth because infection rates were much higher in non-sterile than sterile treatment seedlings. Sterilization of soil extracts also may have resulted in a nutrient pulse, which would have heightened differences between sterilization treatments. A nutrient pulse arising from sterilization is consistent with increased growth for two species in sterilized soil extract under high light, where nutrients could constrain growth

(Kobe 2006). Counter to this interpretation, however, the nutrient pulse from sterilization of field soil (20% of the potting medium for all seedlings) would have overwhelmed any nutrient contribution from extract sterilization. Extract culturing by mature trees of different species may be confounded with an underlying template of soil chemistry that determines tree species occurrence. However, soils were collected from a narrow range of fertility conditions (moraine sites). The study species also occur across a similar range of mineral-bound nutrients (presumably uninfluenced by species occupancy) with divergent exchangeable nutrient pools (Finzi et al 1998a), which could be modified by species occupancy.

Conclusions- Our results add to accumulating evidence that feedbacks between plants and soil micro-organisms could influence plant community dynamics, as supported in temperate (Packer and Clay 2000 and 2003, Reinhart et al. 2005) and tropical (Hood et al. 2004, Bell et. al 2006, McCarthy-Neumann and Kobe, *in press*; Chapter 2) forest as well as grassland (Mills and Bever 1998, Klironomos 2002, Bezemer 2006, Casper and Castelli 2007, Kardol et al 2007) and dune (van der Putten et al. 1993) ecosystems. However, feedbacks in this study occurred primarily between mature trees (not seedlings) and soil and encompassed both biotic (*Fusarium*) and abiotic (possibly through differences in base cation availability) factors that have the potential to differentially impact seedling performance. Additionally, these plant-soil interactions were specific to species pairs with conspecific and heterospecific feedbacks occurring at similar strength across species. Contrary to expectation, effects of the microbial treatment on seedling performance manifested more strongly in high rather than low light. Plant-soil interactions affected seedling performance by as much as 43%, a magnitude of effect that

would be expected to influence community dynamics. The complex nature of these interactions, however, likely diminishes their ability to enhance tree species coexistence via Janzen-Connell processes, especially since the establishment of heterospecific seedlings was not always favored.

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Table 3.1. List of significant seedling life span, total mass and integrated seedling performance responses for all study species to: soil source (tree species culturing soil), soil extract (non-sterile vs. sterile), and irradiance (2% vs. 22% full sun) (condensed from Appendices I-M and Table 3.2).

Source of variation	Species	Life span§	Total mass‡	Integrated Seedling Performance‡
Soil Source	*Ar*	No significant difference	Ar vs. As (-16%, $P = 0.06$) Ar vs. Qr (-18%, $P = 0.07$)	Ar vs. Qr in L light (-15%, $P = 0.10$)
	As	No significant difference	As vs. Fa in L light (-22%, $P = 0.05$) As vs. Fa in H light (+31%, $P = 0.003$) As vs. Qr in H light (+23%, $P = 0.03$)	As vs. Fa in L light (-17%, $P = 0.05$) Ar vs. Fa in H light (+24%, $P = 0.003$)
	Fa	Fa vs. Qr (1.84, P value = 0.10)	No significant difference	No significant difference
	Qr	No significant difference	Qr vs. Ar (+12%, $P = 0.03$) Qr vs. Fa (+10%, $P = 0.06$)	Qr vs. Ar in L light(+10%, $P = 0.04$) Qr vs. As in L light(+11%, $P = 0.10$) Qr vs. Fa in L light (+11%, $P = 0.10$)
Soil Extract	*Ar*	No significant difference	N-S vs S in H light (-23%, $P = 0.01$)	NA
	As	No significant difference	No significant difference	NA

Table 3.1. (Ctd)

	Fa	N-S vs. S (2.38, *P* value = 0.07)	N-S vs S in H light (-23%, *P* = 0.04)	NA
	Qr	No significant difference	N-S vs S (-12%, *P* = 0.01)	NA
Irradiance	*Ar*	No significant difference	L vs. H (-97% *P* = 0.000)	NA
	As	No significant difference	L vs. H (-87%, *P* = 0.000)	NA
	Fa	No significant difference	L vs. H (-94%, *P* = 0.000)	NA
	Qr	No significant difference	L vs. H (-67%, *P* = 0.000	NA

Note: §Hazard ratios > 1 indicate reduction in days to mortality of study species. ‡Values are the negative (-) or positive (+) percent difference in seedling response between conspecific and heterospecific tree soil sources, non-sterile and sterile extract, and low and high light. Description of abbreviations: L = low light and H = high light; N-S = non-sterile and S = sterile; Ar = *Acer rubrum*, As =*Acer saccharum*, Fa = *Fraxinus americana*, and Qr = *Quercus rubra*; NA = not applicable since analyses was not conducted. *P* values for multiple comparisons have been adjusted with a Holm adjustment.

Table 3.2. Reciprocal effects (percent difference in integrated seedling performance [(mean total mass x mean life span) / (days of experiment)]) of plant-soil feedbacks relative to a tap water control for each study species integrated across extract treatment and at a) high and b) low irradiance levels.

a) High Light	Species of Adult Culturing the Soil			
Species of seedling responding to soil	*Acer rubrum*	*Acer saccharum*	*Fraxinus americana*	*Quercus rubra*
Acer rubrum	**-22%** (-33, -12%)	-5% (-20, 10%)	-13% (-23, -3%)	-6% (-20, 7%)
Acer saccharum	-30% (-43, -13%)	**-19%** (-29, -11%)	-43% (-54, -33%)	-37% (-53, -20%)
Fraxinus americana	-37% (-46, -27%)	-38% (-49, -27%)	**-33%** (-43, -22%)	-42% (-54, -30%)
Quercus rubra	-16% (-26, -8%)	-15% (-28, -5%)	-15% (-26, -5%)	**-10%** (-21, -1%)

b) Low Light	Species of Adult Culturing the Soil			
Species of seedling responding to soil	*Acer rubrum*	*Acer saccharum*	*Fraxinus americana*	*Quercus rubra*
Acer rubrum	**-18%** (-28, -7%)	-4% (-17, 10%)	-13% (-22, -3%)	-1% (-13, 13%)
Acer saccharum	-17% (-28, -7%)	**-20%** (-28, -11%)	-3% (-14, 5%)	-12% (-21, -4%)
Fraxinus americana	-19% (-30, -6%)	-16% (-27, -4%)	**-8%** (-21, 10%)	-21% (-34, -5%)
Quercus rubra	-20% (-27, -14%)	-20% (-28, -14%)	-21% (-28, -14%)	**-10%** (-18, -3%)

Note: Effect of conspecific cultured soil on seedlings is in bold. Bootstrap devised 95% CI are in parentheses.

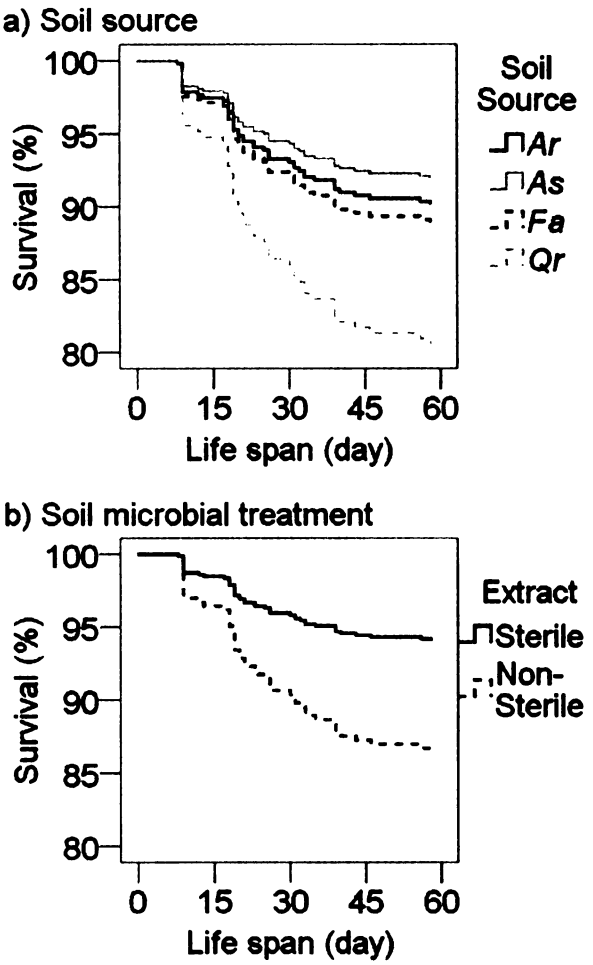
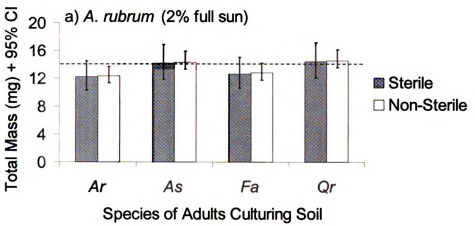
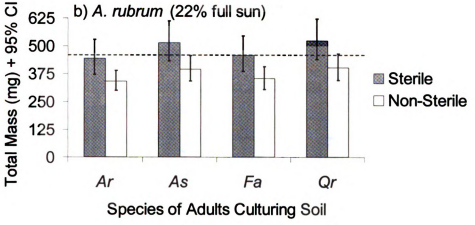


Figure 3.1. Survival curves for *F. americana* seedlings by (a) soil source (tree species culturing soil: *Qr* = *Q. rubra*, *Ar* = *A. rubrum*, *As* = *A. saccharum* and *Fa* = *F. americana*) and (b) soil microbial treatment (sterile vs. non-sterile extract). Survival curves end at 58 days since no *F. americana* seedlings died after that date.

Figure 3.2. Total final mass by soil source (tree species culturing soil) and extract (sterile vs. non-sterile) for each study species in low and high light (*A. rubrum* (*Ar*) a-b, *A. saccharum* (*As*) c-d, *F. americana* (*Fa*) e-f and *Q. rubra* (*Qr*) g-h). Dotted line represents seedling mass when grown with tap water and is shown for reference only, and were not included in statistical analysis.


a) A. rubrum (2% full sun)
Total Mass (mg) + 95% CI
20
16
12
8
4
0
Ar
As
Fa
Qr
Species of Adults Culturing Soil
Sterile
Non-Sterile


b) A. rubrum (22% full sun)
Total Mass (mg) + 95% CI
625
500
375
250
125
0
Ar
As
Fa
Qr
Species of Adults Culturing Soil
Sterile
Non-Sterile

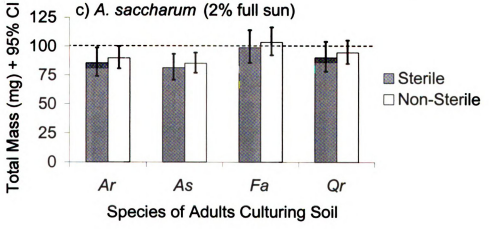

c) A. saccharum (2% full sun)
Total Mass (mg) + 95% CI
125
100
75
50
25
0
Ar
As
Fa
Qr
Species of Adults Culturing Soil
Sterile
Non-Sterile

Figure 3.2. (Ctd)

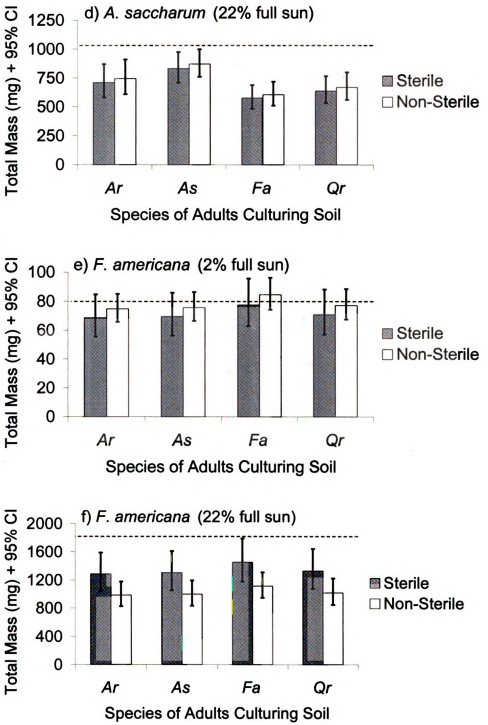


Figure 3.2. (Ctd)

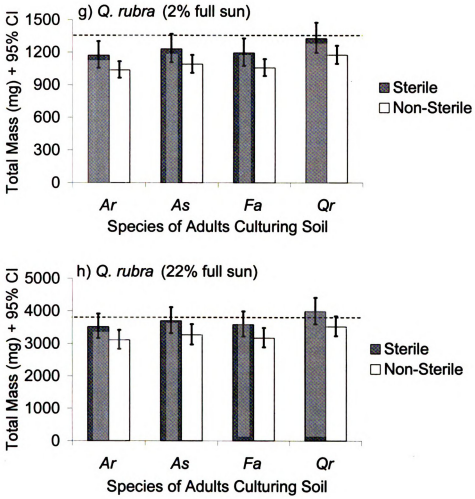
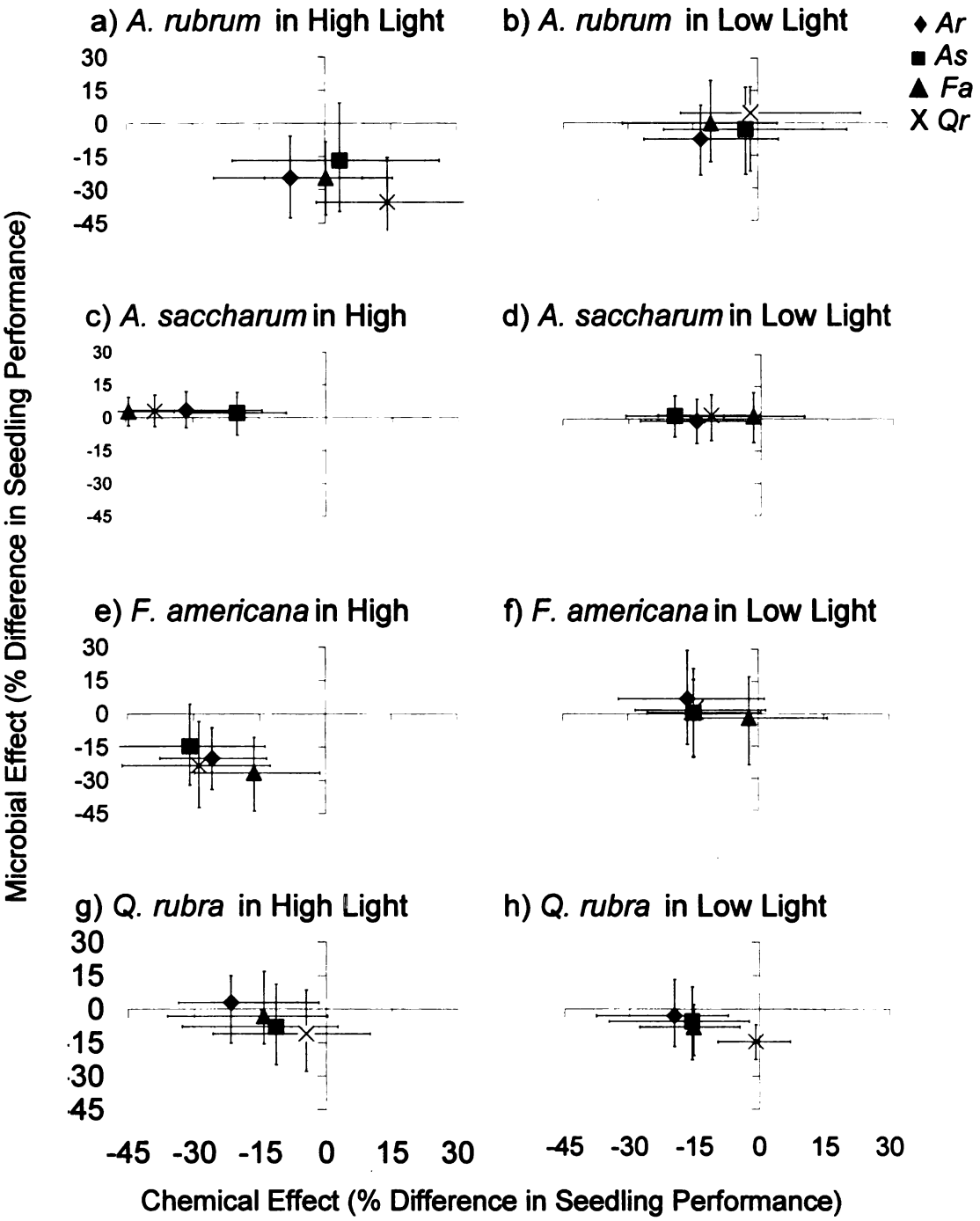


Figure 3.3. Relationship between chemical [sterile extract / tap water] and microbial [(un-sterile extract / tap water) – (sterile / tap water)] effects in soil extracts "cultured" by different species of adult on seedling performance [(mean total mass x mean life span) / (days of experiment)]) for each study species in high and low light. *Ar* = *A. rubrum*, *As* = *A. saccharum*, *Fa* = *F. americana*, and *Qr* = *Q. rubra*. Bootstrap devised 95% CI included.


a) A. rubrum in High Light
b) A. rubrum in Low Light
♦ Ar
■ As
▲ Fa
X Qr
c) A. saccharum in High
d) A. saccharum in Low Light
e) F. americana in High
f) F. americana in Low Light
g) Q. rubra in High Light
h) Q. rubra in Low Light
30
15
0
-15
-30
-45
Microbial Effect (% Difference in Seedling Performance)
Chemical Effect (% Difference in Seedling Performance)

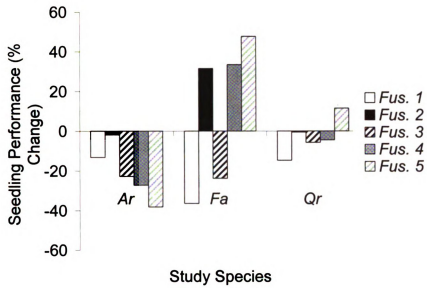


Figure 3.4. Change in performance [(mean total mass x mean life span) / Days in Experiment] between seedlings in the sterile control versus each of the *Fusarium* morphotypes for each study species. *Ar* = *A. rubrum*, *Fa* = *F. americana* and *Qr* = *Q. rubra*.

CHAPTER FOUR

CONSPECIFIC TREE-SOIL FEEDBACKS REDUCE SURVIVORSHIP AND GROWTH OF TROPICAL TREE SEEDLINGS

Abstract

The Janzen-Connell (J-C) Model proposes that host-specific enemies could maintain high tree species diversity by reducing seedling survivorship near conspecific adults and/or at high conspecific seedling densities. Negative feedback between plant and soil communities could be an important mechanism of such non-competitive distance and density-dependent (NCDD) mortality. In a shade-house experiment, we assessed: 1) life span and growth responses for six species of tropical tree seedlings (*Apeiba membranacea*, *Colubrina spinosa*, *Pentaclethra macroloba*, *Prestoea decurrens*, *Iriartea deltoidea* and *Virola koschnyi*) to extracts taken from soils that had been cultured by each of these species; 2) soil microbes as the mechanism creating these plant-soil feedbacks; and 3) whether low light availability increased species vulnerability to pathogens. Species-specific feedbacks between trees and soil influenced life span and/or growth for all species. Supporting the J-C Model, three of the six species had decreased seedling performance when grown with extract cultured by con- vs. all hetero-specific individuals. *I. deltoidea* had mixed results, performing better in two and worse in one hetero-versus

con-specific cultured soil. *C. spinosa* performed worse in con- vs. one of the hetero-specific soils. Chemical rather than biotic soil factors primarily mediated these plant-soil feedbacks. Shading did not increase vulnerability to soil micro-organisms in seedlings of any species. These results, along with parallel prior research in temperate forests, suggest that plant-soil feedbacks are an important component of seedling dynamics in both temperate and tropical forests, but would be more likely to enhance species coexistence in tropical forests because negative conspecific feedbacks were more pronounced for tropical than temperate tree species.

Key words: Density-dependence; distance-dependence; irradiance; host-specificity; Janzen-Connell; plant-soil feedback; soil microbes; species coexistence; tropical forests.

Introduction

Identifying the mechanisms that maintain species richness is a central question in plant community ecology because competitively dominant species are expected to exclude inferior species (Gause 1934). Janzen (1970) and Connell (1971) hypothesized that competitive exclusion could be precluded by host-specific enemies that reduce seed and/or seedling survivorship near conspecific adults and/or at high conspecific densities. Such non-competitive distance or density-dependent (NCDD) mortality would favor establishment of heterospecific individuals, thus promoting species coexistence.

Negative feedback, whereby individual plants "culture" the local soil microbial community in which they grow to the detriment of themselves and other conspecific

individuals (van der Putten et al. 1993, Mills and Bever 1998, Klironomos 2002, Bezemer et al 2006, Casper and Castelli 2007, Kardol et al 2007), may be an important mechanism that could create NCDD mortality and/or reduced growth. Natural enemies such as soil pathogens are likely effective agents in creating this type of feedback since many of these pathogens show host specialization, short generation times, high fecundity, long persistence in soil, and more limited dispersal than their hosts (Gilbert 2002). In a prior study, we found that a majority of 21 tropical tree species experienced reductions in seedling life span and/or growth when inoculated with non-sterile vs. sterile soil extract cultured by conspecific adults and seedlings (McCarthy-Neumann and Kobe *in press*; Chapter 2). However, whether these soil microbe-mediated feedbacks between individual plants were more detrimental in con- than hetero-specific interactions was not investigated and thus host-specificity of these feedbacks is unknown.

Traditionally in studies of NCDD, all heterospecific individuals have been lumped into a single heterospecific category (e.g. "far" distance) (Augspurger and Kelly 1984, Packer and Clay 2000 and 2003; Hood et al 2004). However, tree species vary in many characteristics (e.g., resource allocation to defense vs. growth) and soil-mediated effects of mature individuals on seedlings could be species specific as well (McCarthy-Neumann and Kobe, *in review*; Chapter 3).

Irradiance may also mediate disease-induced NCDD processes. Seedlings of many tropical species experience higher disease related mortality at low than high light (Augspurger 1983, Augspurger 1984, Augspurger and Kelly 1984, Hood et al. 2004). Four mechanisms (tissue compensation, faster lignification, unfavorable conditions for soil micro-organisms, and through increased AMF colonization) have been proposed for

how seedlings could be less affected by disease in high light (Augspurger 1990, Borowicz 2001, Gehring 2003). However, for temperate species, disease reduced seedling performance for some species regardless of light availability and for others only in high light (McCarthy-Neumann and Kobe, *in review*; Chapter 3).

Although the primary focus in this paper is on soil pathogens in NCDD processes, there are other important plant-soil feedbacks (Ehrenfeld et al. 2005). For example, in a temperate greenhouse experiment (McCarthy-Neumann and Kobe, *in review*; Chapter 3) species of adult culturing soil influenced seedling performance, but the effect was manifested in both non-sterile and sterile soil extracts, suggesting a species-induced change in soil chemistry (possibly base cation availability). Additionally, plant-soil feedbacks could be created when a particular plant species is associated with the formation of mycorrhizal networks (Booth 2004), production of allelochemicals (Stinson et al 2006), alterations to soil physical properties (Rillig et al. 2002) and nutrient availability (Finzi et al. 1998a-b).

It is often assumed, but rarely tested, that mechanisms underlying community dynamics in tropical versus temperate forests are different. In particular, the Janzen-Connell Model assumes that NCDD processes are less pronounced in temperate versus tropical forests because temperate forests have a less diverse tree community along with lower rainfall and greater seasonality, which could result in an overall lower abundance of enemies and disproportionately fewer specialist enemies. Over the past 30+ years, the focus of NCDD studies has been primarily on tropical tree species and communities. Among the few studies that have been conducted in temperate forests, there is accumulating evidence of NCDD effects on seedling mortality and growth (Packer and

Clay 2000, Hille Ris Lambers et al. 2002, Packer and Clay 2003, McCarthy-Neumann and Kobe, *in review*; Chapter 3). However, we are not aware of any studies that use parallel experiments in both temperate and tropical forests, which would enable more direct comparisons.

The purpose of this study was to examine mortality and growth responses of seedlings of six tropical tree species to: species culturing the soil (adult species from which soil was collected with further culturing by a high density of seedlings of the same species in the greenhouse), presence of microbial pathogens, and light level. Specifically, we tested the following hypotheses: H1) Soil cultured by conspecific individuals reduces seedling survival and/or growth more than soil cultured by heterospecific individuals. H2) Sterilization of soil extracts enhances survival and growth due to the elimination of soil microbes. H3) Higher irradiance reduces effects of pathogen infection. Additionally, by comparing results here to a parallel study (McCarthy-Neumann and Kobe, *in review*; Chapter 3) we assess whether tropical species exhibit greater sensitivity to NCDD processes than temperate species.

Materials and Methods

Species – To test these hypotheses, we assessed seedling survivorship and growth responses for six tropical tree species (*Apeiba membranacea*, *Colubrina spinosa*, *Iriartea deltoidea*, *Pentaclethra macroloba*, *Prestoea decurrens* and *Virola koschnyi*) to extracts taken from soils that had been cultured by each of the six species. These species were selected because they vary in abundance class and seedling shade tolerance; we previously investigated their seedling responses to microbes extracted from soil cultured

by conspecific but not heterospecific adults and seedlings (McCarthy-Neumann and Kobe, *in press*; Chapter 2).

Field Site – This research was conducted at La Selva Biological Station (Sarapiquí Region, Costa Rica) which is operated by the Organization for Tropical Studies. La Selva is a 1,510 ha reserve of diverse (> 400 tree species), wet tropical forest receiving ~ 4000 mm of rain annually with a mean annual temperature of 25.8° C (Hartshorn and Hammell 1994).

Soil culturing by conspecific versus heterospecific individuals (H1)–To test the effect on seedling performance of soils cultured by conspecific vs. heterospecific trees we collected soil cores beneath each of the study species and further cultured the soil by planting a high seedling density of the same species in intact soils cores in shadehouses. We removed a 10-cm diameter by 30-cm deep soil core within 1 m from the bole of four adults for each species in December 2005. Sampled trees were randomly selected from adults with a diameter at breast height at $\geq 75^{th}$ percentile for that species located in 5 mapped stands. To minimize potential for multi-species culturing of soil, we randomly selected adults for which no individuals >5, or >9, or > 20 cm DBH of the other 5 species were located within 5, 10, or 20 m, respectively, from the focal tree. Seeds were collected within 5 m from the trail system throughout La Selva. Seeds were surface-sterilized (0.6% NaOCl solution), rinsed with DI water and weighed prior to planting. To further culture the soils, each core was planted with 4 germinating conspecific seeds, a density that was maintained for 13 weeks in a shadehouse at 1% full sun. Before microbial extractions, roots were cut and mixed with the soil and aboveground seedling portions

were discarded. All culturing treatments were kept separate through the extraction process.

Effect of sterilization on pathogen infection (H2)–Soil micro-organisms < 20 μm were extracted from cultured soil using a wet-sieving method adapted from Klironomos (2002). For each extraction, 40 g of soil was blended with 250 ml of water for 30 seconds. The liquid suspension was washed through 250, 45 and 20-μm analytical sieves with tap water, keeping the extract to ≤ 800 ml. Sieves were cleaned ultrasonically for 5 min between each extraction, which at least minimized if not eliminated contamination between treatments. To test for microbial effects on seedling performance (H2), planted seedling pots were inoculated with autoclaved (20 min at 121 °C) versus unsterilized soil extract.

Planting methods and effect of irradiance on pathogen infection(H3)– Within 5 days of obtaining extract (which was stored at 4 °C), seeds with newly emerged radicles were planted in a 1:4 mixture of sterilized field soil and commercial peat moss (Nutripeat, Sun Grow Horticulture Canada Ltd, Vancouver, BC, Canada). Field soil was collected from a common pit in a residual, secondary forest at La Selva and was autoclaved for 2 h at 121°C followed by 2 d incubation and a second autoclaving. Lethal temperatures (≥ 121°C) were confirmed at the center of each soil bag. Each seedling received 100 ml of non-sterilized or sterilized extract. We did not add arbuscular mycorrhizal spores (collected on the 45 μm sieve) because they enhanced seedling mortality under similar conditions in a previous experiment (McCarthy-Neumann and Kobe, unpublished data). To test for irradiance effects (H3) seedlings were grown at 1% and 5% full sun. Light levels were confirmed with paired PAR measurements in the open

and at each bench in the shade houses. PAR was measured on a uniformly overcast day with a LI-COR 250A quantum sensor (LI-COR, Lincoln, NE USA).

Experimental Treatments, Seedling Measurements and Harvesting – To summarize, experimental treatments consisted of species of tree culturing the soil, sterilization and, irradiance level. Seedlings were also grown with just tap water addition as an additional control. The 3,084 seedlings were randomly assigned to 10 benches and were allocated among treatments as detailed in Appendix S.

Twice a week for 11 weeks (March to July 2006), emergence and survival were censused, and seedlings were watered (~50 ml of DI) by hand. We assigned date of death as the first census with total leaf and/or stem tissue necrosis, at which time dead seedlings were harvested. To isolate pathogens, roots were rinsed of soil in DI water, surface sterilized for 1 min with 0.6% NaOCl and rinsed with sterilized water. Cross-sections from the leading edge of the disease lesion were plated on water agar amended with Ampicillin. Isolates were identified to genus. To determine live mass, we harvested seedlings surviving to the end of the experiment, washed soil from roots, and oven-dried living tissue at 70°C to constant mass.

Statistical Analysis – Life span was analyzed with survival analysis (SPSS v. 15.0, SPSS Inc, Chicago, IL) and includes both pre- and post- aboveground emergence stages. Cox proportional hazards regression (Cox and Oakes 1984) tested relative effects of soil source (species culturing soil), sterilization, light availability, and initial seed mass on mortality.

For growth responses, we tested for main treatment effects and their interactions with split-plot ANCOVA, split for light (treatments = soil source, sterilization and

irradiance level), using bench as a blocking factor (SPSS). Because seed mass can influence seedling size, estimated dried embryo mass (based on regressions of dry embryo mass to fresh seed mass developed from 20-40 randomly selected seeds for each species) was a covariate. Data were natural-log transformed when errors were not normal. We ran full models (main treatments, bench, covariate and interactions) for each dependent variable and species to test the assumption that covariate effects were independent of treatment effects; interaction terms were removed when $P > 0.05$. If either terms for bench, covariate or the interaction between main treatments had $P > 0.25$ (Bancroft 1964), then the highest order term with the highest P value was removed and the analysis was run with a reduced model. This process was repeated until all terms with $P > 0.25$ were removed. Adjusted means were compared when the covariate was retained and raw means when the model was reduced to ANOVA. When the main effect of soil source was significant (analyses were considered significant at $P < 0.10$ because of the experiment's short duration) a Holm adjustment was used to compare the conspecific to each of the 5 heterospecific soil sources for each species. Differences between treatment means were assessed and P values calculated through degree of overlap in 95% confidence intervals (Austin and Hux 2002).

To summarize reciprocal effects, we compared percent difference in integrated seedling performance [(mean total mass x mean life span) / (days of experiment)] for each species pair in soil extract (combined sterile and non-sterile treatments) relative to tap water averaged across irradiance levels. To determine the relative effects of microbial versus chemical factors in non-sterile soil extracts, we also calculated percent difference in integrated seedling performance for each species pair: tree species culturing through

chemical effect alone [= seedling performance in sterile extract / seedling performance in tap water] and tree species culturing though soil micro-organisms alone [= seedling performance in non-sterile extract – seedling performance in sterile extract / seedling performance in tap water]. We estimated 95% confidence intervals for integrated seedling performance metrics by bootstrapping 3000 data sets for each study species (sampling with replacement), and analyzing each data set as described above using R (R Development Core Team 2008).

Results

Conspecific culturing had greater influence on seedling performance than heterospecific culturing (H1) – Tree species culturing soil (i.e., soil source) affected survivorship in 4 (Figure 4.1a-d and Table 4.1; Appendix T) and total mass in 5 of 6 species (Figure 4.2, Table 4.1; Appendix U). Conspecific cultured soil disproportionately decreased seedling survival and/or growth relative to heterospecific soil.

Seedlings of both *C. spinosa* and *P. decurrens* had shorter life span with conspecific versus heterospecific soil extract. Results for *I. deltoidea* were mixed with life span either longer or shorter in conspecific versus heterospecific cultured soil extract depending upon the particular species of heterospecific. In contrast, life span for *A. membranacea* seedlings was longer with soil extract cultured by conspecific vs. heterospecific individuals. Life span for *P. macroloba* and *V. koshnyi* seedlings did not vary based on species culturing the soil (Table 4.1).

Total mass for *C. spinosa* seedlings was greater with soil extract cultured by conspecific vs. *I. deltoidea* individuals but only in high light. In contrast, seedlings of *I.*

deltoidea, *P. macroloba*, and *V. koschnyi* all had lower mass with soil extract cultured by conspecific vs. at least some if not all heterospecific individuals (Table 4.1 and Figure 4.2). However, the difference between *V. koshnyi* seedling mass in extracts cultured by conspecific vs. *I. deltoidea* and *P. decurrens* individuals only occurred in non-sterile extract. The ANCOVA model assumptions for *P. decurrens* could not be met when cotyledon mass was included in total mass since > 10% of seedlings lost cotyledons prior to harvest. We present results for *P. decurrens* seedlings without cotyledon mass for all seedlings and with cotyledon mass for the subset of seedlings retaining cotelydons. Excluding cotyledon mass, *P. decurrens* total mass was lower in conspecific vs. heterospecific (*I. deltoidea* and *V. koschnyi*) cultured non-sterile soil extract in high light. When including cotyledon mass, however, total mass for *P. decurrens* was greater in conspecific vs. heterospecific (*C. spinosa* and *I. deltoidea*) soil sources in low light. These apparently conflicting results are due to greater retention of cotyledon mass in *P. decurrens* cultured soil extract (results not shown). The inclusion or exclusion of cotyledon mass did not change results for species.

Sterilization minimally influences seedling performance for one species (H2) – Sterilization of soil extracts did not influence life span or growth in most species, with the exception of *P. macroloba*, where the non-sterile extract decreased life span (Figure 4.3, Table 4.1; Appendix T), but increased total mass of surviving seedlings relative to sterilized extract across all soil sources (Figure 4.2g-h, Table 4.1; Appendix U). The majority of dead seedlings that were identified as diseased were infected by *Fusarium* (36%), *Rhizoctonia* (29%) and *Phoma* (22%).

Irradiance does not interact with pathogen infection (H3) – Light availability did not influence pathogen effects (i.e., no significant light x sterilization interactions) on life span or growth for any species. Under higher light, life span was longer for all species except *I. deltoidea* and *V. koschnyi,* which were the most shade tolerant of the species (Figure 4.4, Table 4.1; Appendix T) and mass was greater for all species except *I. deltoidea* (Figure 4.2; Appendix U).

Within each species, initial seed mass did not influence life span but was positively associated with total seedling mass for *P. macroloba*, *P. decurrens*, and *V. koschnyi*. Random assignment of seedlings to the high-light bench nearest to the shadehouse door affected final seedling mass for *C. spinosa*, *P. macroloba* and *P. decurrens*; exclusion of this bench (with slightly higher light levels) eliminated the bench effect, but did not change significance or percent differences for seedling mass, Thus, all data were used in the analysis and are reported.

Integrated seedling performance – Plant-soil feedbacks can influence performance of a given species in con- vs. hetero-specific cultured soils (within rows of Table 4.2; significant comparisons highlighted in Table 4.1), as well as which species of seedling is most affected by soil cultured by a given species (within columns of Table 4.2). Taking the conventional approach of comparing performance in con- vs. hetero-specific soils, conspecific soils were never beneficial and were more likely to be detrimental than at least four heterospecific soils for *P. macroloba*, *P. decurrens* and *V. koschnyi* seedlings. *I. deltoidea* seedlings performed better with culturing by *P. macroloba* and *P. decurrens*, worse under culturing by *C. spinosa,* and performed equally well under *A. membranacea* and *V. koschnyi* culturing relative to conspecific soils. *C.*

spinosa seedlings performed better under *I. deltoidea* than conspecific culturing, but heterospecific culturing by the other four species did not differ from conspecific culturing.

From an alternative perspective of interspecific comparisons of seedling performance in soils cultured by a given study species, conspecific seedlings were not necessarily disadvantaged relative to heterospecific seedlings (Table 4.2). For instance, three species had mixed results with conspecific seedlings less affected than some heterospecific seedlings (*C. spinosa* vs. *A. membranacea*, $P = 0.03$; *I. deltoidea* vs. *A. membranacea*, $P = 0.03$ and *C. spinosa*, $P = 0.007$ and *P. macroloba* vs. *A. membranacea*, $P = 0.02$) and more affected than other heterospecific seedlings (*C. spinosa* vs. *P. macroloba*, $P < 0.001$, *P. decurrens*, $P \leq 0.001$ and *V. koschnyi* $P \leq 0.001$; *I. deltoidea* vs. *P. macroloba*, $P \leq 0.001$ and *V. koschnyi*, $P \leq 0.001$ and *P. macroloba* vs. *I. deltoidea*, $P \leq 0.001$ and V. koschnyi, $P \leq 0.001$). Whereas conspecific seedlings had better performance than heterospecific seedlings (*A. membranacea* $P \leq 0.001$ and *I. deltoidea* $P = 0.02$ in *V. koschnyi* cultured soils. Only *A. membranacea* and *P. decurrens* cultured soils were more detrimental to performance of conspecific than heterospecific seedlings [(*C. spinosa*, $P \leq 0.001$, *P. macroloba*, $P \leq 0.001$, *P. decurrens*, $P = 0.007$, *and V. koschnyi*, $P \leq 0.001$ vs *A. membranacea*) and (*C. spinosa*, $P = 0.001$, *I. deltoidea*, $P \leq 0.001$, *P. macroloba*, $P \leq 0.05$, and *V. koschnyi*, $P \leq 0.001$ vs. *P. decurrens*)]

In general, chemical factors in soil extracts influenced seedling performance more than biotic factors (Figure 4.5). The response of *P. macroloba* seedlings to all soil extracts (non-significant for *C. spinosa* soils) arose from chemical factors, with greater negative response to con- vs. hetero-specific culturing. For *P. decurrens* seedlings, both

chemical and microbial factors were detrimental in conspecific cultured soils, but heterospecific cultured soils benefited performance through (either chemical or microbial factors, depending on soil source) *V. koschnyi* seedlings performed relatively well in heterospecific soils due to chemical or microbial factors. Chemical factors primarily influenced how *I. deltoidea* seedlings performed; *P. macroloba* and *P. decurrens* soils were beneficial whereas *C. spinosa* soils were detrimental relative to conspecific soils. Likewise, chemical factors were detrimental to *C. spinosa* performance with the greatest negative effect from *I. deltoidea* soils. Soil extracts decreased *A. membranacea* performance (which was similar regardless of soil source) primarily through chemical factors.

Seedling performance for *A. membranacea*, *I. deltoidea* and *P. macroloba* are based on effects of soil source regardless of extract treatment or light. For the other species either extract or irradiance treatments interacted with soil source effects. Thus, differences in performance among the different sources of soil extracts are restricted to high light for *C. spinosa*, non-sterile extract treatment for *V. koschnyi*, and non-sterile extract treatment in high light for *P. decurrens* seedlings (based on mean mass excluding cotyledon mass).

Discussion

Our experiment revealed a complex web of interactions among tree species, where the dominant mode of soil feedbacks were mediated through chemical rather than biotic influences, and where negative feedbacks from conspecific individuals were stronger than all heterospecific feedbacks for half of the species. In addition, most

heterospecific seedlings had better performance than conspecific seedlings in soils influenced by a given species. However, we only investigated the species-specific effects of plant-soil feedbacks for six species; if we had added more species, we may have found other species with greater negative influence, usurping the primacy of conspecific effects on the three most common species.

Conspecific culturing had larger influence on seedling performance than heterospecific culturing (H1) – Three of the six species (*P. macroloba*, *P. decurrens* and *V. koschnyi*) had decreased seedling performance when grown with extract cultured by con- vs. all hetero-specific individuals. Whereas *I. deltoidea* had mixed results, performing better in two and worse in one hetero-versus con-specific cultured soil, and only *C. spinosa* performed worse in con- vs. one of the hetero-specific soils. None of the soil sources here were uniformly beneficial or detrimental to seedlings of all species. Likewise, the relative influences of biotic and chemical factors from a given soil source could change depending upon the species of responding seedlings; however, chemical effects in soil extracts generally influenced seedling performance much more than biotic effects. As a whole, these results support fairly specific interactions between pairs of species that are mediated mainly through chemical factors with conspecific culturing tending to be more detrimental than heterospecific soil culturing.

An assumption of the Janzen-Connell (J-C) Model is that maintaining species diversity requires that NCDD processes are more prevalent in species that are common versus those that are rare, thereby constraining the abundance of common species. In this study, species that are common as adults from the site where soil was collected (e.g. *Pentaclethra macroloba*, *Prestoea decurrens*, and *Virola koschnyi*) tended to perform

worse in conspecific vs. heterospecific cultured soil extracts. Thus, tree species coexistence could possibly be maintained with these processes even though chemical factors are the main mechanism for reduced performance in the soil and not host-specific pathogens as assumed by the J-C Model.

There have been numerous studies, like this one, supporting the J-C prediction that a focal species seedling performance is reduced near versus far distances from conspecific adults (Augspurger 1983a-b, 1984, Augspurger and Kelly 1984, Packer and Clay 2000, Hood et al. 2004, Bell et al. 2006). In contrast, there have been no studies in tropical forests that have provided direct evidence that a given species, whose seedlings are disadvantaged in the presence of con- versus hetero-specific adults, would more likely have its adults replaced by hetero- than con-specific seedlings; leading to greater local plant diversity than expected in the absence of NCDD processes. In temperate forests, there is some evidence that heterospecific seedlings have lower mortality due to soil pathogens than *P. serotina* seedling in soils cultured by *P. serotina* adults (Packer and Clay 2000). In our study, heterospecific seedlings were more likely to have better performance than conspecific seedlings in soils influenced by a given individual. However, for many species the results were mixed so that some heterospecific seedlings were favored whereas others were disfavored relative to the conspecific seedling. The lack of a consistent disadvantage to conspecific versus heterospecific seedling recruitment, in this study, may place some limits on the extent that these chemically mediated plant-soil feedbacks can enhance tree species coexistence in this wet tropical forest. In addition, although *V. koschnyi* seedlings did worse in conspecific cultured soils the heterospecific seedlings had relatively poorer performance. Thus, knowledge of the

relative benefits or costs of 'escape' from conspecific adults on seedling recruitment does not translate into knowledge of which species' seedlings are favored for recruitment near a particular tree species. But in combination these two approaches provide a better understanding of how plant-soil feedbacks (or other mechanisms producing NCDD) contribute to tree species coexistence.

Contrary to expectation, the effect of soil culturing on seedling performance presumably reflects a chemical rather than a biotic process, since responses to soil culturing were similar in non-sterile and sterile soil extracts. If effects of culturing had been due only to biotic agents, then we would have expected negligible differences among soil origins for the extracts that had been sterilized. In a parallel temperate experiment, differences in extract cation availability may have been partially responsible for observed species – specific feedbacks between tree adults and seedlings (McCarthy-Neumann and Kobe, *in review*; Chapter 3). However, there are other possible chemical factor(s) that could mediate plant-soil feedbacks. For instance tannins in leaf litter can decrease nutrient cycling through decreased decomposition, inhibition of nitrification and/or changes in microbial activity (Kraus et al 2003). Additionally, leaf litter leachates of some species can suppress seedling performance of other species' seedlings (Stinson et al. 2006). Our results provide strong motivation for investigating the chemical factors that could vary among soils cultured by different species of mature trees and their potential impact on seedling dynamics.

Our soil source results would not have been interpretable or significant for half of our study species if we had lumped all soils cultured by heterospecific individuals into one treatment (e.g. "far" treatments in Augspurger 1984, Packer and Clay 2000 and 2003,

Hood et al 2004). For instance, *A. membranacea* life span and *C. spinosa* mass do not appear to be influenced by soil source when comparing conspecific to a lumped heterospecific grouping (results not shown). However, soil source does influence seedling performance for these species but the effect varies among the heterospecific species in magnitude and whether the effect is beneficial or detrimental relative to conspecific derived soil (Figure 4.1 and 4.2c-d).

Sterilization minimally influences seedling performance for one species (H2) – Diseased dead seedlings for all species were infected by a variety of soil fungal pathogens (*Fusarium, Rhizoctonia and Phoma*). However, only one species, *P. macroloba*, responded to the non-sterile extract with a decrease in life span and a slight increase in mass (Figure 4.4 and 4.6g-h). This result was surprising, because in a prior study seedling performance for the majority of 21 tropical tree species differed significantly between sterile and non-sterile extract treatments at low light (McCarthy-Neumann and Kobe, *in press*; Chapter 2). In particular, the non-sterile extract from conspecific soil reduced either life span or total mass for three of the species represented here (*A. membranacea*, *P. macroloba* and *P. decurrens*) and increased life span for *I. deltoidea*. We obtain consistent results between these two studies when we restrict our current data-set to compare life span and total mass only for conspecific cultured extract at low light. For instance, life span is reduced in the non-sterile vs. sterile extract treatment for *P. macroloba* (3%, $\chi 2 = 3.94$, df = 1,48, $P < 0.05$) and mass is reduced for *A. membranacea* (25%, $F_{1,14} = 2.06$, $P < 0.10$) and *P. decurrens* (25%, $F_{1,33} = 3.98$, $P < 0.10$) seedlings. The response of seedlings to non-sterile extract cultured by various

species was highly diverse (Figure 4.5), and may be partially responsible for the lack of a widespread effect of soil microbes on seedling performance in this study.

Irradiance does not interact with pathogen infection (H3) – Contrary to expectation, increased irradiance did not ameliorate the negative effect of soil microbes on seedling performance. Indeed *P. macroloba's* life span and growth were affected by soil microbes without regard to irradiance level, whereas soil origin differences for *P. decurrens* seedling growth were only different in the non-sterile treatment at high light. Tissue compensation and faster lignification are likely not operating here since they would have led to lower disease effects at high light with our methodology. However, this study may not have adequately tested whether unfavorable conditions for soil micro-organisms or increased AMF colonization reduce disease effects at high light since we minimized abiotic differences between light treatments and excluded AMF spores from microbial extracts. Differences in methodology may explain why our results contradict studies that did allow abiotic factors (e.g. soil moisture and temperature) and/or AMF colonization to vary between irradiance levels (Augspurger 1984, Augspurger and Kelly 1984, Hood et al. 2004).

Plant-soil interactions affected seedling performance by as much as 60%, which is similar in magnitude to the effect (~0-130%) that an increase in light from 1% to 5% full sun had on mean seedling mass for the same species. This moderate increase in light encompasses the endpoints typically encountered from understory to small tree fall gaps in forests at La Selva Biological Station (Chazdon and Fetcher 1984). Thus, plant-soil feedbacks could have a large influence (perhaps similar in magnitude to that of varying irradiance) on community dynamics and composition in tropical forests.

Caveats. — Excluding the filtrate component > 20 μm in soil extracts may have underestimated the role of soil microbes in plant-soil feedbacks, by eliminating some pathogens that could be present in field soil. In addition, extract culturing by trees of different species may be confounded with an underlying template of soil chemistry that determines tree species occurrence. Lastly, our study tested only the simultaneous effect of plant-soil feedbacks and light availability. Interspecific competition, another process critical in recruitment dynamics, has been shown to influence whether plant-soil feedbacks occur for grassland species (Casper and Castelli 2007, Kardol et al 2007), and a variety of other biological processes may interact with plant-soil feedbacks as well.

Comparison between tropical and temperate forests.—There is accumulating evidence of NCDD seedling mortality and reduced growth (Packer and Clay 2000, Hille Ris Lambers et al. 2002, Packer and Clay 2003) in temperate systems which have called into question the long-standing assumption of the Janzen-Connell Model that NCDD processes are less pronounced in temperate vs. tropical forests. We wanted to explicitly test this assumption by directly comparing seedling responses to NCDD processes between tropical and temperate species with parallel experiments designed to enable direct comparison. We found that soil-mediated (likely through chemical factors in the soil) species – specific feedbacks between tree adults and seedlings were ubiquitous in both temperate and tropical systems. However, the feedbacks between temperate species were largely idiosyncratic and were not consistent with the Janzen-Connell Model since soil cultured by heterospecific species were more likely to decrease seedling performance than soil cultured by conspecific individuals. In addition, soils cultured by a particular species did not necessarily improve heterospecific seedling performance relative to

conspecific seedlings (McCarthy-Neumann and Kobe, *in review*; Chapter 3). In contrast, in the tropical forest, species that were common as adults from the site where soil was collected tended to perform worse in conspecific vs. heterospecific cultured soil extract, and soils cultured by a particular species were more likely to favor heterospecific performance relative to conspecific seedling performance. These results suggest that chemical mediated plant-soil feedbacks are an important component of seedling dynamics in both temperate and tropical forests; that these feedbacks can create complex interactions between tree species, and currently there is more evidence for these feedbacks enhancing species coexistence in tropical than temperate forests.

Acknowledgements

We thank Mindy McDermott, Marisol Luna, Ademar Hurtado, Martin Cascante, Edgar Vargas and David Neumann for their invaluable assistance in the field, greenhouse and laboratory. We also thank Mike Walters, John Klironomos, Andy Jarosz, David Rothstein and Tom Baribault for comments on earlier drafts of this manuscript. The Organization for Tropical Studies and the staff at La Selva Biological Station provided logistic support. This research was funded by the National Science Foundation (DEB 0235907).

Table 4.1. List of significant seedling life span and total mass responses for all study species to: soil source (tree species culturing soil), soil extract (non-sterile vs. sterile), and irradiance (1% vs. 5% full sun) (condensed from Appendices T & U and Table 4.2).

Source of variation	Species	Life span§	Total mass‡	Integrated Seedling Performance‡
Soil Source	*Am*	Am vs. Vk (1.59, $P = 0.03$)	No significant difference	No significant difference
	Cs	Cs vs. Am (0.35, $P = 0.002$) Cs vs. Pm (0.50, $P = 0.02$) Cs. vs. Pd (0.56, $P = 0.05$) Cs. vs. Vk (0.36, $P = 0.002$)	Cs vs. Id in H light (+65%, $P = 0.03$)	Cs vs. Id in H light (+40%, $P = 0.004$)
	Id	Id vs. Cs (2.52, $P = 0.000$) Id vs. Pm (0.13, $P = 0.000$) Id vs. Pd (0.27, $P = 0.001$)	Id vs. Pm (-45%, $P = 0.000$) Id vs. Pd (-17%, $P = 0.10$)	Id vs. Cs (+31%, $P = 0.003$) Id vs. Pm (-69%, $P = 0.000$) Id vs. Pd (-38%, $P = 0.000$)
	Pm	No significant difference	Pm vs. Am (-14%, $P = 0.02$) Pm vs. Cs (-14%, $P = 0.04$) Pm vs. Pd (-11%, $P = 0.06$) Pm vs. Vk (-12%, $P = 0.06$)	Pm vs. Am (-13%, $P = 0.03$) Pm vs. Cs (-11%, $P = 0.10$) Pm vs. Pd (-9%, $P = 0.05$) Pm vs. Vk (-11%, $P = 0.10$)
	Pd•	Pd vs. Vk (0.26, $P = 0.003$)	Pd vs. Id in N-S extract and H light (-46%, $P = 0.01$) Pd vs. Vk in N-S extract and H light (-37%, $P = 0.10$)	Pd vs. Am in N-S extract and H light (-33%, P = 0.08) Pd vs. Cs in N-S extract and H light (-30%, $P = 0.01$) Pd vs. Id in N-S extract and H light (-54%, $P = 0.000$) Pd vs. Pm in N-S extract and H light (-31%, $P = 0.04$) Pd vs. Vk in N-S extract and H light (-42%, $P = 0.005$)

Table 4.1. (Ctd)

	Pd	Pd vs. Vk (0.26, $P = 0.003$)	Pd vs. Cs in L light (+8%, $P = 0.01$) Pd vs. Id in L light (+9%, $P = 0.005$)	NA
	Vk	No significant difference	Vk vs. Am (-25% $P = 0.000$) Vk vs. Cs (-31% $P = 0.000$) Vk vs. Id in N-S extract (-34%, $P = 0.000$) Vk vs. Pm (-24% $P = 0.000$) Vk vs. Pd in N-S extract (-24%, $P = 0.01$)	Vk vs. Am in N-S extract (-25%, P = 0.002) Vk vs. Cs in N-S extract (-20%, $P = 0.002$) Vk vs. Id in N-S extract (-27%, $P = 0.000$) Vk vs. Pm in N-S extract (-10%, $P = 0.02$) Vk vs. Pd in N-S extract (-18%, $P = 0.01$)
Soil Extract	*Am*	No significant difference	No significant difference	NA
	Cs	No significant difference	No significant difference	NA
	Id	No significant difference	No significant difference	NA
	Pm	N-S vs. S (2.78, $P = 0.004$)	N-S vs S (+5%, $P = 0.09$)	NA
	Pd●	No significant difference	No significant difference	NA
	Pd	No significant difference	No significant difference	NA
	Vk	No significant difference	No significant difference	NA
Irradiance	*Am*	L vs. H (2.65, $P = 0.000$)	L vs. H (-61%, $P = 0.000$)	NA
	Cs	L vs. H (2.43, $P = 0.000$)	L vs. H (-45%, $P = 0.000$)	NA
	Id	No significant difference	No significant difference	NA
	Pm	L vs. H (3.87, $P = 0.001$)	L vs. H (-27%, $P = 0.001$)	NA
	Pd●	L vs. H (2.17, $P = 0.002$)	L vs. H (-14% $P = 0.002$)	NA
	Pd	L vs. H (2.17, $P = 0.002$)	No significant difference	NA
	Vk	No significant difference	L vs. H (-15%, $P = 0.000$)	NA

Table 4.1. (Ctd)

Note: §Hazard ratios > 1 indicate reduction in days to mortality of study species. ‡Total mass values are the negative (-) or positive (+) percent difference in seedling response between conspecific and heterospecific tree soil sources, non-sterile and sterile extract, and low and high light. • *Prestoea decurrens* seedling mass did not include cotyledon mass. *Prestoea decurrens* seedling mass did not include cotyledon mass. Description of abbreviations: L = low light and H = high light; N-S = non-sterile and S = sterile; Am = *Apeiba membranacea*, Cs =*Colubrina spinosa*, Id = *Iriartea deltoidea*, Pm = *Pentaclethra macroloba*, Pd = *Prestoea decurrens*, and Vk = *Virola koschnyi*, NA = not applicable since analyses was not conducted. *P* values for multiple comparisons have been adjusted with a Holm adjustment.

Table 4.2. Reciprocal effects (percent difference in integrated seedling performance [(mean total mass x mean life span) / (days of experiment)]) of plant-soil feedbacks for each study species integrated across extract treatments and irradiance level.

	Species of Adult Culturing the Soil					
Species of Seedling Responding to Soil	*Apeiba membranacea*	*Colubrina spinosa*	*Iriartea deltoidea*	*Pentaclethra macroloba*	*Prestoea decurrens*	*Virola koschnyi*
Apeiba membranacea	**-40%** (-53, -26%)	-43% (-54, -31%)	-43% (-53, -33%)	-36% (-44, -26%)	-36% (-47, -24%)	-47% (-59, -32%)
Colubrina spinosa	3% (-13, 22%)	**-15%** (-32, 7%)	-55% (-67, -41%)	-22% (-38, -5%)	-8% (-28, 15%)	-13% (-26, 4%)
Iriartea deltoidea	-28% (-40, -16%)	-56% (-69, -44%)	**-25%** (-38, -12%)	44% (33, 54%)	13% (4, 22%)	-26% (-37, -15%)
Pentaclethra macroloba	-8% (-4, -3%)	-10% (-19, -1%)	-15% (-22, -9%)	**-21%** (-27, -14%)	-12% (-18, -5%)	-9% (-15, -4%)
Prestoea decurrens	1% (-22, 21%)	-1% (-16, 11%)	21% (10, 34%)	-1% (-20, 15%)	**-32%** (-53, -13%)	10% (-6, 23%)

Table 4.2. (Ctd)

Virola koschnyi	20%	20%	27%	10%	18%	**-5%**
	(10, 25%)	(11, 26%)	(19, 35%)	(1, 22%)	(5, 29%)	(-15, 5%)

Note: Results for *C. spinosa* feedbacks are from high light, *V. koschnyi* feedbacks from non-sterile extract treatment, and *P. decurrens* feedbacks are from non-sterile extract treatment in high light. Effect of conspecific cultured soil on seedlings is in bold. Bootstrap derived 95% CI are in parentheses.

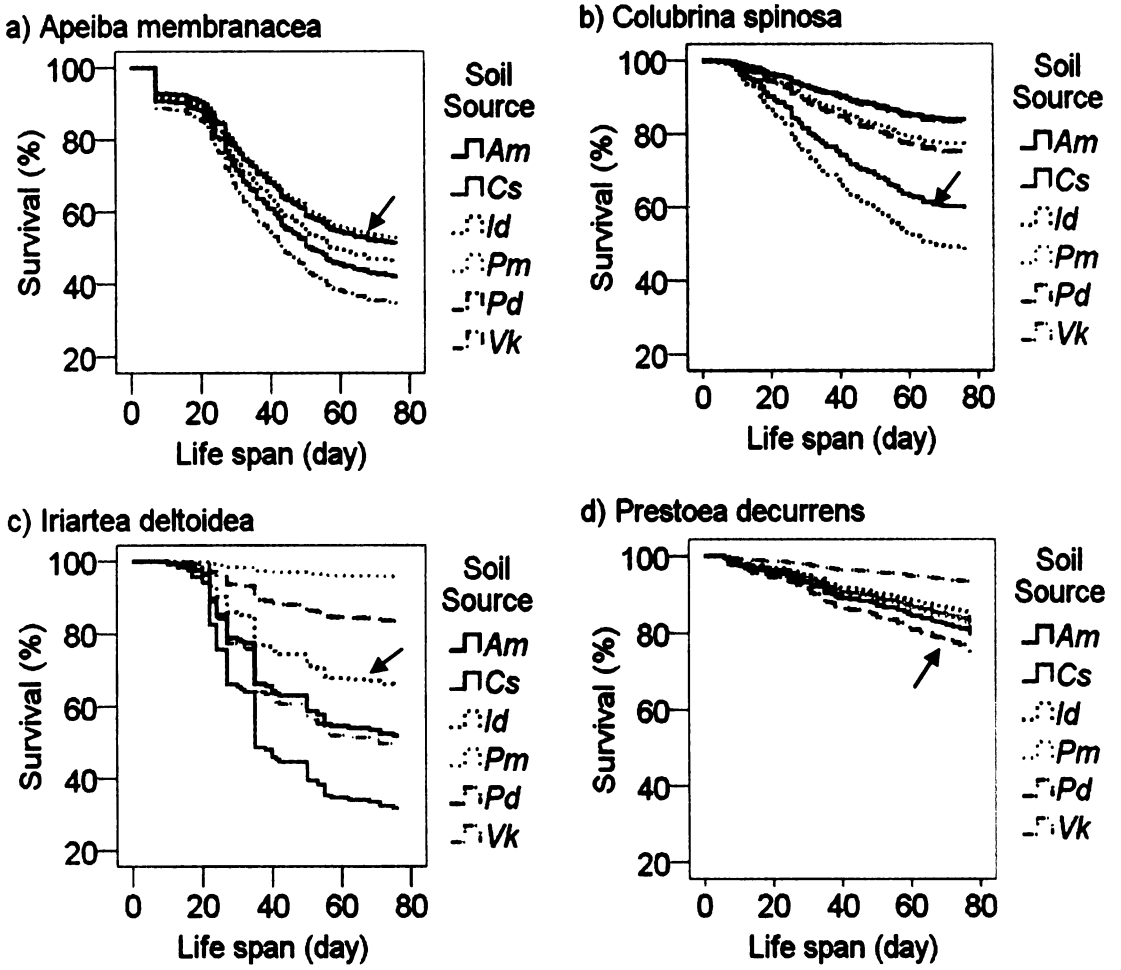
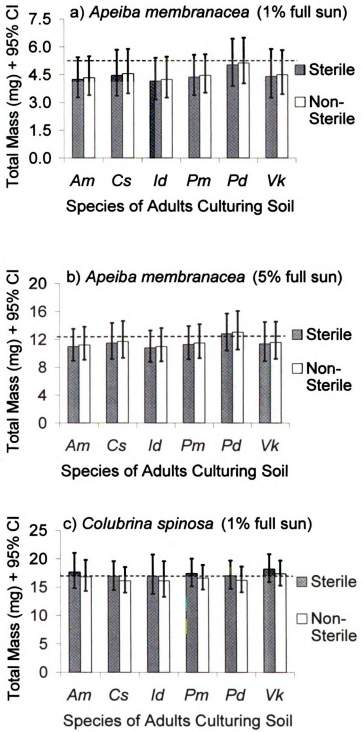


Figure 4.1. Survival curves for study species [a) *Apeiba membranacea*, b) *Colubrina spinosa*, c) *Iriartea deltoidea*, and d) *Prestoea decurrens*] with significant effects of soil source (tree species culturing soil: *Am* = *Apeiba membranacea*, *Cs* = *Colubrina spinosa*, *Id* = *Iriartea deltoidea*, *Pm* = *Pentaclethra macroloba*, *Pd* = *Prestoea decurrens* and *Vk* = *Virola koschnyi*). Arrows indicate seedling response to conspecific cultured soil source.

Figure 4.2. Total final mass by soil source (tree species culturing soil) and extract (sterile vs. non-sterile) for each study species in low and high light (*Apeiba membranacea* (*Am*) a-b, *Colubrina spinosa* (*Cs*) c-d, *Iriartea deltoidea* (*Ia*) e-f, *Pentaclethra macroloba* (*Pm*) g-h, *Prestoea decurrens* (*Pd*) seedling mass without cotyledon mass i-j and seedling mass with cotyledon mass k-l, and *Virola koschnyi* (*Vk*) m-n). Dotted line represents seedling mass when grown with tap water and is shown for reference only, and were not included in statistical analysis.

Figure 4.2. (Ctd)



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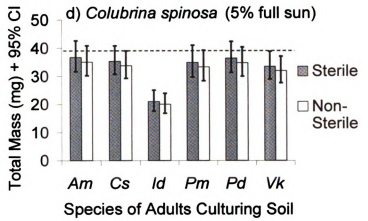

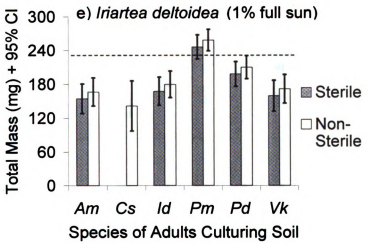

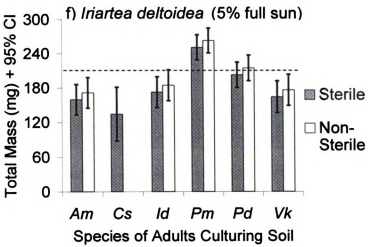

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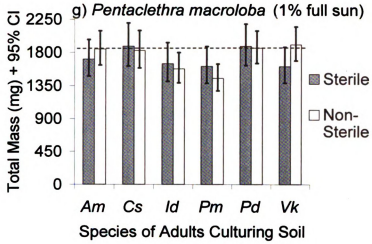

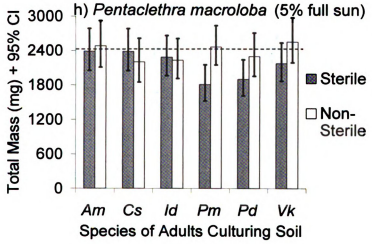

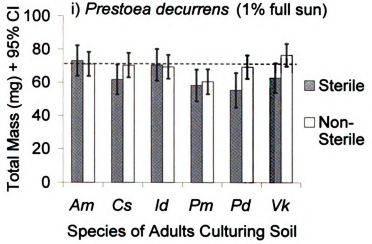

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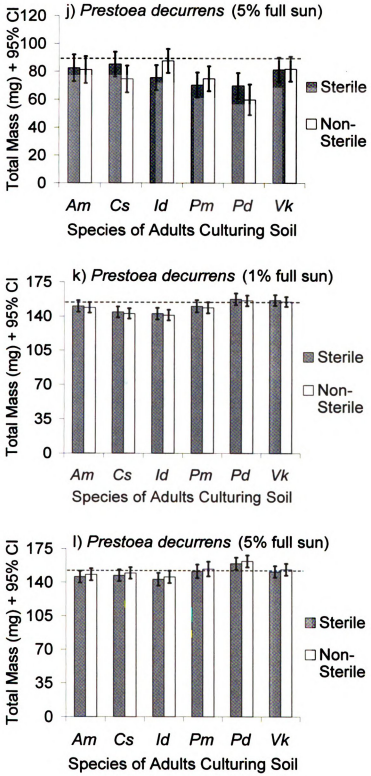

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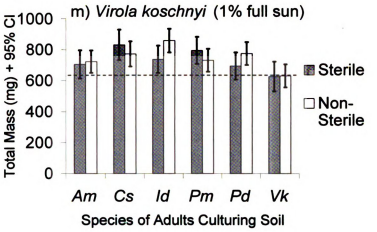
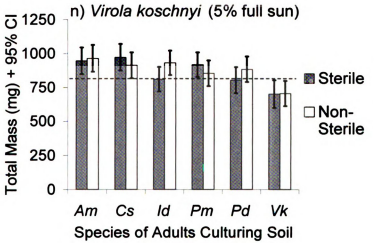




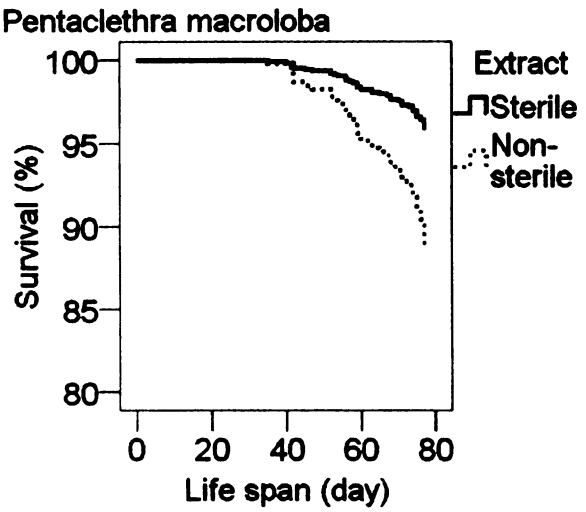


Figure 4.3. Survival curve for *Pentaclethra macroloba* seedlings by soil microbial treatment (sterile vs. non-sterile extract) and integrated across soil source and irradiance level.

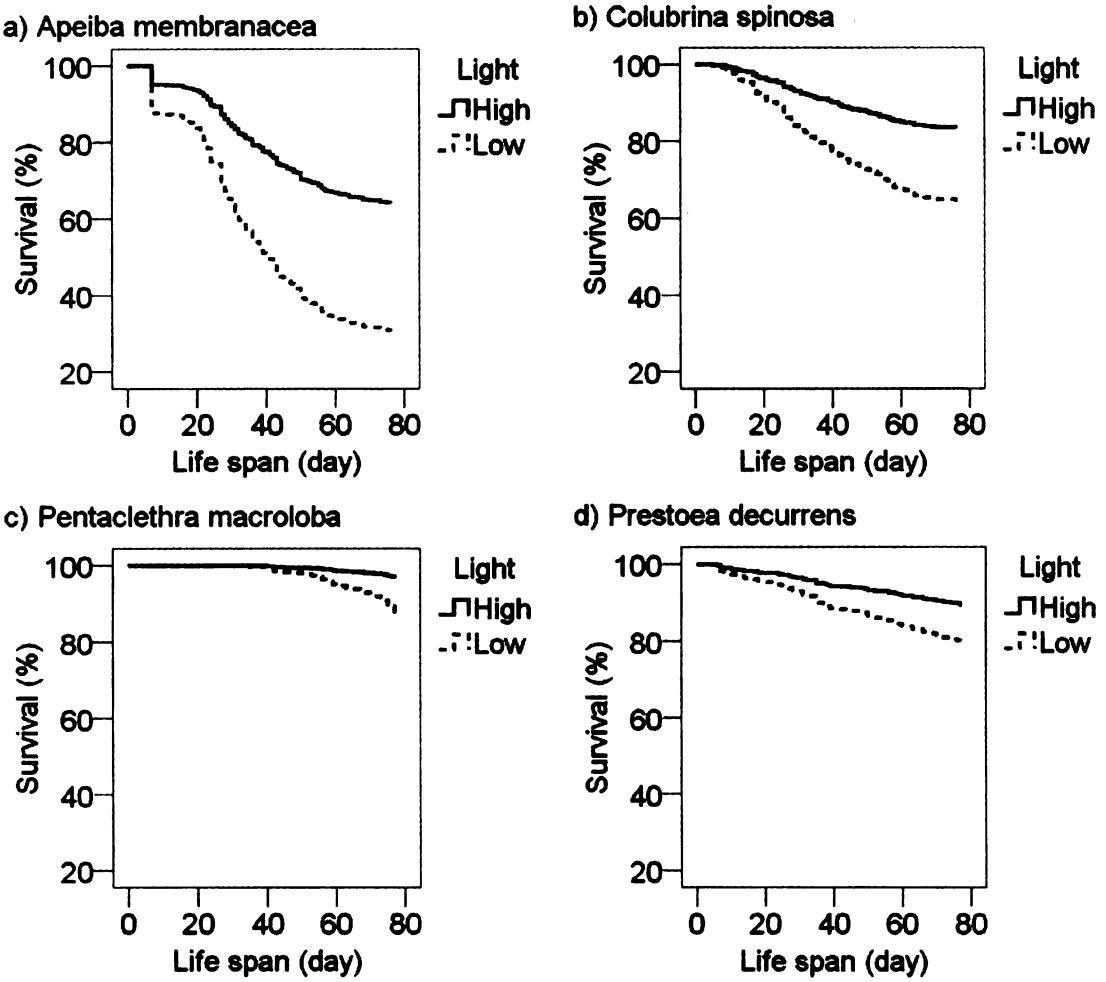


Figure 4.4. Survival curves for study species [a) *Apeiba membranacea*, b) *Colubrina spinosa*, c) *Pentaclethra macroloba*, and d) *Prestoea decurrens*] with significant effect of light level (high light; 5% full sun vs. low light; 1% full sun) and integrated across soil source and soil microbial treatment.

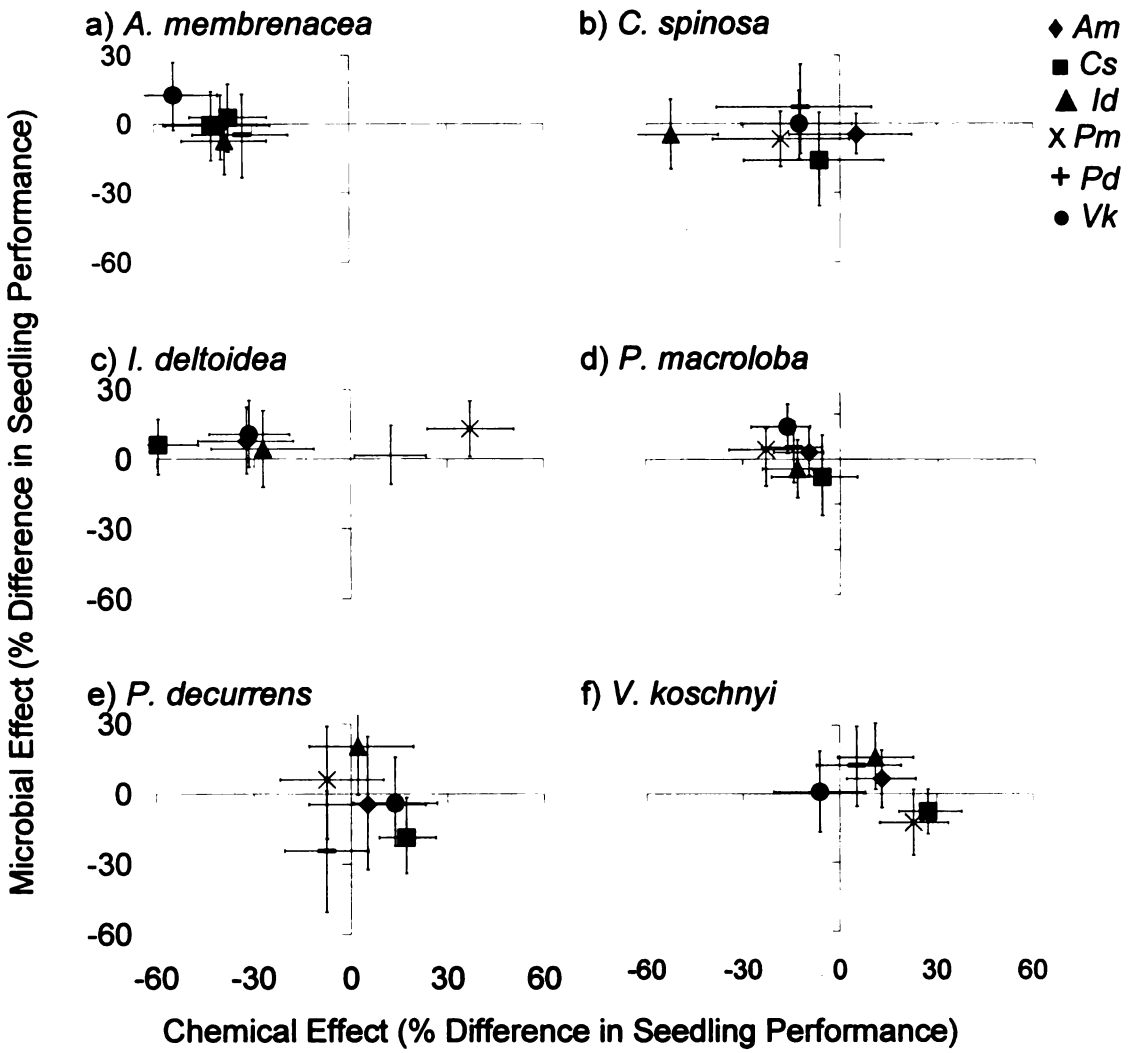


Figure 4.5. Relationship between chemical [sterile extract / tap water] and microbial [(un-sterile extract / tap water) – (sterile / tap water)] effects in soil extracts "cultured" by different tree species on seedling performance [(mean total mass x mean life span) / (days of experiment)]) for each study species integrated across irradiance levels, except for *C. spinosa* and *P. decurrens* seedlings whose results are only from high light. *Am* = *Apeiba membranacea*, *Cs* = *Colubrina spinosa*, *Id* = *Iriartea deltoidea*, *Pm* = *Pentaclethra macroloba*, *Pd* = *Prestoea decurrens* and *Vk* = *Virola koschnyi*). Bootstrap devised 95% CI included.

CHAPTER FIVE

CONCLUSION

My goal in this dissertation was to test the major assumptions of the Janzen-Connell (J-C) Model using plant-soil feedbacks as the particular agent for creating differences in tree seedling performance. In this chapter I will briefly outline the major results from my dissertation and suggest avenues of research that may answer questions raised by the research.

Plant-Soil Feedbacks - The preceding chapters have documented that species-specific plant-soil feedbacks influence both temperate and tropical seedling performance which may in turn affect tree community dynamics and composition. The Janzen-Connell Model can be placed within the larger context of negative feedbacks between plants and the soil in which they grow. Surprisingly, the effect of adult culturing on seedling performance occurred in both non-sterile and sterile soil extracts presumably reflecting a chemical rather than a microbial process. This effect may be mediated by tree species induced differences in base cation availability, at least for the temperate species since the amount of base cations in the temperate soil extracts varied depending upon the species of tree that cultured the soil. This is a novel mechanism for producing NCDD mortality and/or reduced growth. Experiments linking soil chemical factors with species-specific feedbacks between tree adults and seedlings are still needed to fully ascertain which

chemical factors are responsible for creating these feedbacks and documenting the full extent of these feedbacks on a variety of tree species.

Distance and Density-Dependent Processes - In the temperate study (chapter 3), I determined that plant-soil feedbacks created by different species of adults culturing the soil were generally not influenced by the species of seedling culturing the soil nor the density of those seedlings. Thus, my results suggest that plant-soil feedbacks are more important in creating distance (e.g. conspecific vs. heterospecific) rather than density-dependent effects on seedling performance. The lack of a density effect on seedling performance in my study differs from the positive relationship between seedling density and disease induced seedling mortality documented by other researchers (Packer and Clay 2000, Bell et al. 2006). Conflicting results among studies might arise because of different methodologies, especially if density enhances disease transmission through close proximity of roots, which was eliminated in our extraction-based study.
For temperate species heterospecific and conspecific adult interactions with the soil had similar prevalence and magnitude of influence on seedling performance, contrary to J-C expectations. For tropical species, however, negative feedbacks from conspecific individuals were stronger than heterospecific feedbacks for the majority of the species (chapter 4).

I find evidence in both chapters 3 and 4 that a species-specific approach was more appropriate than simply comparing seedling responses to near vs. far conditions. In addition, by comparing species-pair interaction to these plant-soil feedbacks, I was able to assess whether reduced seedling performance from non-competitive distance-dependent mortality and/or growth in a particular species contributed to greater success

of heterospecific seedlings. Surprisingly, the large negative impact that conspecific cultured soil had on a focal species' seedlings did not necessarily result in heterospecific seedlings having relative better performance. Future work should concentrate not only on the relative benefits to seedling recruitment of 'escape' from conspecific adults, but also the relative recruitment success among species dispersed into neighborhoods influenced by heterospecific adults. In combination, these two approaches to NCDD processes will provide a better understanding of how well plant-soil feedbacks enable species coexistence.

Host-Specificity – Soil pathogens decreased seedling performance for three temperate and one tropical species, but this negative biotic effect was consistent regardless of which species of adult had cultured the soil, which indicates that these soil pathogens may not exhibit host-specificity. When investigating pathogen host-specificity in the temperate species I found that infection by five *Fusarium* morphotypes, isolated from roots of dead seedlings, affected seedling performance differentially among these species but were neither strictly host-specific nor strictly generalist.

Seedlings of the study species did not react uniformly to any of the soils cultured by a particular species (i.e. no soil extract was either the most beneficial or the most detrimental for all of the tree species). This result suggests that the chemical factors mediating the plant-soil feedbacks documented in my dissertation differentially influence seedlings of different species, which is akin to pathogen host-specificity. Future research is necessary not only to determine which chemical factors are responsible for creating these feedbacks, but also how they affect these species differently and whether there are

any species traits that make them more likely to be susceptible to these chemically mediated plant-soil feedbacks.

Light and Disease Interaction - Irradiance levels did not influence response or susceptibility to disease for my tropical study species, but the negative effect of soil microbes on growth occurred only in high light for two of my temperate study species. These results suggest that growth for some species is so severely constrained in shade that the effect of soil microbes were not manifested, but when light limitation is alleviated microbial impacts can become apparent. Light availability did not affect how seedlings responded to soil extract cultured by conspecific vs. heterospecific adults in a consistent manner; for most species feedbacks were consistent regardless of light availability, for a few species there were greater negative effects on seedling mass from conspecific than heterospecific cultured soils in low light and for a few other species this pattern occurred only in high light.

By simultaneously testing the effects of plant-soil feedbacks and light availability on seedling performance in both temperate and tropical species, I can compare the likely impact that these two mechanisms have on community dynamics and composition. Plant-soil interactions affected seedling performance by as much as 40% in the temperate and 60% in the tropical forest, a magnitude of effect that would be expected to influence community dynamics and composition. Light availability, primarily through changes in mean seedling mass, had a much larger impact (between 90-3000% increase from 2% to 22% full sun with temperate species and 0-130% increase from 1% to 5% full sun with tropical species) on seedling performance. Thus, plant-soil feedbacks may be similar in impact to moderate increases in light availability that occur frequently in the forest

understorey, but play a secondary role to the increases in light availability created from less frequently forming large canopy gaps in tree community dynamics.

Species' Abundance – In the second chapter working with 21 tropical tree species, I demonstrated that susceptibility to soil microbes cultured by conspecific individuals co-varied with a species' seedling shade intolerance rather than local abundance. Thus, rather than soil micro-organisms causing negative feedbacks that constrain the abundance of common species, my results suggest that soil micro-organisms may exaggerate seedling shade tolerance differences among species which in turn may influence species coexistence through enhancing light gradient partitioning. I then used six of these tropical species (which varied in shade tolerance and abundance) to test the relative effect of conspecific vs. heterospecific cultured soils on seedling performance. My results (outlined in chapter 4) suggest that common species are more likely to experience greater negative effects on seedling performance from conspecific than heterospecific cultured soils than species that are locally rare. A major assumption of the J-C Model is that maintaining species diversity requires that NCDD processes are more prevalent in species that are common versus those that are rare. Thus, tree species coexistence could possibly be maintained through plant-soil feedbacks even though chemical factors are the main mechanism for reduced performance in the soil and not host-specific pathogens as assumed by the Janzen-Connell model. Additional species (ranging in local species abundance) need to be tested for the relative effect of plant-soil feedbacks by conspecific vs. heterospecific adults before a general consensus can be reached that these feedbacks are more detrimental to locally common species.

Temperate vs. Tropical Forests – Implicit in the J-C Model is the assumption that NCDD processes are less pronounced in temperate vs. tropical forests. I explicitly tested this assumption in this dissertation and that soil-mediated (likely through chemical factors in the soil) species – specific feedbacks between tree adults and seedlings were ubiquitous in both temperate and tropical systems. However, the feedbacks between temperate species were largely idiosyncratic and were not consistent with the Janzen-Connell hypothesis since soil cultured by a heterospecific species was more likely to decrease seedling performance than soil cultured by conspecific adults. In addition, soils cultured by a particular temperate species do not necessarily improve heterospecific seedling performance relative to conspecific seedlings (chapter 3). In contrast, tropical species that were common as adults from the site where soil was collected tended to perform worse in conspecific vs. heterospecific cultured soil extracts (chapter 4). In addition, with tropical species, heterospecific seedlings were more likely to have better performance than conspecific seedlings in soils influenced by a given individual. These results suggest that chemical mediated plant-soil feedbacks are an important component of community seedling dynamics in both temperate and tropical forests; that these feedbacks can create complex interactions between tree species, and currently there is more evidence for these feedbacks enhancing species coexistence in tropical than temperate forests. However, more work needs to focus on the relative success in seedling recruitment among species dispersed into neighborhoods influenced by adults of different tree species. In addition, studies need to begin explicitly testing whether, over time, plant diversity is greater than expected with plant-soil feedbacks (either biotic or abiotic

mediated) compared with random survival of seeds and seedlings in both temperate and tropical forests.

In summary, this work has demonstrated that negative plant-soil feedbacks are important in both temperate and tropical forests, but these feedbacks primarily are mediated not from host-specific enemies, but from chemical feedbacks that differentially affect seedlings of different species. Not all species do better with soils cultured by conspecific versus heterospecific adults, but tropical species that are locally common appear to be more likely to be affected in this manner. My dissertation research has raised as many questions as it has answered. I plan on investigating some of these questions, and I hope that the results presented in this dissertation will also stimulate more research into the role that plant-soil feedbacks (particularly those mediated by chemical factors) have on tree community dynamics.

Appendix A. Kaplan-Meier analysis of seedling life span compared between microbial vs. sterilized microbial extract for all study species.

Species	Extract	Life span (std. error)	Breslow, χ	*P* value
Luehea seemannii (Intolerant / Common)	Microbial	**38.90 (4.51)**	4.25*	*P* = *
	Sterile	**50.60 (4.13)**		
Neea psychotroides (Intolerant / Common)	Microbial	60.53 (3.92)	1.70•	*P* = NS
	Sterile	65.72 (2.29)		
Apeiba membranacea (Intolerant / Intermediate)	Microbial	49.51 (4.40)	0.43•	*P* = NS
	Sterile	52.47 (4.10)		
Colubrina spinosa (Intolerant / Intermediate)	Microbial	63.77 (2.65)	0.31•	*P* = NS
	Sterile 1	63.10 (2.84)		
Guatteria diospyroides (Intolerant / Intermediate)	Microbial	66.61 (2.03)	1.19	*P* = NS
	Sterile	63.33 (2.70)		
Psychotria panamensis (Intolerant / Intermediate)	Microbial	66.64 (2.41)	0.35	*P* = NS
	Sterile 1	68.00 (1.97)		
Castilla elastica (Intolerant / Rare)	Microbial	68.05 (1.95)	1.00	*P* = NS
	Sterile	70.00 (0.00)		
Coussarea hondensis (Intermediate / Common)	Microbial	**47.57 (4.90)**	3.19•	*P* = †
	Sterile	**54.60 (4.19)**		
Pentaclethra macroloba (Intermediate / Common)	Microbial	70.00 (0.00)	1.00	*P* = NS
	Sterile	68.90 (1.08)		
Prestoea descurrens (Intermediate / Common)	Microbial	**59.02 (4.61)**	2.83•	*P* = †
	Sterile	**65.81 (2.87)**		
Quararibea bracteolosa (Intermediate / Rare)	Microbial	43.47 (6.47)	0.08•	*P* = NS
	Sterile	47.73 (7.64)		
Trophis racemosa (Intermediate / Rare)	Microbial	59.78 (4.18)	0.31•	*P* = NS
	Sterile	61.92 (3.78)		
Vochysia ferruginea (Intermediate / Rare)	Microbial	50.97 (5.08)	0.28•	*P* = NS
	Sterile	52.07 (4.76)		
Capparis pittieri (Tolerant / Common)	Microbial	63.70 (2.54)	0.07•	*P* = NS
	Sterile	63.17 (2.83)		
Euterpe precatoria (Tolerant / Common)	Microbial	64.07 (2.73)	1.37	*P* = NS
	Sterile	55.30 (4.45)		
Iriartea deltoidea (Tolerant / Common)	Microbial	**65.45 (2.52)**	4.32*	*P* = *
	Sterile	**57.23 (3.94)**		
Welfia regia (Tolerant / Common)	Microbial	**70.00 (0.00)**	2.81*	*P* = †
	Sterile	**66.70 (2.12)**		
Virola koschnyi (Tolerant / Intermediate)	Microbial	70.00 (0.00)	1.00	*P* = NS
	Sterile	65.892 (3.97)		
Dussia macrophyllata (Tolerant / Rare)	Microbial	53.79 (6.89)	0.29	*P* = NS
	Sterile	48.93 (6.88)		
Miconia affinis (Tolerant / Rare)	Microbial	96.90 (3.91)	1.31*	*P* = NS
	Sterile	85.92 (6.42)		

Appendix A. (Ctd)

Stryphnodendron microstachyum (Tolerant / Rare)	Microbial Sterile	56.67 (4.35) 47.07 (4.73)	1.14•	P = NS

Notes: Seedlings that did not emerge are part of the survival analysis with their death date given as the mean number of days prior to emergence. "•" indicates that the Breslow statistic for effect of microbial extract was adjusted for the effect of adult culturing the soil. "*" indicates that the Breslow statistic for effect of microbial extract was adjusted for the effect of bench. Degrees of freedom for all species are 1. Significance is shown as: NS, not significant; † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. When the extract treatment is significant ($P \leq 0.10$) the means between the microbial and sterile treatment means are in bold.

Appendix B. Analysis of covariance results for the effects of inoculum treatment on seedling extract; adult = location where soil was collected for use in extraction; bench = location that

Species (Shade Tolerance / Adult Abundance Classification)	Dependent Variable	Inoculum (1)			
		N	*F*	*P*	Means (Std. er Microbial vs. S
Luehea seemannii (Intolerant / Common)	Root Mass	22	0.44	NS	0.06 (0.09) vs.
	Stem Mass	23	0.79	NS	0.34 (0.03) vs.
	Leaf Mass	23	0.48	NS	0.42 (0.06) vs.
	Total Mass	23	0.16	NS	0.83 (0.16) vs.
	Root Length	23	0.06	NS	0.46 (0.16) vs.
	Root Surface Area	23	0.29	NS	0.06 (0.02) vs.
	Height	23	0.52	NS	16.33 (1.21) vs.
Neea psychotroides (Intolerant / Common)	Root Mass	48	0.03	NS	2.38 (0.23) vs.
	Stem Mass	48	0.92	NS	3.98 (0.21) vs.
	Leaf Mass	48	2.44	NS	12.72 (0.84) vs.
	Total Mass	48	2.09	NS	19.06 (1.13) vs.
	Root Length	48	0.29	NS	12.56 (1.03) vs.
	Root Surface Area	48	0.08	NS	2.08 (0.18) vs.
	Height	48	0.67	NS	32.17 (1.15) vs.
Apeiba membranacea (Intolerant / Intermediate)	Root Mass	20	0.10	NS	0.68 (0.07) vs.
	Stem Mass	21	0.69	NS	1.54 (0.08) vs.
	Leaf Mass	21	6.38	*	**2.26 (0.22) vs.**
	Total Mass	20	4.26	†	**4.61 (0.30) vs.**
	Root Length	20	0.00	NS	3.27 (0.50) vs.
	Root Surface Area	20	0.08	NS	0.54 (0.07) vs.
	Height	21	0.02	NS	23.15 (2.27) vs.
Colubrina spinosa (Intolerant / Intermediate)	Root Mass	47	0.06	NS	2.87 (0.18) vs.
	Stem Mass	47	0.79	NS	5.70 (0.31) vs.
	Leaf Mass	47	0.59	NS	13.62 (1.00) vs.
	Total Mass	47	0.87	NS	22.09 (1.39) vs.
	Height	47	2.38	NS	56.58 (2.96) vs.
Guatteria diospyroides (Intolerant / Intermediate)	Root Mass	36	3.02	†	**5.20 (0.28) vs.**
	Stem Mass	36	0.18	NS	10.19 (0.31) vs.
	Leaf Mass	36	0.85	NS	5.58 (0.88) vs.
	Total Mass	36	0.59	NS	21.34 (0.97) vs.
	Root Length	36	1.81	NS	19.89 (1.30) vs.
	Root Surface Area	36	1.43	NS	3.75 (0.21) vs.
	Height	36	0.34	NS	74.38 (2.79) vs.
Psychotria panamensis (Intolerant / Intermediate)	Root Mass	47	0.05	NS	3.63 (0.16) vs.
	Stem Mass	47	0.28	NS	5.80 (0.27) vs.
	Leaf Mass	47	0.16	NS	9.49 (0.60) vs.
	Total Mass	47	0.00	NS	18.87 (0.64) vs.
	Root Length	46	0.00	NS	26.09 (1.15) vs.

stem height and root morphology. Inoculum = microbial vs. sterilized microbial
igs were randomly assigned to during the experiment; covariate = initial seed mass.

	Source of Variation (df)							
	Adult (3)		Bench (5)		Covariate (1)		Adult x Covariate (1)	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
.07)	—	—	—	—	2.45	NS	—	—
.02)	—	—	—	—	54.55	***	—	—
.05)	—	—	—	—	23.98	***	—	—
.12)	—	—	—	—	17.33	***	—	—
.13)	—	—	—	—	41.74	***	—	—
.02)	—	—	—	—	67.97	***	—	—
(0.97)	—	—	—	—	—	—	—	—
.22)	4.25	**	—	—	2.37	NS	—	—
.20)	3.19	*	1.66	NS	—	—	—	—
(0.79)	9.66	***	—	—	—	—	—	—
(1.06)	9.65	***	—	—	—	—	—	—
(0.97)	8.07	***	—	—	—	—	—	—
.17)	2.62	†	—	—	—	—	—	—
(1.10)	—		—		—	—	—	—
.06)	2.31	NS	—	—	—	—	—	—
.07)	—	—	—	—	26.53	***	—	—
.20)	1.72	NS	—	—	2.04	NS	—	—
.25)	—	—	—	—	10.33	†	—	—
.41)	—	—	—	—	—	—	—	—
.05)	—	—	—	—	—	—	—	—
(2.04)	3.82	*	—	—	—	—	—	—
.19)	—	—	4.69	**	13.77	**	—	—
.32)	—	—	—	—	4.41	*	—	—
(1.06)	—	—	—	—	—	—	—	—
(1.49)	—	—	—	—	1.95	NS	—	—
(3.16)	—	—	—	—	1.58	NS	—	—
.29)	—	—	—	—	15.38	***	—	—
0.32)	—	—	—	—	22.80	***	—	—
.92)	3.07	*	—	—	—	—	—	—
(1.03)	—	—	—	—	16.34	***	—	—
(1.38)	—	—	—	—	13.45	***	—	—
.23)	—	—	—	—	16.56	***	—	—
(2.89)	4.40	*	—	—	—	—	—	—
.16)	—	—	6.47	***	42.55	***	—	—
.26)	—	—	—	—	3.44	†	—	—
.59)	—	—	—	—	5.29	*	—	—
(0.63)	—	—	3.47	**	20.11	***	—	—
(1.11)	1.98	NS	2.99	*	57.57	**	—	—

Appendix B. (Ctd)

	Root Surface Area	46	0.94	NS	4.15 (0.18) vs
	Height	47	0.55	NS	48.46 (1.57) t
Castilla elastica	Root Mass	59	0.27	NS	17.78 (0.86) t
(Intolerant / Rare)	Stem Mass	59	0.03	NS	34.35 (1.01) t
	Leaf Mass	58	5.83	*	**79.56 (3.14)** t
	Total Mass	58	3.76	†	**131.54 (4.16)** t
	Root Length	58	7.07	**	**103.41 (4.92)** t
	Root Surface Area	58	3.07	†	**17.06 (0.85)** t
	Height	59	0.20	NS	114.33 (2.59) t
Coussarea hondensis	Root Mass	36	0.05	NS	15.43 (1.55) vt
(Intermediate / Common)	Stem Mass	36	3.84	†	**36.37 (1.75)** vt
	Leaf Mass	36	0.31	NS	42.09 (1.64) vt
	Total Mass	36	1.37	NS	93.43 (2.15) vt
	Root Length	36	0.10	NS	22.84 (2.40) vt
	Root Surface Area	36	0.31	NS	4.22 (0.38) vst
	Height (final – initial)	36	0.91	NS	10.52 (1.51) vt
Pentaclethra macroloba	Root Mass	58	0.28	NS	299.17 (16.87)
(Intermediate / Common)	Stem Mass	58	0.72	NS	569.90 (40.48)
	Leaf Mass	58	1.59	NS	726.10 (51.47)
	Cotyledon Mass*	58	1.41	NS	176.82 (96.54)
	Total Mass	58	3.92	†	**1760.56 (138.1**
	Root Length	58	0.34	NS	206.64 (15.27)
	Root Surface Area	58	0.33	NS	41.48 (2.72) vs
	Height	58	0.02	NS	370.3 (16.76) v
Prestoea descurrens	Root Mass	48	0.06	NS	16.52 (0.87) vs
(Intermediate / Common)	Stem Mass	48	7.52	**	**13.51 (0.82)** vt
	Leaf Mass	48	7.00	*	**19.73 (1.56)** vs
	Cotyledon Mass	47	6.74	*	**60.70 (4.25)** vt
	Total Mass	48	1.89	NS	109.26 (2.83) v
	Root Length	48	0.99	NS	32.76 (1.89) v
	Root Surface Area	48	0.54	NS	5.92 (0.32) vs
	Height	48	2.32	NS	53.91 (2.80) v
Quararibea bracteolosa	Root Mass	15	0.05	NS	38.90 (5.05) v
(Intermediate / Rare)	Stem Mass	15	1.09	NS	47.20 (5.37) v
	Leaf Mass	15	3.21	†	**82.08 (14.22)**
	Total Mass	15	3.04	NS	175.56 (18.02
	Height	15	0.07	NS	64.45 (3.84) v
Trophis racemosa	Root Mass	51	4.05	*	**4.09 (0.21) vs**
(Intermediate / Rare)	Stem Mass	51	0.14	NS	10.29 (0.41) v
	Leaf Mass	51	0.12	NS	22.35 (0.94) v
	Cotyledon Mass	51	0.15	NS	13.94 (1.38) v
	Total Mass	51	0.00	NS	50.68 (2.02) v
	Root Length	51	0.64	NS	16.92 (0.78) v
	Root Surface Area	51	1.88	NS	2.98 (0.13) vs
	Height	51	2.38	NS	76.40 (2.28) v

4.15( ) (0.19)	1.83	NS	2.50	*	15.35	***	—	—
48.46 .10 (1.54)	—	—	—	—	—	—	—	—
17.78 .36 (0.78)	4.52	**	—	—	54.11	***	—	—
34.35 .12 (1.01)	2.92	*	—	—	76.03	***	—	—
79.56 0.42 (3.21)	—	—	—	—	48.91	***	—	—
131.54 43.35 (4.24)	—	—	—	—	67.97	***	—	—
103.41 21.73 (4.83)	1.74	NS	—	—	46.47	***	—	—
17.06 0.14 (0.83)	1.61	NS	—	—	41.14	***	—	—
114.3 15.95 (2.59)	2.01	NS	—	—	22.13	***	—	—
15.43 3.93 (1.47)	—	—	—	—	20.10	***	—	—
36.37 9.14 (1.67)	—	—	—	—	65.37	***	—	—
42.09 .85 (1.53)	—	—	2.43	†	88.02	***	—	—
93.43 5.88 (2.01)	—	—	4.52	**	228.81	***	—	—
22.84 .80 (2.27)	—	—	—	—	12.62	***	—	—
4.22 .3 (0.37)	—	—	1.53	NS	4.57	*	—	—
10.52 .19 (1.36)	2.67	†	2.42	†	—	—	—	—
299.1 311.81 (16.87)	—	—	1.59	NS	19.32	***	—	—
569.9 618.55 (40.48)	—	—	—	—	19.95	***	—	—
726.1 817.94 (51.47)	—	—	—	—	11.93	***	—	—
176.8 374.61 (110.87)	—	—	—	—	—	—	—	—
1760.5 vs. 2148.90 (138.90)	1.67	NS	1.53	NS	12.24	***	—	—
206.64 219.18 (15.27)	—	—	—	—	2.73	NS	—	—
41.48 .69 (2.73)	1.83	NS	—	—	3.98	†	—	—
370.3 73.4 (16.76)	—	—	—	—	11.91	***	—	—
16.52 .82 (0.80)	—	—	—	—	3.34	†	—	—
13.51 0.40 (0.72)	1.69	NS	—	—	9.29	**	—	—
19.73 3.03 (1.36)	2.52	†	—	—	1.58	NS	—	—
60.70 8.35 (3.79)	2.32	†	—	—	11.35	*	—	—
109.26 03.90 (2.66)	—	—	—	—	48.23	***	—	—
32.76 .16 (1.66)	2.61	†	—	—	—	—	—	—
5.92 .2 (0.29)	1.66	NS	—	—	—	—	—	—
53.91 .45 (2.51)	4.50	**	—	—	—	—	—	—
38.90 .44 (4.73)	—	—	—	—	—	—	—	—
47.20 .30 (5.02)	—	—	—	—	—	—	—	—
82.08 15.22 (13.66)	2.71	NS	—	—	—	—	—	—
175.58 218.55 (16.86)	—	—	—	—	—	—	—	—
64.45 .11 (3.69)	4.96	*	—	—	—	—	—	—
4.09 .8 (0.20)	1.80	NS	2.13	†	12.45	**	—	—
10.29 .08 (0.39)	4.75	**	—	—	19.96	***	5.99	**
22.35 .79 (0.90)	5.34	**	—	—	37.97	***	8.34	***
13.94 .19 (1.33)	4.89	**	—	—	6.77	*	6.35	***
50.68 .73 (1.95)	9.56	***	—	—	36.4	***	13.21	***
16.92 .77 (0.74)	4.64	**	2.12	†	—	—	—	—
2.98 .2 (0.13)	—	—	—	—	3.22	NS	—	—
76.40 .49 (2.19)	4.56	**	—	—	16.39	***	—	—

Appendix B. (Ctd)

Vochysia ferruginea	Root Mass	40	0.42	NS	2.92 (0.17) vs. 2.1
(Intermediate / Rare)	Stem Mass	40	0.01	NS	7.27 (0.22) vs. 7.1
	Leaf Mass	40	0.00	NS	15.78 (0.31) vs. 1
	Total Mass	40	0.19	NS	26.04 (0.50) vs. 2
	Root Length	40	2.38	NS	4.00 (0.32) vs. 3.3
	Root Surface Area	40	2.06	NS	0.86 (0.05) vs. 0.7
	Height (final – initial)	40	1.00	NS	11.15 (.847) vs. 1
Capparis pittieri	Root Mass	43	1.94	NS	76.53 (6.23) vs. 8
(Tolerant / Common)	Stem Mass	43	0.57	NS	109.66 (6.81) vs. 1
	Leaf Mass	43	0.77	NS	395.57 (20.68) vs.
	Total Mass	43	1.01	NS	580.16 (29.26) vs.
	Root Length	42	0.28	NS	26.95 (2.32) vs. 2
	Root Surface Area	42	1.65	NS	9.37 (0.57) vs. 10.
	Height	43	0.18	NS	79.82 (2.75) vs. 81
Euterpe precatoria	Root Mass	45	0.83	NS	36.08 (1.65) vs. 3
(Tolerant / Common)	Stem Mass	45	0.88	NS	13.25 (0.55) vs. 12
	Leaf Mass	45	0.43	NS	80.18 (5.37) vs. 85
	Cotyledon Mass	45	0.37	NS	137.49 (10.81) vs.
	Total Mass	45	0.07	NS	266.45 (5.22) vs. 2
	Root Length	45	1.77	NS	32.99 (1.75) vs. 36
	Root Surface Area	45	1.55	NS	6.97 (0.32) vs. 7.5
	Height	45	0.03	NS	100.55 (6.02) vs. 1
Iriartea deltoidea	Root Mass	48	5.56	*	**116.94 (6.19) vs. 1**
(Tolerant / Common)	Stem Mass	48	2.59	NS	96.10 (4.55) vs. 10
	Cotyledon Mass	48	1.76	NS	2821.98 (32.34) vs
	Total Mass	48	0.49	NS	3034.63 (28.83) v
	Root Length	48	2.49	NS	42.53 (2.90) vs. 49
	Root Surface Area	48	3.69	NS	14.56 (0.78) vs. 16
	Height	48	1.05	NS	33.04 (1.33) vs. 35
Welfia regia	Root Mass	56	0.94	NS	187.10 (6.94) vs.
(Tolerant / Common)	Stem Mass	56	0.22	NS	47.23 (2.40) vs. 48
	Leaf Mass	56	0.19	NS	296.00 (15.97) vs.
	Cotyledon Mass	56	1.56	NS	25.92 (3.10) vs. 20
	Total Mass	56	1.04	NS	555.79 (21.41) vs.
	Root Length	56	0.97	NS	211.78 (11.03) vs.
	Root Surface Area	56	1.23	NS	37.31 (1.79) vs. 34
	Height	56	1.16	NS	146.89 (5.93) vs. 1
Virola koschnyi	Root Mass	28	1.83	NS	176.93 (13.50) vs.
(Tolerant / Intermediate)	Stem Mass	28	1.54	NS	483.66 (24.71) vs.
	Leaf Mass	28	3.68	†	**263.11 (16.57) vs.**
	Cotyledon Mass	28	0.95	NS	7.90 (2.09) vs. 4.9
	Total Mass	28	0.12	NS	933.05 (39.19) vs.
	Height	28	1.71	NS	216.67 (6.52) vs. 2

2 (0.17)	6)	1.89	NS	—	—	18.70	***	—	—
7 (0.22)	2)	—	—	2.19	†	13.83	***	—	—
78 (0.5 | .28)	2.15	NS	—	—	108.75	***	—	—
04 (0.5 | .46)	2.55	†	—	—	105.56	***	—	—
0 (0.32 | 2)	—	—	—	—	—	—	—	—
6 (0.05 | 5)	—	—	—	—	2.06	NS	—	—
15 (1.8 | 847)	—	—	—	—	—	—	—	—
53 (6.2 | .09)	8.27	***	2.80	*	54.88	***	11.08	***
9.66 (6.9	(6.81)	8.06	***	—	—	57.02	***	7.33	**
5.57 (20	7 (20.66)	10.80	***	—	—	67.07	***	12.10	***
0.16 (3	4 (29.23)	11.43	***	—	—	80.29	***	12.68	***
.95 (2.2 | 132)	—	—	—	—	14.36	***	—	—
37 (0.5 | 57)	1.54	NS	—	—	18.88	***	—	—
.82 (2.7 | 69)	—	—	—	—	27.53	***	—	—
.08 (1.6 | .76)	—	—	—	—	—	—	—	—
.25 (0.5 | .58)	1.80	NS	—	—	—	—	—	—
.18 (5.3 | .74)	—	—	—	—	—	—	—	—
7.49 (10	(11.56)	—	—	—	—	5.36	*	—	—
6.45 (5.	(5.58)	—	—	—	—	34.57	***	—	—
.99 (1.7 | 87)	—	—	—	—	—	—	—	—
97 (0.3	)	—	—	—	—	—	—	—	—
0.55 (6.	(6.37)	1.69	NS	—	—	3.43	†	—	—
6.94 (6.	(7.02)	—	—	—	—	11.06	***	—	—
.10 (4.5 | .27)	3.02	*	—	—	8.59	**	—	—
321.98 (2	.09 (37.51)	3.81	*	—	—	100.33	***	—	—
034.63 (2	.60 (33.45)	3.10	*	—	—	152.50	***	—	—
2.53 (2.6 | 36)	2.42	†	—	—	4.26	*	—	—
4.56 (0.7 | 91)	2.64	†	—	—	6.60	*	—	—
3.04 (1.3 | 51)	—	—	—	—	—	—	—	—
37.10 (9.	(7.47)	—	—	—	—	43.19	***	—	—
7.23 (2. | 58)	—	—	—	—	16.22	***	—	—
96.00 (1	(17.18)	—	—	—	—	13.84	***	—	—
5.92 (3.1 | 13)	—	—	—	—	—	—	—	—
55.79 (21	(23.03)	—	—	—	—	38.40	***	—	—
11.78 (1.	(11.86)	—	—	—	—	21.82	***	—	—
7.31 (1.7 | 13)	—	—	—	—	24.97	***	—	—
46.89 (5. | 5.37)	—	—	—	—	12.11	**	—	—
76.93 (1	(13.98)	—	—	—	—	6.38	*	—	—
83.66 (2	(26.09)	2.91	†	—	—	2.74	NS	3.55	*
63.11 (1	(17.15)	—	—	—	—	5.16	*	—	—
		—	—	—	—	3.17	†	—	—
.90 (2.0	(41.37)	3.46	*	—	—	8.62	*	4.02	*
33.05 (9 | .74)	—	—	—	—	—	—	—	—
16.67 (1									

Appendix B. (Ctd)

Dussia macrophyllata (Tolerant / Rare)	Root Mass	19	0.26	NS	209.45 923.03)
	Stem Mass	19	4.86	*	**306.00 (27.59)**
	Leaf Mass	19	0.00	NS	269.38 (33.69)
	Total Mass	19	1.03	NS	784.17 (71.94)
	Root Length	19	0.71	NS	70.28 (7.68) vs.
	Root Surface Area	19	0.01	NS	21.34 (1.80) vs.
	Height	19	0.30	NS	263.04 (17.07)
Miconia affinis (Tolerant / Rare)	Root Mass	40	1.50	NS	0.02 (0.01) vs. (
	Stem Mass	44	1.24	NS	0.06 (0.01) vs. (
	Leaf Mass	46	0.04	NS	0.16 (0.02) vs. (
	Total Mass	38	0.51	NS	0.26 (0.03) vs. (
	Root Length	36	0.47	NS	0.64 (0.09) vs. (
	Root Surface Area	36	0.94	NS	0.07 (0.01) vs. (
	Height	46	0.66	NS	7.64 (0.42) vs. 8
Stryphnodendron microstachyum (Tolerant / Rare)	Root Mass	35	0.44	NS	4.05 (0.24) vs. 3
	Stem Mass	35	0.15	NS	12.32 (0.72) vs.
	Leaf Mass	35	1.86	NS	20.02 (1.30) vs.
	Total Mass	35	1.57	NS	36.37 (1.69) vs.
	Root Length	34	0.11	NS	11.86 (0.80) vs.
	Root Surface Area	34	0.29	NS	2.00 (0.16) vs. 1
	Height	35	0.01	NS	110.72 (6.52) vs

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. N = to contain that term due to a non-significant ($P > 0.10$) effect on the dependent variable. Root, s and root surface area in cm^2. Means have been rounded up. Significance is shown as: NS, not inoculum is significant ($P \leq 0.10$) the microbial and sterile treatment means are in bold.

209.45	91.50 (24.43)	—	—	—	—	16.55	***	—	—
306.00	**11.97 (29.32)**	3.39	†	—	—	28.26	***	—	—
269.38	70.80 (35.74)	—	—	—	—	5.45	*	—	—
784.17	71.14 (76.33)	—	—	—	—	18.98	***	—	—
70.28	9 (8.15)	—	—	—	—	4.42	†	—	—
21.34	4 (1.91)	—	—	—	—	5.59	*	—	—
263.04	48.59 (18.14)	5.64	*	—	—	6.59	*	—	—
0.02	(0.00)	1.80	NS	—	—	—	—	—	—
0.06	(0.01)	4.14	*	2.49	†	—	—	—	—
0.16	(0.02)	—	—	2.47	*	—	—	—	—
0.26	(0.03)	3.29	*	2.55	†	—	—	—	—
0.64	(0.08)	—	—	—	—	—	—	—	—
0.07	(0.01)	—	—	—	—	—	—	—	—
7.64	(0.43)	—	—	—	—	—	—	—	—
4.05	(0.30)	—	—	5.26	**	—	—	—	—
12.32	8 (0.89)	—	—	—	—	2.14	NS	—	—
20.02	0 (1.59)	—	—	2.92	*	4.47	*	—	—
36.37	0 (2.07)	—	—	2.98	*	4.84	*	—	—
11.86	7 (0.94)	—	—	3.88	**	—	—	—	—
2.00	0.19)	—	—	1.63	NS	3.84	†	—	—
110.72	1.42 (7.98)	—	—	—	—	8.17	**	—	—

ersion 1
variable
own as
re in bo

umber of seedlings in the model. "—"denotes that the ANCOVA model does not
leaf, cotyledon and total mass measurements are in mg; root length and height in cm
nificant; † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. When the main effect of

Appendix C. Analysis of covariance results for the effects of inoculum treatment on organ mass, s
microbial extract; adult = location where soil was collected for use in extraction; bench = location
plant mass.

Species (Shade Tolerance / Adult Abundance Classification)	Dependent Variable	Treatment (1)			
		N	*F*	*P*	Means (Std. error) Microbial vs. Sterile
Luehea seemannii (Intolerant / Common)	Root Mass	22	0.07	NS	0.09 (0.04) vs. 0.10 (0.03)
	Stem Mass	22	0.82	NS	0.34 (0.02) vs. 0.32 (0.02)
	Leaf Mass	22	0.94	NS	0.48 (0.02) vs. 0.51 (0.02)
	Root Length	22	1.60	NS	0.34 (0.12) vs. 0.53 (0.09)
	Root Surface Area	22	3.06	†	**0.04 (0.02) vs. 0.08 (0.01)**
Neea psychotroides (Intolerant / Common)	Root Mass	48	0.55	NS	2.55 (0.16) vs. 2.39 (0.15)
	Stem Mass	48	0.00	NS	4.20 (0.14) vs. 4.19 (0.13)
	Leaf Mass	48	0.41	NS	13.80 (0.20) vs. 14.00 (0.19)
	Root Length	48	5.66	*	**13.35 (0.70) vs. 11.03 (0.66)**
	Root Surface Area	48	1.64	NS	2.26 (0.12) vs. 2.06 (0.11)
Apeiba membranacea (Intolerant / Intermediate)	Root Mass	20	3.32	†	**0.79 (0.06) vs. 0.66 (0.05)**
	Stem Mass	20	0.28	NS	1.61 (0.10) vs. 1.54 (0.08)
	Leaf Mass	20	1.76	NS	2.68 (0.12) vs. 2.89 (0.09)
	Root Length	20	0.02	NS	3.62 (0.46) vs. 3.04 (0.37)
	Root Surface Area	20	0.12	NS	0.58 (0.07) vs. 0.55 (0.05)
Colubrina spinosa (Intolerant / Intermediate)	Root Mass	47	2.14	NS	3.02 (0.13) vs. 2.74 (0.14)
	Stem Mass	47	0.05	NS	5.93 (0.17) vs. 5.98 (0.18)
	Leaf Mass	47	0.70	NS	14.03 (0.23) vs. 14.32 (0.25)
Guatteria diospyroides (Intolerant / Intermediate)	Root Mass	36	0.21	NS	5.56 (0.24) vs. 5.72 (0.25)
	Stem Mass	36	2.03	NS	10.40 (0.39) vs. 9.60 (0.41)
	Leaf Mass	36	1.29	NS	5.73 (0.48) vs. 6.52 (0.50)
	Root Length	36	0.00	NS	21.44 (1.24) vs. 21.32 (1.28)
	Root Surface Area	36	0.09	NS	3.98 (0.19) vs. 3.90 (0.20)
Psychotria panamensis (Intolerant / Intermediate)	Root Mass	55	0.05	NS	3.79 (0.16) vs. 3.74 (0.15)
	Stem Mass	55	0.22	NS	5.69 (0.20) vs. 5.82 (0.19)
	Leaf Mass	55	0.11	NS	9.68 (0.21) vs. 9.58 (0.21)
	Root Length	55	0.16	NS	27.29 (1.09) vs. 26.69 (1.07)
	Root Surface Area	55	0.00	NS	4.46 (0.21) vs. 4.44 (0.20)
Castilla elastica (Intolerant / Rare)	Root Mass	58	3.73	†	**18.71 (0.54) vs. 17.18 (0.58)**
	Stem Mass	58	6.00	*	**35.59 (0.67) vs. 33.25 (0.67)**
	Leaf Mass	58	7.40	**	**83.17 (0.80) vs. 86.28 (0.81)**
	Root Length	57	2.34	NS	108.40 (4.64) vs. 118.45 (4.9
	Root Surface Area	57	0.55	NS	17.85 (0.79) vs. 18.68 (0.78)

eight and root morphology allocation. Inoculum = microbial vs. sterilized
eedlings were randomly assigned to during the experiment; covariate = total

Source of Variation (df)									
Adult (3)		Treatment x Adult (3)		Bench (5)		Covariate (1)		Adult x Covariate (1)	
F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
3.04	†	—	—	—	—	76.63	***	—	—
4.57	*	—	—	—	—	18.74	**	14.30	***
5.23	*	—	—	—	—	63.37	***	3.95	*
1.84	NS	—	—	—	—	44.96	***	—	—
—	—	—	—	—	—	119.56	***	—	—
—	—	—	—	—	—	58.09	***	—	—
—	—	—	—	—	—	89.58	***	—	—
—	—	—	—	—	—	1220.4	***	—	—
—	—	—	—	2.56	*	71.30	***	—	—
4.84	**	—	—	—	—	62.91	***	—	—
—	—	—	—	—	—	15.48	***	—	—
—	—	—	—	—	—	19.16	***	—	—
—	—	—	—	—	—	75.93	***	—	—
—	—	—	—	—	—	6.12	*	—	—
—	—	—	—	—	—	2.72	NS	—	—
7.05	**	—	—	3.15	*	52.67	***	—	—
—	—	—	—	—	—	107.06	***	—	—
2.90	*	—	—	—	—	763.09	***	—	—
1.42	NS	1.57	NS	—	—	22.57	***	—	—
—	—	—	—	—	—	3.91	†	—	—
1.00	NS	1.97	NS	—	—	68.92	***	—	—
1.64	NS	—	—	—	—	23.07	***	—	—
1.44	NS	—	—	—	—	32.86	***	—	—
2.27	†	1.86	NS	—	—	41.33	***	—	—
—	—	—	—	—	—	18.51	***	—	—
0.90	NS	1.66	NS	—	—	260.73	***	—	—
1.66	NS	—	—	—	—	37.28	***	—	—
1.39	NS	1.85	NS	—	—	24.14	**	—	—
4.33	**	1.99	NS	1.56	NS	107.59	***	—	—
4.63	**	—	—	2.05	†	186.30	***	—	—
0.32	NS	1.49	NS	3.25	*	834.78	***	—	—
2.05	NS	—	—	—	—	56.30	***	—	—
—	—	—	—	2.48	*	45.66	***	—	—

Appendix C. (Ctd)

Coussarea hondensis (Intermediate / Common)	Root Mass	36	0.37	NS	15.79 (1.39) vs. 14.61 (1.31
	Stem Mass	36	2.22	NS	38.19 (2.42) vs. 41.71 (2.12
	Leaf Mass	36	4.13	†	**43.45 (1.19) vs. 38.14 (1.45**
	Root Length	36	0.01	NS	21.14 (2.30) vs. 18.72 (2.93
	Root Surface Area	35	0.95	NS	4.35 (0.36) vs. 3.86 (0.35)
Pentaclethra macroloba (Intermediate / Common)	Root Mass	58	0.53	NS	313.29 (15.36) vs. 297.28 (1
	Stem Mass	58	0.12	NS	602.67 (34.76) vs. 585.78 (3
	Leaf Mass	58	0.05	NS	764.80 (43.36) vs. 779.23 (4
	Cotyledon Mass	58	0.03	NS	266.49 (75.55) vs. 284.95 (7
	Root Length	58	0.06	NS	215.22 (13.71) vs. 210.60 (1
	Root Surface Area	58	0.23	NS	43.49 (2.43) vs. 41.82 (2.43
Prestoea descurrens (Intermediate / Common)	Root Mass	45	0.42	NS	17.47 (0.74) vs. 16.83 (0.66
	Stem Mass	45	5.97	*	**14.40 (0.77) vs. 16.92 (0.69**
	Leaf Mass	45	3.30	†	**22.62 (1.29) vs. 25.77 (1.16**
	Cotyledon Mass	45	3.10	†	**50.97 (2.13) vs. 45.93 (1.91**
	Root Length	45	0.08	NS	36.42 (1.56) vs. 35.83 (1.39
	Root Surface Area	45	0.38	NS	6.49 (0.26) vs. 6.28 (0.23)
Quararibea bracteolosa (Intermediate / Rare)	Root Mass	14	0.01	NS	37.95 (2.54) vs. 37.64 (2.17
	Stem Mass	14	0.00	NS	51.31 (4.50) vs. 51.51 (3.86
	Leaf Mass	14	0.00	NS	115.41 (3.03) vs. 115.52 (2.6
Trophis racemosa (Intermediate / Rare)	Root Mass	51	2.69	NS	4.13 (0.21) vs. 4.59 (0.20)
	Stem Mass	51	0.46	NS	10.09 (0.31) vs. 9.79 (0.31)
	Leaf Mass	51	0.01	NS	22.11 (0.65) vs. 22.03 (0.63
	Cotyledon Mass	51	0.15	NS	13.00 (1.44) vs. 12.20 (1.41
	Root Length	51	0.86	NS	16.75 (0.76) vs. 17.73 (0.74
	Root Surface Area	51	1.84	NS	3.01 (0.12) vs. 3.25 (0.12)
Vochysia ferruginea (Intermediate / Rare)	Root Mass	40	0.27	NS	2.88 (0.16) vs. 2.78 (0.14)
	Stem Mass	40	0.06	NS	7.23 (0.17) vs. 7.29 (0.17)
	Leaf Mass	40	0.25	NS	15.67 (0.19) vs. 15.79 (0.17
	Root Length	40	0.66	NS	3.91 (0.31) vs. 3.58 (0.29)
	Root Surface Area	40	1.33	NS	0.85 (0.05) vs. 0.78 (0.04)
Capparis pittierin (Tolerant / Common)	Root Mass	43	2.33	NS	59.49 (2.09) vs. 63.81 (2.05
	Stem Mass	43	0.05	NS	110.96 (3.02) vs. 111.88 (2.9
	Leaf Mass	43	0.19	NS	376.64 (3.53) vs. 374.56 (3.3
	Root Length	42	0.05	NS	27.44 (2.37) vs. 28.20 (2.37
	Root Surface Area	42	0.46	NS	9.52 (0.48) vs. 9.98 (0.48)
Euterpe precatoria (Tolerant / Common)	Root Mass	45	0.83	NS	36.08 (1.65) vs. 38.28 (1.76
	Stem Mass	45	0.68	NS	13.09 (0.54) vs. 12.45 (0.56
	Leaf Mass	45	0.95	NS	78.92 (4.82) vs. 85.86 (5.13
	Cotyledon Mass	45	0.33	NS	136.12 (6.05) vs. 131.11 (6.3
	Root Length	45	1.77	NS	32.99 (1.75) vs. 36.39 (1.87
	Root Surface Area	45	1.55	NS	6.97 (0.32) vs. 7.56 (0.35)

—	—	—	—	—	—	33.46	***	—	—
1.87	NS	—	—	—	—	74.42	***	—	—
1.02	NS	2.39	NS	—	—	267.66	***	—	—
0.85	NS	1.57	NS	—	—	15.34	**	—	—
—	—	—	—	—	—	11.57	**	—	—
—	—	—	—	—	—	39.20	***	—	—
—	—	—	—	—	—	49.28	***	—	—
—	—	—	—	—	—	4.71	***	—	—
—	—	—	—	—	—	53.88	***	—	—
—	—	—	—	—	—	18.40	***	—	—
—	—	—	—	—	—	26.78	***	—	—
—	—	—	—	—	—	5.80	*	—	—
—	—	—	—	—	—	14.34	***	—	—
—	—	—	—	—	—	2.81	NS	—	—
—	—	—	—	—	—	95.25	***	—	—
—	—	—	—	—	—	5.19	*	—	—
—	—	—	—	—	—	3.81	†	—	—
—	—	—	—	—	—	28.36	***	—	—
—	—	—	—	—	—	8.96	*	—	—
—	—	—	—	—	—	174.03	***	—	—
—	—	—	—	2.50	*	14.57	***	—	—
—	—	—	—	—	—	60.57	***	—	—
2.62	†	—	—	—	—	120.68	***	—	—
—	—	—	—	—	—	10.19	**	—	—
3.81	*	1.58	NS	—	—	7.46	**	—	—
0.45	NS	1.51	NS	—	—	11.00	**	—	—
1.50	NS	—	—	—	—	29.14	***	—	—
—	—	—	—	1.89	NS	43.87	***	—	—
1.78	NS	—	—	—	—	360.78	***	—	—
3.39	*	—	—	—	—	5.65	*	3.91	*
3.09	†	—	—	1.67	NS	13.63	*	3.54	†
4.56	*	—	—	8.54	***	225.11	***	5.94	**
2.43	†	—	—	114.50	***	—	—	—	—
1.87	NS	—	—	1.53	NS	1095.0	***	—	—
—	—	—	—	—	—	12.29	***	—	—
—	—	—	—	—	—	43.08	***	—	—
—	—	—	—	—	—	—	—	—	—
1.57	NS	1.91	NS	—	—	1.75	NS	—	—
0.47	NS	1.64	NS	1.48	NS	12.34	**	—	—
0.46	NS	1.50	NS	—	—	109.99	***	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—

Appendix C. (Ctd)

Iriartea deltoidea (Tolerant / Common)	Root Mass	48	5.84	*	**115.90 (6.70) vs. 140.42 (7**
	Stem Mass	48	3.15	†	**95.60 (4.69) vs. 108.36 (5.4**
	Cotyledon Mass	48	5.95	*	**2816.91 (10.40) vs. 2778.0 (12.05)**
	Root Length	48	3.13	†	**42.02 (2.99) vs. 50.13 (3.47**
	Root Surface Area	48	4.21	*	**14.45 (0.83) vs. 17.04 (0.96**
Welfia regia (Tolerant / Common)	Root Mass	56	0.17	NS	183.64 (4.50) vs. 180.89 (4
	Stem Mass	56	0.37	NS	46.86 (2.18) vs. 48.86 (2.37
	Leaf Mass	56	1.04	NS	283.89 (6.06) vs. 293.39 (6
	Cotyledon Mass	56	1.56	NS	25.92 (3.10) vs. 20.24 (3.33
	Root Length	56	0.37	NS	207.22 (9.40) vs. 199.22 (9.
	Root Surface Area	56	1.49	NS	36.77 (1.31) vs. 34.35 (1.43
Virola koschnyi (Tolerant / Intermediate)	Root Mass	29	1.06	NS	181.62 (11.13) vs. 198.18 (
	Stem Mass	29	4.45	*	**491.07 (14.77) vs. 445.97 (**
	Leaf Mass	29	3.55	†	**269.61 (11.85) vs. 301.91 (**
	Cotyledon Mass	29	1.63	NS	8.30 (2.03) vs. 4.55 (2.10)
Dussia macrophyllata (Tolerant / Rare)	Root Mass	19	0.00	NS	201.19 (21.53) vs. 200.68 (2
	Stem Mass	19	1.83	NS	290.61 (28.72) vs. 232.06 (3
	Leaf Mass	19	0.01	NS	271.73 (24.49) vs. 268.19 (2
	Cotyledon Mass	19	0.58	NS	220.43 (59.01) vs. 288.08 (6
	Root Length	19	0.53	NS	69.09 (7.06) vs. 61.37 (7.50
	Root Surface Area	19	0.06	NS	20.90 (1.77) vs. 21.53 (1.87
Miconia affinis (Tolerant / Rare)	Root Mass	38	1.96	NS	0.02 (0.00) vs. 0.03 (0.00)
	Stem Mass	38	0.40	NS	0.06 (0.00) vs. 0.06 (0.00)
	Leaf Mass	38	0.02	NS	0.16 (0.00) vs. 0.16 (0.01)
	Root Length	30	0.66	NS	0.66 (0.07) vs. 0.73 (0.06)
	Root Surface Area	30	0.94	NS	0.07 (0.01) vs. 0.08 (0.001)
Stryphnodendron microstachyum (Tolerant / Rare)	Root Mass	35	0.49	NS	4.22 (0.37) vs. 3.86 (0.32)
	Stem Mass	35	0.32	NS	11.52 (0.81) vs. 12.06 (0.99
	Leaf Mass	35	0.16	NS	18.74 (0.56) vs. 18.38 (0.69
	Root Length	34	0.11	NS	11.86 (0.80) vs. 12.27 (0.94
	Root Surface Area	34	0.00	NS	1.97 (0.21) vs. 1.96 (0.23)

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. N = total contain that term due to a non-significant ($P > 0.10$) effect on the dependent variable. Root, stem cm^2. Means have been rounded up. Significance is shown as: NS, not significant; † $P \leq 0.10$; * $P \leq 0.10$) the microbial and sterile treatment means are in bold.

—	—	—	—	—	—	2.73	NS	—	—
4.06	*	—	—	—	—	5.26	*	—	—
2.66	†	—	—	—	—	1327.6	***	—	—
2.61	†	—	—	—	—	—	—	—	—
3.11	*	—	—	—	—	1.65	NS	—	—
—	—	—	—	—	—	177.62	***	—	—
1.98	NS	—	—	—	—	21.19	***	—	—
1.21	NS	1.63	NS	—	—	434.77	***	—	—
—	—	—	—	—	—	—	—	—	—
1.63	NS	—	—	—	—	59.27	***	—	—
2.10	NS	—	—	2.31	†	66.40	***	—	—
—	—	—	—	—	—	22.12	**	—	—
—	—	—	—	—	—	76.37	**	—	—
—	—	—	—	—	—	5.13	†	—	—
—	—	—	—	—	—	5.38	†	—	—
—	—	—	—	—	—	19.11	***	—	—
3.02	NS	1.69	NS	—	—	21.31	***	—	—
—	—	—	—	—	—	22.26	***	—	—
—	—	—	—	—	—	23.06	***	—	—
0.80	NS	1.75	NS	—	—	5.90	*	—	—
—	—	—	—	—	—	5.21	*	—	—
—	—	—	—	—	—	23.90	***	—	—
—	—	—	—	—	—	96.36	***	—	—
1.63	NS	—	—	—	—	501.61	***	—	—
4.00	*	—	—	—	—	53.47	***	—	—
2.47	†	—	—	—	—	37.77	***	—	—
0.21	NS	1.80	NS	3.58	*	—	—	—	—
1.48	NS	—	—	—	—	11.57	**	—	—
—	—	—	—	—	—	202.68	***	—	—
—	—	—	—	3.88	**	—	—	—	—
4.15	*	—	—	2.84	*	—	—	—	—

er of seedlings in the model. "—" denotes that the ANCOVA model does not
and cotyledon measurements are in mg; root length in cm and root surface area in
)5; ** $P \leq 0.01$; *** $P \leq 0.001$. When the main effect of inoculum is significant (P

Appendix D. Analysis of covariance results for the effects of inoculum treatment on root morphol bole of 4 different adults per species) where soil was collected for use in extraction; bench = locat mass.

Species (Shade Tolerance / Adult Abundance Classification)	Dependent Variable	Treatment (1)			
		N	*F*	*P*	Mean (Std. error) Microbial vs. Sterile
Neea psychotroides	Root Length	48	1.12	NS	12.87 (0.80) vs. 11.
(Intolerant / Common)	Root Surface Area	48	0.01	NS	2.14 (0.11) vs. 2.13
Apeiba membranacea	Root Length	20	0.01	NS	3.44 (0.42) vs. 3.50
(Intolerant / Intermediate)	Root Surface Area	20	0.33	NS	0.57 (0.04) vs. 0.60
Guatteria diospyroides	Root Length	36	0.07	NS	21.29 (0.94) vs. 20.9
(Intolerant / Intermediate)	Root Surface Area	36	0.40	NS	4.00 (0.14) vs. 3.87
Psychotria panamensis	Root Length	57	0.28	NS	27.12 (1.01) vs. 26.3
(Intolerant / Intermediate)	Root Surface Area	57	0.02	NS	4.38 (0.18) vs. 4.41
Castilla elastica	Root Length	58	7.65	**	**105.73 (3.63) vs. 11**
(Intolerant / Rare)	Root Surface Area	58	4.10	*	**17.42 (0.53) vs. 18.9**
Coussarea hondensis	Root Length	34	0.49	NS	22.94 (2.33) vs. 20.6
(Intermediate / Common)	Root Surface Area	34	0.66	NS	3.65 (0.50) vs. 3.26
Pentaclethra macroloba	Root Length	58	0.24	NS	209.83 (11.87) vs. 2
(Intermediate / Common)	Root Surface Area	58	0.08	NS	42.32 (1.97) vs. 43.0
Prestoea descurrens	Root Length	48	0.63	NS	33.93 (1.24) vs. 35.2
(Intermediate / Common)	Root Surface Area	48	0.44	NS	6.07 (0.20) vs. 6.25
Trophis racemosa	Root Length	51	0.21	NS	16.93 (0.75) vs. 17.4
(Intermediate / Rare)	Root Surface Area	51	0.28	NS	3.06 (0.108) vs. 3.14
Vochysia ferruginea	Root Length	40	0.94	NS	4.00 (0.37) vs. 3.55
(Intermediate / Rare	Root Surface Area	40	0.64	NS	0.83 (0.03) vs. 0.79
Capparis pittierin	Root Length	42	0.09	NS	27.27 (2.53) vs. 28.3
(Tolerant / Common)	Root Surface Area	42	0.53	NS	9.51 (0.47) vs. 9.99
Euterpe precatoria	Root Length	45	3.41	†	**33.09 (1.13) vs. 36.1**
(Tolerant / Common)	Root Surface Area	45	2.47	NS	7.03 (0.18) vs. 7.45
Iriartea deltoidea	Root Length	48	0.69	NS	46.44 (1.61) vs. 44.3
(Tolerant / Common)	Root Surface Area	48	1.99	NS	15.76 (0.23) vs. 15.2
Welfia regia	Root Length	56	0.35	NS	208.21 (9.53) vs. 19
(Tolerant / Common)	Root Surface Area	56	1.10	NS	36.78 (1.41) vs. 34.3
Dussia macrophyllata	Root Length	19	0.70	NS	69.07 (5.98) vs. 61.4
(Tolerant / Rare)	Root Surface Area	19	0.02	NS	21.08 (1.22) vs. 21.3
Miconia affinis	Root Length	32	0.30	NS	0.73 (0.07) vs. 0.68
(Tolerant / Rare)	Root Surface Area	32	0.02	NS	0.08 (0.01) vs. 0.07

noculum = microbial vs. sterilized microbial extract; adult = location (near the
t seedlings were randomly assigned to during the experiment; covariate = root

	Source of Variation (df)							
	Adult (3)		Treatment x Adult (3)		Bench (5)		Covariate (1)	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
75)	3.78	*	—	—	—	—	30.37	***
)	2.38	†	—	—	—	—	68.44	***
)	2.53	†	—	—	—	—	9.69	**
)	7.53	**	—	—	—	—	25.94	***
99)	—	—	—	—	—	—	52.47	***
)	0.06	NS	1.80	NS	—	—	74.34	***
00)	—	—	—	—	—	—	64.80	***
)	—	—	—	—	—	—	73.99	***
3.50)	1.78	NS	—	—	—	—	127.64	***
51)	3.73	*	—	—	2.24	†	143.49	***
19)	—	—	—	—	—	—	8.84	**
)	2.58	†	—	—	—	—	19.41	***
(11.94)	0.17	NS	1.77	NS	—	—	36.73	***
98)	5.36	**	1.93	NS	1.77	NS	52.29	***
10)	3.32	*	—	—	—	—	55.48	***
)	2.74	†	1.66	NS	—	—	71.83	***
72)	3.97	*	1.66	NS	—	—	9.14	**
06)	—	—	—	—	—	—	24.49	***
)	0.58	NS	1.63	NS	—	—	17.59	***
)	—	—	—	—	—	—	53.67	***
53)	—	—	—	—	—	—	6.04	*
)	—	—	—	—	—	—	47.03	***
21)	—	—	—	—	4.64	**	48.45	***
)	—	—	—	—	4.42	**	80.71	***
38)	3.12	*	—	—	—	—	117.38	***
27)	10.33	***	—	—	—	—	573.32	***
10.26)	—	—	—	—	—	—	47.90	***
55)	1.57	NS	—	—	1.43	NS	51.54	***
32)	—	—	—	—	—	—	14.78	***
29)	—	—	—	—	—	—	27.24	***
	1.50	NS	—	—	—	—	37.15	***
	1.52	NS	—	—	—	—	37.72	***

Appendix D. (Ctd)

Stryphnodendron microstachyum (Tolerant / Rare)	Root Length	34	0.71	NS	11.31 (0.72) vs. 12
	Root Surface Area	34	1.34	NS	1.80 (0.14) vs. 1.91

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. N = total contain that term due to a non-significant ($P > 0.10$) effect on the dependent variable. Root length Significance is shown as: NS, not significant; † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ treatment means are in bold.

(0.86)	—	—	—	—	—	—	23.41	***
12)	8.81	***	1.68	NS	—	—	34.04	***

ıber of seedlings in the model. "—" denotes that the ANCOVA model does not
measured in cm and root surface area in cm^2. Means have been rounded up.
hen the main effect of inoculum is significant ($P \leq 0.10$) the microbial and sterile

Appendix E. Meta-analysis results for the effect (size and magnitude) of inoculum treatment on mass, ste
categories in each of 5 different local species characteristics. Data for meta-analysis derived from ANCOVA an

Species Categories[a]	N[b,c]	Root Mass Effect Size (2 Std error)	Root Mass Q_B[d,e]	Root Mass *P* value	Stem Mass Effect Size (2 Std error)	Stem Mass Q_B[d,e]	Stem Mass *P* value	Leaf Mass Effect Size (2 Std error)	Leaf Mass Q_B[d,e]	Leaf Mass *P* value	Ro Effect Size (2 Std error)
Overall	21	-0.15 (0.14)			-0.08 (0.14)			-0.21 (0.14)			-0.13 (0.15)
Shade Intolerant	7	-0.14 (0.23)			-0.08 (0.23)			-0.34 (0.23)			-0.21 (0.26)
Shade Intermediate	6	-0.12 (0.25)	0.09	NS	-0.29 (0.25)	4.71	†	-0.26 (0.26)	3.38		-0.04 (0.26)
Shade Tolerant	8	-0.17 (0.22)			0.09 (0.22)			-0.04 (0.24)			-0.13 (0.24)
Adult - Rare	7	-0.12 (0.25)			0.15 (0.24)			-0.15 (0.24)			-0.15 (0.26)
Adult – Intermediate	5	-0.20 (0.29)	0.21	NS	-0.04 (0.29)	6.00	*	-0.28 (0.29)	0.50		-0.15 (0.37)
Adult – Common	9	-0.14 (0.20)			-0.24 (0.20)			-0.22 (0.21)			-0.11 (0.20)
Seedling – Rare	6	-0.22 (0.27)			0.12 (0.26)			-0.20 (0.26)			-0.32 (0.28)
Seedling – Intermediate	8	-0.26 (0.23)	3.07	NS	-0.07 (0.23)	3.85	NS	-0.34 (0.25)	1.64		-0.23 (0.24)
Seedling – Common	7	0.00 (0.22)			-0.22 (0.22)			-0.12 (0.22)			0.10 (0.24)
Basal Area – Rare	7	-0.25 (0.23)			-0.11 (0.23)			-0.23 (0.23)			-0.21 (0.26)
Basal Area – Intermediate	8	-0.05 (0.22)	1.50	NS	-0.05 (0.23)	0.17	NS	-0.27 (0.23)	0.90		-0.08 (0.23)
Basal Area – Common	6	-0.15 (0.25)			-0.07 (0.25)			-0.10 (0.28)			-0.10 (0.27)
Seed Size – Small	4	-0.07 (0.30)			0.02 (0.30)			-0.28 (0.30)			0.10 (0.31)
Seed Size – Medium	8	-0.15 (0.21)	0.37	NS	-0.16 (0.21)	1.05	NS	-0.18 (0.21)	0.27		-0.25 (0.22)
Seed Size – Large	9	-0.19 (0.22)			-0.04 (0.22)			-0.21 (0.24)			-0.12 (0.24)

Notes: [a]Localized species Characteristics for all 21 species (Table 1). Shade tolerance classifi (shade t seedling mortality at 1% full-sun and with zero conspecific seedling density. Adult abundanc sification Seedling abundance classification (rare <20, intermediate 20-200, common >200 standing de of seedlin 0.06 – 0.25, common > 0.25 total adult mass (m^2/ha)). Seed size classification (small <0.03. m 0.03 – category. [c]Number of species is reduced for leaf mass since *Iriratea* seedlings had no leaves for root le used in the analysis. [d]A statistics that measures the homogeneity of effect sizes between cate [e]*df* = 2 0.10; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

organ mass, stem height and root morphology for 21 tropical tree species grouped into 3
m ANCOVA analysis using initial seed mass as a covariate (Appendix B).

lue	Root Length			Root Surface Area			Height		
	Effect Size (2 Std error)	Q_B [d,e]	*P* value	Effect Size (2 Std error)	Q_B [d,e]	*P* value	Effect Size (2 Std error)	Q_B [d,e]	*P* value
	-0.13 (0.15)			-0.17 (0.14)			-0.15 (0.14)		
	-0.21 (0.26)			-0.28 (0.26)			-0.18 (0.23)		
	-0.04 (0.26)	0.79	NS	-0.06 (0.26)	1.31	NS	-0.19 (0.25)	0.51	NS
	-0.13 (0.24)			-0.17 (0.24)			-0.08 (0.22)		
	-0.15 (0.26)			-0.14 (0.26)			-0.18 (0.24)		
	-0.15 (0.37)	0.08	NS	-0.28 (0.37)	0.41	NS	-0.28 (0.29)	1.65	NS
	-0.11 (0.20)			-0.16 (0.20)			-0.06 (0.20)		
	-0.32 (0.28)			-0.28 (0.28)			-0.14 (0.26)		
	-0.23 (0.24)	6.15	*	-0.30 (0.24)	4.78	†	-0.201 (0.23)	0.60	NS
	0.10 (0.24)			0.04 (0.24)			-0.09 (0.22)		
	-0.21 (0.26)			-0.30 (0.26)			-0.30 (0.23)		
	-0.08 (0.23)	0.62	NS	-0.14 (0.23)	1.56	NS	-0.09 (0.22)	2.86	NS
	-0.10 (0.27)			-0.08 (0.27)			-0.03 (0.25)		
	0.10 (0.31)			-0.04 (0.31)			-0.17 (0.30)		
	-0.25 (0.22)	3.22	NS	-0.22 (0.22)	0.94	NS	-0.22 (0.21)	1.27	NS
	-0.12 (0.24)			-0.19 (0.24)			-0.05 (0.22)		

n (shade tolerant <10.5%, shade intermediate 10.5-20% and shade intolerant >20%
ssification (rare < 3, intermediate 3-10, common >10 individuals (>5 cm DBH)/ha).
of seedling (≤ 5 yrs old) /ha/year. Basal area classification (rare < 0.06, intermediate
um 0.03 – 0.30, large > 0.30 mg of dry seed mass. [b]Number of species in each
for root length and root surface area since *Colubrina*, *Quararibea* and *Virola* are not
s. [e]*df* = 2 for all comparisons. Significance is shown as: NS, not significant; † *P* ≤

Appendix F. Meta-analysis results for the effect (size and magnitude) of inoculum treatment on mass, ste categories in each of 5 different local species characteristics. Data for meta-analysis derived fr ANCOVA an

Species Categories[a]	N[b,c]	Root Mass Effect Size (2 Std error)	Q_B[d,e]	P-value	Stem Mass Effect Size (2 Std error)	Q_B[d,e]	*P* value	Leaf Mass Effect (2 Std error)	Q_B[d,e]
Overall	21	0.01 (0.14)			0.03 (0.14)			-0.16 (0.14)	
Shade Intolerant	7	0.24 (0.23)			0.19 (0.23)			-0.31 (0.24)	
Shade Intermediate	6	0.05 (0.25)	8.36	*	-0.13 (0.25)	3.30	NS	-0.01 (0.25)	2.90
Shade Tolerant	8	-0.24 (0.22)			0.01 (0.22)			-0.14 (0.24)	
Adult - Rare	7	0.02 (0.25)			0.19 (0.25)			-0.15 (0.25)	
Adult – Intermediate	5	0.11 (0.29)	0.85	NS	0.17 (0.29)	5.51	†	-0.27 (0.29)	0.87
Adult – Common	9	-0.05 (0.20)			-0.14 (0.20)			-0.10 (0.21)	
Seedling – Rare	6	-0.01 (0.27)			0.21 (0.27)			-0.18 (0.27)	
Seedling – Intermediate	8	-0.12 (0.23)	2.30	NS	0.01 (0.23)	2.73	NS	-0.30 (0.25)	2.56
Seedling – Common	7	0.13 (0.22)			-0.08 (0.22)			-0.03 (0.22)	
Basal Area – Rare	7	-0.04 (0.23)			-0.05 (0.23)			-0.15 (0.23)	
Basal Area – Intermediate	8	0.11 (0.23)	1.45	NS	0.14 (0.23)	1.77	NS	-0.14 (0.23)	0.10
Basal Area – Common	6	-0.08 (0.25)			-0.03 (0.25)			-0.20 (0.28)	
Seed Size – Small	4	0.07 (0.31)			0.04 (0.31)			-0.20 (0.31)	
Seed Size – Medium	8	0.13 (0.21)	4.14	NS	0.01 (0.21)	0.05	NS	-0.13 (0.21)	0.15
Seed Size – Large	9	-0.18 (0.22)			0.04 (0.22)			-0.17 (0.24)	

Notes: [a]Localized species characteristics for all 21 species (Table 1). Shade tolerance classificat (shade seedling mortality at 1% full-sun and with zero conspecific seedling density. Adult abundance c ification Seedling abundance classification (rare <20, intermediate 20-200, common >200 standing densi of seedli 0.06 – 0.25, common > 0.25 total adult mass (m^2/ha)). Seed size classification (small <0.03, me n 0.03 category. [c]Number of species is reduced for leaf mass since *Irviratea* seedlings had no leaves ar or root used in the analysis. [d]A statistics that measures the homogeneity of effect sizes between categor e df = 0.10; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

organ mass, stem height and root morphology for 21 tropical tree species grouped into 3
m ANCOVA analysis using total plant mass as a covariate (Appendix C).

Leaf Mass Size	Q_B[d,e]	*P* value	Root Length Effect Size (2 Std error)	Q_B[d,e]	*P* value	Root Surface Area Effect Size (2 Std error)	Q_B[d,e]	*P* value
			-0.02 (0.15)			-0.04 (0.15)		
2.90		NS	0.06 (0.26) 0.05 (0.26) -0.15 (0.24)	1.79	NS	0.00 (0.26) 0.09 (0.26) -0.17 (0.24)	2.23	NS
0.87		NS	-0.14 (0.26) 0.13 (0.38) 0.00 (0.20)	1.53	NS	-0.13 (0.26) 0.07 (0.37) -0.01 (0.20)	0.83	NS
2.56		NS	-0.31 (0.29) -0.06 (0.24) 0.21 (0.24)	7.74	*	-0.29 (0.29) -0.11 (0.24) 0.21 (0.24)	7.77	*
0.10		NS	-0.05 (0.27) 0.07 (0.23) -0.12 (0.27)	1.15	NS	-0.08 (0.27) 0.02 (0.23) -0.08 (0.27)	0.45	NS
0.15		NS	0.20 (0.32) -0.07 (0.22) -0.09 (0.24)	2.34	NS	0.05 (0.32) -0.02 (0.22) -0.11 (0.24)	0.65	NS

inoculum
categories in
rived from

urface Area Q_B[d,e]	*P* value
).55	NS
).73	NS
2.74	NS
2.22	NS
1.19	NS

)n (shade tolerant <10.5%, shade intermediate 10.5-20% and shade intolerant >20%
ıssification (rare < 3, intermediate 3-10, common >10 individuals (>5 cm DBH)/ha).
/ of seedling (≤ 5 yrs old) /ha/year. Basal area classification (rare < 0.06, intermediate
um 0.03 – 0.30, large > 0.30 mg of dry seed mass. [b]Number of species in each
for root length and root surface area since *Colubrina*, *Quararibea* and *Virola* are not
es. [e]*df* = 2 for all comparisons. Significance is shown as: NS, not significant; † *P* ≤

ade tolerance
shade
cific seedling
nmon >10
<20,
s old) /ha/year.

Appendix G. Meta-analysis results for the effect (size and magnitude) of inoculum treatment on root morphology for 21 tropical tree species grouped into 3 categories in each of 5 different local species characteristics. Data for meta-analysis derived from ANCOVA analysis using root mass as a covariate (Appendix D).

Species Categories[a]	N[b]	Root Length			Root Surface Area		
		Effect Size (2 SE)	Q_B[d,e]	*P* value	Effect Size (2 SE)	Q_B[d,e]	*P* value
Overall	17	-0.03 (0.15)			-0.04 (0.15)		
Shade Intolerant	5	-0.07 (0.27)			-0.13 (0.27)		
Shade Intermediate	5	-0.02 (0.26)	0.11	NS	-0.01 (0.26)	0.55	NS
Shade Tolerant	7	-0.01 (0.24)			0.00 (0.24)		
Adult - Rare	6	-0.13 (0.26)			-0.13 (0.26)		
Adult – Intermediate	3	0.09 (0.37)	1.04	NS	0.01 (0.37)	0.73	NS
Adult – Common	8	-0.01 (0.20)			0.00 (0.20)		
Seedling – Rare	4	-0.29 (0.30)			-0.24 (0.30)		
Seedling – Intermediate	7	-0.02 (0.24)	4.26	NS	-0.04 (0.24)	2.74	NS
Seedling – Common	6	0.11 (0.24)			0.08 (0.24)		
Basal Area – Rare	5	0.00 (0.26)			-0.04 (0.26)		
Basal Area – Intermediate	8	-0.09 (0.23)	0.42	NS	-0.15 (0.23)	2.22	NS
Basal Area – Common	4	0.02 (0.28)			0.13 (0.28)		
Seed Size – Small	4	0.23 (0.34)			0.08 (0.33)		
Seed Size – Medium	7	-0.15 (0.22)	3.39	NS	-0.13 (0.22)	1.19	NS
Seed Size – Large	6	-0.03 (0.24)			0.00 (0.24)		

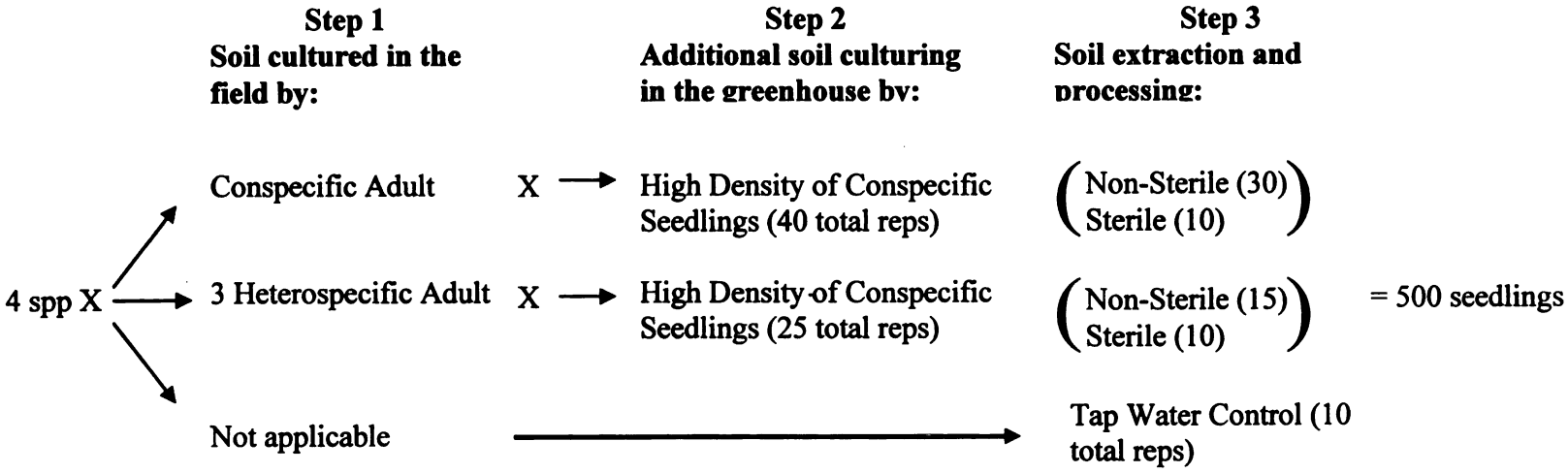
Notes: [a]Localized species characteristics for all 21 species (Table 1). Shade tolerance classification (shade tolerant <10.5%, shade intermediate 10.5-20% and shade intolerant >20% seedling mortality at 1% full-sun and with zero conspecific seedling density. Adult abundance classification (rare < 3, intermediate 3-10, common >10 individuals (>5 cm DBH)/ha). Seedling abundance classification (rare <20, intermediate 20-200, common >200 standing density of seedling (≤ 5 yrs old) /ha/year.

Appendix G. (Ctd)

Basal area classification (rare < 0.06, intermediate 0.06 – 0.25, common > 0.25 total adult mass (m^2/ha)). Seed size classification (small <0.03, medium 0.03 – 0.30, large > 0.30 mg of dry seed mass. [b]Number of species in each category. [c]Number of species is reduced for root length and root surface area since *Colubrina*, *Luehea*, *Quararibea* and *Virola* are not used in the analysis. [d]Statistic that measures the homogeneity of effect sizes between categories. [e]$df = 2$ for all comparisons. Significance is shown as: NS, not significant; † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

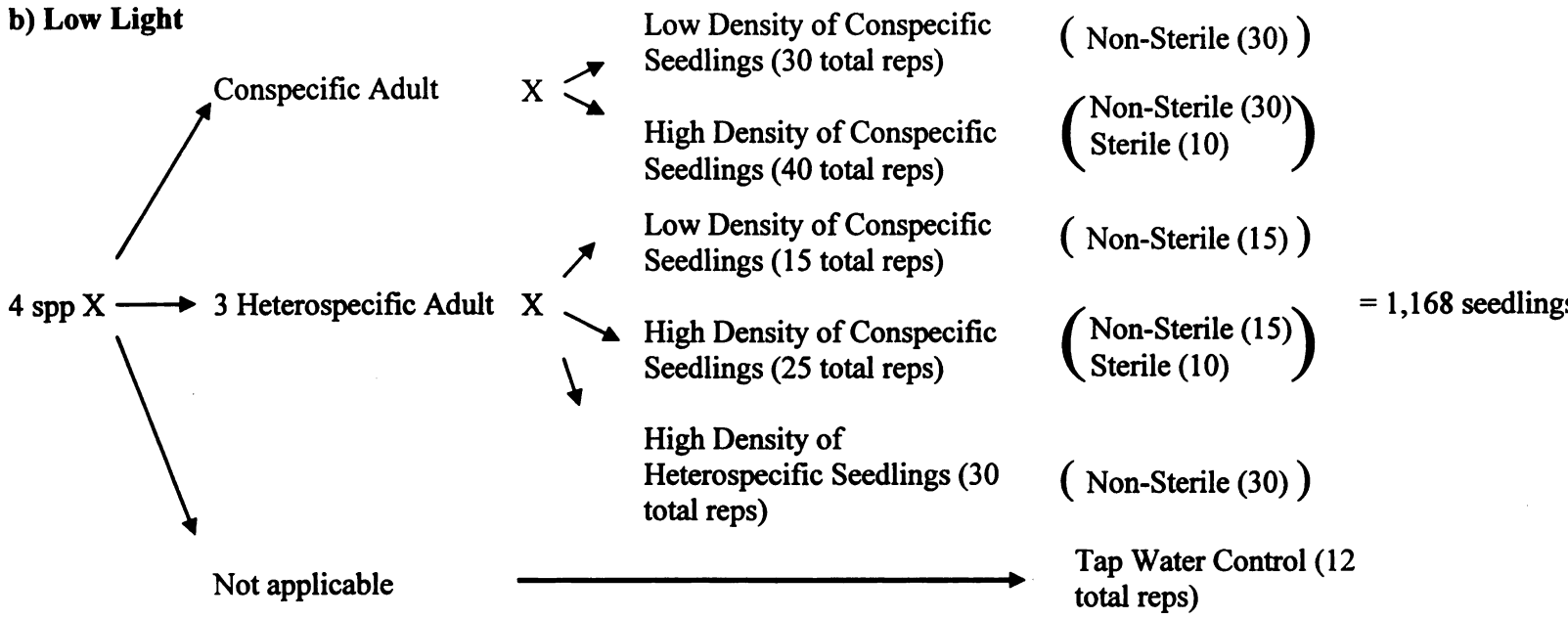
Appendix H. Experimental design and allocation of temperate seedlings to treatments.

a) High Light



Appendix H (Ctd)

b) Low Light



Appendix I. Hazards ratios for the effects of light availability (2% vs. 22% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil) and initial seed mass on seedling mortality estimated by the Cox regression model.

Species	Source of variation	df	Parameter estimate	SE	Wald χ^2	*P* value	Hazard ratio
A. rubrum	$Light_{(Low)}$	1	0.25	0.37	0.44	0.51	1.28
	$Extract_{(Non\text{-}Sterile)}$	1	0.41	0.45	0.85	0.36	1.51
	$Source_{(A.\ saccharum)}$	1	-0.25	0.42	0.35	0.55	0.78
	$Source_{(F.\ americana)}$	1	-0.22	0.42	0.29	0.59	0.80
	$Source_{(Q.\ rubra)}$	1	-0.36	0.43	0.71	0.40	0.70
	Seed Mass	1	0.20	0.23	0.72	0.40	1.22
A. saccharum	$Light_{(Low)}$	1	1.67	1.04	2.59	0.11	5.32
	$Extract_{(Non\text{-}Sterile)}$	1	1.04	0.76	1.90	0.17	2.83
	$Source_{(A.\ rubrum)}$	1	0.54	0.62	0.76	0.25	1.72
	$Source_{(F.\ americana)}$	1	-0.33	0.72	0.22	0.38	0.72
	$Source_{(Q.\ rubra)}$	1	0.70	0.61	1.32	0.64	2.01
	Seed Mass	1	-0.06	0.02	9.87	0.002	0.94
F. americana	$Light_{(Low)}$	1	0.02	0.33	0.01	0.94	1.02
	$Extract_{(Non\text{-}Sterile)}$	1	0.87	0.48	3.25	**0.07**	2.38
	$Source_{(A.\ rubrum)}$	1	-0.13	0.43	0.09	0.77	0.88
	$Source_{(A.\ saccharum)}$	1	-0.34	0.47	0.52	0.47	0.72
	$Source_{(Q.\ rubra)}$	1	0.61	0.37	2.67	**0.10**	1.84
	Seed Mass	1	0.03	0.03	1.15	0.29	1.03
Q. rubra	$Light_{(Low)}$	1	-0.32	0.49	0.42	0.52	0.73
	$Extract_{(Non\text{-}Sterile)}$	1	-0.72	0.51	2.02	0.16	0.49
	$Source_{(A.\ rubrum)}$	1	-0.75	0.81	0.86	0.36	0.47
	$Source_{(A.\ saccharum)}$	1	0.07	0.59	0.02	0.90	1.08
	$Source_{(F.\ americana)}$	1	-0.41	0.70	0.35	0.56	0.66
	Seed Mass	1	-0.00	0.00	0.82	0.37	1.00

Notes: Seedlings that did not emerge are part of the analysis with their death date given as the mean number of days prior to emergence. Total number of seedlings in model for each species was 395 except for *Q. rubra* (N=304). Effects of low light vs. high light, non-sterile vs. sterile extract, soil source (reference category is always the study species) and initial seed mass on mortality. Significant *P*-values are shown in bold. Hazard ratios > 1 indicate reduction in days to mortality of study species. For quantitative covariates (e.g. initial seed mass), subtracting 1 from the hazard ratio and multiplying by 100 (i.e. $100(e\beta - 1)$) gives the per cent change in the hazard of mortality associated with a 1-unit increase in the covariate, controlling for effects of other covariates.

Appendix J. Split-plot analysis of covariance results for the effects of light availability (2% vs. 22% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil), bench and covariate (initial seed mass) on *A. rubrum* seedling mass and stem height.

	Source of Variation (df)															
Dependent Variable	Light (1)			Extract (1)			Soil Source (3)			Light * Extract (1)			Bench (6)		Covariate (1)	
	F	P	Means (95% CI) L vs. H	F	P	Means (95% CI) N-S vs. S	F	P	Means (95% CI) Ar vs. As, Fa, Qr	F	P	Means (95% CI) L/N-S, L/S, H/N-S, H/S	F	P	F	P
Total Mass (mg)	1339.8	****	NA	3.9	**	NA	2.7	**	29.1 (26.2-32.4) vs. 33.8 (30.2-37.7) 30.1 (27.0-33.5) 34.3 (30.8-38.2)	4.6	**	13.3 (12.5-14.2) 13.3 (11.4-15.5) 371.7 (331.3-417.4) 483.5 (414.9-563.4)	2.4	**	8.4	****
Root Mass (mg)	1020.5	****	1.5 (1.4-1.6) vs. 68.7 (61.0-77.4)	0.0	NS	3.9 (3.6-4.2) vs. 3.9 (3.4-4.5)	3.6	**	3.4 (3.0-3.9) vs. 4.3 (3.8-4.9) 3.6 (3.2-4.2) 4.3 (3.8-4.9)	—	—	—	2.7	**	15.3	****
Stem Mass (mg)	1117.4	****	NA	3.6	*	NA	3.1	**	7.7 (6.9-8.5) vs. 8.8 (7.9-9.9) 7.2 (6.5-8.1) 8.4 (7.6-9.4)	7.0	***	3.9 (3.7-4.2) 3.7 (3.2-4.4) 63.2 (56.3-71.0) 84.9 (72.8-99.1)	2.0	**	5.9	**
Leaf Mass (mg)	1167.4	***	NA	5.9	**	NA	2.1	*	17.3 (15.4-19.4) vs. 19.8 (17.6-22.3) 18.7 (16.7-21.0) 20.6 (18.4-23.2)	4.0	**	7.7 (7.2-8.2) 7.9 (6.7-9.3) 238.4 (211.0-269.3) 323.1 (274.8-380.3)	2.6	**	7.4	***
Height (mm)	378.0	***	NA	3.7	*	NA	0.7	NS	65.2 (61.8-68.8) vs. 66.6 (62.5-71.1) 66.6 (62.5-70.9) 69.0 (64.7-73.6)	13.8	****	114.5 (108.1-121.4) 138.0 (127.9-148.9) 55.8 (53.5-58.1) 52.6 (48.9-56.8)	1.7	NS	—	—

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 269 (height) and 349 (all other dependent variables). All dependent variables were natural log transformed. "—"denotes that the ANCOVA model does not contain that term due to a non-significant ($P > 0.25$ for covariate, bench and interactions between main terms) effect on the dependent variable. Description of abbreviations: L = low light and H = high light; N-S = non-sterile and S = sterile; Ar = *A. rubrum*, As = *A. saccharum*, Fa = *F. americana* and Qr = *Q. rubra*. Significance shown as follows: NS, not significant, $P > 0.10$; *$P \leq 0.10$; **$P \leq 0.05$; *** $P \leq 0.01$; ****$P \leq 0.001$.

Appendix K. Split-plot analysis of covariance results for the effects of light availability (2% vs. 22% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil), bench and covariate (initial seed mass) on *A. saccharum* seedling mass and stem height.

	Source of Variation (df)											
Dependent Variable	Light (1)			Extract (1)			Soil Source (3)			Light * Source (3)		
	F	P	Means (95% CI) L vs. H	F	P	Means (95% CI) S vs. S	N-	F	P	Means (95% CI) As vs. Ar, Fa, Qr	F	P	Means (95% CI) L/As, L/Ar, L/FA, L/Qr vs. H/As, H/Ar, H/Fa, H/Qr
Total Mass (mg)	253.9	****	NA	0.7	NS	154.9 (147.2-162.9) vs. 147.7 (133.9-162.9)		6.0	****	NA	6.5	***	83.2 (74.7-92.7) 87.5 (78.1-98.2) 101.2 (89.9-113.9) 92.3 (82.4-103.4) vs. 850.6 (741.7-975.5) 727.8 (600.0-881.8) 590.5 (501.2-696.5) 653.3 (550.6-775.1)
Root Mass (mg)	112.3	****	NA	0.9	NS	34.4 (31.9-37.0) vs. 31.7 (27.3-36.8)		1.5	NS	NA	4.7	***	16.7 (14.2-19.7) 18.4 (15.5-21.8) 19.7 (16.7-23.3) 18.6 (15.6-22.1) vs. 269.6 (219.6-331.3) 186.4 (142.5-242.0) 161.1 (125.3-207.3) 173.3 (134.4-223.2)
Stem Mass (mg)	189.9	****	NA	0.1	NS	43.9 (39.9-48.4) vs. 44.5 (42.4-46.8)		5.7	****	NA	5.2	***	27.6 (24.8-30.7) 28.6 (25.6-32.1) 34.4 (30.7-38.7) 30.4 (27.2-34.0) vs. 166.7 (145.6-190.8) 133.0 (109.9-160.8) 126.2 (107.3-148.4) 135.6 (114.5-160.5)
Leaf Mass (mg)	442.4	****	NA	0.4	NS	70.5 (67.1-74.0) vs. 68.1 (61.9-74.9)		6.8	****	NA	4.8	***	37.4 (33.6-41.6) 38.8 (34.7-43.3) 43.8 (39.0-49.2) 41.9 (37.5-46.8) vs. 395.8 (346.2-452.6) 381.1 (315.8-460.4) 294.7 (250.9-346.2) 332.6 (281.5-393.5)
Height (mm)•	50.6	****	86.7 (83.5-89.9) vs. 106.4 (101.5-111.5)	0.1	NS	95.7 (92.7-98.8) vs. 96.4 (91.4-101.7)		2.6	*	92.2 (87.6-97.0) vs. 90.0 (61.7-131.4) 97.0 (64.5-139.4) 92.7 (54.9-148.8)	—	—	NA

Appendix K. (Ctd)

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 369. All dependent variables were natural log transformed. "—"denotes that the ANCOVA model does not contain that term due to a non-significant ($P > 0.25$ for covariate, bench and interactions between main terms) effect on the dependent variable or due to the assumption of homogenous slopes not being violated ($P > 0.05$ for interactions between main terms and covariate). "•" indicates that linear regression analysis was used rather than ANCOVA due interaction term between the treatment and covariate terms being significant. Means provided are from the linear regression analysis. Description of abbreviations: L = low light and H = high light; N-S = non-sterile and S = sterile; Ar = *A. rubrum*, As = *A. saccharum*, Fa = *F. americana* and Qr = *Q. rubra*. Significance shown as follows: NS, not significant, $P > 0.10$; *$P \leq 0.10$; **$P \leq 0.05$; *** $P \leq 0.01$; ****$P \leq 0.001$.

Appendix K Ctd. Split-plot analysis of covariance results for the effects of light availability (2% vs. 22% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil), bench and covariate (initial seed mass) on *A. saccharum* seedling mass and stem height.

Dependent Variable	*Source of Variation (df)*					
	Bench (6)		Covariate (1)		Source * Covariate (3)	
	F	P	F	P	F	P
Total Mass (mg)	6.2	****	104.5	****	5.3	****
Root Mass (mg)	8.1	****	57.9	****	—	—
Stem Mass (mg)	4.8	****	91.9	****	5.6	****
Leaf Mass (mg)	4.1	****	98.7	****	6.0	****
Height (mm)	—	—	15.4	****	3.1	**

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 369. All dependent variables were natural log transformed. "—"denotes that the ANCOVA model does not contain that term due to a non-significant ($P > 0.25$ for covariate, bench and interactions between main terms) effect on the dependent variable or due to the assumption of homogenous slopes not being violated ($P > 0.05$ for interactions between main terms and covariate). Significance shown as follows: NS, not significant, $P > 0.10$; *$P \leq 0.10$; **$P \leq 0.05$; *** $P \leq 0.01$; ****$P \leq 0.001$.

Appendix L) Split-plot analysis of covariance results for the effects of light availability (2% vs. 22% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil), bench and covariate (initial seed mass) on *F. americana* seedling mass and stem height.

	Source of Variation (df)															
Dependent Variable	Light (1)			Extract (1)			Soil Source (3)			Light * Extract (1)			Bench (6)		Covariate (1)	
	F	P	Means (95% CI) L vs. H	F	P	Means (95% CI) N-S vs. S	F	P	Means (95% CI) Fa vs. Ar, As, Qr	F	P	Means (95% CI) L/N-S, L/S vs. H/N-S, H/S	F	P	F	P
Total Mass (mg)	667.4	****	NA	1.4	NS	NA	0.9	NS	161.1 (141.8-183.1) vs. 142.6 (125.1-162.6) 144.4 (126.5-164.9) 147.3 (128.2-169.2)	5.2	**	77.9 (72.1-84.4) 71.5 (59.6-86.1) vs. 1026.6 (892.5-1180.9) 1340.8 (1116.6-1610.0)	2.0	*	4.4	**
Root Mass (mg)	458.4	****	NA	0.3	NS	NA	2.1	*	33.4 (27.9-39.6) vs. 28.2 (23.6-33.8) 28.5 (23.8-34.5) 24.5 (20.3-30.0)	3.7	*	13.6 (12.2-15.2) 11.8 (9.1-15.2) vs. 286.9 (237.5-347.2) 373.5 (290.0-483.0)	2.2	**	5.7	**
Stem Mass (mg)	704.3	****	NA	2.2	NS	NA	2.1	*	41.7 (36.6-47.0) vs. 35.2 (31.2-39.6) 37.0 (32.8-42.1) 34.5 (30.3-39.3)	6.0	**	21.2 (19.7-22.8) 19.7 (16.6-23.5) vs. 188.7 (165.3-215.3) 250.9 (211.2-298.3)	1.6	NS	7.3	***
Leaf Mass (mg)	637.9	****	NA	1.8	NS	NA	0.8	NS	81.4 (71.2-93.0) vs. 74.9 (65.4-85.9) 74.2 (64.6-85.2) 83.7 (72.4-96.7)	4.7	**	41.1 (37.9-44.6) 38.4 (31.7046.5) vs. 524.8 (453.0-607.3) 696.5 (575.4-843.0)	1.0	*	2.0	NS
Height (mm)	237.3	****	NA	2.3	NS	NA	1.5	NS	95.3 (88.8-102.3) vs. 85.6 (78.5-93.2) 90.8 (84.6-97.4) 93.6 (87.3-100.3)	4.5	**	75.4 (71.6-79.5) 73.6 (67.3-80.6) vs. 155.7 (145.3-166.8) 179.5 (164.0-196.4)	1.7	NS	3.2	*

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 256 (height) and 334 (all other dependent variables). All dependent variables were natural log transformed. Description of abbreviations: L = low light and H = high light; N-S = non-sterile and S = sterile; Ar = *A. rubrum*, As = *A. saccharum*, Fa = *F. americana* and Qr = *Q. rubra.*

Appendix M. Split-plot analysis of covariance results for the effects of light availability (2% vs. 22% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil), bench and covariate (initial seed mass) on *Q. rubra* seedling mass and stem height.

	Source of Variation (df)													
Dependent Variable	Light (1)				Extract (1)			Soil Source (3)			Light * Extract (1)			
	F	P	Means (95% CI) vs. H	L	F	P	Means (95% CI) N-S vs. S	F	P	Means (95% CI) Qr vs. Ar, As, Fa	F	P	Means (95% CI) L/N-S, L/S, H/N-S, H/S
Total Mass (mg)	373.9	****	1156.3 (1097.7-1218.0) vs. 3470.3 (3248.7-3707.1)		7.4	***	1430.8 (1377.5-1486.2) vs. 1618.1 (1493.7-1752.9)	2.6	**	1642.5 (1528.4-1765.2) vs. 1451.0 (1346.1-1562.4) 1523.9 (1412.3-1644.2) 1477.3 (1370.6-1594.0)	—	—	NA
Root Mass (mg)	296.7	****	NA		8.7	***	NA	2.6	**	642.9 (584.6-707.0) vs. 568.5 (514.4-628.9) 543.5 (491.3-601.8) 642.9 (584.6-707.0)	3.6	*	395.8 (373.5-419.1) 421.6 (373.5-419.1) 1435.1 (1296.0-1589.2) 1916.0 (1657.4-2212.8)
Stem Mass (mg)	147.7	****	NA		4.7	**	309.8 (297.1-323.1) vs. 338.7 (315.1-363.6)	2.2	*	NA	—	—	NA
Leaf Mass (mg)	471.7	****	441.0 (416.5-466.8) vs. 1131.2 (1052.6-1215.6)		1.9	NS	683.1 (651.4-716.4) vs. 731.1 (671.3-796.3)	3.7	**	777.9 (719.9-840.5) vs. 678.6 (625.0-737.4) 717.0 (658.9-780.2) 658.8 (606.2-718.0)	—	—	NA
Height (mm)	27.6	****	NA		0.3	NS	168.9 (163.4-174.7) vs. 166.0 (156.5-176.2)	4.1	***	NA	—	—	NA

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 284 (stem) and 364 (all other dependent variables). All dependent variables were natural log transformed. "—"denotes that the ANCOVA model does not contain that term due to a non-significant ($P > 0.25$ for covariate, bench and interactions between main terms) effect on the dependent variable or due to the assumption of homogenous slopes not being violated ($P > 0.05$ for interactions between main terms and covariate). Description of abbreviations: L = low light and H = high light; N-S = non-sterile and S = sterile; .Ar = *A. rubrum*, As = *A. saccharum*, Fa = *F. americana* and Qr = *Q. rubra.* Significance shown as follows: NS, not significant, $P > 0.10$; *$P \leq 0.10$; **$P \leq 0.05$; *** $P \leq 0.01$; ****$P \leq 0.001$.

Appendix M Ctd. Split-plot analysis of covariance results for the effects of light availability (2% vs. 22% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil), bench and covariate (initial seed mass) on *Q. rubra* seedling mass and stem height.

	Source of Variation (df)											
	Light * Source (3)			Extract * Source (3)			Bench (6)		Covariate (1)		Source * Covariate (3)	
Dependent Variable	F	P	Means (95% CI) L/Qr, L/Ar, L/As, L/Fa vs. H/Qr, H/Ar, H/As, H/Fa	F	P	Means (95% CI) N-S/Qr, N-S/Ar, N-S/As, N-S/Fa vs. S/Qr, S/Ar, S/As, S/Fa	F	P	F	P	F	P
Total Mass (mg)	—	—	NA	—	—	NA	2.0	*	343.4	****	—	—
Root Mass (mg)	—	—	NA	—	—	NA	2.1	*	241.9	****	—	—
Stem Mass (mg)	2.7	*	274.0 (253.7-296.2) 228.8 (207.1-296.2) 261.9 (237.7-288.6) 293.5 (265.9-324.1) vs. 676.5 (613.4-746.2) 519.0 (460.4-585.8) 646.1 (570.2-732.2) 575.4 (511.2-648.1)	—	—	NA	2.9	**	278.7	****	2.8	**
Leaf Mass (mg)	—	—	NA	—	—	NA	—	—	260.8	****	—	—
Height (mm)	5.0	***	153.3 (142.1-165.4) 136.7 (126.3-147.9) 154.0 (142.1-166.8) 177.0 (163.1-192.1) vs. 196.4 (179.7-214.7) 166.2 (149.8-184.3) 196.4 (176.2-218.7) 169.2 (152.7-187.7)	1.9	NS	NA	—	—	113.5	****	—	—

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 284 (stem) and 364 (all other dependent variables). All dependent variables were natural log transformed. "—"denotes that the ANCOVA model does not contain that term due to a non-significant ($P > 0.25$ for covariate, bench and interactions between main terms) effect on the dependent variable or due to the assumption of homogenous slopes not being violated ($P > 0.05$ for interactions between main terms and covariate). Description of abbreviations: L = low light and H = high light; N-S = non-sterile and S = sterile; Ar = *A. rubrum*, As = *A. saccharum*, Fa = *F. americana* and Qr = *Q. rubra*. Significance shown as follows: NS, not significant, $P > 0.10$; *$P \leq 0.10$; **$P \leq 0.05$; *** $P \leq 0.01$; ****$P \leq 0.001$.

Appendix N. Analysis of variance results for the effect of soil source (tree species culturing soil) on base cations (combined Ca, K, and Mg), total organic C, total N, and C:N in the soil extracts.

Source of Variation	F	*P*	Means (95% CI)
Base Cations (ppm)	11.15	**0.000**	Ar = 259.39 (245.96-272.83) As = 288.79 (275.36-302.22) Fa = 302.14 (288.16-316.12) Qr = 255.49 (242.05-268.92)
Total Organic C (mg/L)	14.24	**0.000**	Ar = 5.47 (4.46-6.48) As = 4.22 (3.21-5.23) Fa = 4.28 (3.21-5.23) Qr = 8.17 (7.16-9.18)
Total N (mg/L)	1.00	0.41	Ar = 2.30 (1.68-2.91) As = 2.54 (1.68-2.91) Fa = 2.54 (1.92-3.15) Qr = 2.22 (1.61-2.84)
C:N	16.91	**0.000**	Ar = 2.37 (1.92-2.91) As = 1.71 (1.39-2.11) Fa = 1.48 (1.18-1.86) Qr = 3.81 (3.10-4.69)

Notes: Tests were performed using the Type III sum of squares from SPSS version 15. Degree of freedom for all cation models is 3,47 and for total organic C, total N, and C:N ratios are 3,19. Description of abbreviations: Ar = *A. rubrum*, As = *A. saccharum*, Fa = *F. americana* and Qr = *Q. rubra*.

Appendix O. Kaplan-Meier analysis of the effect of inoculum type (Five *Fusarium* morphotypes and control) on seedling life span for *A. rubrum*, *F. americana* and *Q. rubra*.

Species	Treatment	Days alive (std. error)	Breslow, χ^{2c}	*P* value
A. rubrum	Control	56.30 (1.66)	17.99	**0.003**
	Fusarium 1	52.30 (3.19)		
	Fusarium 2	56.10 (1.85)		
	Fusarium 3	55.55 (2.39)		
	Fusarium 4	45.85 (4.19)		
	Fusarium 5	51.35 (3.26)		
F. americana	Control	39.65 (4.91)	4.06	0.54
	Fusarium 1	31.50 (4.46)		
	Fusarium 2	42.90 (4.19)		
	Fusarium 3	36.70 (4.38)		
	Fusarium 4	36.80 (4.46)		
	Fusarium 5	41.75 (4.99)		
Q. rubra	Control	58.00 (0.00)	5.00	0.42
	Fusarium 1	58.00 (0.00)		
	Fusarium 2	57.40 (0.63)		
	Fusarium 3	58.00 (0.00)		
	Fusarium 4	58.00 (0.00)		
	Fusarium 5	58.00 (0.00)		

Notes: Seedlings that did not emerge are part of the survival analysis with their death date given as the mean number of days prior to emergence. $df = 5, 120$ for each species.

Appendix P. Mixed effects analysis of covariance results for the fixed effects of inoculum type (Control vs. *Fusarium* morphotype 1-5), random effect of bench and covariate (initial seed mass) on *A. rubrum* seedling mass and stem height.

	Source of Variation (*df*)							
	Inoculum (5)				Bench (1)		Covariate (1)	
Dependent Variable	*F*	*P*	Treatment	Means (95% CI)	*F*	*P*	*F*	*P*
Total (mg)	2.7	**0.03**	Control	14.6 (12.3-17.3)	9.8	**0.002**	—	—
			Fusarium 1	13.6 (11.4-16.3)				
			Fusarium 2	14.4 (12.1-17.0)				
			Fusarium 3	11.4 (9.6-13.6)				
			Fusarium 4	13.0 (10.4-16.3)				
			Fusarium 5	9.9 (8.1-12.0)				
Root (mg)	3.1	**0.01**	Control	1.7 (1.4-2.1)	9.4	**0.007**	—	—
			Fusarium 1	1.5 (1.2-1.9)				
			Fusarium 2	1.4 (1.1-1.8)				
			Fusarium 3	1.0 (0.7-1.3)				
			Fusarium 4	1.4 (1.0-1.9)				
			Fusarium 5	1.0 (0.7-1.3)				
Stem (mg)	2.6	**0.03**	Control	3.3 (2.9-3.7)	5.8	**0.02**	—	—
			Fusarium 1	2.9 (2.4-3.3)				
			Fusarium 2	3.2 (2.7-3.6)				
			Fusarium 3	2.6 (2.1-3.0)				
			Fusarium 4	2.8 (2.3-3.3)				
			Fusarium 5	2.4 (1.9-2.8)				
Leaves (mg)	2.7	**0.03**	Control	7.4 (5.8-9.4)	9.5	**0.003**	—	—
			Fusarium 1	6.8 (5.2-8.8)				
			Fusarium 2	7.4 (5.7-9.4)				
			Fusarium 3	5.1 (3.9-6.6)				
			Fusarium 4	6.5 (4.7-9.0)				
			Fusarium 5	4.2 (3.1-5.7)				
Cotyledon (mg)	1.5	0.21	Control	2.2 (1.8-2.5)	—	—	2.6	0.11
			Fusarium 1	2.1 (1.7-2.4)				
			Fusarium 2	2.4 (2.1-2.7)				
			Fusarium 3	2.7 (2.3-3.0)				
			Fusarium 4	2.3 (1.9-2.8)				
			Fusarium 5	2.6 (2.2-2.9)				
Height (cm)	1.5	0.19	Control	46.5 (42.6-50.4)	—	—	—	—
			Fusarium 1	43.6 (39.5-47.8)				
			Fusarium 2	47.6 (43.7-51.5)				
			Fusarium 3	42.9 (38.9				
			Fusarium 4	42.5 (37.2				
			Fusarium 5	40.8 (36.4				

Appendix P. (Ctd)

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 99. All dependent variables except stem and cotyledon mass and stem height were transformed with natural log function. "—" denotes that the ANCOVA model does not contain that term due to a non-significant ($P > 0.25$ for covariate and for random factor of bench) effect on the dependent variable.

Appendix Q. Mixed effects analysis of covariance results for the fixed effects of inoculum type (Control vs. *Fusarium* morphotype 1-5), random effect of bench and covariate (initial seed mass) on *F. americana* seedling mass and stem height.

	Source of Variation (*df*)							
	Inoculum (5)				Bench (1)		Covariate (1)	
Dependent Variable	*F*	*P*	Treatment	Means (95% CI)	*F*	*P*	*F*	*P*
Total (mg)	2.5	**0.04**	Control	40.5 (30.4-53.9)	3.8	**0.06**	2.9	**0.10**
			Fusarium 1	32.5 (22.1-47.6)				
			Fusarium 2	49.3 (37.2-65.2)				
			Fusarium 3	33.4 (23.8-46.6)				
			Fusarium 4	58.3 (41.8-81.0)				
			Fusarium 5	56.8 (43.8-73.4)				
Root (mg)	1.8	0.13	Control	4.8 (3.7-6.3)	5.2	**0.03**	5.5	**0.02**
			Fusarium 1	3.7 (2.4-5.3)				
			Fusarium 2	6.6 (5.1-8.5)				
			Fusarium 3	4.2 (3.0-5.7)				
			Fusarium 4	5.3 (3.8-7.2)				
			Fusarium 5	5.0 (3.9-6.3)				
Stem (mg)	2.9	**0.02**	Control	8.8 (7.0-11.0)	4.4	**0.04**	5.3	**0.03**
			Fusarium 1	7.4 (5.4-10.0)				
			Fusarium 2	9.8 (7.9-12.2)				
			Fusarium 3	7.9 (6.1-10.3)				
			Fusarium 4	12.6 (9.8-16.2)				
			Fusarium 5	11.9 (9.8-14.5)				
Leaves (mg)	2.8	**0.03**	Control	19.0 (12.3-28.9)	4.8	**0.03**	—	—
			Fusarium 1	13.6 (7.4-24.2)				
			Fusarium 2	22.8 (14.9-34.6)				
			Fusarium 3	10.2 (5.9-17.1)				
			Fusarium 4	28.1 (17.2-45.8)				
			Fusarium 5	27.5 (18.7-40.3)				
Cotyledon (mg)	1.2	0.33	Control	8.6 (6.9-10.2)	—	—	6.4	**0.02**
			Fusarium 1	8.6 (6.3-10.8)				
			Fusarium 2	9.8 (8.2-11.5)				
			Fusarium 3	9.1 (7.2-11.0)				
			Fusarium 4	10.4 (8.4-12.3)				
			Fusarium 5	10.8 (9.3-12.4)				
Height (cm)	1.7	0.15	Control	74.1 (63.9-84.3)	2.7	0.11	—	—
			Fusarium 1	60.2 (46.6-73.8)				
			Fusarium 2	72.4 (62.3-82.4)				
			Fusarium 3	67.4 (55.6-79.1)				
			Fusarium 4	81.4 (69.4-93.3)				
			Fusarium 5	80.2 (70.8-89.5)				

Appendix Q. (Ctd)

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 57. All dependent variables except cotyledon mass and stem height were transformed with natural log function. "—"denotes that the ANCOVA model does not contain that term due to a non-significant ($P > 0.25$ for covariate and for random factor of bench) effect on the dependent variable.

Appendix R. Mixed effects analysis of covariance results for the fixed effects of inoculum type (Control vs. *Fusarium* morphotype 1-5), random effect of bench and covariate (initial seed mass) on *Q. rubra* seedling mass and stem height.

Dependent Variable	Source of Variation (*df*)									
	Inoculum (5)				Bench (1)		Covariate (1)		Inoculum * Covariate (1)	
	F	*P*	Treatment	Means (95% CI)	*F*	*P*	*F*	*P*	*F*	*P*
Total (mg)	2.2	**0.06**	Control	1314.1 (1160.5-1467.7)	3.2	**0.08**	106.6	**0.000**	—	—
			Fusarium 1	1120.7 (967.2-1274.1)						
			Fusarium 2	1321.0 (1167.4-1474.6)						
			Fusarium 3	1238.8 (1088.3-1389.2)						
			Fusarium 4	1255.3 (1101.7-1409.0)						
			Fusarium 5	1465.6 (1316.0-1615.1)						
Root (mg)•	2.2	**0.06**	Control	295.5 (262.1-334.5)	6.8	**0.01**	132.9	**0.000**	2.6	**0.03**
			Fusarium 1	289.1 (-96.5-680.2)						
			Fusarium 2	312.2 (262.1-356.8)						
			Fusarium 3	278.8 (250.9-306.7)						
			Fusarium 4	303.5 (3.1-609.5)						
			Fusarium 5	334.5 (295.5-368.0)						
Stem (mg)	2.7	**0.02**	Control	263.5 (236.5-290.5)	6.3	**0.01**	83.3	**0.000**	—	—
			Fusarium 1	207.5 (180.5-234.5)						
			Fusarium 2	225.0 (197.9-252.0)						
			Fusarium 3	240.6 (214.1-267.1)						
			Fusarium 4	241.9 (214.9-269.0)						
			Fusarium 5	265.5 (239.2-291.9)						
Leaves (mg)	1.9	**0.10**	Control	694.8 (613.1-776.4)	—	—	104.6	**0.000**	—	—
			Fusarium 1	525.0 (443.4-606.5)						
			Fusarium 2	619.5 (537.9-701.2)						
			Fusarium 3	645.2 (517.0-680.4)						
			Fusarium 4	598.7 (517.0-680.4)						

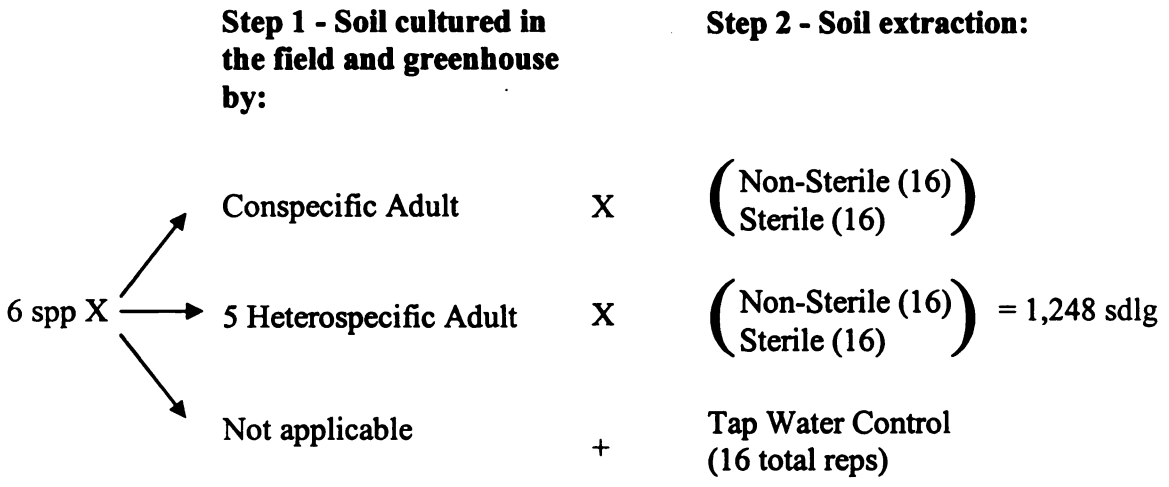
Appendix R. (Ctd)

			Fusarium 5	639.5 (559.9-719.0)						
Height (cm)	2.2	**0.06**	Control	189.0 (169.2-208.8)	—	—	13.0	**0.000**	—	—
			Fusarium 1	163.6 (143.8-183.5)						
			Fusarium 2	157.8 (138.0-177.6)						
			Fusarium 3	183.1 (163.7-202.6)						
			Fusarium 4	157.6 (137.7-177.4)						
			Fusarium 5	186.7 (167.4-206.0)						

Notes: Tests were performed using the Type III sum of squares from SPSS version 14. Total number of seedlings in model = 116. "—"denotes that the ANCOVA model does not contain that term due to a non-significant ($P > 0.25$ for covariate and for random factor of bench) effect on the dependent variable or due to the assumption of homogenous slopes not being violated ($P > 0.05$ for interactions between fixed factor and covariate). "•" indicates that linear regression analysis was used rather than ANCOVA due interaction term between the treatment and covariate terms being significant. Means provided are from the linear regression analysis.

Appendix S. Experimental design and allocation of tropical seedlings to treatments.

a) High Light



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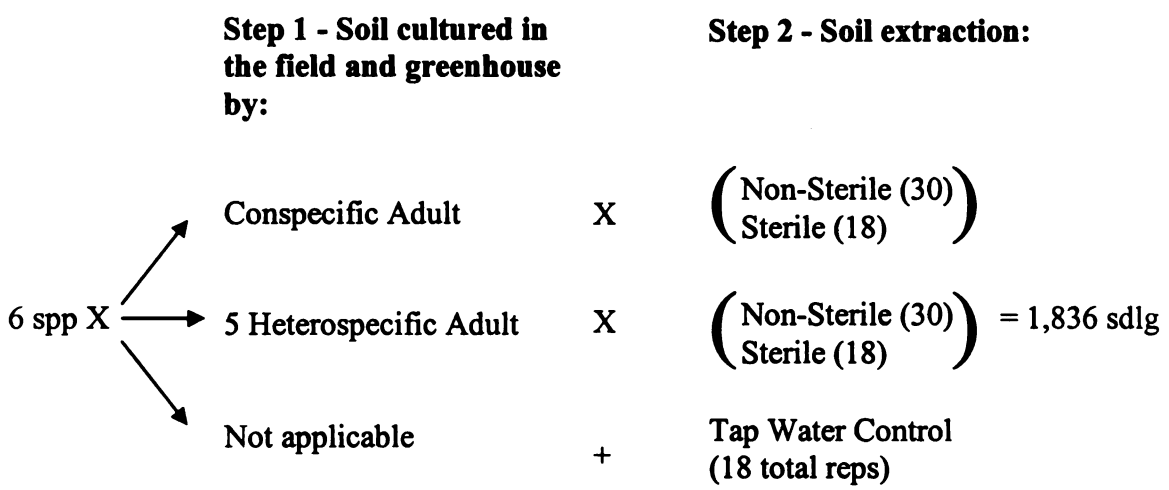
b) Low Light



Appendix T. Hazards ratios for the effects of light availability (1% vs. 5% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil) and initial seed mass on seedling mortality estimated by the Cox regression model.

Species	Source of Variation	df	Parameter estimate	SE	Wald χ^2	*P* value	Hazard ratio
Apeiba membranacea	$Light_{(Low)}$	1	0.97	0.15	44.76	**0.000**	2.65
	$Extract_{(Non\text{-}Sterile)}$	1	-0.03	0.13	0.05	0.83	0.97
	$Source_{(Colubrina)}$	1	0.26	0.23	1.33	0.25	1.30
	$Source_{(Iriartea)}$	1	0.15	0.21	0.48	0.49	1.16
	$Source_{(Pentaclethra)}$	1	-0.04	0.22	0.04	0.85	0.96
	$Source_{(Prestoea)}$	1	0.27	0.22	1.51	0.22	1.30
	$Source_{(Virola)}$	1	0.46	0.21	4.97	**0.03**	1.59
	Seed Mass	1	0.04	0.03	1.87	0.17	1.05
Colubrina spinosa	$Light_{(Low)}$	1	0.89	0.20	19.97	**0.000**	2.43
	$Extract_{(Non\text{-}Sterile)}$	1	-0.28	0.17	2.76	0.10	0.75
	$Source_{(Apeiba)}$	1	-1.06	0.34	10.01	**0.002**	0.35
	$Source_{(Iriartea)}$	1	0.34	0.23	2.12	0.15	1.40
	$Source_{(Pentaclethra)}$	1	-0.69	0.29	5.77	**0.02**	0.50
	$Source_{(Prestoea)}$	1	-0.58	0.30	3.88	**0.05**	0.56
	$Source_{(Virola)}$	1	-1.03	0.33	9.83	**0.002**	0.36
	Seed Mass	1	0.01	0.00	2.22	0.15	1.01
Iriartea deltoidea	$Light_{(Low)}$	1	-0.21	0.16	1.72	0.19	0.81
	$Extract_{(Non\text{-}Sterile)}$	1	-0.01	0.16	0.01	0.93	0.99
	$Source_{(Apeiba)}$	1	-0.01	0.24	0.00	0.97	0.99
	$Source_{(Colubrina)}$	1	0.93	0.23	16.00	**0.000**	2.52
	$Source_{(Pentaclethra)}$	1	-2.08	0.51	16.41	**0.000**	0.13
	$Source_{(Prestoea)}$	1	-1.30	0.38	11.78	**0.001**	0.27
	$Source_{(Virola)}$	1	0.10	0.23	0.19	0.66	1.11
	Seed Mass	1	0.00	0.00	1.70	0.19	1.00
Pentaclethra macroloba	$Light_{(Low)}$	1	1.36	0.41	11.03	**0.001**	3.87
	$Extract_{(Non\text{-}Sterile)}$	1	1.02	0.35	8.29	**0.004**	2.78
	$Source_{(Apeiba)}$	1	-0.27	0.54	0.25	0.62	0.77
	$Source_{(Colubrina)}$	1	0.56	0.45	1.55	0.21	1.75
	$Source_{(Iriartea)}$	1	0.23	0.47	0.23	0.63	1.25
	$Source_{(Prestoea)}$	1	-0.00	0.50	0.00	0.99	1.00
	$Source_{(Virola)}$	1	-0.50	0.57	0.77	0.38	0.61

Appendix T (Ctd)

Prestoea decurrens	$Light_{(Low)}$	1	0.78	0.26	9.24	**0.002**	2.17
	$Extract_{(Non\text{-}Sterile)}$	1	0.26	0.26	1.07	0.30	1.30
	$Source_{(Apeiba)}$	1	-0.23	0.33	0.50	0.48	0.79
	$Source_{(Colubrina)}$	1	-0.40	0.34	1.42	0.23	0.67
	$Source_{(Iriartea)}$	1	-0.53	0.35	2.27	0.13	0.59
	$Source_{(Pentaclethra)}$	1	-0.35	0.34	1.04	0.31	0.71
	$Source_{(Virola)}$	1	-1.34	0.46	8.71	**0.003**	0.26
	$Extract_{(Non\text{-}Sterile)}$ * $Light_{(Low)}$	1	-0.69	0.51	1.83	0.18	0.50
Virola koschnyi	$Light_{(Low)}$	1	-0.19	0.46	0.17	0.68	0.83
	$Extract_{(Non\text{-}Sterile)}$	1	-0.18	0.46	0.16	0.69	0.83
	$Source_{(Apeiba)}$	1	0.90	0.72	1.56	0.21	2.45
	$Source_{(Colubrina)}$	1	0.00	0.96	0.00	1.00	1.00
	$Source_{(Iriartea)}$	1	-0.82	1.17	0.50	0.48	0.44
	$Source_{(Pentaclethra)}$	1	0.27	0.83	0.11	0.75	1.31
	$Source_{(Prestoea)}$	1	0.43	0.77	0.31	0.58	1.54
	Seed Mass	1	0.00	0.00	2.34	0.13	1.00

Notes: Effects of low light vs. high light, non-sterile vs. sterile extract, soil source (reference category is always the study species) and initial seed mass on mortality. Total number of seedlings in model for each species was 480 except for *Apeiba membranacea* = 469, *Colubrina spinosa* = 471 and *Iriartea deltoidea* = 454. Hazard ratios > 1 indicate reduction in days to mortality of study species. For quantitative covariates (e.g. initial seed mass), subtracting 1 from the hazard ratio and multiplying by 100 (i.e. $100(e\beta - 1)$) gives the per cent change in the hazard of mortality associated with a 1-unit increase in the covariate, controlling for effects of other covariates.

Appendix U. Split-plot analysis of covariance results for the effects of light availability (1% vs. 5% full sun), soil extract (non-sterile vs. sterile), soil source (tree species culturing soil), bench and covariate (initial seed mass) on seedling mass (mg) for each species.

1) *A. membranacea*

Source of Variation	*df*	F	P	Means (95% CI)
Light	1	95.80	**0.000**	L = 4.5 (3.9 – 5.2) vs. H = 11.6 (10.5 – 12.8)
Extract	1	0.06	0.82	N-S = 8.7 (6.0 – 7.5) vs. S = 6.6 (5.8 – 7.5)
Soil Source	5	0.41	0.84	Am = 6.4 (5.2 – 7.7) vs. Cs = 6.7 (5.4 – 8.3) Id = 6.2 (5.1 – 7.7) Pm = 6.5 (5.4 – 7.9) Pd = 7.5 (6.1 – 9.0) Vk = 6.6 (5.2 – 8.3)
Bench	8	1.30	0.25	NA
Covariate	1	4.62	**0.03**	NA

2) *C. spinosa*

Source of Variation	*df*	F	P	Means (95% CI)
Light	1	42.12	**0.000**	NA
Extract	1	1.15	0.29	N-S = 21.3 (20.2 – 22.5) vs. S = 22.3 (20.9 – 23.9)
Soil Source	5	3.38	**0.005**	NA
Light * Soil Source	5	2.89	**0.01**	L/Cs = 16.5 (13.9 – 19.6) vs. L/Am = 17.2 (15.1 – 19.6) L/Id = 16.5 (12.2 – 20.1) L/Pm = 17.0 (14.9 – 19.3) L/Pd = 16.6 (14.5 – 19.0) L/Vk = 17.7 (15.7 – 20.1) H/Cs = 34.6 (29.8 – 41.0) vs. H/Am = 36.0 (31.5 – 41.0) H/Id = 20.5 (17.3 – 24.4) H/Pm = 34.1 (29.2 – 39.9) H/Pd = 35.6 (30.9 – 41.1) H/Vk = 32.8 (28.5 – 37.7)
Bench	8	4.96	**0.000**	NA

Appendix U. (Ctd)

3) *I. deltoidea*

Source of Variation	*df*	F	P	Means (95% CI)
Light	1	0.30	0.58	L = 181.9 (169.2 – 194.5) vs. H = 186.9 (171.3 – 202.6)
Extract	1	1.78	0.18	N-S = 190.4 (177.1 – 203.8) vs. S = 178.4 (163.6 – 193.2)
Soil Source	5	14.32	**0.000**	Id = 176.2 (154.0 – 198.4) vs. Am = 162.9 (140.3 – 185.6) Cs = 138.1 (95.1 – 182.0) Pm = 254.6 (237.7 – 271.5) Pd = 206.5 (188.8 – 224.1) Vk = 168.1 (144.5 – 191.8)

4) *P. macroloba*

Source of Variation	*df*	F	P	Means (95% CI)
Light	1	24.08	**0.001**	L = 1729.4 (1661.1 – 1800.5) vs. H = 2256.0 (2155.6 – 2361.1)
Extract	1	2.98	**0.09**	N-S = 1967.0 (1889.1 – 2048.0) vs. S = 1880.8 (1797.6 – 1967.9)
Soil Source	5	2.06	**0.07**	Pd = 1741.9 (1641.1 – 1879.8) vs. Am = 2015.7 (1876.1 – 2165.8) Cs = 2020.1 (1871.8 – 2180.0) Id = 1847.4 (1713.7 – 1991.6) Pm = 1957.6 (1818.9 – 2106.8) Vk = 1974.0 (1835.7 – 2122.6)
Light * Extract	1	2.12	0.15	NA
Light * Soil Source	5	1.34	0.25	NA
Extract * Soil Source	5	1.24	0.29	NA
Light * Extract * Soil Source	5	1.41	0.22	NA
Bench	8	3.06	**0.002**	NA
Covariate	1	278.61	**0.000**	NA

5) •*P. decurrens* (without cotyledon mass)

Source of Variation	*df*	F	P	Means (95% CI)
Light	1	18.76	**0.002**	NA
Extract	1	1.93	0.17	NA
Soil Source	5	6.08	**0.000**	NA
Light * Extract	1	3.41	**0.07**	NA
Light * Soil Source	5	0.78	0.56	NA
Extract * Soil Source	5	0.62	0.69	NA

Appendix U. (Ctd)

Light * Extract * Soil Source	5	2.53	**0.03**	L/N-S/Pd = 69.3 (62.3 – 76.4) L/N-S/Am = 71.0 (63.7 – 78.2) L/N-S/Cs = 70.4 (63.0 – 77.8) L/N-S//Id = 69.4 (62.2 – 76.6) L/N-S/Pm = 60.4 (52.9 – 68.0) L/N-S/Vk = 76.5 (69.7 – 83.3) L/S/Pd = 16.5 (13.9 – 19.6) L/S/Am = 73.0 (63.8 – 82.3) L/S/Cs = 61.6 (52.7 – 70.6) L/S//Id = 70.6 (61.0 – 80.1) L/S/Pm = 58.2 (48.5 – 67.8) L/S/Vk = 62.7 (53.8 – 71.7) H/N-S/Pd = 74.7 (65.8 – 83.6) H/N-S/Am = 81.1 (71.6 – 90.7) H/N-S/Cs = 74.5 (64.9 – 84.1) H/N-S//Id = 87.4 (78.8 – 96.1) H/N-S/Pm = 74.7 (65.8 – 83.6) H/N-S/Vk = 81.6 (72.7 – 90.5) H/S/Pd = 69.6 (60.4 – 78.8) H/S/Am = 82.5 (72.9 – 92.1) H/S/Cs = 85.1 (76.2 – 94.0) H/S//Id = 75.5 (66.6 – 84.4) H/S/Pm = 70.2 (61.2 – 79.1) H/S/Vk = 81.2 (72.6 – 89.9)
Bench	8	1.71	**0.09**	NA
Covariate	1	75.56	**0.000**	NA

6) §*P. decurrens* (seedlings without cotyledon's at harvest were excluded from analysis)

Source of Variation	*df*	F	P	Means (95% CI)
Light	1	0.85	0.36	NA
Extract	1	0.07	0.79	N-S = 150.4 (148.2 – 152.6) vs. S = 150.0 (147.6 – 152.4)
Soil Source	5	6.11	**0.000**	NA
Light * Extract	1	1.39	0.24	NA
Light * Soil Source	5	2.78	**0.02**	L/Pd = 156.7 (151.4 – 162.0) vs. L/Am = 149.6 (144.4 – 154.7) L/Cs = 143.5 (136.6 – 147.2) L/Id = 141.9 (143.9 – 155.0) L/Pm = 149.5 (143.9 – 155.0) L/Vk = 155.5 (150.8 – 160.3)

Appendix U. (Ctd)

				H/Pd = 152.8 (145.8 – 159.7) vs. H/Am = 160.9 (155.1 – 166.6) H/Cs = 147.0 (141.2 – 152.7) H/Id = 148.5 (142.9 – 154.0) H/Pm = 144.4 (138.3 – 150.5) H/Vk = 152.3 (146.8 – 157.8)
Covariate	1	582.71	**0.000**	NA

7) *V. koshnyi*

Source of Variation	*df*	F	P	Means (95% CI)
Light	1	37.76	**0.000**	L = 739.6.0 (712.7 – 766.5) vs. H = 867.5 (835.0 – 900.1)
Extract	1	0.66	0.42	NA
Soil Source	5	3.70	**0.003**	NA
Light * Soil Source	5	1.40	0.23	NA
Extract * Soil Source	5	2.23	**0.05**	N-S/Vk = 667.4 (601.1– 733.8) vs. N-S/Am = 843.7 (777.1 – 910.4) N-S/Cs = 842.9 (771.0 – 914.8) N-S/Id = 895.1 (829.4 – 960.9) N-S/Pm = 792.7 (725.6 – 856.8) N-S/Pd = 829.1 (763.1 – 895.1) S/Vk = 663.5 (579.5 – 747.2) vs. S/Am = 826.0 (748.8 – 902.9) S/Cs = 902.3 (819.1 – 985.1) S/Id = 774.6 (702.2 – 846.7) S/Pm = 856.5 (799.8 – 928.8) S/Pd = 748.9 (673.4 – 824.1)
Covariate	1	70.31	**0.000**	NA
Soil Source * Covariate	1	4.27	**0.001**	NA

Notes: Tests were performed using the Type III sum of squares from SPSS version 15. Total number of seedling in model for *A. membranacea* = 204, *C. spinosa* = 333, *I. deltoidea* = 277, *P. macroloba* = 425, •*P. decurrens* (without cotyledon mass) = 390, §P. decurrens (seedlings without cotyledon's at harvest were excluded from analysis) = 345, and *V. koschnyi* = 460. Seedling mass for *A. membranacea*, *C. spinosa*, and *P. macroloba* were natural log transformed. *I. deltoidea* seedling mass did not include cotyledon mass. Description of abbreviations: L = low light and H = high light; N-S = non-sterile and S = sterile; Am = *Apeiba membranacea*, Cs =*Colubrina spinosa*, Id = *Iriartea deltoidea*, Pm = *Pentaclethra macroloba*, Pd = *Prestoea decurrens*, and Vk = *Virola koschnyi*.

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