Interactions among tree species and pathogen-suppressive bacteria in tropical forest soils

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Dedication:

To John Kinder, my Mom, and my Grandparents

ABSTRACT:

In tropical forests, soil-borne plant pathogens are thought to regulate diversity by driving negative plant-soil feedbacks and promoting density-dependent mortality of seedlings close to parent trees. Pathogens interact with a myriad of other organisms in soil that have the potential to moderate plant disease and in turn its effects on community composition. Studies that consider interactions among microbial groups in the context of plant-soil feedbacks are relatively rare. In order to understand how microbes influence diversity in tropical forests, we need to move beyond the assumption of one-to-one feedbacks between plants and specialized pathogens and consider the potential importance of multiple soil functional groups and environmental heterogeneity in microbe-microbe and plant-microbe interactions. In my dissertation, I investigate antibiotic-producing soil bacteria (genus: *Streptomyces*) in tropical forest soils. I focus on biotic and abiotic characteristics that influence variation in pathogen suppression across the landscape and among individual trees, and address the potential consequences for plant community composition.

In Chapters 1 and 2, I explored *Streptomyces* communities in tropical dry forests in Costa Rica and asked whether variation was related to soil gradients and/or tree species. There was substantial variation in plant-soil-microbe relationships. In general, variation among tree species was not as great as variation among individual trees. In Chapter 3, I investigated the potential for *Streptomyces* to influence seedling biomass and plant-soil feedbacks in a tropical moist forest in Panama. Seedling biomass was

significantly affected by a three-way interaction between seedling species, soil source species (adult tree species), and densities of pathogen-suppressive *Streptomyces* in soil. Overall, results suggest that unique relationships among antibiotic-producing *Streptomyces*, pathogens, and abiotic resources among soils from different adult trees influence seedling biomass and may mediate the strength and specificity of plant-soil feedbacks in tropical forest soils. Future studies which account for multi-dimensional interactions among soils, microbes, and plants will allow us to better understand how aboveground diversity is regulated by diverse microbes in soil.

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INTRODUCTION:

Antibiotic-producing bacteria are a ubiquitous group of soil microbes in many terrestrial ecosystems. One genus that is well-known for the production of diverse antibiotics and other secondary metabolites is *Streptomyces*. *Streptomyces* play an important role in medicine as a significant source of naturally-derived antibiotics (Hopwood 2007). However, *Streptomyces* have been relatively overlooked as a microbial functional group in the community and ecosystem ecology literature. *Streptomyces* encompass a diversity of life history strategies, ranging from endophytes to plant pathogens to non-obligate plant-associated bacteria (Siepke et al. 2012), and have been linked to a variety of microbially-mediated soil functions including disease suppression (Kinkel et al. 2011, 2012), decomposition (Crawford 1978, McCarthy and Williamson 1992, Mahadeven and Crawford 1997), phosphate solubilization, and bioremediation of heavy metals (Schrey and Tarkka 2008, Franco-Correa et al. 2010). In my dissertation, I focus on the role of antibiotic-producing *Streptomyces* in tropical forest soils.

High densities, frequencies, and diversities of antibiotic-producing *Streptomyces* have long been associated with disease suppressive soils in agricultural systems (Kinkel et al. 2012 and references therein). The potential for *Streptomyces* to suppress soil-borne diseases of plants in unmanaged ecosystems could have significant implications for processes that influence plant community composition and diversity as well as biodiversity-ecosystem functioning relationships (Latz et al. 2012, Latz et al. 2016).

Much of what we know about the ecology and evolution of *Streptomyces* in soil comes from agricultural systems and experimental grasslands (Kinkel et al. 2012) and some work in temperate forests (Lehr et al. 2008, Jayasinghe 2008, Bontemps et al. 2013). Plant host identity, community composition, and soil physicochemical characteristics have been shown to influence pathogen-suppressive capacities of *Streptomyces* communities in temperate ecosystems (Bakker et al. 2010, 2013, Felice et al. 2014, Kinkel et al. 2011, Schlatter et al. 2015). More information is needed on the factors that structure inhibitory phenotypes across different ecosystems and latitudes in order to understand how *Streptomyces* might influence community dynamics and ecosystem functioning as well as their potential use in restoration, bioremediation, and biological control in diverse locations.

Tropical forests are hotspots for theories on the role of pathogens in the maintenance of plant diversity (Gillett 1962, Janzen 1970, Connell 1971) as well as studies that test those theories (Carson et al. 2004). However, very little is known about pathogen-suppressive bacteria and their interactions with pathogens and plants in tropical forest ecosystems. My dissertation work addresses this gap in knowledge by examining variation in functional capabilities of *Streptomyces* communities in tropical forest soils, investigating the biotic and abiotic factors that structure variation in inhibitory phenotypes within and among sites, and exploring the potential consequences for tropical plant communities.

In all three Chapters, I used a culture-based functional assay to quantify pathogen-inhibitory capacities of soil-borne *Streptomyces* (SI Fig. 0.1; Herr 1959, Wiggins and

Kinkel 2005). Despite the predicted importance of soil-borne pathogens in tropical forests and the substantial tropical community ecology literature devoted to this topic (reviewed in Gilbert 2005), corresponding soil microbiology and plant pathology research is extremely limited. Collections of confirmed pathogen strains of tree species are unavailable for most tropical forest ecosystems. Therefore, in order to test pathogen-suppressive capacities of *Streptomyces* communities in tropical forest soils, I used strains isolated from temperate agricultural ecosystems as indicators of antibiotic production (SI Table 0.1). The data collected estimate total *Streptomyces* densities in soil as well as the abundance of pathogen-suppressive *Streptomyces* (inhibitor densities and proportions) and the strength of antibiotic-mediated pathogen inhibition (inhibition zone size), which represent distinct inhibitory phenotypes.

The degree of antagonism among *Streptomyces* communities is predicted to depend on biotic and abiotic factors that influence competitive dynamics and interaction phenotypes among soil microbes, including landscape-scale heterogeneity in soil and/or vegetative characteristics as well as local heterogeneity in the microbial resource pool provided by inputs from individual plants (Kinkel et al. 2011). The specific goals of Chapter 1 were to determine whether pathogen-suppressive capacities of soil-borne *Streptomyces* communities varied across a tropical dry forest landscape in Costa Rica, and to test whether functional variation was related to soil nutrient availability (Becklund et al. 2014). Chapter 2 built on this study by examining *Streptomyces* communities beneath individual focal trees in the same two sites in order to gain further insight into factors that structure inhibitory phenotypes between sites and among coexisting tree

species. Chapter 3 tests for linkages among pathogen-suppressive capacities of *Streptomyces* communities, indices of soil disease potential, and seedling biomass and asks whether *Streptomyces* play a role in plant-soil feedbacks among five tree species in a tropical moist forest in Panama.

Negative plant-soil feedbacks are thought to promote species coexistence when the net effects of soil microbes or abiotic soil characteristics disproportionately disadvantage conspecifics relative to heterospecifics (Bever et al. 1997). Evidence of negative feedback is often attributed to the accumulation of specialized pathogens in soil beneath host plants (Kulmatiski et al. 2008, Mangan et al. 2010, Bever et al. 2015). By mediating interactions between pathogens and plants as well as among soil microbes themselves (Bakker et al. 2014), *Streptomyces* have the potential to moderate the net effects of soils on the relative performance of plant competitors via plant-soil feedbacks (Fig. 0.1). *Streptomyces* have the potential to mediate the strength and specificity of feedbacks through direct effects on plant hosts via the production of signaling molecules (Linares et al. 2006), or indirect effects through suppression of soil-borne pathogens (Bakker et al. 2014, Schlatter and Kinkel 2014). Variation in the distribution, degree, and specificity of antibiotic production and pathogen inhibition among *Streptomyces* communities in soil could influence tree species coexistence.

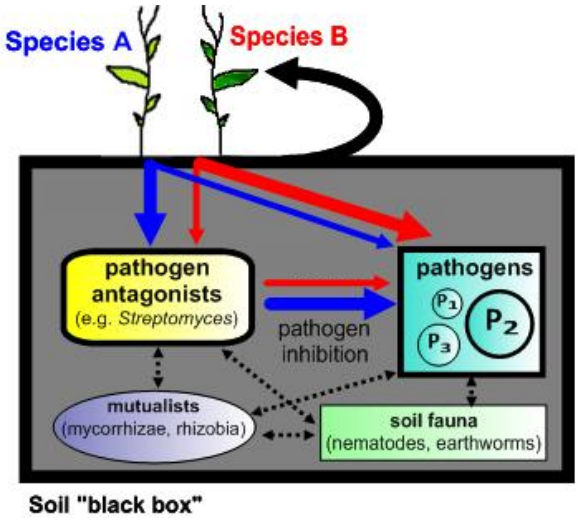

Figure 0.1. Plant-soil feedbacks occur when plants influence microbial community composition and/or activity, and these changes positively or negatively affect plant performance. Colored lines indicate differential effects of plant species A and B on *Streptomyces*, pathogens, and their interactions, which could feedback directly or indirectly on plant growth or relative performance (black arrow). Line thickness indicates the *strength* of effects. *Specificity* of pathogen inhibition by *Streptomyces* (illustrated as differential inhibition of pathogen populations P1 – P3) has the potential to promote species coexistence if it leads to greater intraspecific than interspecific effects on plant species. Dotted arrows show interactions among *Streptomyces* and other belowground organisms that could also influence net effects of soils on plants. *Streptomyces* may exacerbate or ameliorate existing feedbacks (either positive or negative), generate new feedbacks, or have no net effect.

Chapter 1

Landscape-scale variation in pathogen-suppressive bacteria
in tropical dry forest soils of Costa Rica

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**Abstract is available in Spanish in the online version of this publication in Biotropica*

ABSTRACT:

Bacteria in the genus *Streptomyces* are ubiquitous in soil and are well known for their production of diverse secondary metabolites, including antibiotics that can inhibit soil-borne plant pathogens and suppress disease. Pathogen-suppressive soil bacteria have the potential to influence plant community composition and diversity, but remain relatively unexplored in tropical forest soils. To estimate the potential for disease suppression among *Streptomyces* communities in tropical dry forests, we cultured soil-borne *Streptomyces* from plots in two forests in Northwestern Costa Rica (Santa Rosa and Palo Verde) and quantified antibiotic-mediated pathogen inhibition against three plant pathogens. The potential for pathogen inhibition and disease suppression by *Streptomyces* was highly variable across the landscape. Densities of pathogen-suppressive *Streptomyces* varied by over ten-fold and were correlated with soil nutrients across the plots. In particular, *Streptomyces* communities became more pathogen-suppressive as labile soil P decreased. Inhibitor densities were significantly higher in Santa Rosa than Palo Verde, which may be related to differences in soil texture and/or

plant community composition between the two forests. Our findings suggest potential differences in the degree and specificity of antibiotic-mediated disease suppression in tropical dry forest soils of Costa Rica, and highlight the need for further studies on the drivers of pathogen-suppressive phenotypes as well as the consequences of spatially-variable pathogen inhibition for plant community composition in tropical forest ecosystems.

INTRODUCTION:

Soil microbes influence plant community diversity, productivity, invasion, and ecosystem functioning (Klironomos 2002, Wardle 2002, Reed & Martiny 2007, Bever et al. 2010, Schnitzer et al. 2010, Schimel & Schaeffer 2012). However, the specific microbial taxa, phenotypes, and interactions mediating plant-microbe linkages remain poorly understood (Westover & Bever 2001, Morris et al. 2007, Bever et al. 2012, Hodge & Fitter 2012). Soil-borne plant pathogens are hypothesized to be important in structuring seedling recruitment and local plant diversity in many ecosystems (Mordecai 2011) and have received considerable attention in tropical forest ecology (Gillett 1962, Janzen 1970, Connell 1971, Freckleton & Lewis 2006). Density-dependent seedling mortality (Bell et al. 2006, Bagchi et al. 2014), Janzen-Connell spacing distributions (Augspurger 1983, Packer & Clay 2000), and negative plant-soil feedbacks (Mangan et al. 2010, Liu et al. 2012) have all been attributed to the activity of soil-borne pathogens, yet it remains

uncertain whether pathogens alone exhibit sufficient specificity to promote tree species diversity in tropical forests (Augspurger & Wilkinson 2007, Gallery et al. 2007, Terborgh 2012). Plant-soil feedback experiments have provided a useful framework for testing the net effects of belowground biota on plants (Bever et al. 1997, Van der Putten et al. 2013), but a significant step forward will be to open the microbial 'black box' and examine the microbial phenotypes and interactions that are expected to influence disease across plant species. Soil-borne pathogens exist within a complex microbial community, yet very little is known about how interactions with other soil microbes influence the effects of pathogens on plants.

Pathogen-suppressive bacteria have the potential to regulate the activities of pathogens and moderate the impacts of disease on plant populations and communities (Raaijmakers & Weller 1998, Garbeva et al. 2004, Kinkel et al. 2011, 2012). A particularly important group of pathogen antagonists are bacteria in the genus *Streptomyces* (650+ species; Euzéby 2012). *Streptomyces* are gram-positive, filamentous bacteria that are ubiquitous in soil and are prolific producers of antibiotics and other secondary metabolites. *Streptomyces* and other antibiotic-producing bacteria can influence plant fitness by producing plant growth hormones (Sadeghi et al. 2012), stimulating plant defenses (Jousset et al. 2011), or reducing plant disease through resource competition with pathogens as well as antibiotic-mediated pathogen inhibition (Yuan & Crawford 1995, Samac et al. 2003, Wiggins & Kinkel 2005, Perez et al. 2008). Numerous *Streptomyces* have been shown to inhibit a wide range of plant pathogens, including bacteria and fungi (Liu et al. 1995, Chamberlain & Crawford 1999, Bakker et

al. 2010), Oomycetes (Jones & Samac 1996, Xiao et al. 2002), and parasitic plant nematodes (Samac & Kinkel 2001). Antibiotics are thought to mediate species interactions among soil microbes by acting as weapons or potentially signaling molecules against competitors (Linares et al. 2006, Yim et al. 2007). Pathogen-suppressive bacteria are predicted to influence plant-soil feedbacks both directly and indirectly, by mediating interactions among plants, pathogens, and the broader soil community (Kinkel et al. 2011; Bakker et al. 2013b, 2014).

Streptomyces have been used extensively in agriculture as biological control agents against plant pathogens (Millard & Taylor 1927, Liu et al. 1995, Kinkel et al. 2012). However, very little is known about the distribution and ecology of pathogen-suppressive *Streptomyces* in non-agricultural soils, even though such knowledge could have significant implications for plant community ecology. An area for future research involves exploring whether *Streptomyces* and other antibiotic-producing bacteria mediate the effects of pathogens on plants across diverse plant hosts, ecosystems, and spatial scales.

Studies aimed at antibiotic discovery frequently cite tropical forest soils as hotspots of phylogenetically and phenotypically diverse antibiotic-producing bacteria (Wang et al. 1999, Cardona et al. 2009, Qin et al. 2009), but basic research on *Streptomyces* in both tropical and temperate forests is lacking. In temperate grasslands a number of factors can influence the potential for disease suppression among *Streptomyces* communities, including host plant identity, plant community richness and composition, and soil nutrient availability (Bakker et al. 2013a). Similarly, the high tree

species diversity and often nutrient deficient soils characteristic of tropical forests are likely to have significant and interacting effects on *Streptomyces* community functions, especially their abilities to suppress plant pathogens. The quantity and diversity of soil resources are hypothesized to influence selection for antagonistic phenotypes among soil microbes (Schlatter et al. 2009, Kinkel et al. 2011, 2012). In particular, low nutrient availability and/or diversity represent potentially competitive environments that may favor antibiotic production and select for highly antagonistic communities. On the other hand, soils with greater diversities of resources are predicted to produce niche-differentiated, less antagonistic microbial communities (Kinkel et al. 2011, Schlatter & Kinkel 2014a).

As a first step toward understanding the potential impacts of *Streptomyces* on plant communities, information is needed on variation in the potential for pathogen inhibition and disease suppression and whether these functions vary geographically and across environmental gradients. In this study we examined *Streptomyces* community function (i.e. pathogen suppression) across tropical dry forest sites that vary in soil fertility, texture, and plant community composition. Our specific objectives were to (1) quantify variation in *Streptomyces* densities and pathogen inhibitory capacities across 18 tropical dry forest plots that vary in soil properties, and (2) test for relationships between pathogen suppression and soil edaphic properties across the landscape. We collected soils from plots in two sites in Northwestern Costa Rica (Santa Rosa and Palo Verde), and used a culture-based functional assay to quantify two important measures of pathogen-suppressive potential among *Streptomyces* communities: 1) the frequency of

antibiotic-producing *Streptomyces* in soil (because not all *Streptomyces* produce antibiotics, quantifying both density and the proportion of the *Streptomyces* community that produce antibiotics is informative), and 2) the intensity or strength of inhibition against three plant pathogens (the bacterium *Streptomyces scabies,* and the fungi *Fusarium oxysporum* and *Rhizoctonia solani*). By assessing antagonistic activity of *Streptomyces* in vitro against standard plant pathogens, this method estimates the potential for *Streptomyces* communities to suppress diverse indigenous soil-borne pathogens and promote disease-suppression in these soils. Furthermore, by documenting landscape-scale variation in the functional characteristics of *Streptomyces* communities, this study provides a necessary first step towards understanding their potential effects on plant community dynamics in tropical forests.

METHODS:

SITE DESCRIPTION AND SOIL COLLECTION.— Soils were collected in July 2009 from eighteen 20 x 50-m plots located in Área de Conservación Guanacaste – Sector Santa Rosa (10°50' N, 85°40' W; n = 12) and in Área de Conservación Arenal-Tempisque – Parque Nacional Palo Verde (10°21' N, 85°21' W; n = 6) in Northwestern Costa Rica. Santa Rosa and Palo Verde are separated by approximately 100 kilometers and differ in soil parent material and edaphic characteristics. Detailed site descriptions can be found in Powers and Peréz-Aviles (2013) and Gillespie *et al.* (2000); a brief summary is provided here. Mean annual temperature at both sites is 25° C, and mean annual precipitation is

1765 mm in Santa Rosa and 1444 mm in Palo Verde (Becknell & Powers 2014). Both sites experience large interannual variation in rainfall and are characterized by a 5-6 month dry season lasting roughly from December to May (Becknell & Powers 2014). Plots were established in 2007 and are located in secondary tropical dry forests at various stages of regeneration from pasture (Powers et al. 2009). Stand ages ranged from 5-60 years at the time of sampling. Management strategies to promote forest regeneration vary among plots and have included cattle grazing and fire suppression. Plots vary in tree species diversity and composition and span a soil fertility gradient from rocky nutrient-poor soils in oak-dominated Santa Rosa plots to more clayey, nutrient-rich sites in Palo Verde (Kissing & Powers 2010), with more typical, species rich Santa Rosa sites being intermediate in soil fertility. The 18 plots we sampled were chosen from a larger number of plots throughout the region (Powers et al. 2009, Becknell & Powers 2014) and were broadly representative of variation in soils and forest ages across the landscape (Powers & Perez 2013). In each plot two replicate soil cores were collected to a depth of 20 cm using a Dutch auger after scraping away surface litter. The air-dried soil samples from each plot were pooled, pushed through a 2-mm sieve, and stored at room temperature (≈ 25 °C) prior to microbial and soil analyses.

STREPTOMYCES DENSITIES AND PATHOGEN INHIBITORY CAPACITIES.— To quantify pathogen inhibitory capacities of *Streptomyces* communities present in soil, we used a modification of the method described by Herr (Herr 1959, Wiggins & Kinkel 2005) against three plant pathogens. This method involves culturing environmental samples of

Streptomyces on selective media and overlaying *Streptomyces* colonies with a suspension of pathogens in order to quantify (1) the frequency of *Streptomyces* within each community that are capable of inhibiting pathogen growth, and (2) the intensity of pathogen inhibition by *Streptomyces*, as indexed by the size of inhibition zones resulting from antibiotic production.

Briefly, soil suspensions consisting of one gram soil in 10 mL sterile water were shaken for one hour at 175 rpm at 4°C. Serial dilutions were plated onto 1% water agar (WA) and overlaid with 5 mL Starch Casein Agar (SCA) to promote the growth of *Streptomyces*. *Streptomyces* colonies were counted after three days of growth at 28°C and total *Streptomyces* density (CFU/g soil) was determined for each sample. Morphological characteristics were used to discriminate between *Streptomyces* and other bacteria. On SCA medium, colonies exhibiting characteristic *Streptomyces* morphology have smooth margins and are opaque and generally chalky or powdery in appearance due to extensive sporulation. The following day, plates were overlaid with a pathogen suspension (see below) to determine the inhibitory activity of *Streptomyces* colonies against the three plant pathogens. Three replicate plates were assayed for each sample-dilution-pathogen combination.

PREPARATION OF PATHOGEN SUSPENSIONS.— To index the potential for *Streptomyces* communities to suppress diverse soil-borne pathogens, pathogen suppression was quantified against a pathogenic species of *Streptomyces* (*Streptomyces scabies* Strain 87, pathogenic on potato; Liu et al. 1995) and two fungal pathogens (*Fusarium oxysporum*

[Isolate 08-78] and *Rhizoctonia solani* [Isolate 48 Ag-2-1]). These genera occur in temperate and tropical soils and are documented pathogens of tropical plants (Old 2000, Anderson et al. 2002, Ploetz 2006). Pathogen overlays were conducted as described above. Briefly, *F. oxysporum* was grown on 1/2x potato dextrose agar (PDA). Two-week old cultures were cut into approximately 1-cm^2 pieces, added to sterile water, and blended in a sterile Waring blender. Four mL of the resulting mixture was added to 100 mL molten PDWA (potato dextrose water agar; 1.2 g Potato Dextrose broth and 5 g agar in 500 mL distilled water) and mixed thoroughly using a magnetic stir bar. Ten mL of this suspension was overlaid onto dilution plates and plates were incubated at room temperature. *R. solani* was grown in 25 mL Czapek-Dox broth (0.875 g CZB in 25 mL DI H_2O) for 7 days at room temperature and then blended with molten CZB amended with agar (1% final concentration). Plates were overlaid with 10 mL of this suspension and incubated at room temperature. For *S. scabies* overlays, ten mL of SCA was overlaid onto plates and allowed to dry. Subsequently, these plates were overlaid with 150 µL of an *S. scabies* spore suspension in 20% glycerol solution and incubated at 28°C. Overlaid plates were incubated for three days.

DATA COLLECTION.— Inhibitory *Streptomyces* (also referred to as "inhibitors") showed inhibition of pathogen growth after three days. Inhibitors were counted. In addition, for each inhibitor two perpendicular measures of inhibition zone radius were taken from the colony edge and averaged to yield mean zone size per inhibitor. Inhibition zones with a radius <1 mm were counted and assigned a zone size of 0.5 mm for the calculation of

mean zone size. Partial inhibition of pathogen growth was noted, and analyses were run separately with partial inhibitors included and excluded. Results were consistent in both cases, therefore only the results from analyses including partial inhibitors are reported.

There are two important characteristics of the *Streptomyces* communities that are quantified by this assay: inhibitor frequency and inhibitor intensity. Inhibitor density (colony forming units [CFU] /g soil) and the proportion of inhibitors (inhibitor density divided by total *Streptomyces* density) measure the frequency of pathogen-suppressive *Streptomyces* in a given soil sample. Mean inhibition zone size (averaged per plate and per soil sample) measures the intensity of pathogen inhibition. Larger zone sizes indicate greater pathogen suppression, which reflects either the quantity of antibiotics produced or the efficacy of antibiotics against a given target.

SOIL ANALYSES.— Soil particle size distribution (% sand, silt, clay), total carbon (C), total nitrogen (N), and labile soil phosphorus (P) and potassium (K) were determined for each soil sample. Exchangeable K was analyzed at the University of Minnesota Research Analytical Lab using NH_4OAc extraction and elemental determination by ICP-AES. Bray 1-P was measured using acid-fluoride extractions at a 1:5 soil-to-solution ratio (Bray and Kurtz 1945). Five mL of Bray's extracting solution (0.03 N NH_4F and 0.025 N HCl) was added to one g of soil and samples were shaken for one minute and filtered through Whatman No. 42 filter paper. Inorganic P was analyzed colorimetrically on a microplate reader at 630 nm (Robertson 1999). These methods estimate labile forms of P and K and are used to represent plant- and microbe- available nutrients. Total soil C and

N were measured on ground samples via dry combustion (Costech 4010 Elemental Analyzer at the University of Minnesota). Particle-size distribution was measured using the hydrometer method.

DATA ANALYSES.— We used analysis of variance (ANOVA) and Fisher's least-significant-difference test (LSD) to test whether *Streptomyces* community characteristics (*Streptomyces* density, inhibitor density, proportion of inhibitors, and mean zone size) were significantly different between the two sites (Santa Rosa and Palo Verde) and against the three plant pathogens (*S. scabies, F. oxysporum*, and *R. solani*). Correlation analyses were used to determine whether pathogen inhibitory characteristics of *Streptomyces* communities among plots were related to soil characteristics (% C, % N, Bray's P, exchangeable K, and % sand, % silt, % clay), and to evaluate relationships in inhibitory capacities of *Streptomyces* communities against different pathogens. Soil exchangeable K, *Streptomyces* densities, and inhibitor densities were log-transformed prior to analyses. Inhibitor densities (CFUs/g soil) were calculated from the number of inhibitors per plate plus one, to avoid an undefined term after log transformation. Proportions of inhibitors were arc-sine square-root transformed prior to analysis to normalize the variance. Statistical analyses were performed in JMP (JMP 10, SAS Institute, Inc.).

RESULTS:

VARIATION IN *STREPTOMYCES* DENSITIES AND PATHOGEN INHIBITORY CAPACITIES ACROSS THE LANDSCAPE.— *Streptomyces* densities and pathogen inhibitory capacities varied extensively among soils from the 18 tropical dry forest plots (Fig. 1.1). Inhibitor densities averaged across the three plant pathogens varied by more than an order of magnitude among plots, ranging from 2.11 x 10^4 colony forming units (CFU) per gram soil to 5.70 x 10^5 CFU/g soil. The proportion of inhibitors comprising *Streptomyces* communities ranged from ~3% to nearly 70%, suggesting substantial differences in the potential for disease suppression in these soils.

Plots within Santa Rosa spanned the entire range of variation in both total *Streptomyces* densities and inhibitor densities (Fig. 1.1, closed circles), whereas *Streptomyces* communities in Palo Verde (open circles) were much less variable in terms of pathogen-suppressive capacities. Mean total *Streptomyces* densities did not differ significantly between the two tropical dry forest sites (Santa Rosa = 7.44 x 10^5 CFU/g, and Palo Verde = 6.09 x 10^5 CFU/g; $R^2 = 0.03$, $F = 0.43$, $P = 0.52$). However, Santa Rosa soils harbored significantly higher inhibitor densities (2.71 x 10^5 CFU/g) than soils from Palo Verde (5.49 x 10^4 CFU/g; $R^2 = 0.46$, $F = 13.52$, $P = 0.0020$). Mean inhibition zone sizes did not differ between the two sites ($R^2 = 0.01$, $F = 0.15$, $P = 0.704$).

INHIBITION OF DIVERSE PLANT PATHOGENS BY ANTIBIOTIC-PRODUCING *STREPTOMYCES*— *Streptomyces* capable of inhibiting one or more plant pathogens were detected in every plot. In fact, out of the 18 plots there was only one instance in which inhibition of all three plant pathogens was not observed. The densities of *Streptomyces* that were

suppressive to the bacterial and fungal pathogens studied here were highly correlated among soils from the 18 plots (Fig. 1.2), suggesting that inhibitory *Streptomyces* communities have the potential for broad-spectrum pathogen inhibition and disease suppression. Inhibitor densities were not correlated with mean inhibition zone size for any of the three pathogen targets ($R = 0.41$, $P = 0.095$).

Among all plots, inhibitor densities were significantly higher against the bacterial pathogen *S. scabies* than against fungal pathogens ($R^2 = 0.17$, $F = 5.37$, $P = 0.0077$; $P = 0.0061$ and 0.0009, respectively, LSD). In Santa Rosa, *Streptomyces* capable of inhibiting *S. scabies* comprised greater proportions of communities than inhibitors of the fungal pathogens (Fig. 1.3a), but *S. scabies* inhibitors also tended to produce smaller inhibition zones (Fig. 1.3b), reflecting less intense pathogen inhibition. By contrast, in Palo Verde proportions of inhibitors and inhibition intensity were similar against the three pathogen targets (Fig. 1.3a, b), suggesting potential differences in the specificity of pathogen inhibition and disease suppression between the two sites.

STREPTOMYCES DENSITIES AND PATHOGEN SUPPRESSION IN RELATION TO SOIL PROPERTIES—Among all plots there was a marginally significant positive correlation between the proportion of inhibitors in soil and percent sand ($R = 0.44$, $P = 0.065$). We found no significant relationships between soil characteristics and total *Streptomyces* densities among the 18 plots. By contrast, the abundance and activity of pathogen-suppressive *Streptomyces* were significantly correlated with soil nutrient availability. The frequency of inhibitors (inhibitor densities, proportions) tended to be highest under

low soil nutrient conditions (Fig. 1.4a, b), whereas the intensity of pathogen inhibition (mean zone size) was highest under high soil nutrient conditions (Fig. 1.4c, d). In particular, densities of pathogen-suppressive *Streptomyces* were negatively correlated with labile soil P ($R = -0.50$, $P = 0.033$); *Streptomyces* communities were more pathogen-suppressive as soil P decreased. On the other hand, the intensity of pathogen suppression (mean inhibition zone size) was positively correlated with soil P ($R = 0.58$, $P = 0.0114$), K ($R = 0.61$, $P = 0.0067$), percent C ($R = 0.55$, $P = 0.017$), and percent N ($R = 0.49$, $P = 0.0402$). Soil P, K, C and N were all highly correlated among plots (Pearson correlation coefficients from 0.61 to 0.96, $P < 0.007$ in all cases), therefore it is possible that the relationships detected between soil P and inhibitor frequencies reflect a broader influence of overall soil fertility.

DISCUSSION:

Pathogen-suppressive *Streptomyces* are widespread in tropical dry forest soils of Costa Rica and the potential for disease suppression varies extensively across the landscape. Densities of pathogen-suppressive *Streptomyces* varied by more than 10-fold among soils from the 18 plots (Fig. 1.1) and differed significantly between two tropical dry forest locations (Santa Rosa and Palo Verde; Table 1.1). *Streptomyces* and other antibiotic producing soil bacteria have the potential to mediate plant-pathogen interactions and influence community- and ecosystem-level processes across diverse ecosystems, yet the importance of this microbial functional group is rarely considered

outside of agricultural systems. Table 1.1 reports total and inhibitory (antibiotic-producing) *Streptomyces* densities from selected studies spanning a variety of ecosystems and locations. The *Streptomyces* densities we observed in tropical dry forest soils of Costa Rica are broadly consistent with densities observed in diverse locations across the globe, which highlights the need for future research exploring the consequences of pathogen inhibition for plant community dynamics.

Variation in *Streptomyces* community function may be influenced by environmental factors spanning a variety of spatial scales, ranging from local microbial community composition and edaphic characteristics to plant host, community composition, and geographic context (Kinkel et al. 2011, Bakker et al. 2013a, Schlatter & Kinkel 2014b). Previous work in temperate grasslands and agricultural fields has demonstrated significant variation in *Streptomyces* inhibitory and resource-use phenotypes at small spatial scales, suggesting local-scale adaptation of *Streptomyces* communities (Davelos et al. 2004, Bakker et al. 2013a, Schlatter et al. 2013). However, less is known about variation in *Streptomyces* densities and associated disease suppression across large spatial scales and distinct habitat types (Schlatter & Kinkel 2014b). Our study is among the first to examine differences in the abundance and pathogen-suppressive potential of indigenous soil *Streptomyces* communities across regional spatial scales, and is notable in the significant correlations observed between antibiotic production and nutrient availability across a tropical dry forest landscape. We suggest several possible explanations for the substantial variation in *Streptomyces*

community function and discuss the potential implications of these findings for tropical forest communities.

ANTIBIOTIC-MEDIATED PATHOGEN SUPPRESSION AND SOIL NUTRIENT AVAILABILITY.— Antibiotics are broadly recognized as secondary metabolites that mediate microbial species interactions, especially in the context of resource competition among *Streptomyces* as well as between *Streptomyces* and distantly related bacteria and fungi that share similar soil niches (Raaijmakers et al. 2009, Bakker et al. 2013b). The quantity, diversity, and accessibility of soil carbon (C) and nutrients are predicted to influence microbial life history strategies, growth efficiencies, and the balance between costs and benefits of antibiotic production (Kinkel et al. 2011). Our findings support the prediction that antibiotic production may be a successful competitive strategy when resource competition is intense, due to low resource availability (i.e. soil C, nutrients) and/or high microbial densities. We found modest evidence for selective enrichment of pathogen-suppressive phenotypes at high *Streptomyces* population densities (Fig. 1.1; slope > 1), which is consistent with the prediction that high densities increase the frequency and diversity of *Streptomyces* interactions and result in a greater competitive advantage conferred by antibiotic-production (Davelos et al. 2004, Kinkel et al. 2011).

Consistent with the predicted link between antibiotic production and resource competition, we found that the pathogen-suppressive capacities of *Streptomyces* communities were broadly correlated with soil nutrient availability. While total *Streptomyces* densities did not vary with soil P, inhibitory *Streptomyces* densities were

generally higher in low-P than high-P soils (Fig. 1.4a). A recent study by Turner and Wright shows evidence of microbial P limitation in a lowland tropical forest in Panama (2014) and P limitation of microbial growth and metabolism may be widespread across tropical forest soils (Cleveland et al. 2002, Waring et al. 2013). We hypothesize that the higher densities and proportions of inhibitors in low-P soils reflects a competitive advantage conferred by antibiotic production when soil resources, including P, are scarce.

In contrast to the negative relationship between inhibitor densities and soil resources, the intensity of pathogen suppression was greatest in isolates from high-nutrient sites (Fig. 1.4c and d). In particular, soils with low C and nutrients were associated with less intense pathogen inhibition (*i.e.* smaller mean zone sizes per antibiotic producer). This could reflect the costs of antibiotics, specifically stoichiometric and/or metabolic constraints on the quantity or types of antibiotics that can be produced in nutrient-poor soils. While low-nutrient soils may select for higher densities or proportions of antibiotic-producing *Streptomyces*, high-nutrient soils may select for *Streptomyces* isolates having greater pathogen-suppressive capacities.

DIFFERENCES IN *STREPTOMYCES* COMMUNITIES BETWEEN SANTA ROSA AND PALO VERDE.—Despite the significant relationships between pathogen suppression and soil nutrients across the landscape, we observed site-specific differences in *Streptomyces* community function that could not be explained by soil nutrients alone. The negative relationship between inhibitor densities and soil nutrients was less pronounced in Palo Verde than Santa Rosa (Fig. 1.4a and b, open vs. filled circles). Furthermore, inhibitor

densities were nearly ten times higher in Santa Rosa than Palo Verde, despite similar densities of total *Streptomyces*. It is likely that other unmeasured aspects of soil chemistry or biology influence the densities of antibiotic-producing *Streptomyces* and potentially moderate the relationships between soil nutrients and pathogen suppression. In particular, soil texture varies among the plots and is a critical characteristic of the microbial habitat. Soil texture has the potential to affect fungal to bacterial ratios, water holding capacity, and soil carbon and nutrient availability (De Vries et al. 2012, Silver et al. 2000), and can also affect rates of antibiotic diffusion and/or microbial access to carbon and nutrients (Carson et al. 2010). Thus, we hypothesize that the sandier soils of Santa Rosa produce a more competitive environment and an increased benefit of antibiotic production to *Streptomyces* populations.

Tree species community composition also varies among plots (Powers et al. 2009) and could influence inhibitor densities. We expect that edaphic characteristics and plant community composition interactively shape antagonism among *Streptomyces* communities, consistent with other studies of controls on bacterial community function (Marschner et al. 2001, Berg & Smalla 2009, Bakker et al. 2014). Our experimental design did not permit a direct test of the effects of tree species or forest composition on *Streptomyces* densities and phenotypes, but this remains a promising area for further study.

DISEASE-SUPPRESSION BY SOIL-BORNE *STREPTOMYCES* AND POTENTIAL CONSEQUENCES FOR PLANT COMMUNITY COMPOSITION.— Our results suggest that inhibitory *Streptomyces*

communities can suppress diverse fungal and bacterial pathogens across the tropical dry forest soils of Northwestern Costa Rica. Furthermore, the differences in pathogen inhibition between Santa Rosa and Palo Verde suggest the potential for *Streptomyces* to differentially inhibit diverse plant pathogens and promote spatially heterogeneous disease development across the landscape. This variation in *Streptomyces* community function could have important consequences for seedling establishment, secondary succession, and plant community composition. Peña and colleagues (1994) found that applying antagonistic *Streptomyces* to seeds reduced damping-off disease and increased germination and survival rates of the tropical tree species *Casuarina equisetifolia* under nursery conditions. However, to our knowledge the relationship between antibiotic-producing soil bacteria and plant disease has not been characterized in tropical forest soils. Pathogen-mediated negative plant-soil feedbacks are predicted to contribute to tree species diversity and community composition in tropical forests as well as many other ecosystems (Packer and Clay 2000, Petermann et al. 2008, Liu et al. 2012, Van der Putten et al. 2013). Therefore a compelling question for additional study is whether pathogen-suppressive bacteria mediate plant-soil feedbacks by influencing the severity, specificity, and spatial extent of soil-borne disease development (Kinkel et al. 2011, Bakker et al. 2013b).

ANTIBIOTIC-PRODUCING BACTERIA: AN UNDEREXPLORED FUNCTIONAL GROUP IN TROPICAL ECOSYSTEMS.— Disease suppression is only one of many functions that can be performed by *Streptomyces* and other antibiotic-producing bacteria in soil (Seipke et al. 2012,

Chater et al. 2010). In addition to their potential to mediate interactions between plants and pathogens, *Streptomyces* can form intimate associations with a myriad of other organisms, ranging from mycorrhizae (Schrey et al. 2012) to mutualistic ants and their fungal partners (Currie et al. 2003, Fernández-Marín et al. 2013). *Streptomyces* are also well-known for their abilities to degrade complex C substrates including lignin, cellulose, and chitin (Pometto III & Crawford 1986, Mahadevan & Crawford 1997). Spatial variation in resource-use characteristics could have substantial consequences for biogeochemical cycling, yet the contribution of *Streptomyces* to C cycling and nutrient availability in forest soils remains largely unknown (Jayasinghe & Parkinson 2008, Bontemps et al. 2013).

A better understanding of the ecology and distribution of indigenous pathogen-suppressive bacteria will open the door for more effective agricultural management, forestry practices, and conservation efforts in the region, as well as more targeted approaches to antibiotic discovery from tropical forest soils. We caution that the correlative nature of this study limits interpretations regarding the causes of variation in pathogen suppression. Future studies are needed to disentangle the biotic and abiotic factors influencing pathogen suppression and its consequences in tropical forests at a variety of spatial scales.

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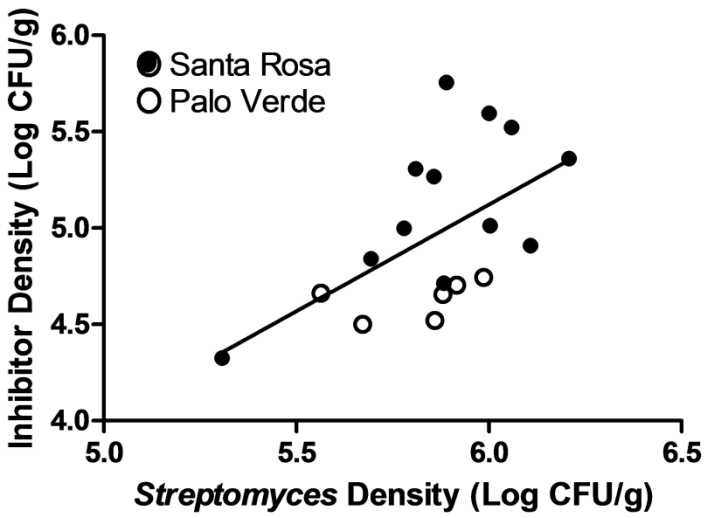

Figure 1.1. Extensive variation in total and pathogen-suppressive *Streptomyces* densities in soils across a tropical dry forest landscape. The relationship between total *Streptomyces* density (log[CFU/g]) and inhibitor density (log[CFU/g]) is plotted for 18 tropical dry forest plots. Filled circles denote samples from Santa Rosa plots (n = 12) and open circles denote samples from Palo Verde (n = 6). Regression line is fit to all plots (slope = 1.08, $R^2 = 0.316$, P = 0.0153). The slope of the regression line suggests selective enrichment of inhibitors with increasing *Streptomyces* densities.

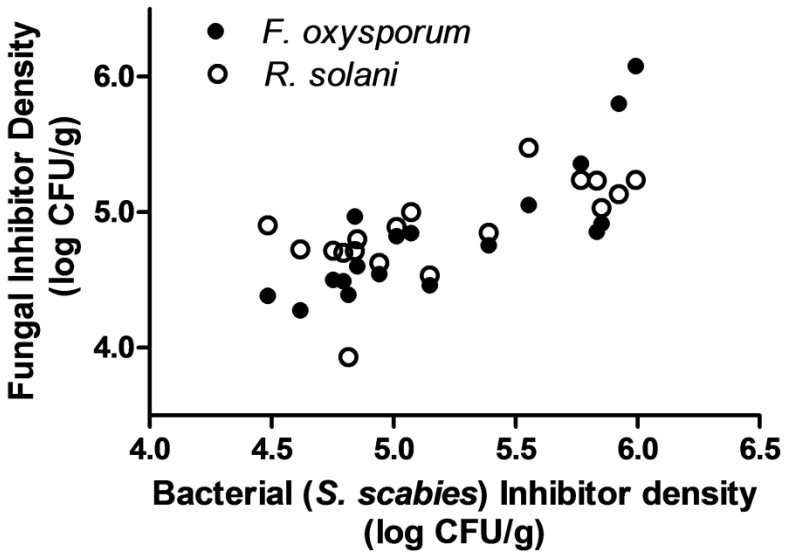

Figure 1.2. Densities of bacterial and fungal inhibitors are positively correlated among 18 tropical dry forest plots. Densities of *Streptomyces* (log[CFU/g]) that inhibit the bacterial pathogen *S. scabies* and fungal pathogens **(a)** *F. oxysporum* (closed symbols; R = 0.8114, P < 0.0001), or **(b)** *R. solani* (open symbols; R = 0.6618, P = 0.0028). Each dot represents inhibitor densities from one plot (average of three replicate plates per pathogen). Pearson correlation coefficients and associated *P* values are provided.

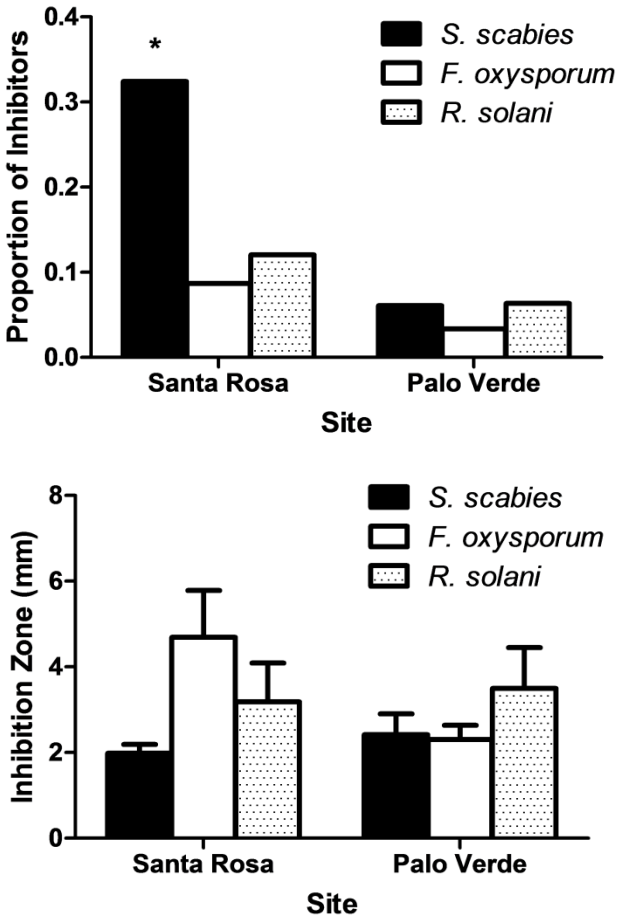

Figure 1.3. Proportions of inhibitors (inhibitor density / total *Streptomyces* density) and mean inhibition zone sizes against three plant pathogens (*Streptomyces scabies, Fusarium oxysporum,* and *Rhizoctonia solani*) in Santa Rosa and Palo Verde. **(a)** There were significantly higher proportions of inhibitors against *S. scabies* in Santa Rosa (1-way ANOVA, $P < 0.05$). **(b)** *Streptomyces* communities produced larger inhibition zones against fungal pathogens relative to zones produced against the pathogenic bacterial congener *S. scabies*.

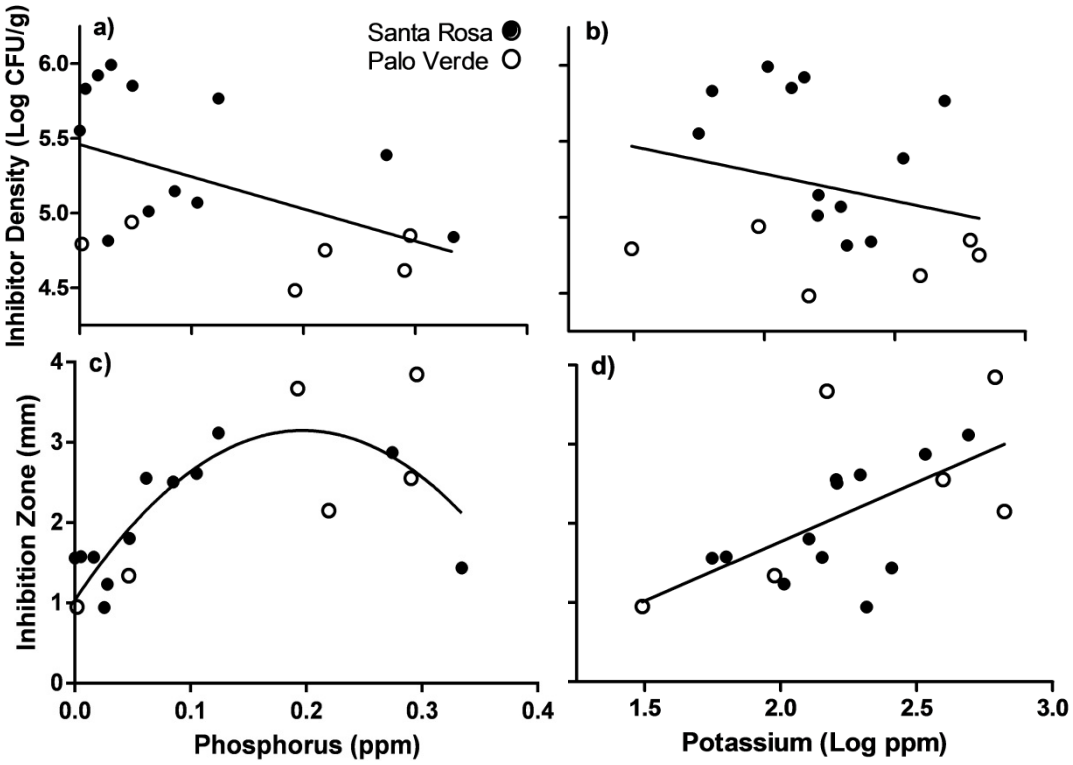

Figure 1.4. Relationships between pathogen-suppressive *Streptomyces* (inhibitor density [a, b] or mean inhibition zone size [c,d]) and soil nutrients (P or log K) across 18 tropical dry forest plots. Filled circles denote samples from Santa Rosa plots (n = 12) and open circles denote samples from Palo Verde (n = 6). Linear (a,b,d) and polynomial (c) regression lines were fit to maximize R^2 values. Inhibitor densities against *S. scabies* (log CFU/g soil) decreased with soil P (panel a; $R^2 = 0.25$, $P = 0.0331$) and soil K (panel b; not significant). Inhibition zone sizes (mm) increased with soil P (panel c; $R^2 = 0.64$, $P < 0.0001$) and soil K (panel d; $R^2 = 0.38$, $P = 0.0067$).

TABLE 1.1. Average densities of indigenous soil-borne Streptomyces *taken from selected studies. Inhibitor density reflects* Streptomyces *capable of antibiotic-mediated inhibition of the plant pathogen* S. scabies*. Proportion data indicate the proportion of the total* Streptomyces *community that inhibit* S. scabies*. Studies used similar methodology for selectively culturing* Streptomyces *from soil. The densities from Semêdo et al. 2001 are reported as Actinomycetes, densities from all other studies are* Streptomyces*.*

Location	**Ecosystem**	***Streptomyces* density** (CFU/g soil)	**Inhibitor density** (CFU/g soil)	**Proportion**	**Citation**
Costa Rica	Tropical dry forest				
	Santa Rosa	7.44×10^5	2.08×10^5	0.32	This study
	Palo Verde	6.09×10^5	4.91×10^4	0.06	
Brazil	Atlantic forests	1.9×10^6	-	-	Semêdo *et al.* 2001
	Cerrado	7.7×10^5	-	-	
Panama	Tropical forests*	4.43×10^5	6.06×10^4	-	D. Schlatter, unpubl. data
Hawaii, USA	Montane forest	1.19×10^5	-	-	D. Schlatter, unpubl. data
Minnesota, USA	Prairie	1.55×10^6	4.36×10^5	0.30	Bakker *et al.* 2013**
Minnesota, USA	Prairie	1.62×10^5	-	-	Davelos *et al.* 2004

Minnesota, USA	Prairie	7.22×10^4	-	-	Schlatter *et al.* 2013
Minnesota, USA	Agricultural fields				
	alfafa	1.24×10^6	6.49×10^5	0.53	Wiggins & Kinkel 2005
	corn	1.34×10^6	6.49×10^5	0.49	
	potato	1.54×10^6	8.78×10^5	0.56	
Texas, USA	Agricultural fields				
	sorghum	1.5×10^6	-	-	Blanco *et al.* 2007
	watermelon	0.9×10^6	-	-	

**Streptomyces* densities were averaged across evergreen, semideciduous, and montane forest sites. Inhibitor densities were averaged across evergreen and montane sites only.

***Values reported in Bakker *et al.* 2013 reflect average inhibitor densities against three indicator strains.

Chapter 2

Tree species effects on pathogen-suppressive capacities
of soil bacteria across two tropical dry forests in Costa Rica

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ABSTRACT:

Antibiotic-producing bacteria in the genus *Streptomyces* can inhibit soil-borne plant pathogens and have the potential to mediate the impacts of disease on plant communities. Little is known about how antibiotic production varies among soil communities in tropical forests, despite a long history of interest in the role of soil-borne pathogens in these ecosystems. Our objective was to determine how tree species and soils influence variation in antibiotic-mediated pathogen suppression among *Streptomyces* communities in two tropical dry forest sites (Santa Rosa and Palo Verde). We targeted tree species that co-occur in both sites and used a culture-based functional assay to quantify pathogen-suppressive capacities of *Streptomyces* communities beneath 50 focal trees. We also measured host-associated litter and soil element concentrations as potential mechanisms by which trees may influence soil microbes. Pathogen-suppressive capacities of *Streptomyces* communities varied within and among tree species, and inhibitory phenotypes were significantly related to soil and litter element concentrations. Average proportions of inhibitory *Streptomyces* in soils from the same tree species varied

between 1.6 and 3.3-fold between sites. Densities and proportions of pathogen-suppressive bacteria were always higher in Santa Rosa than Palo Verde. Our results suggest that spatial heterogeneity in the potential for antibiotic-mediated disease suppression is shaped by tree species, site, and soil characteristics, which could have significant implications for understanding plant community composition and diversity in tropical dry forests.

INTRODUCTION:

Soil microbes play critically important roles in regulating plant community composition, diversity, and ecosystem functioning (Wardle 2002, Bever et al. 2010, Bardgett and Van der Putten 2014). However, our understanding of the specific microbes and mechanisms underlying plant-soil relationships remains limited by "black box" approaches to belowground ecology, which often ignore the microbe-microbe and environment-plant-microbe interactions that collectively shape aboveground-belowground linkages and their community and ecosystem-level consequences (Friesen et al. 2011, Benitez et al. 2013). Focusing on specific microbial traits and their variation relative to environmental characteristics can provide insight into the complex network of interactions linking microbes to macroscale patterns and processes such as biodiversity-ecosystem functioning relationships and plant-soil feedbacks (Kraus et al. 2014). In particular, more studies are needed on the activity and abundance of specific microbial functional groups,

especially free-living, plant-associated microbes, which have received far less attention than N-fixing bacteria, mycorrhizal fungi, and other symbionts.

Antibiotic-producing bacteria can mediate microbe-microbe and plant-microbe interactions and have substantial effects on microbial community structure and function as well as plant performance, population dynamics, and community composition (Latz et al. 2012, Bakker et al. 2014, Vaz Jauri and Kinkel 2014, Schlatter et al. 2015). *Streptomyces* are gram-positive, filamentous bacteria that are common in soil and are renowned for their diverse and prolific production of antibiotics and other secondary metabolites (Schrey and Tarkka 2008). In temperate grasslands, antibiotic production among *Streptomyces* has been suggested to influence plant-soil feedbacks through a combination of direct effects of pathogen suppression on plant disease as well as indirect effects of antibiotic production on overall microbial community structure (Bakker et al. 2014).

To date, studies on the ecology and evolutionary dynamics of *Streptomyces* are concentrated primarily in temperate ecosystems, especially agricultural systems and experimental grasslands (Liu et al. 1995, Becker et al. 1997, Davelos et al. 2004a,b, Blanco et al. 2007, Kinkel et al. 2012). However, two recent studies of *Streptomyces* biogeography found that phenotypes related to antibiotic-mediated inhibition, resistance, and resource use varied substantially among a global collection of *Streptomyces* isolates, and that local selection pressures play an important role in generating functional variation in these traits (Schlatter and Kinkel 2014a, Kinkel et al. 2014). Furthermore, phylogenetically similar *Streptomyces* isolates from diverse locations are often highly

differentiated in function (Davelos et al. 2004c, Becklund et al. 2014, Schlatter and Kinkel 2014a, Vaz-Jauri and Kinkel 2014). This suggests that measuring microbial traits and the biotic and abiotic factors associated with them is critical to understanding the roles of *Streptomyces* and other microbial taxa in different ecosystems.

Soils are characterized by a high degree of spatial heterogeneity, which can have profound impacts on microbial interactions and competitive phenotypes. Plant species and soil characteristics interactively shape the microbial habitat by determining the abundance and availability of soil resources (Binkley and Giardina 1998, Berg and Smalla 2009). Plant hosts can influence soils – and in turn soil microbes – through differences in nutrient uptake and use as well as through the quantity and composition of above and belowground litter inputs and root exudates (Saetre and Baath 2000, Wardle 2002, Hartmann et al. 2009, Hobbie 2015). A recent meta-analysis found that soil properties beneath individual plants varied by an average of 41% across a range of latitudes and climates, suggesting that individual plant effects on soils are common in many ecosystems and may vary in strength with biome type and land-use history (Waring et al. 2015a). In addition to localized effects of individual trees on soil biogeochemistry and nutrient cycling (Zinke 1962), variation in soil physicochemical properties is also structured at larger spatial scales due to differences in parent material and landscape gradients in soil nutrients and texture (Ettema and Wardle 2002).

Antibiotic production can enhance resource acquisition among microbial producers by reducing populations of competitors, making it a successful strategy when competition for shared soil resources is intense. Theory predicts that spatial

heterogeneity in soil resources will impact rates of antagonism as a function of both the *diversity* and *abundance* of plant- and soil-derived resources (Schlatter et al. 2009, Kinkel et al. 2011, 2012), namely carbon and nutrients. Lower resource diversity is predicted to exacerbate microbial competition and select for more antagonistic bacterial communities, whereas soils with a greater variety of resources are predicted to result in more niche-differentiated, less antagonistic microbial communities. Resource abundance is predicted to have an interacting effect on antagonism depending on the plant diversity of the ecosystem as well as the spatial scale, seasonality, and longevity of plant-microbe associations.

Tropical forests harbor some of the most biogeochemically heterogeneous soils of any terrestrial ecosystem (Townsend et al. 2008), but studies of *Streptomyces* community function are currently lacking in tropical forests. Plant-derived resource inputs in tropical forests are both diverse and patchily distributed, due to the high phylogenetic, functional, and structural diversity of plant species. At the scale of individual trees, the complexity of the microbial resource pool is subject to effects of the plant host as well as potentially strong neighborhood effects due to the high degree of overlap in canopy and root litter inputs (Powers et al. 2004, Jones et al. 2011). Unlike temperate ecosystems in which microbes are typically assumed to be C-limited, microbes in tropical forest soils may be limited by P or other nutrients (Cleveland et al. 2002, Waring et al. 2014, Turner and Wright 2014) and may compete directly with plants for limiting resources. All of these factors confound predictions regarding microbial resource availability and pathogen

suppression in tropical forest soils, and provide further motivation for studies of microbial function across tree species in these ecosystems.

Pathogens are predicted to promote species coexistence in tropical forests and are a cornerstone of theories such as the Janzen-Connell hypothesis (Janzen 1970, Connell 1971), and the potential for antibiotic-producing bacteria to moderate pathogen effects remains a critical gap in knowledge. Information is needed on the abundance and activity of antibiotic-producing bacteria among tree species in tropical forests. In previous work we demonstrated extensive landscape-scale variation in pathogen-suppressive capacities of *Streptomyces* communities across tropical dry forests in Costa Rica, and found that the frequency and intensity of inhibition were related to soil P and K availability (Becklund et al. 2014). Here, we expand on our previous work by measuring pathogen-inhibitory capacities of *Streptomyces* communities associated with individual tropical dry forest trees in the same two sites. If variation in antibiotic production is also structured by host trees and/or species (as we predict), this would suggest the potential for *Streptomyces* to influence composition and diversity in these forests.

The objective of this study was to examine relationships among tree species, soil physicochemical factors, and *Streptomyces* community function in two tropical dry forest sites in Costa Rica (Santa Rosa and Palo Verde). Our specific aims were to characterize the effects of tree species and site on (1) soil and litter elements, and (2) soil-borne *Streptomyces* densities and pathogen-suppressive capacities associated with plant hosts. We targeted focal tree species that differ with respect to leaf habit and functional traits (Table 2.1; Powers and Tiffin 2010) because associated variation in the quantities and

qualities of detrital inputs to soil could influence competitive dynamics and antagonistic phenotypes among *Streptomyces* communities. In order to explore possible mechanisms underlying tree species effects on *Streptomyces* communities, we investigated (3a) whether differences in pathogen suppression were related to variation in litter or soil elements (or litter-soil relationships) and (3b) whether tree species effects on *Streptomyces* were consistent between the two sites, which differ in soil fertility, texture, and plant and fungal community composition (Powers et al. 2009, Waring et al. 2015b). These contrasting site characteristics have the potential to influence selective pressures for antibiotic production in soil (Kinkel et al. 2011) and therefore could mediate tree species effects on pathogen suppression in these tropical forest sites.

METHODS:

STUDY SITES: We worked in two secondary tropical dry forests separated by ~80 km in Northwestern Costa Rica – Sector Santa Rosa in the Área de Conservación Guanacaste and Parque Nacional Palo Verde in the Área de Conservación Arenal-Tempisque, which we refer to as Santa Rosa (SR) and Palo Verde (PV). Mean annual precipitation in SR is ~1765 mm and in PV is ~1444 mm (Becknell and Powers 2014). Both sites have mean annual temperatures of ~25 °C and experience a pronounced dry season that typically lasts from December through May. PV soils are clayey and relatively nutrient-rich compared to the sandy, nutrient-poor soils of SR (Powers et al. 2009). Soils arise from

diverse parent materials, and nutrient availability and soil moisture tend to be higher at PV.

SAMPLE COLLECTION AND PROCESSING: We selected five tree species that differ in functional traits and are common at both sites (Table 2.1): *Calycophyllum candidiscimun*, the nitrogen-fixing legume *Gliricidia sepium*, *Luehea candida*, *Sideroxylon capiri*, and *Tabebuia ochraceae*. Tree species are referred to by genus names throughout. In January 2008, soil and litter samples were collected from five trees per species per site for a total of 50 focal trees. Focal trees were separated by at least 100 m. Diameter at breast height of trees ranged from 8 cm to 138 cm. For each species, samples were collected from both sites during the same week. Beneath each tree, four soil samples were collected with a 2.5 cm diameter punch tube corer at 0-10 cm depth from the cardinal directions at locations that were approximately equidistant between the bole and the crown dripline. Replicate soil cores were composited to yield one bulk sample per tree. Soil samples were air-dried for at least 4 days. Within one week of soil collection, host-specific leaf litter was collected beneath each sampled tree. We collected at least five recently senesced leaves per tree, free of signs of herbivory. Leaves were bulked per tree and oven dried at approximately 60° C for at least 3 days.

Soil and litter samples were shipped to the University of Minnesota for analyses and stored at room temperature. Total element concentrations (Al, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, Zn) in litter and soil were digested in hot nitric acid and quantified by ICP-AES at the Research Analytical Laboratory, University of Minnesota

(http://ral.cfans.umn.edu). Litter Ni concentrations and soil Mo concentrations were below detection limits and were excluded from statistical analyses.

ESTIMATING *STREPTOMYCES* DENSITIES AND PATHOGEN-SUPPRESSIVE CAPACITIES: We used modified Herr's assays to culture soil-borne *Streptomyces* and quantify pathogen-suppressive capacities of *Streptomyces* communities from the 50 focal trees (Herr 1959, Wiggins and Kinkel 2005). Soil samples were shaken in sterile deionized water (1 air-dried g soil in 10 mL water) for one hour at 175 rpm and 4° C. Serial dilutions were plated onto 1% water agar and overlaid with 10 mL starch casein agar (Kuster and Williams 1964). This approach selects for *Streptomyces* by taking advantage of their filamentous growth form. Plates were incubated at 28° C for 3 days. *Streptomyces* colonies were identified based on morphology, and counted on every plate to determine total *Streptomyces* density per sample (colony-forming units [CFU] per gram soil). In order to quantify the inhibitory capacities of isolated *Streptomyces*, plates were overlaid with another layer of starch casein agar (5 mL) and 150 µl of one of three *Streptomyces* indicator strains. Three replicate plates were assessed for each sample by dilution x overlay combination. Previous work shows that suppression of *Streptomyces* isolates is a good predictor of suppression of bacterial and fungal plant pathogens (Wiggins and Kinkel 2005). The indicator strains we used included a plant pathogen (*S. scabies* strain 87, pathogenic on potato; Liu et al. 1995) and two non-pathogenic *Streptomyces* isolates (*Streptomyces* sp. 4-21 and sp. 1324). These isolates were selected because they are

phenotypically diverse in resource use and antagonistic profiles (Davelos et al. 2004c) and thus create an index of the breadth and specificity of pathogen suppression.

Plates were incubated for 3 days at 28° C to allow growth of the overlaid indicator strain. Colonies surrounded by a clear zone with no growth of the overlay strain were counted and designated as inhibitory (i.e. antibiotic-producing) *Streptomyces*. For every inhibitor, two perpendicular measures of zone size were taken from the colony edge to the end of the inhibition zone. Zone radius (mm) was averaged per inhibitor. Inhibition zone size was then averaged per plate and again across replicate plates to calculate mean zone size per soil sample. For zones with a radius of less than 1 mm, a value of 0.5 mm was used for calculation of mean zone size.

For each soil sample x overlay combination, four *Streptomyces* community characteristics were determined: total *Streptomyces* density (CFU/g soil), density of inhibitory *Streptomyces* (also referred to as "inhibitors"), proportion of inhibitors (inhibitor density/total *Streptomyces* density) and average inhibition zone size. Inhibitor density and proportion measure the frequency of antibiotic-producing *Streptomyces* in soil, whereas inhibition zone size measures the magnitude of effect against the isolate being inhibited. *Streptomyces* densities and inhibitor densities were log-transformed prior to analyses.

STATISTICAL ANALYSES: We used Principle Components Analysis (PCA) to examine variation in litter and soil element composition among tree species and between sites. Loading scores are reported in SI Table 2.1. In order to test for influences on PCA

scores, we conducted two-way ANOVAs with litter or soil PC1 as the response variable and tree species, site, and their interaction as predictor variables. Elements were also tested individually for significant differences between sites and among tree species (one-way ANOVA with Tukey's HSD post-hoc contrasts). In addition to testing for tree species and site effects on litter and soil element composition separately, we used correlation analysis to test whether trees with high concentrations of elements in litter were also associated with higher element concentrations in soil. Furthermore, because large site differences in soil chemistry may obscure the effects of trees or tree species on their own soils, we also tested for correlations between litter and soil elements within each site individually. Pearson correlation coefficients are reported unless otherwise noted.

We also used two-way ANOVAs to test for effects of tree species, site, and their interaction on *Streptomyces* community characteristics. When significant effects were observed, Tukey's post-hoc tests were used to examine multiple contrasts. In addition, correlation analyses were used to evaluate the consistency of tree species effects on pathogen-suppressive capacities in the two sites. For each *Streptomyces* community characteristic (inhibitor density and zone size against each of the three indicator strains), we plotted the mean value per tree species in PV vs. SR, and used Spearman rank correlation coefficients to test for significant correlations among tree species between the two sites.

We used nonmetric multidimensional scaling (NMDS) to investigate whether differences in inhibitory phenotypes among *Streptomyces* communities were related

systematically to variation in soil or litter elements between sites or among tree species. NMDS ordinations were based on a Bray-Curtis similarity matrix and were conducted using the metaMDS function in the "Vegan" package in R. NMDS plots illustrate dissimilarity in litter and soil element composition among tree species. *Streptomyces* community characteristics (total *Streptomyces* densities, inhibitor densities, inhibition zone sizes, and inhibitor proportions against the three indicator isolates) were fit to separate NMDS ordinations of soil and litter element concentrations using the envfit function in Vegan. Significant vectors ($P \leq 0.05$) were plotted. Vectors reflect variation in the direction and magnitude of change in the specified trait across the plot. Vector length is proportional to the strength of correlation between the ordination and the specified variable. Envfit was also used to fit centroids to tree species and site in order to determine whether these factors influenced the clustering of soil and litter element concentrations among trees. NMDS stress levels were < 0.2, which suggests that ordinations were a good fit to the data.

In order to further explore relationships between soil, litter, and *Streptomyces*, we tested for correlations between pathogen inhibition and individual litter and soil element concentrations across tree species in the two sites. We acknowledge that there is considerable collinearity among elements in these sites (see Waring et al. 2015c), which could influence the correlations detected between *Streptomyces* characteristics and soil and litter elements. We also tested for relationships between average values of leaf functional traits (measured previously by J. Powers, unpublished data) and average values of *Streptomyces* community characteristics among the five tree species (N = 5).

The functional traits tested were foliar and litter specific leaf area (SLA), N:P content, and leaf toughness (each measured towards the beginning and end of the wet season). Statistical analyses were performed in JMP (JMP 11, SAS Institute Inc., Cary, NC, USA) and R version 3.2.1 software (R core team, 2014).

RESULTS:

DIFFERENCES IN SOIL AND LITTER ELEMENTS AMONG TREE SPECIES AND BETWEEN SITES:

Soil element composition differed between SR and PV but did not differ among tree species (Fig. 2.1a). The first two axes of the PCA accounted for 60.8% of the variation in soil elements. Soil PC1 (41.4%), did not differ among tree species but was significantly different between sites ($F = 9.22$, $P = 0.0042$). The interaction between tree species and site was not significant. B, Mg, and Cr were the largest contributors to PC1 (SI Table 2.1a). When each element was examined individually, seven (Ca, Cr, K, Mg, Na, Ni, P) differed significantly between the two sites (Table 2.2), with higher mean element concentrations in soils from PV than from SR. None of the 13 soil elements differed significantly among tree species; however, when each site was examined separately we detected significant differences in soil K ($R^2 = 0.48$, $P = 0.0088$), Cr ($R^2 = 0.47$, $P = 0.0096$), and Al ($R^2 = 0.37$, $P = 0.048$) among tree species in SR but not in PV.

In contrast to the trend for soil elements, overall litter element composition differed among tree species but not between sites (Fig. 2.1b). The first two axes of the litter PCA explained 39.8 % variance in litter element concentrations. Litter PC1

(21.6%) was highly correlated with Mn, Al, and Fe (SI Table 2.1b) and was significantly associated with tree species ($F = 24.19$, $P < 0.0001$) but not site. The species by site interaction was not significant; however, in SR litter from *Sideroxylon* and *Gliricidia* trees were more similar to each other than to litter from conspecifics in PV (Fig. 2.1b). Of the five focal species, *Luehea* and *Tabebuia* appeared to have the most distinct litter chemistry (relative to PC1 and PC2, respectively). When litter elements were examined individually, all elements except Ca showed significant differences among tree species (Table 2.3), while only three elements exhibited significant differences in concentrations between sites (Al, Ca, Mo were significantly higher in PV; data not shown). Collectively, these results suggest that total soil element concentrations beneath trees are primarily driven by differences between sites, whereas litter element composition depends primarily on tree species identity.

When we tested for correlations between the concentration of individual elements in litter and soil across all 50 trees, we found no evidence that trees with higher litter elements were associated with soils with higher element concentrations. However, when examining each tree species separately, litter and soil K were significantly positively correlated among *Calycophyllum* trees ($R^2 = 0.53$, $P = 0.017$) and *Gliricidia* trees ($R^2 = 0.62$, $P = 0.0062$), meaning that for these species, trees with higher litter K were also associated with soils characterized by high K concentrations. Significant positive correlations were also observed between litter and soil Ca among *Gliricidia* ($R^2 = 0.64$, $P = 0.0055$) and *Sideroxylon* trees ($R^2 = 0.61$, $P = 0.0081$) and litter and soil B (*Gliricidia* only; $R^2 = 0.56$, $P = 0.013$). By contrast, litter and soil Fe were negatively correlated

among *Calycophyllum* trees ($R^2 = 0.48$, $P = 0.025$). In the case of Mg, litter and soil concentrations were positively correlated among *Luehea* trees ($R^2 = 0.51$, $P = 0.02$) but were negatively correlated for *Sideroxylon* ($R^2 = 0.84$, $P = 0.0002$), potentially reflecting differences in life history strategies, growth requirements, or habitat preferences of these species.

When trees from all five species were analyzed together within each site (N=25), litter and soil P concentrations were positively correlated in the more nutrient-rich PV ($R^2 = 0.18$, $P = 0.034$) but there was no significant relationship in SR. When litter-soil relationships were examined for each individual tree species in each site, litter and soil Zn were positively correlated among *Gliricidia* trees in PV ($R^2 = 0.92$, $p = 0.0095$). Soil and litter Na were also positively correlated for *Gliricidia* ($R^2 = 0.97$, $P = 0.0025$) and *Calycophyllum* trees ($R^2 = 0.86$, $P = 0.023$), but only in SR.

PATHOGEN-SUPPRESSIVE POTENTIAL AMONG *STREPTOMYCES* COMMUNITIES: Among the 50 trees, total *Streptomyces* densities in soil varied by two orders of magnitude (1.09×10^4 to 1.09×10^6 CFU/g soil; average = 2.09×10^5 CFU/g soil). Mean densities of inhibitory *Streptomyces* also varied more than 100-fold, ranging from 3.68×10^2 to 8.05×10^4 CFU/g soil averaged across three indicator strains. The average proportion of inhibitory (antibiotic-producing) *Streptomyces* within communities was 14%, and ranged from less than 1% to over 75%. Among inhibitors, mean inhibition zone size varied ten-fold among trees (average = 2.73 mm). These results suggest substantial differences in the potential for disease suppression associated with tropical dry forest trees.

Mean inhibitor densities averaged across the three indicator strains did not differ significantly among the five tree species (1-way ANOVA, $P > 0.05$). However, examining the three indicator strains separately revealed considerable variation in specific antagonistic capacities among host-associated *Streptomyces* communities (Fig. 2.2). Most trees (47 out of 50) harbored *Streptomyces* capable of suppressing the growth of all three indicator strains. Two *Calycophyllum* trees (one from each site) lacked inhibitors against 1324, and one *Gliricidia* tree in PV had no inhibitors against strain 87.

There were significant differences in pathogen-suppressive capacities between SR and PV. Although total *Streptomyces* densities were similar in both sites (Fig. 2.2), trees in SR harbored soil communities with over twice as many inhibitors than those in PV (2.24×10^4 vs. 1.00×10^4 CFU/g soil, respectively; Student's t-test, $P < 0.05$). Similarly, the average proportion of *Streptomyces* capable of producing antibiotics differed significantly between sites, with an average of 19% inhibitors (± 0.03 SE) in SR as compared to 9% (± 0.01 SE) in PV. Zone sizes of inhibitors were also greater in SR, although this difference was not statistically significant.

Across all soil samples, inhibitor densities against the three indicator strains were positively correlated ($R > 0.70$ and $P < 0.0001$ in all cases). This suggests the potential for broad-spectrum pathogen suppression, and that soils identified as highly disease suppressive by this method are also likely to be effective at suppressing diverse pathogen targets *in situ.*

Mean inhibitor densities were significantly different across sites (Table 2.4), but the effect of tree species and the species by site interaction were not significant. When

we tested for effects of tree species, site, and their interaction on inhibition against each indicator strain separately (Table 2.4), there was a significant main effect of site but not tree species on inhibitor densities against 4-21. There was also a significant interaction between the effects of tree species and site on inhibitor densities against this strain, which appeared to be driven by significantly higher densities of inhibitory *Streptomyces* in soils associated with *Luehea* trees in SR than PV (Tukey's HSD, $P < 0.05$; Fig. 2.2; Fig. 2.3d). Results varied for the other two indicator strains.

In order to compare the relative magnitude of tree species effects on inhibitory phenotypes between the two sites, we tested for correlations between *Streptomyces* community characteristics among the five tree species in SR and PV (Fig. 2.3). Mean zone sizes and inhibitor densities against 1324 were positively correlated among tree species in SR and PV (Fig. 2.3e,f). Despite differences between the sites (e.g. soil type, forest community composition), the effects of tree species on pathogen-suppressive capacities against this strain were consistent between SR and PV. No significant correlations were observed for inhibition against the other two indicator strains, suggesting that tree species effects on inhibitor density and zone size against these indicators were inconsistent between sites. In general, there were higher inhibitory capacities among *Streptomyces* in SR than PV for most tree species (Fig. 2.3a-d), as noted previously. *Streptomyces* communities associated with *Gliricidia* trees were a notable exception, with much larger inhibition zones against strain 4-21 in PV than SR (Fig. 2.3c).

RELATIONSHIPS AMONG *STREPTOMYCES* COMMUNITIES AND LITTER AND SOIL ELEMENTS: NMDS analyses show that tree species and site effects on pathogen-suppressive capacities of *Streptomyces* communities were driven in part by variation in soil and litter elements (Figs. 2.4 and 2.5). Site, but not tree species, was significantly correlated with the soil element ordination (Fig. 2.4; $R^2 = 0.40$; $P = 0.001$), which is consistent with interpretation of the PCA (Fig. 2.1a). Inhibition zone size against indicator strain 87 was positively correlated with variation in soil elements across the plot (Fig. 2.4). In the case of litter elements, tree species were clearly differentiated by litter element composition in both PV (Fig. 2.5a; $R^2 = 0.84$; $P < 0.0001$) and SR (Fig. 2.5b; $R^2 = 0.75$, $P < 0.0001$). In PV, we detected only marginally significant correlations between the litter element ordination and inhibition against strain 4-21 (Fig. 2.5a). In SR, on the other hand, inhibitor density and zone size against strain 1324 were both strongly associated with variation in litter elements among hosts (Fig. 2.5b).

Across all 50 trees, there were few consistent relationships between individual elements and *Streptomyces* community characteristics. Mean inhibitor densities were negatively correlated with Zn concentrations in litter ($R^2 = 0.15$, $P = 0.0049$) and in soil ($R^2 = 0.17$, $P = 0.0026$). Mean zone sizes of inhibitors were positively correlated with soil Zn ($R^2 = 0.29$, $P < 0.0001$). Inhibitor proportions were negatively correlated with Ca in both litter and soil ($R^2 = 0.09$, $P = 0.039$; $R^2 = 0.11$, $P = 0.016$). Concentrations of P in soil were negatively correlated with inhibitor density ($R^2 = 0.08$, $P = 0.039$) and positively correlated with inhibition zone size ($R^2 = 0.16$, $P = 0.0045$).

When we examined each tree species in each site separately, we found much stronger correlations between litter elements and *Streptomyces* characteristics. Inhibitory phenotypes and litter elements were highly correlated for *Calycophyllum* and *Sideroxylon* trees in SR (R^2 from 0.81 to 0.94, *P* values from 0.036 to 0.0057) and for *Tabebuia* and *Gliricidia* trees in PV (R^2 = 0.79 to 0.98, $P < 0.0001$ to 0.045). Pathogen suppression among *Streptomyces* communities beneath *Luehea* trees was correlated with litter elements in both sites, but the specific elements and the direction of relationships varied (R^2 from ± 0.77 to 0.96, *P* values from 0.043 to 0.0031).

There were highly significant correlations between average functional traits and *Streptomyces* community characteristics per tree species. Across the five focal species, foliar SLA was positively correlated with average *Streptomyces* densities (N = 5; R^2 = 0.79, $P = 0.045$) and negatively correlated with inhibitor proportions ($R^2 = 0.80$, $P = 0.042$). Average foliar N:P at the end of the wet season was positively correlated with inhibitor densities ($R^2 = 0.88$, $P = 0.019$) and zone sizes ($R^2 = 0.94$, $P = 0.0071$).

DISCUSSION:

We found substantial variation in the pathogen-suppressive capacities of soil-borne *Streptomyces* associated with tropical dry forest trees. Both tree species and site structured inhibitory capacities of *Streptomyces* communities. The frequency and intensity of antibiotic-mediated inhibition were related to variation in litter and soil element concentrations. In particular, our results suggest that site sets the context for

microbe-microbe and plant-microbe interactions by influencing relationships between host-specific litter and *Streptomyces* community function. Site differences between SR and PV may influence *Streptomyces* communities directly by altering selection pressures on antibiotic production, or indirectly by promoting intraspecific variation in tree species traits and/or litter-soil relationships, which in turn influence pathogen-suppressive capacities of host-associated *Streptomyces*. Taken together, our findings show that variation in inhibitory phenotypes among *Streptomyces* communities is a function of local-scale interactions mediated by individual trees as well as regional variation in tree-soil relationships across distinct forest sites.

DIFFERENCES IN *STREPTOMYCES* COMMUNITIES BETWEEN SITES AND AMONG TREE SPECIES: Despite similar total *Streptomyces* densities, there were significantly more pathogen-suppressive *Streptomyces* beneath trees in SR than in PV. This trend is consistent with previous work in these forests in which pathogen suppression was measured at the plot scale rather than in soils associated with individual trees (Becklund et al. 2014). Soil nutrient availability is likely a major factor generating the higher inhibitor densities in SR. We predict that the sandy, nutrient-poor soils of SR impose a more competitive microbial environment in which there is stronger selection for antibiotic production. Compared to the clayey, more nutrient-rich soils of PV, antagonism is expected to be a much more successful strategy in SR soils where the costs of antibiotic production may be outweighed by the benefits of enhanced nutrient acquisition. Differences in particle size distribution and volumetric water content between the sites could impact diffusion

rates of extracellular metabolites and substrates as well as the frequencies and outcomes of microbial interactions (Carson et al. 2010, Vos et al. 2013), but it is unclear the extent to which these factors interact with nutrient availability to influence *Streptomyces* inhibitory phenotypes at these sites.

Differences in inhibitor densities against the three indicator strains varied within and among tree species, which may confer soils with varying degrees of specificity in the potential for antibiotic-mediated disease suppression. Our findings suggest that tree species effects on *Streptomyces* communities are related to interspecific variation in litter elemental concentrations and leaf functional traits. This is consistent with previous studies that have linked the identity and distribution of leaf litter inputs to soil nutrient cycling and microbial characteristics (Mitchell et al. 2010, Aponte et al. 2013, Uriarte et al. 2015).

The chemical composition of roots as well as the quantity of above and belowground litter inputs are also likely to impact soil microbial communities, and the relative importance of these factors can vary within and among sites and ecosystem types (Freschet et al. 2013, Prescott and Grayston 2013). A recent study in a tropical moist forest on Barro Colorado Island (BCI) found that the species identity of roots was only weakly correlated with microbial community composition, suggesting that the ephemeral and patchy nature of root distributions relative to trees may lessen their impact on microbial diversity compared to aboveground inputs at this site (Barberan et al. 2015). Leaf litter from different tree species have been shown to create distinct chemical signatures beneath trees on BCI (Donoso et al. 2010), however, there is mixed support for

whether host-specific litter chemistry translates into distinct soil chemistry beneath individual trees (Powers et al. 2004, Marichal et al. 2011, Gei and Powers 2013). Fanin et al. (2014) found strong effects of host-specific litter on microbial biomass, community structure, and function in soils in a tropical forest in French Guiana. Namely, species-specific effects of litter on soil microbes were mediated by differences in C leachates. The degree to which trees and host-specific litter affect soil characteristics and biota depends on the site, soil property, and microbial taxon (Donoso et al. 2010, Barberan et al. 2015, Waring et al. 2015a).

RELATIONSHIPS AMONG HOST-ASSOCIATED ELEMENTS AND *STREPTOMYCES* COMMUNITY CHARACTERISTICS: Total soil element concentrations were generally higher in PV than SR, which is consistent with previous studies (Powers et al. 2009) and is likely driven by differences in parent material and other soil forming factors between the sites. Litter elements, on the other hand, tended to be similar among trees of the same species, reflecting interspecific variation in functional characteristics. We found that site modified the relationships between host-specific litter inputs and soil element concentrations. In particular, three soil elements (Al, Cr, K) varied significantly among tree species in nutrient-poor SR but not PV. Although site was the most important factor explaining variation in soil elements beneath trees, there was considerable intra- and interspecific variation within each site. By determining baseline levels of soil nutrients, site characteristics can impact resource acquisition and allocation strategies of both plants

and microbes and affect the nature of the plant-microbe associations that develop (Waring et al. 2015b).

Relationships among soil and litter elements and inhibitory phenotypes varied among tree species and were not consistent between the two sites. Effect sizes were consistently greatest when examining *Streptomyces*-element correlations for tree species in each site separately. This suggests that differences between SR and PV mediate the effects of tree species on pathogen suppression. Differences in forest composition (i.e. plant richness or relative abundance) and local neighborhood could be contributing to antagonistic phenotypes associated with tree species in the two sites, both of which can have stronger impacts on soil microbes than plant species identity alone (Bakker et al. 2013a,b; Barberan et al. 2015). Our results suggest that relationships between litter and soil elements differ between SR and PV and may contribute to differential effects of tree species on local soil chemistry and pathogen-suppressive capacities of *Streptomyces* communities.

Soil and litter element and micronutrient concentrations were frequently correlated with total *Streptomyces* densities as well as the frequency and intensity of pathogen suppression. Micronutrients play an important role as cofactors in microbial enzymes (Wackett et al. 2004) and can regulate the production of antibiotics and other secondary metabolites in *Streptomyces* and other microbes (Abbas and Edwards 1989, Weinberg 1990). Zn can be especially important for the production of certain antibiotics in *Streptomyces*, but it can also lead to toxicity or growth inhibition depending on its concentration in soil (Shirokikh et al. 2015). Some antibiotics can act as chelating agents

and accumulate heavy metals from soils, which can be associated with concomitant changes in plant uptake (Haferburg and Kothe 2007, Sessitsch et al. 2013). This could provide an explanation for why significant relationships were detected for some tree species but not others.

Litter and soil elements were most frequently related to *Streptomyces* inhibitory phenotypes among *Gliricidia* trees. This could be related to *Gliricidia's* symbiosis with N-fixing bacteria or other life history characteristics associated with legumes, such as high leaf C and N (Powers and Tiffin 2010). Powers and Salute (2011) found that the addition of P and Zn to *Gliricidia* litter increased decomposition rates, and we found that low concentrations of these elements in soil were associated with higher densities and proportions of inhibitors but smaller average zone sizes. These results suggest that P and Zn may be important regulators of plant-soil-microbe relationships for this tree species. This could result from microbial P limitation in these soils or reflect changes in C inputs related to decomposition of *Gliricidia* litter. Unfortunately, we did not measure soil C or N, and these elements should be examined in future studies in order to further explore interactions among *Streptomyces*, N-fixing bacteria, and legumes in these forests.

Our results suggest that analyzing only average trait values across the three indicator strains would have masked biologically important nuances in the potential specificity of disease suppression among *Streptomyces* communities. The relative importance of tree species and site effects on pathogen suppression varied depending on the inhibitor strain being tested (Fig. 2.3). Against two of the three indicators, pathogen-suppressive capacities of *Streptomyces* communities appeared to be primarily affected by

site. Against the third indicator we detected host-specific differences in inhibition that were maintained across distinct forest ecosystems. Furthermore, we found that tree species and site characteristics can influence different aspects of pathogen suppression by selecting for different types of antagonism (strength of inhibition vs. frequency of inhibitors) or inhibitory capacities against different pathogen targets (Figs. 2.4 and 2.5).

CONSEQUENCES OF SPATIALLY-HETEROGENEOUS PATHOGEN SUPPRESSION IN TROPICAL FORESTS: The Janzen-Connell hypothesis predicts that host-specific pathogens promote plant species diversity by disproportionately reducing the survival of seedlings near conspecific adults (Janzen 1970, Connell 1971). However, pathogens do not necessarily need to be strict specialists in order to promote plant diversity, as long as they have differential effects across tree species (Sedio and Ostling 2013). Recent work suggests that mechanisms underlying diversity-promoting effects may be complex, and can be driven by multiple enemies including generalist pathogens and seed predators and interacting functional groups above- and belowground (Visser et al. 2011, Hersh et al. 2012, Fricke et al. 2014, Garzon-Lopez et al. 2015). Accounting for beneficial plant mutualists can also have dramatic effects on the predicted impacts of soil-borne microbes on plant communities (Mangan et al. 2010, Liang et al. 2015). Our study demonstrates the potential importance of considering the enemies of plant enemies such as pathogen-suppressive bacteria in efforts to understand plant community composition in tropical forests.

The impacts of antibiotic-producing *Streptomyces* on plant-pathogen interactions and community composition will depend on the degree of variation in pathogen-suppressive capacities across plant hosts as well as the efficacy of antibiotics produced by *Streptomyces* against pathogens and other microbes *in situ*. We found a wide range of variation in pathogen-suppressive capacities among plant hosts in tropical dry forests of Costa Rica. The differences observed in pathogen-inhibitory phenotypes reflect variation in the potential for disease suppression to develop in association with different tree species, which could have important implications for seedling recruitment, tree species diversity, and plant community composition in these forests. Further work is needed to connect differences in pathogen-suppressive capacities to disease and growth dynamics among tropical plant species.

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Figure 2.1. Principal Components Analyses of total element concentrations in (a) soil and (b) leaf litter associated with five tree species. Each point is the average (± SE) of n = 5 trees. (a) Soil nutrients cluster by site and reflect underlying differences in total soil nutrients between Santa Rosa (triangles) and Palo Verde (circles). (b) Litter nutrients cluster by tree species (differentiated by shading), reflecting species-specific differences in plant inputs to the microbial resource pool. Black = *Gliricidia*; grey = *Sideroxylon*; white = *Luehea*; dotted = *Tabebuia*; striped = *Calycophyllum*

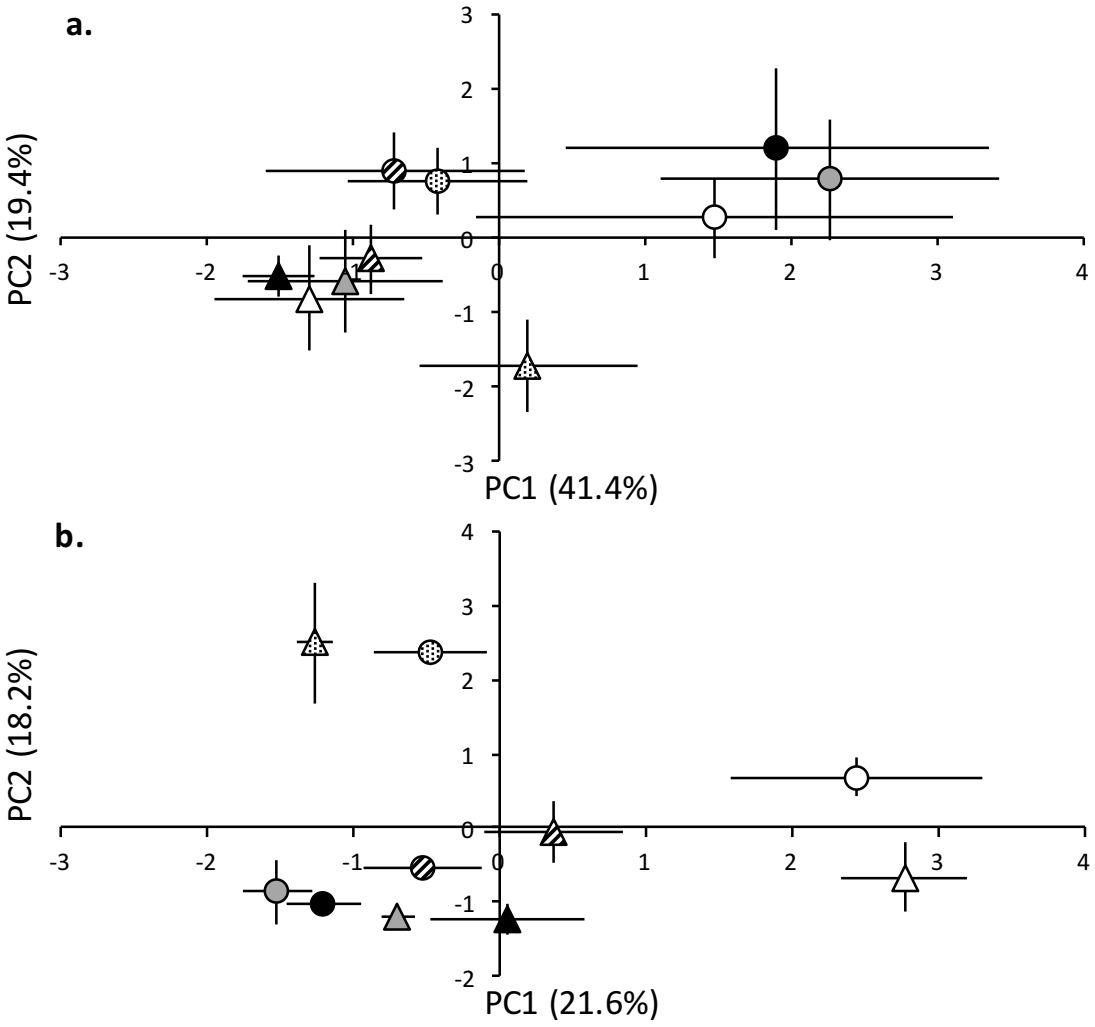

Figure 2.2. Total *Streptomyces* densities (solid grey bars; "Total Streps") and inhibitor densities (patterned bars) against three indicator strains (*S. scabies* [87], 4-21, and 1324) in soils from five tree species in (a) Palo Verde and (b) Santa Rosa. Bars depict average *Streptomyces* densities (Log CFU/g soil) ± SE from n = 5 trees per species per site. Tree species codes: *Calycophyllum* (CALYCA), *Gliricidia* (GLIRSE), *Luehea* (LUEHCA), *Sideroxylon* (SIDECA), *Tabebuia* (TABEOC)

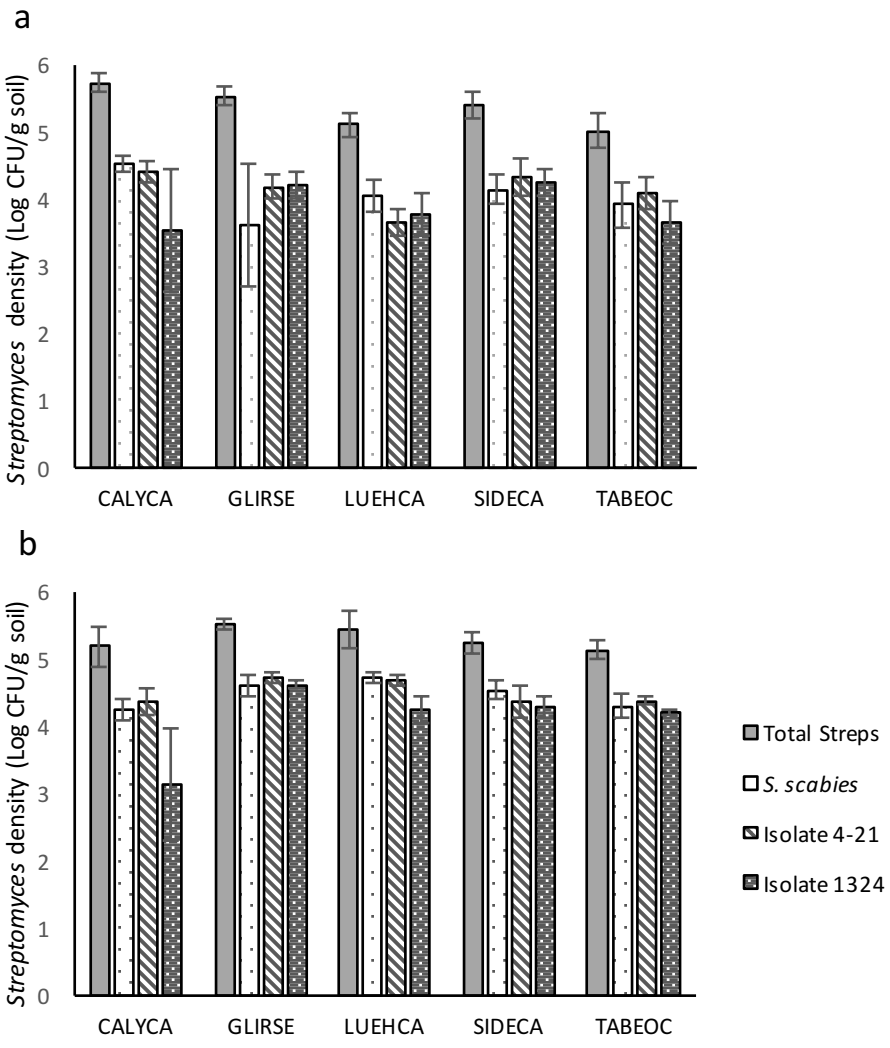

Figure 2.3. Correlations between pathogen-suppressive capacities of *Streptomyces* communities beneath five tree species in two sites. Inhibition zone size (left-hand panels: a, c, e) and average inhibitor densities (right-hand panels: b, d, f) are shown. Each dot represents one tree species (mean ± SE), plotted by taking the average value across five trees per species in Palo Verde (x-axis) and Santa Rosa (y-axis). Inhibition zones and inhibitor densities were measured against three *Streptomyces* indicator strains: *S. scabies* (a and b), isolate 4-21 (c and d), and isolate 1324 (e and f). Spearman correlation coefficients and p-values are shown. A significant correlation (panels e and f) indicates that tree species have consistent effects on pathogen-suppressive capacities in both sites. The dotted 1:1 line reflects identical values in the two sites. Black = *Gliricidia*; grey = *Sideroxylon*; white = *Luehea*; dotted = *Tabebuia*; striped = *Calycophyllum*

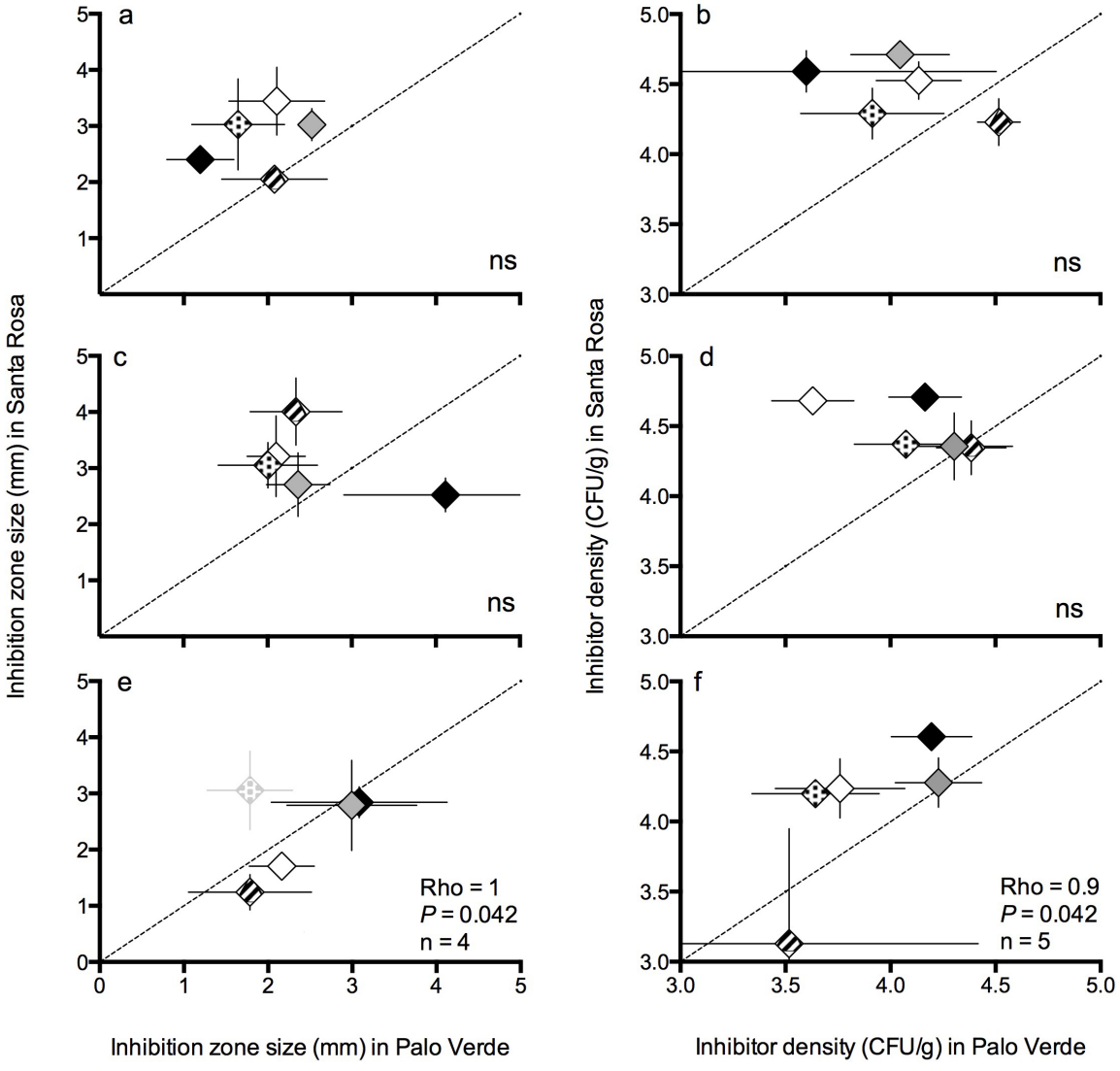

Figure 2.4. NMDS ordination of soil element concentrations (stress = 0.144) associated with five tree species in Palo Verde (PV; circles) and Santa Rosa (SR; triangles). Inhibition zone size against indicator strain 87 ("ZoneA") was significantly correlated with the soil element ordination ($r^2 = 0.146$, $P = 0.027$). Vector direction indicates more intense inhibition among *Streptomyces* communities in SR than PV. Black = *Gliricidia*; grey = *Sideroxylon*; white = *Luehea*; dotted = *Tabebuia*; striped = *Calycophyllum*

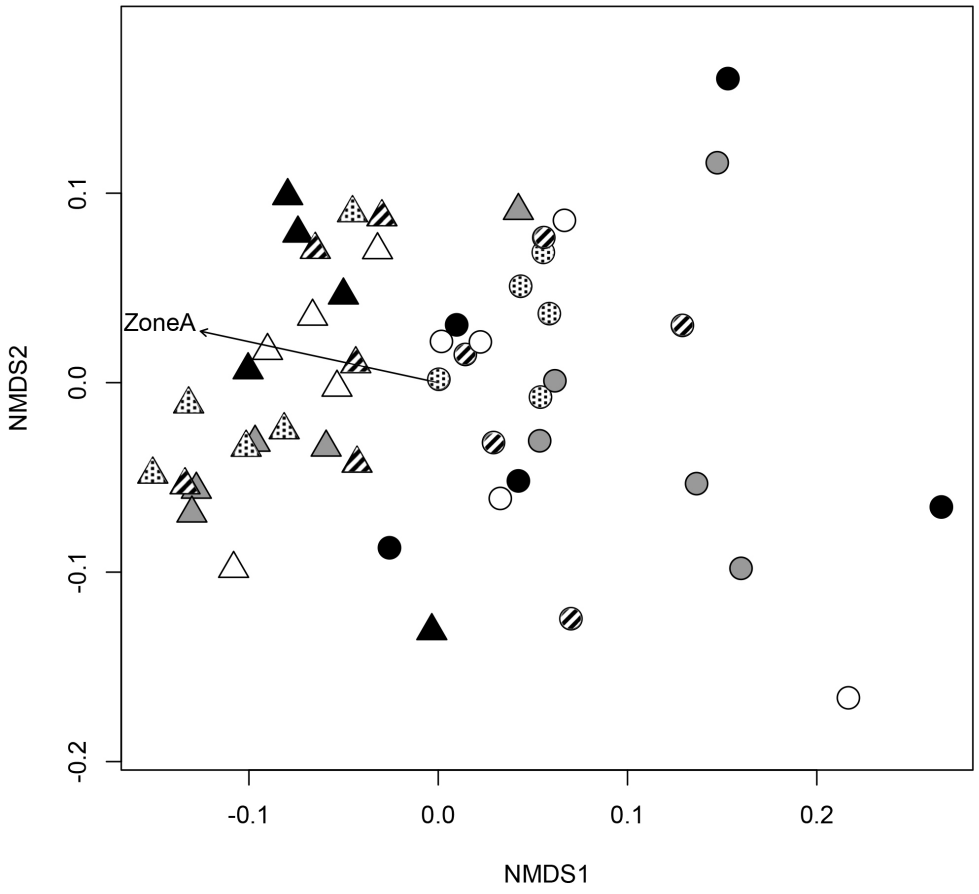

Figure 2.5. NMDS ordination of litter element concentrations fitted with vectors of *Streptomyces* community characteristics in (a) Palo Verde (PV; stress = 0.145) and (b) Santa Rosa (SR; stress = 0.176). Vectors indicate significant relationships between the litter element ordination and (a) inhibitor densities and zone sizes against strain 4-21 ("InhibitorB": $r^2 = 0.26$, $P = 0.045$; "ZoneB": $r^2 = 0.23$, $P = 0.058$) and (b) inhibitor densities and zone sizes against strain 1324 ("InhibitorC": $r^2 = 0.26$, $P = 0.020$; "ZoneC": $r^2 = 0.29$, $P = 0.028$). Symbol fill is the same as Figure 2.4.

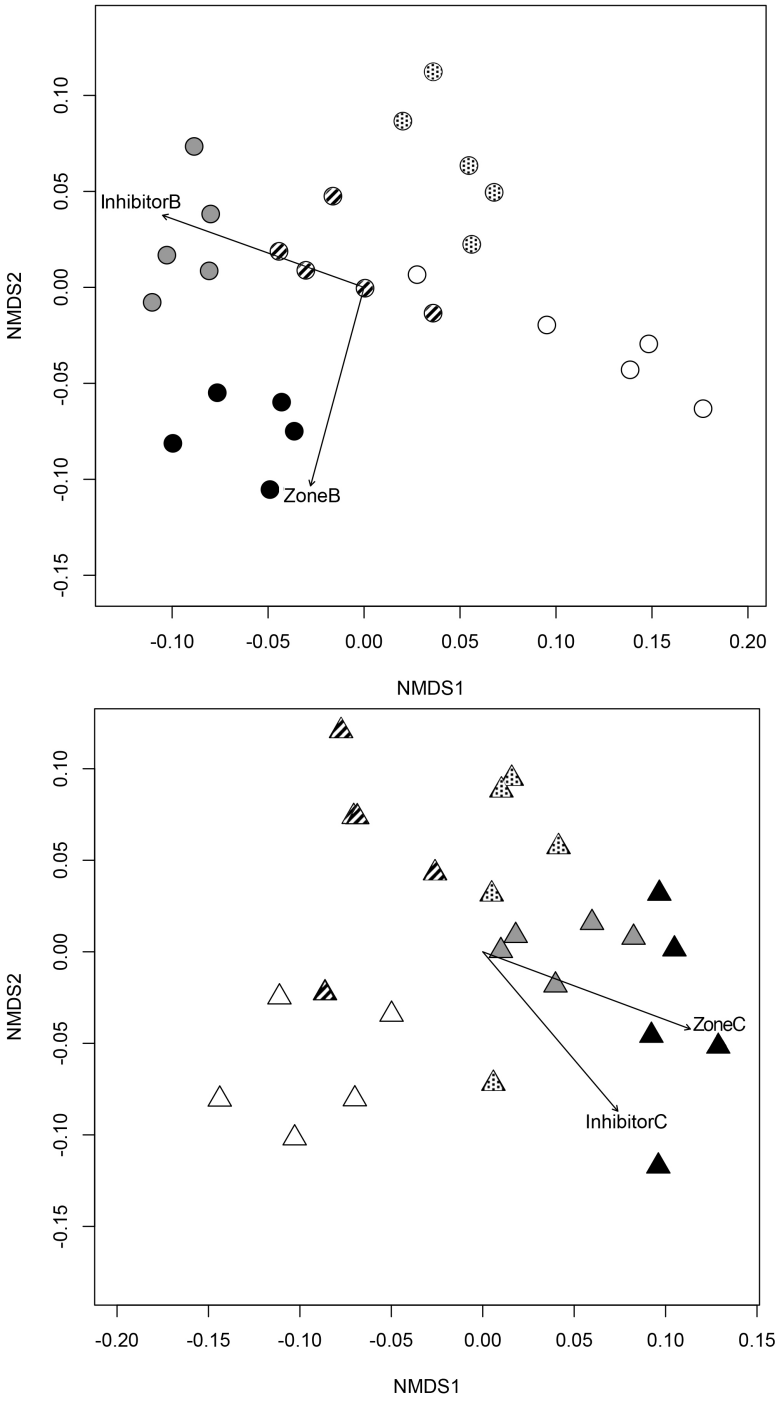

Table 2.1. Focal tree species and life history traits

Tree species	Family	Leaf habit	Nitrogen-fixer
Calycophyllum candidiscimun	Rubiaceae	deciduous	no
Gliricidia sepium	Fabaceae (Papilionoide)	deciduous	yes
Luehea candida	Tiliaceae	semideciduous	no
Sideroxylon capiri	Sapotaceae	evergreen	no
Tabebuia ochraceae	Bignoniaceae	deciduous	no

Table 2.2. Average soil element concentrations (mg kg^{-1}) in Santa Rosa and Palo Verde. Values represent means (± SE) of n = 25 trees. T-tests were used to test for significant differences between sites. Bolded elements indicate significant differences ($P < 0.05$; values in the site with the higher element concentration are bolded). Soil Mo was below detection limits and was therefore excluded

	Santa Rosa		**Palo Verde**	
Al	53278	(2163)	53595	(2271)
B	14.34	(1.04)	16.63	(1.25)
Ca	7395	(263)	**26013**	**(3849)**
Cr	9.26	(0.66)	**39.93**	**(10.55)**
Cu	56.24	(1.85)	56.72	(2.61)
Fe	35481	(2008)	34387	(1797)
K	858.95	(71.55)	**1225.31**	**(157.90)**
Mg	1817.9	(126.4)	**5864.0**	**(921.9)**
Mn	1059.19	(209.73)	885.25	(98.03)
Na	1134.12	(82.70)	**2000.90**	**(173.01)**
Ni	10.14	(1.53)	**35.84**	**(10.21)**
P	223.79	(16.73)	**383.06**	**(42.25)**
Zn	54.33	(3.89)	60.99	(6.14)

Table 2.3 Litter element concentrations (mg kg^{-1}) of five tree species. Values represent means (± SE) per tree species (n = 10 trees, averaged across two sites). For each element, letters represent significant differences (Tukey's HSD) in litter element concentrations among species ($P < 0.05$). Litter Ni was below detection limits and was therefore excluded. Tree species codes: *Calycophyllum* (CALYCA), *Gliricidia* (GLIRSE), *Luehea* (LUEHCA), *Sideroxylon* (SIDECA), *Tabebuia* (TABEOC)

	Al	B	Ca	Cr	Cu	Fe
CALYCA	68.23 (18.84) AB	53.29 (4.45) B	20261 (1561)	0.63 (0.02) B	9.4 (0.59) AB	75.41 (11.02) B
GLIRSE	49.02 (14.66) B	86.01 (8.4) A	17762 (1208)	0.62 (0.02) B	3.45 (0.16) C	126.89 (12.97) A
LUEHCA	127.6 (19.97) A	44.39 (2.09) B	17911 (934)	0.68 (0.04) B	9.58 (0.45) A	128.65 (11.54) A
SIDECA	40.3 (2.63) B	61.54 (5.64) B	22604 (2198)	0.62 (0.04) B	6.16 (0.49) BC	66.51 (4.76) B
TABEOC	79.04 (14.82) AB	84.5 (3.66) A	17907 (1183)	1.43 (0.28) A	12.32 (1.65) A	84.98 (8.62) B

K	Mg	Mn	Mo	Na	P	Zn
10903 (1044) A	2979 (165) B	17.35 (1.86) CD	8.19 (0.33) AB	777.66 (184.05) A	612.78 (53.82) AB	14.93 (1.03) A
7087 (961) BC	3982 (201) A	34.95 (3.28) B	7.14 (0.52) B	405.9 (89.24) B	419.58 (34.95) B	5.58 (0.41) B
9442 (906) AB	2694 (303) B	185.27 (35.33) A	6.68 (0.38) B	198.27 (9.92) B	634.68 (33.65) AB	16.01 (2.27) A
5520 (659) C	2747 (207) B	16.79 (1.97) D	8.21 (0.35) AB	352.46 (46.06) B	781.73 (188.58) AB	6.24 (0.39) B
5666 (693) C	4500 (172) A	27.55 (2.26) BC	8.75 (0.3) A	187.79 (21.42) B	771.82 (58.3) A	18.48 (1.56) A

Table 2.4. Two-way ANOVA table examining the effects of tree species and site on inhibitor densities. Mean inhibitor density was averaged across the three indicator strains; results are also reported for each isolate individually (87; 4-21; 1324)

Source	**DF**	**SS**	**F**	***P***
Mean inhibitor density				
Species	4	-	-	ns
Site	1	1.53	6.0057	0.0187
Species*Site	4	-	-	ns
Inhibitor density (87)				
Species	4	-	-	ns
Site	1	2.28	3.99	0.0527
Species*Site	4	-	-	ns
Inhibitor density (4-21)				
Species	4	-	-	ns
Site	1	1.78	10.89	0.002
Species*Site	4	1.91	2.92	0.03
Inhibitor density (1324)				
Species	4	-	-	ns
Site	1	-	-	ns
Species*Site	4	-	-	ns

Supplementary Information for this Chapter can be found in Appendix 2

Chapter 3

Pathogen-suppressive bacteria have potential
to mediate plant-soil feedbacks in a tropical moist forest

ABSTRACT:

Plant-soil feedbacks occur when plants influence microbial community composition and/or activity, and these changes positively or negatively affect performance of the host plant or its offspring. Microbially-mediated plant-soil feedbacks may promote species coexistence and thus local plant diversity in many ecosystems. Nevertheless, we know very little about the soil microbes or interactions that drive plant-soil feedbacks. We conducted a fully-reciprocal plant-soil feedback experiment in a tropical forest in Panama in order to test whether interactions between antibiotic-producing soil bacteria and pathogens influence plant community composition. Seedlings of five tree species were planted in a shadehouse experiment in soils collected beneath conspecific and heterospecific adults in the field (n = 5 trees/species). Soil disease potential, pathogen-suppressive capacities of *Streptomyces* communities, and C, N, P, and K were measured in the 25 soils in which seedlings were planted. Seedlings were harvested after six months and total seedling biomass was used to calculate direct and net plant-soil feedbacks.

Plant-soil feedbacks varied among the five seedling species. *Virola* seedlings experienced positive feedback and were significantly larger when grown in soil from conspecific adults than in any other soil source. By contrast, *Tabebuia* seedlings had higher biomass when grown in soils from other tree species relative to growth in

Tabebuia soils (negative feedback). Soil C, N, P, and K did not differ significantly among soils from the five tree species and had no significant effects on seedling biomass. Our results suggest that pathogen-suppressive *Streptomyces* promote variation in seedling biomass and have the potential to mediate plant-soil feedbacks. Inhibitory *Streptomyces* had both positive and negative effects on seedling biomass, and the strength and direction of these effects varied among seedling species and soil sources. In *Ormosia* and *Tabebuia* soils, densities of pathogen-suppressive bacteria were significantly negatively correlated with soil disease potential, further suggesting that *Streptomyces* effects on feedbacks were related to interactions with plant pathogens.

INTRODUCTION:

Soil-borne plant pathogens are predicted to regulate plant community diversity in many ecosystems (Alexander 2010, Bever et al. 2015, Mordecai 2011, 2015). Studies of spatial patterns of seedling survival in the vicinity of conspecifics provide support for a role of pathogens in the coexistence of tree species (Janzen 1970, Connell 1971, Packer and Clay 2000). The Janzen-Connell hypothesis predicts that natural enemies will promote local species diversity through heightened seedling mortality close to parents, which allows for recruitment of locally rare plant species and thereby promotes species coexistence. So-called "Janzen-Connell effects" have been documented in a number of ecosystems, especially tropical forests (Carson et al. 2004, Comita et al. 2014) and temperate grasslands (Petermann et al. 2008, Klironomos 2002). Janzen-Connell spatial

distributions are thought to result primarily from negative feedbacks with plant pests (Box 3.1a), especially specialized soil-borne pathogens that can accumulate beneath adult trees and lead to reduced growth or survival of conspecific seedlings in home soils relative to performance in soils from other tree species (Augspurger 1984, Gilbert et al. 1994).

In order for plant-soil feedbacks to influence diversity, soils must vary in their effects on heterospecific seedling species. This can happen is through the activity of specialized microbes that accumulate in soil under specific plant hosts and disproportionately affect seedlings of that species relative to heterospecific seedlings in the same soil. Another possibility that has received much less attention is that net feedback effects result from interactions between specialized pathogens (or mutualists) and other microbes in soil.

Both species-specific positive and negative plant-soil feedbacks have been documented among tropical tree species (Mangan et al. 2010a, b), but most studies have focused on negative feedbacks due to their potential to promote species coexistence in highly diverse tropical forests (Terborgh 2012, Bagchi et al. 2014). There is general consensus in the literature that soil-borne pathogens play an important role in tropical forests ecosystems (Gillett 1962, Gilbert 2002), but the involvement of soil-borne pathogens in feedbacks has rarely been tested directly and there is insufficient evidence that pathogens alone are specialized enough to produce the species-specific effects required for negative feedbacks to promote diversity (Augspurger and Wilkinson 2007).

Pathogens do not interact with plants in isolation, but rather within the dynamic and spatially heterogeneous context of the larger soil community and abiotic

environment. Plant pathologists have long recognized that the outcome of plant-pathogen interactions depends not only on pathogenicity and host resistance, but also on spatial and temporal variability in biotic and abiotic environmental conditions (often referred to as the "disease triangle;" Barrett et al. 2009, Francl 2011). The importance of abiotic context (e.g. light, moisture) in regulating plant-pathogen interactions has been fairly well recognized (Augspurger 1984, Smith and Reynolds 2015), and there is growing recognition of a potential role for co-infection by multiple pathogens of varying specificities (Hersh et al. 2012, Benitez et al. 2013, Sedio et al. 2014). However, studies of biotic interactions between pathogens and other microbial functional groups remain less common, despite their important contributions to our understanding of how species interactions affect plant-soil feedbacks and species coexistence (Morris 2007, Fitzsimmons et al. 2010, Liang et al. 2015, Van der Putten et al. 2016).

Free-living, plant-associated bacteria can have significant effects on plant performance and fitness, and one group that has received relatively little attention in the context of plant-soil feedbacks is plant growth-promoting bacteria (Raajimakers 2009, Kinkel et al. 2011, Latz et al. 2016). This includes bacteria that indirectly affect plants through antibiotic-mediated pathogen suppression, as well as through effects of antibiotics on overall microbial community structure and function in soil (Bakker et al. 2014, Schlatter et al. 2015). *Streptomyces* is a common genus of soil bacteria that is well known for the production of diverse antibiotics and their role in pathogen inhibition and disease suppression have been well-documented in agricultural systems (Liu et al. 1995, Kinkel et al. 2012). Very little is known, however, about interactions between *Streptomyces* and soil-borne pathogens in natural ecosystems. Variation in the diversity

and functional characteristics of soil-borne *Streptomyces* communities have been documented at very fine spatial scales (i.e. < 1m; Davelos et al. 2004), at the landscape scale (Becklund et al. 2014), and at biogeographic scales within and among diverse habitats globally (Schlatter et al. 2014). However, outside of agricultural systems and experimental grasslands, information is lacking on how functional variation among *Streptomyces* relates to soil-borne disease, plant performance, or plant community dynamics.

We predict that antibiotic-producing soil bacteria mediate plant-soil feedbacks in tropical forest soils by promoting variation in the effects of pathogens on plants. To test this hypothesis, we conducted a fully reciprocal plant-soil feedback experiment on Barro Colorado Island (BCI), Panama. Soils were collected from five tree species in the field (n=5 trees/species) and soils from each tree were used as soil sources in a pot experiment. Every seedling species was planted in soil from each of the 25 focal trees. For each tree, we measured three sets of soil characteristics that we predicted might contribute to plant-soil feedbacks: 1) pathogen-suppressive capacities of *Streptomyces* communities, 2) soil disease potential, and 3) total soil carbon (C), nitrogen (N), and available soil phosphorus (P) and potassium (K). We asked whether these soil biotic and abiotic characteristics – or relationships among them – varied among tree species and individual host trees, and whether they were related to seedling biomass and plant-soil feedbacks after six months of growth.

We predicted that *Streptomyces* communities would mediate the strength, specificity and/or direction of plant-soil feedbacks by promoting variation in seedling biomass (Boxes 3.2 and 3.3). The pathways by which *Streptomyces* could influence

plant-soil feedbacks are numerous. *Streptomyces* have the potential to strengthen or weaken positive and negative feedbacks, or they could have no net effect. *Streptomyces* could weaken negative feedbacks / strengthen positive feedbacks by suppressing pathogens in home soils or by suppressing mutualists in away soils. Similarly, they could strengthen negative feedbacks / weaken positive feedbacks by benefiting seedlings in away soils or having detrimental effects in home soils.

We compared three approaches to analysis of plant-soil feedbacks: 1) direct feedbacks (Box 3.3a), which compare the performance of a single focal species in "home" (i.e. conspecific) vs. "away" (i.e. heterospecific) soil sources, 2) pairwise feedbacks (Box 3.3b, c), which examine the *relative* performance of two or more species across home and away soil sources, and 3) comparison of relative seedling biomass in any two soil sources, regardless of whether they were collected from conspecific or heterospecific trees.

METHODS:

SITE DESCRIPTION AND FOCAL SPECIES: The shadehouse experiment was conducted on Barro Colorado Island (BCI), Panama. BCI is classified as tropical moist forest and receives an average annual rainfall of 2600 mm, most of which falls in the wet season from May to mid-December (Windsor 1990). Seedlings of five focal species were grown in pots filled with live, unsterilized field soil collected beneath adult trees of the same five species (n = 5 trees/species). The focal species used in this experiment were: *Apeiba aspera* (Tiliaceae), *Ormosia macrocalyx* (Fabaceae: Papillionoidideae), *Protium*

tenuifolium (Burseraceae), *Tabebuia guayacan* (Bignoniaceae), and *Virola surinamensis* (Myristicaceae). We targeted species from different families with different functional characteristics (Table 3.1). The final focal tree species were selected based on seed availability and germination success.

SOIL COLLECTION: Soils were collected on BCI at the end of the wet season in October 2013 (N = 5 trees per species). Focal trees that fit the following criteria were selected at random throughout a 25-ha permanent inventory plot: diameter at breast height (dbh) ranged from 30 to 100 cm, no conspecifics ≥ 30 cm dbh were located within 20 m^2, there were at least 20 m between any two focal individuals, the nearest neighboring tree ≥ 30 cm dbh was at least 5 m away, trees were located on relatively flat terrain and at least 3 m from trails, and trees were not located near gaps or colonized by lianas. Four *Ormosia* trees were located elsewhere on BCI due to low abundance in the 25-ha plot.

For each focal tree, four soil samples were collected at 0-10 cm depth at locations approximately 2-m from the trunk in the four cardinal directions (10 cm x 10 cm x 30 cm dimensions; 3 L soil/hole). Shovels were cleaned with 70% ethanol between every tree to prevent cross-contamination of host-specific microbes. The four subsamples per tree were bulked into one composite soil sample and sieved through a steel screen to remove large stones and organic debris. Invertebrates were removed when seen.

Samples were stored in Tupperware containers with loose lids in the dark under ambient conditions until soil had been collected and processed from all 25 trees. Soil from each tree was then mixed thoroughly by hand and a subsample was saved for microbial and edaphic analyses. Immediately prior to planting, sterile sand was added in

a 2.5:1 soil to sand ratio to minimize differences in texture and improve drainage in pots. Sand was washed eight times prior to use to remove nutrients, salts, and particulates and then autoclaved twice. The soil-sand mixture from each tree was used to fill five pots (one for each seedling species).

Soils were not pooled by focal tree species because in previous work we found substantial intraspecific variation in *Streptomyces* densities and inhibitory capacities among host trees (Becklund et al. in press) and we wanted our feedback design for this study to incorporate tree-to-tree level variation in plant-microbe interactions. Throughout the text, "soil source" refers to the tree species origin of the soil in which seedlings were planted (n = 5 tree species).

SEEDLING GERMINATION, TRANSPLANT, AND EXPERIMENTAL CONDITIONS: Seeds were collected from three or more fruiting adults per species. Seeds were collected outside of the 25-ha plot, which ensured that soils used in the feedback experiment did not include parent trees. Seeds were sterilized with 95% ethanol (10 seconds), 15% commercial bleach (2 min), and 70% ethanol (2 min), and then rinsed twice in sterile water and allowed to dry under sterile cheesecloth. Germination treatments were used to improve germination success when necessary (Table 3.1). *Apeiba* seeds were germinated in petri dishes lined with sterile paper towels moistened with sterile, distilled water. For all other species, surface-sterilized seeds were planted in a 1:1 sterile sand-vermiculite mixture (autoclaved twice, at least 24 hours apart) and watered with sterile, distilled water as needed. Seeds were allowed to germinate in a shadehouse at approximately 30% light until transplant into the experiment.

The experiment was conducted in a shadehouse covered with clear plastic sheeting to exclude precipitation and reduce airborne contamination. Shadecloth was used to reduce light levels to those typically found in the forest understory (≈ 2%; Mangan 2010b). Photosynthetically active radiation (PAR) was measured with an Apogee Quantum Sensor throughout the experiment. Average light levels ranged between 1-2% relative to full sun readings collected outside the shadehouse.

We used fully reciprocal plant-soil feedback design in which all seedling species were planted in each soil source. Pre-germinated seedlings were transplanted into treatment soils (one per pot) when seedlings were approximately 3-4 weeks in age. All instruments were sterilized between transplanting sets of seedlings into the 25 soils. In addition, a sterile layer of sand was added to cover the soil surface of each pot in order to reduce the potential for airborne contamination and reduce fungal gnat populations. Five additional seedlings of each species were planted into a sterilized "control" soil collected from a common soil source and mixed with sterile sand (see Supplemental Text SI 3.1, SI Table 3.4).

In order to minimize intraspecific differences in initial seedling biomass, seedlings were selected for transplant based on specific height ranges for each species (*Apeiba* = 1.0 – 2.5 cm; *Ormosia* = 11 – 15 cm; *Protium* = 14.0 -17.0 cm; *Tabebuia* = 2.2 - 3.3 cm), with the exception of *Virola* due to limited availability of germinated seedlings (range = 3.5 - 14.2 cm). Seedlings of *Virola, Ormosia*, and *Protium* had true leaves at the time of transplant. To test whether initial seedling size influenced final plant size at harvest, we harvested extra seedlings at the beginning of the experiment and used them to estimate initial biomass of experimental seedlings (see Supplemental Text SI 3.1 and SI

Table 3.1 for full methods). Estimates of initial seedling biomass in analyses never resulted in significantly improved model fit of seedling biomass at harvest (SI Table 3.2), therefore we present results without this covariate.

Pot locations were randomized every 2-3 weeks to minimize the potential for neighbor effects as well as effects of variation in light levels across the shadehouse. Seedlings were watered with distilled water 2-3 times a week as needed. Due to high transplant mortality of *Tabebuia* and *Apeiba* seedlings, individuals of these species were replanted as needed after approximately one month. Replanted seedlings had at least one true leaf.

Experimental seedlings were harvested approximately 175 days from the date of planting. Roots were harvested and rinsed thoroughly with sterile water to remove debris. Seedling components (stems, leaves, and roots) were dried at 65° C for a minimum of 3 days and then weighed for total biomass.

QUANTIFYING SOIL DISEASE POTENTIAL: We used a modification of the rolled-towel (RT) bioassay (based on Kraft et al. 1990, Williams-Woodward 1998) to index soil disease potential among the 25 soils used in the feedback experiment (see Supplemental Text SI 1.3, Appendix 1). We used three seedling species that vary in seed size and shade tolerance (Table 3.1) to assay soil disease potential: *Apeiba aspera* (Tiliaceae), *Ochroma pyramidale* (Bombacaceae), and *Luehea seemannii* (Tiliaceae). These species were used to create an index of the potential effects of each soil's microbial community on seedling infection (not to estimate susceptibility or tolerance of specific hosts to any particular soil

pathogen). The three species were selected based on rapid germination and sufficient seed availability, which are requirements for this assay.

Seeds were surface-sterilized and pre-germinated in petri dishes lined with sterile paper towels. Approximately 1-2 days after emergence of the radicle, six seedlings without signs of abnormality or physical damage were evenly spaced on treatment soil (approximately 35 mL volume over 19 x 5 cm area), rolled in sterile paper towels pre-moistened with sterile water, and placed in unsealed, ziploc bags. Five replicate bags were prepared for each focal tree x seedling species combination (N = 25 focal trees x 3 seed species = 75 treatment combinations). In addition, five soil-free controls were prepared for each seedling species. Unsealed bags were stored at 26 °C in the dark for eleven days to simulate conditions experienced by C-limited seedlings germinating in the seed bank. Sterile water was sprayed into bags every other day to maintain moist, humid conditions which are optimal for pathogen growth.

For each bag (experimental unit), disease incidence (presence/absence of disease symptoms) and severity were scored for each seedling. Severity was scored on a 0-3 scale according to the extent and severity of symptoms. Disease assessment criteria were based on the range of disease symptoms observed per species and disease categories that could be reproducibly detected. 0 = no symptoms; 1 = minor, localized infection (and/or growth reduction for *Apeiba*); 2 = < 75 % tissue infected (or major growth reduction for *Apeiba*); 3 = > 75 % necrotic tissue/dead seedling. For each replicate (one RT containing six seedlings), we calculated the proportion of diseased seedlings, the average disease severity, and a composite disease index that incorporates both incidence and severity (following Wang et al. 2012): disease index (%) = [(∑scale x number of infected

plants)*(proportion incidence) / (highest scale x total number of plants)] x 100. This index weights the average severity of disease symptoms per bioassay by the proportion of seedlings affected, making it a conservative measure of soil disease potential.

SOIL ABIOTIC CHARACTERISTICS AND PATHOGEN-SUPPRESSIVE CAPACITIES OF *STREPTOMYCES* COMMUNITIES: Samples of the 25 soils used in the feedback experiment were shipped to the University of Minnesota for analysis of soil chemistry and pathogen-suppressive capacities of soil bacteria. Total organic C, total N, Bray's P, and exchangeable K from each of the 25 soil sources were analyzed at the University of Minnesota Soil Testing and Research Analytical Lab (http://ral.cfans.umn.edu/soil-analysis-and-methods/). Soil moisture content (based on soil dry weight) was determined on BCI after drying samples at 105 °C for at least 24 hours.

We used a modified Herr's assay to quantify the abundance and inhibitory activity of soil-borne *Streptomyces* from each of the 25 soils. This method measures total *Streptomyces* densities as well as pathogen-suppressive capacities of antibiotic-producing *Streptomyces* (Herr 1959; Wiggins and Kinkel 2005). Soil samples were air dried under sterile cheesecloth in a biosafety cabinet for approximately 24 hours prior to the start of each assay. Approximately 1 g (dry weight) of each sample was suspended in 9.6 mL sterile distilled water and shaken at 175 rpm at 4 °C for one hour. Serial dilutions were plated on 1% water agar (WA) and then overlaid with 10 mL of starch casein agar (SCA), which is selective for *Streptomyces*. Plates were incubated at 28 °C for three days. *Streptomyces* colonies were identified morphologically and counted on all plates.

Counted plates were then overlaid with a pathogen strain and incubated for an additional three days.

Pathogen suppression was measured against a bacterial and a fungal plant pathogen (*Streptomyces scabies* and *Fusarium oxysporum*; see SI Table 0.1, Appendix 0) in order to create an index of antibiotic-mediated pathogen-inhibitory capacities among *Streptomyces* communities in the tropical soils studied here. These "indicator" pathogens were originally isolated from temperate agricultural systems but inhibition against these strains has been well correlated with suppression of a wide variety of plant diseases caused by bacteria, fungi, viruses, Oomycetes, and nematodes (Liu et al. 1995, Jones and Samac 1996, Samac and Kinkel 2001).

Pathogen inoculum used to overlay plates was prepared as follows: *S. scabies* was grown on Soya Flour Mannitol (SFM; 10 g agar, 10 g Mannitol, 10 g Soya flour, 500 mL H_2O) medium for 7-10 days at 28 °C. Spores were collected with a sterile cotton swab, transferred into 20% glycerol solution, and stored at -80 °C until use. To assay inhibition against *S. scabies*, 5 mL of SCA was added to soil dilution plates and allowed to dry. Subsequently, 150 uL of the *S. scabies* cell suspension was distributed evenly across each plate. Plates were incubated at 28 °C.

F. oxysporum was grown on 1/2X PDA media under white, UVB fluorescent light for approximately 7 days and plates were stored at 4 °C until use. Individual *Fusarium* cultures were cut into approximately 1-cm^2 pieces and blended with 100 mL sterile water using a Waring Blender. Twenty-five mL of the resulting pathogen-water mixture was added to 500 mL Potato Dextrose Water Agar (PDWA) and mixed thoroughly. Each soil dilution plate was overlaid with 10 mL of this suspension. *Fusarium* plates were

incubated at room temperature (approximately 25°C). For a small number of samples, we used a modified overlay method that included chloroform to reduce fast-growing non-*Streptomyces* bacteria that consistently overgrew plates and obscured measurements of *Streptomyces*-mediated pathogen suppression (SI Table 0.2, Appendix 0).

After three days, plates were examined for signs of antibiotic-mediated pathogen suppression. Inhibitory *Streptomyces* (also referred to as inhibitors) were defined as *Streptomyces* colonies surrounded by a clear inhibition zone without pathogen growth. On every plate, inhibitors were counted and digital calipers were used to take two perpendicular measures of inhibition zone radius (mm) for each inhibitor. Zone sizes were averaged per inhibitor and then averaged again across all inhibitors on a plate. Values were averaged across replicate plates to generate mean inhibition zone size for each soil sample. The proportion of inhibitory *Streptomyces* (inhibitor density/total *Streptomyces* density) was calculated for each plate and averaged across replicate plates.

DATA ANALYSES:

Biotic and abiotic soil variation among focal trees:

Total and inhibitory *Streptomyces* densities (colony forming units [CFUs] per gram soil) were calculated for each plate, averaged across replicates, and log-transformed prior to analysis. Zone sizes were square-root transformed to better approximate a normal distribution. Proportions were arcsine-square-root transformed prior to analysis to meet assumptions of normality. One was added to the number of inhibitors per plate to allow log transformation of densities.

We used principal components analyses (PCAs) to examine variation in pathogen-suppressive capacities, soil disease potential, and soil C and nutrients among individual host trees and focal tree species (SI Fig. 3.1). One-way ANOVA was used to test whether *Streptomyces* community characteristics (total *Streptomyces* density, inhibitor densities, zone sizes, and proportions) varied among the five tree species that were used as soil sources in the shadehouse experiment. For RT bioassays, two-way ANOVA with tree as a random effect was used to test for effects of soil source and seed indicator species on soil disease potential. Average disease severity scores per RT unit were used in the two-way ANOVA, but results were similar when proportion of disease incidence and weighted disease index values were analyzed as response variables. Disease severity and index values were then averaged across replicates for each soil source and averages per tree were used in correlation analyses to test for relationships with *Streptomyces* community characteristics. We examined correlations among soils from all focal trees (N = 25) and for each tree species individually (N = 5). Pearson correlation coefficients are reported unless otherwise noted.

Analysis of seedling biomass and plant-soil feedbacks:

We used a mixed model ANCOVA (type II sum of squares) to test for effects of soil source, seedling species, and the soil x seedling interaction on seedling biomass at harvest (Table 3.4). Total seedling biomass was log-transformed to achieve normality prior to analysis. Tree was included as a random effect to account for variation in seedling biomass that was related to the individual focal trees from which soils were collected (N=25). Models including estimates of initial seedling biomass were never

significantly different from models without this term (SI Table 3.2), therefore the final models presented in the results do not include this covariate and *Tabebuia* seedlings are included (for which no initial biomass estimate was calculated due to small size at time of transplant). The largest *Apeiba* was 2.5 standard deviations larger than the second largest seedling of this species and was therefore excluded from analyses as an outlier (Cook's $D > 0.25$). One *Tabebuia* seedling was also excluded due to a very late replanting date.

To test whether variation in soil biotic or abiotic characteristics influenced seedling biomass and/or plant-soil feedbacks, we constructed additional mixed effects models including covariates related to pathogen suppression (total *Streptomyces* density, inhibitor densities, and inhibition zone sizes against bacterial and fungal pathogens) and soil abiotic characteristics (multivariate indices of soil C and N [soil PC1] and P and K [soil PC2]; loading factors in SI Table 3.4). Soil abiotic characteristics did not have significant effects on seedling biomass, either as main effects or through interactions with soil source or seedling species. When effects of added *Streptomyces* variables were significant, we compared the expanded model to the base model described above. Because models included the same random effect but different fixed effects, we used maximum likelihood ratio tests to compare models based on AIC and determine if additional predictor variables resulted in significant improvement over the base model (SI Table 3.7).

We calculated direct feedbacks (Box 3.3a) in order to compare the performance of individual seedling species in conspecific soils and each heterospecific soil source (i.e. four feedback values per seedling species). We also determined an average feedback value across all heterospecific soil sources. For example, feedback for seedling species A

in soil source B was calculated as $BiomassA_A$-$BiomassA_B$/$Max(A_A,A_B)$, following Kulmatiski et al. 2016 (also see Kuebbing et al. 2015 and Brinkmann et al. 2010). Uppercase letters denote seedling species and subscripts denote soil source. Thus, the numerator determines feedback direction by calculating the difference in biomass between home soil and the heterospecific soil source of interest, and the denominator scales this difference by the maximum biomass observed for Species A in either soil source. Positive values indicate that seedlings grew larger when grown in soil from conspecific trees as compared to the other soil source (i.e. positive feedbacks). Negative feedbacks indicate that seedlings grew larger when grown in soil from the other soil species. This feedback metric results in values that range from -1 to 1 and are symmetrical around zero (no difference in growth between soil sources). This means that positive and negative feedbacks of the same value represent equivalent increases and/or reductions in biomass in home soils relative to soils from heterospecifics.

In addition to direct feedbacks, we also calculated pairwise feedbacks for each species pair (Box 3.3b,c) using *a priori* contrasts within the soil source x seedling species interaction in the mixed effects model described above, using the glht function in the R package "multcomp." Pairwise feedbacks were calculated as described in Bever et al. 1997, using the interaction coefficient, defined as $I_s = Biomass(A)_a - Biomass(A)_b - Biomass(B)_a + Biomass(B)_b$. This compares the difference in biomass for species A and B in home and away soil sources.

Finally, we constructed heat maps to compare proportional differences in seedling biomass in each soil source and in soil from each individual focal tree. To facilitate intra- and interspecific comparisons among species that varied greatly in size (both in terms of

mean biomass and range, Table 3.3), we standardized biomass relative to the average biomass for that species across all treatment soils according to the formula $[(A_i - A_{avg})/A_{avg}]*100$ (Brinkmann 2010). Analyses were performed in JMP (JMP Pro 12.0.1, SAS Institute Inc., Cary, NC, USA) and R (R core team, 2014).

RESULTS:

HOST-ASSOCIATED VARIATION IN *STREPTOMYCES* DENSITIES AND PATHOGEN-SUPPRESSIVE PHENOTYPES: Total *Streptomyces* densities in soils from the 25 focal trees varied by almost two orders of magnitude, ranging from 4.79×10^4 to 6.49×10^6 CFU/g soil (mean = 6.49×10^5 CFU/g soil). Inhibitor densities were slightly more variable, ranging from 1.72×10^3 to 7.39×10^5 CFU/g soil against the bacterial plant pathogen *S. scabies* (mean = 6.14×10^4 CFU/g soil) and 4.00×10^3 to 4.24×10^5 CFU/g soil against the fungal plant pathogen *F. oxysporum* (mean = 4.20×10^4 CFU/g soil). The average proportions of *Streptomyces* capable of inhibiting *S. scabies* and *F. oxysporum* were 11% (range: 3.5-29%) and 9.6% (range: 2.2%-51%), respectively. Overall, soils with high densities of bacterial inhibitors against *S. scabies* also had high densities of fungal inhibitors against *F. oxysporum* ($R = 0.75$, $p = 0.0002$, $N = 25$). Inhibition zone sizes against the two pathogens were also positively correlated among samples (Spearman: $R = 0.44$, $p = 0.024$). This suggests that soil communities capable of inhibiting one plant pathogen may also be able to suppress diseases caused by other pathogens in soil.

There was substantial intra- and interspecific variation in the pathogen-suppressive capacities of *Streptomyces* communities associated with individual focal

trees (SI Fig. 3.1a, Table 3.2). Differences among tree species in zone sizes against *S. scabies* were marginally significant ($F_{4,20} = 2.74$, $p = 0.059$) and were highest in *Apeiba* soils and lowest in *Protium* soils (Tukey HSD, $p < 0.05$; Fig. 3.1a). Densities of *Streptomyces* capable of inhibiting *S. scabies* were also marginally significantly different among soil sources ($F_{4,20} = 2.60$, $p = 0.067$), with the highest densities in soils from *Protium* trees and the lowest densities in *Ormosia* soils (Tukey HSD, $p < 0.05$; Fig. 3.1b). *Ormosia* soils harbored the fewest total *Streptomyces* as well as the lowest densities of fungal inhibitory *Streptomyces*, but inhibitors in *Ormosia* soils produced the largest inhibition zone sizes against the fungal pathogen (Fig. 3.1a). Similarly, the smallest inhibition zone sizes against the bacterial pathogen were observed in *Protium* soils, which were associated with the highest densities of bacterial inhibitors. There were no significant differences in total *Streptomyces* densities, inhibitor proportions, or inhibition zone sizes against *F. oxysporum* among soils from the five tree species (1-way ANOVA, $p < 0.05$). Among soils associated with individual focal tree species, there were substantial differences in the inhibitory capacities of *Streptomyces* against the two plant pathogens in *Apeiba* soils (Fig. 1a; inhibition zone sizes) and *Protium* soils (Fig. 3.1b; inhibitor densities); in both cases, pathogen suppression was greater against the bacterial pathogen than the fungal pathogen.

Only one tree (an *Ormosia*) was associated with soils in which inhibitors of *S. scabies* were never detected. Against the fungal pathogen, soils from four out of 25 trees lacked pathogen-suppressive potential (soils from *Protium*, *Apeiba,* and two *Tabebuia* trees). However, a lack of inhibitors on dilution plates does not necessarily imply zero inhibitors in an entire gram of soil. For these soils, predicted inhibitor densities per gram

soil (Fig. 3.1b) reflect the limit of detection of this assay, which depends on the particular dilution used for each sample.

BIOASSAYS OF SOIL DISEASE POTENTIAL: Estimates of soil disease potential varied within and among tree species and across the three seedling species (SI Fig. 3.1b, SI Fig. 3.2). Average disease severity and disease index values were positively correlated for *Luehea* and *Ochroma* seedlings among all soils ($R = 0.43$, $p = 0.03$). The severity of disease symptoms varied among the three seedling species (2-way ANOVA, species main effect: $F_{2, 322} = 115.85$, $p < 0.0001$) and was inversely correlated with seed size (Fig. 3.2a). Disease was significantly higher among *Luehea* seedlings (Tukey HSD, $p < 0.05$), which had the smallest seed mass and on average 2-3x higher disease severity scores than the other species.

The effect of seedling species (and thus seedling size) on the severity of disease symptoms was modified by soil source (2-way ANOVA, seedling species x soil source interaction; Fig. 3.2b). Among *Apeiba* seedlings, which had the largest seeds and the lowest disease overall, the effect of the species x soil interaction increased disease severity in *Ormosia* soils (positive parameter estimate = 0.19 ± 0.06; significantly different from 0). Among *Luehea* seedlings, which had the most disease overall, the species x soil interaction reduced disease severity in *Tabebuia* soils (negative parameter estimate = -0.22 ± 0.06; significantly different from 0).

RELATIONSHIPS BETWEEN PATHOGEN SUPPRESSION AND SOIL DISEASE POTENTIAL: Overall, there were no significant correlations among *Streptomyces* community characteristics and

metrics of soil disease potential among the 25 focal trees. However, the average disease index (averaged across the three RT seedling species) was negatively correlated with mean inhibition zone sizes against bacterial and fungal pathogens in *Tabebuia* soils (Spearman R = -0.90, p = 0.037, N = 5) as well as mean inhibitor densities in *Apeiba* soils (Spearman R = -0.90, p = 0.037, N =5), indicating a general trend of less soil-borne disease in soils with more inhibitors or more intense antibiotic production. When bacterial and fungal pathogen overlays and RT indicator species were examined separately, soils from *Tabebuia*, *Apeiba*, and *Ormosia* trees with higher densities of fungal inhibitors were associated with lower disease indices against at least one of the three seedling species (Fig. 3.3). In *Protium* soils, we found a positive correlation between densities of bacterial inhibitors and the disease index on *Luehea* seedlings (Spearman R = 0.90, p = 0.037, N = 5), but no significant relationships between disease potential and suppression of the fungal pathogen. Relationships between pathogen suppression and disease potential were never significant in *Virola* soils.

SOIL ABIOTIC SOIL CHARACTERISTICS, PATHOGEN-SUPPRESSIVE CAPACITIES, AND DISEASE POTENTIAL: Soil C, N, and nutrient availability varied among soils from focal trees but were not consistently related to tree species (SI Fig. 3.1c; SI Table 3.6). In particular, soil P varied by over an order of magnitude and soil K varied 4.5-fold among soils from the 25 host trees. There were no significant differences in soil moisture concentrations in soils from the five tree species at the time of soil collection (SI Table 3.6). Average disease index values were higher in soils with higher soil moisture content (N = 25; R =

0.45, p = 0.026). Inhibitory capacities of *Streptomyces* communities were not related to soil moisture (p > 0.05).

There were higher *Streptomyces* densities in soils with higher soil K (N = 25; R = 0.42, p = 0.036). Bacterial inhibitors also increased with increasing soil K (R = 0.40, p = 0.046) but produced smaller inhibition zones (R = -0.36, p = 0.087). Average soil disease potential (disease index) increased with soil C (N = 25; R = 0.51, p = 0.0091) and N (R = 0.46, p = 0.019) but was not significantly related to soil P or K availability (p > 0.05). When soils from each tree species were examined individually (N = 5), soil disease potential was highly positively correlated with soil C and N in *Virola* soils (R = 0.92 and p = 0.028 for C; R = 0.92 and p = 0.029 for N) and these trends were marginally significant in *Ormosia* soils (p < 0.07 for both C and N). No significant relationships were detected in soils from the other tree species (p > 0.05).

SEEDLING BIOMASS AND PLANT-SOIL FEEDBACKS: Average seedling biomass after six months of growth in the shadehouse experiment varied significantly among seedling species (Table 3.3). Biomass of *Tabebuia* and *Virola* seedlings varied 25-fold and 12-fold, respectively, across soils from the 25 focal trees. *Protium* seedlings varied least in size (≈ 3-fold variation), compared to roughly 5-fold variation for *Ormosia*, and 8-fold variation for *Apeiba*. There was a significant interaction between the effect of seedling species and soil source on seedling biomass (base model in Table 3.4, significant species x soil source interaction), which suggests the potential for plant-soil feedbacks. In particular, *Virola* seedlings grown in *Virola* soils were significantly larger than all other seedling species x soil combinations (Tukey HSD, p < 0.05). *Tabebuia* seedlings had

significantly lower biomass when grown in *Tabebuia* soils than all other seedling species x soil source combinations (Tukey HSD, $p < 0.05$). These results suggest positive and negative feedbacks for *Virola* and *Tabebuia*, respectively. To further explore the possibility of feedbacks as well as other soil effects on seedlings, we calculated direct and pairwise feedbacks, which focus on differences in seedling biomass between home and away soils (see Box 3.3), as well as a measure of relative biomass for each seedling to allow for comparisons of proportional differences in biomass among species and soil sources, regardless of home vs. away classifications.

We calculated direct feedbacks for each seedling species in each heterospecific soil source, as well as the average direct feedback value across all heterospecific soils. Average direct feedback values ranged from negative to neutral to positive (Table 3.5, "home vs. away"). *Virola* seedlings were consistently almost twice as large when grown in soils from *Virola* trees than in soils from any other species, thus the average feedback value reflected a consistent home soil advantage. For the other four species, however, average feedback values obscured variation in soil effects across different heterospecific tree species and did not reflect consistent trends between home and away soils (Table 3.5).

Tabebuia seedlings were consistently associated with negative direct feedback values (higher biomass in soils from other species), but the "away" soil advantage was more than twice as strong in *Ormosia* soils than in *Virola* soils. Feedback values for *Apeiba* seedlings varied 8-fold across heterospecific soil sources, ranging from neutral/no feedback in *Ormosia* soils to strongly negative feedback in *Virola* soils. For *Ormosia* seedlings, direct feedbacks varied in both strength and direction depending on the

heterospecific soil source. Relative to *Ormosia* biomass in home soils, seedlings were roughly 20% smaller when grown in *Tabebuia* soils (consistent with positive feedback) but were approximately 30% larger when grown in *Virola* soils (consistent with negative feedback). While individually these values suggest feedbacks (positive and negative, respectively), collectively they provide no biological support for plant-soil feedback in *Ormosia* soils, only considerable variation in the effects of heterospecific soils on *Ormosia* biomass. *Protium* seedlings varied least in biomass overall, and soil effects were relatively weak regardless of the specific heterospecific soil source (values all near 0; Table 3.5).

To assess whether soil effects on seedling biomass were species-specific, we calculated pairwise feedbacks (see Box 3.3), which can shed light on the potential for soils to influence species coexistence. Pairwise feedbacks varied considerably in strength and direction and were significantly different from zero for two species pairs (Fig. 3.4). *Virola* soils appeared to have a stronger effect on *Virola* seedlings than on *Protium* seedlings (Fig. 3.5a), which resulted in the significant positive pairwise feedback for these species. By contrast, *Virola* soil conferred a similar biomass advantage to both *Virola* and *Apeiba* seedlings relative to biomass in *Apeiba* soils (Fig. 3.5b), therefore the pairwise feedback for this species pair was not significant. There was a significant negative pairwise feedback for *Ormosia* and *Tabebuia* because although seedlings of both species had higher biomass in *Ormosia* soils than in *Tabebuia* soils, the proportional difference in biomass between home and away soils was much greater for *Tabebuia* seedlings (Fig. 3.5c).

Significant pairwise feedbacks suggest specificity in soil effects between individual species pairs, but the overall specificity of *Tabebuia* and *Virola* feedbacks remained unclear. Standardizing seedling biomass from each individual pot by the overall average per species allowed us to address this issue by comparing variation in relative seedling biomass across the five soil sources and the 25 focal trees (Fig. 3.7). For four of the five species, the average seedling biomass in conspecific soils masked considerable intraspecific variation in responses to soils from individual focal trees (Fig. 3.7a vs. b; home soils indicated by yellow border). The one exception was *Virola* seedlings in *Virola* soils, which is consistent with the positive feedback detected for this species. Because comparisons were not limited to home vs. away soils (as in direct and pairwise feedbacks), we were able to assess overall specificity of this feedback by asking: Do other species also tend to do better in *Virola* soils? Consistent with the increased biomass of *Virola* seedlings in *Virola* soils, *Apeiba* and *Ormosia* seedlings also did better in *Virola* soils relative to species-level averages (Fig. 3.7a). However, relative increases in the biomass of heterospecific seedlings in *Virola* soils were generally smaller and/or less consistent (observed in soils from fewer *Virola* trees; Fig. 3.7b) than biomass responses of *Virola* seedlings to the same soils. *Protium* exhibited a significant positive pairwise feedback with *Virola* (Fig. 3.5a), but analysis of relative biomass revealed that *Protium* seedlings did at least as well or better than the species average in three of the five *Virola* soils (Fig. 3.7, light grey and green bars, respectively).

DO *STREPTOMYCES* COMMUNITIES AFFECT SEEDLING BIOMASS AND CONTRIBUTE TO PLANT-SOIL FEEDBACKS? To test whether *Streptomyces* characteristics (total *Streptomyces*

density, inhibitors of *S. scabies*, inhibitors of *F. oxysporum*, and inhibition zone sizes against these two strains) were associated with plant-soil feedbacks, we independently incorporated each *Streptomyces* variable into the mixed effects model of log-transformed seedling biomass used to calculate pairwise feedbacks (Table 3.4). Models that incorporated *Streptomyces* variables as covariates were always significantly better than the base model without an added *Streptomyces* term (SI Table 3.7). Relationships between *Streptomyces* and seedling biomass varied by seedling species and soil source (soil x seedling x *Streptomyces* interactions; Table 3.4), indicating that the effects of *Streptomyces* communities on biomass depended on both the seedling species and the soil source in which seedlings were planted. Pairwise feedbacks were calculated within the two-way soil x seedling interaction, therefore significant three-way interactions (soil x seedling x *Streptomyces*) suggest that *Streptomyces* densities or pathogen-suppressive capacities have the potential to influence plant-soil feedbacks among these species.

We used parameter estimates to examine the slopes of relationships between seedling biomass and *Streptomyces* community characteristics for each seedling species in each soil source (Fig. 3.6). A significant *Streptomyces* effect means that the parameter estimate (reflecting the slope of the relationship with biomass) was significantly different than zero. Relationships between seedling biomass and *Streptomyces* densities, inhibitor densities, and zone sizes were usually similar (SI Table 3.8), however the direction and significance of relationships sometimes differed between inhibitor densities of bacterial and fungal pathogens (Fig. 3.6a vs. b). For simplicity, in the text below we focus on relationships between biomass and total *Streptomyces* density (Fig. 3.6c).

Streptomyces density had significant effects on seedling biomass in seven out of 24 soil source x seedling species combinations, four of which were positive in direction, reflecting larger seedlings in soils with greater *Streptomyces* densities. The other three significant *Streptomyces* effects were negative *(*for *Tabebuia* and *Virola* seedlings), meaning seedlings were larger in soils with fewer total or pathogen-suppressive *Streptomyces*. Significant relationships between *Streptomyces* and seedling biomass were not evenly distributed among soil sources or seedling species (Fig. 3.6), consistent with the significant soil source x seedling species x *Streptomyces* interactions (Table 3.4). In *Tabebuia* soils, *Streptomyces* densities were significantly related to seedling biomass for three of the five species, but in soils from *Protium* trees *Streptomyces* characteristics were never related to biomass of any seedling species. Across the five soil sources, significant *Streptomyces* effects were most common among *Tabebuia* seedlings (significant relationships with biomass in three of the five soil sources), were less common for *Virola, Ormosia*, and *Protium* seedlings, and were never detected among *Apeiba* seedlings in soils from any of the five tree species. Seedlings also varied in their sensitivity (responsiveness) to *Streptomyces* communities. For a given change in *Streptomyces* densities, *Tabebuia* seedlings tended to exhibit a greater change in biomass than other seedling species (steeper slopes in SI Fig. 3.4; larger parameter estimates in Fig. 3.6).

DISCUSSION:

In order to better understand how microbes influence diversity in tropical forests, we need to move beyond the assumption that feedbacks are driven solely by one-to-one

interactions between plants and specialized pathogens or mutualists (Box 3.1) and consider the potential importance of multiple interacting soil functional groups as well as environmental heterogeneity in microbe-microbe and plant-microbe interactions. Our results suggest that antibiotic-producing bacteria and their potential for interactions with soil-borne pathogens are spatially heterogeneous, and may promote variation in seedling biomass for some seedling species in certain soil sources. By modifying the effects of soils on tropical seedlings in a context-dependent manner (as opposed to a strictly host-specific manner), pathogen-suppressive *Streptomyces* have the potential promote variation in plant-soil feedbacks in tropical forests.

VARIATION IN PLANT-SOIL FEEDBACKS AMONG TREE SPECIES: Plant-soil feedbacks varied among tree species. We found strong evidence for positive feedback in *Virola surinamensis* as well as support for negative feedback in *Tabebuia guayacan*. That is, *Virola* seedlings had significantly higher biomass when grown in soils collected beneath *Virola* trees than in soils from other tree species (positive feedback), whereas *Tabebuia* had higher biomass when grown in soils from heterospecifics than in soils from *Tabebuia* trees (negative feedback).

It was difficult to determine whether the positive and negative feedbacks were specific to *Virola* and *Tabebuia* seedlings relative to biomass responses of other seedling species in the same soils. There were two primary reasons for this: First, focal species varied greatly in average biomass and range (Table 3.3), which made significance of the intra- and interspecific comparisons that generate pairwise feedbacks difficult to interpret (see Fig. 3.5). Second, in most cases, the strength and direction of soil effects on

seedlings varied across heterospecific tree species (Table 3.5, Fig. 3.5 a vs. b), which meant that conclusions regarding feedback specificity changed depending on which heterospecific soil source was used in the home vs. away comparison. To address these issues we calculated a measure of relative seedling biomass to compare proportional differences in seedling size across all soil sources simultaneously, which suggested that feedbacks were somewhat species-specific, in that that they affected *Virola* and *Tabebuia* seedlings more strongly than other species.

One possible explanation for the positive feedback for *Virola* relates to effects of arbuscular mycorrizal fungi (AMF) in *Virola* soils. Previous work on BCI found that *Virola* seedlings were smaller when exposed to AMF communities in soils from parent trees, but benefitted from AMF in soils from conspecific, non-parent trees (Eck, unpublished data). The negative feedback in parent soils could be due to lower AMF colonization or more detrimental or less beneficial effects of the colonizing AMF relative to AMF communities from non-parent soils. In our study, the increased biomass of *Virola* seedlings grown in conspecific soils was consistent across the five *Virola* trees (Fig. 3.7b). Other species also did better in *Virola* soils, but to a lesser degree than *Virola* seedlings. These results are consistent with benefits of *Virola* AMF to all species, but with the greatest benefit conferred on *Virola* seedlings.

POSSIBLE EFFECTS OF *STREPTOMYCES*, PATHOGENS, AND STREPTOMYCES-PATHOGEN INTERACTIONS ON SEEDLING BIOMASS AND DISEASE: There were significant three-way interactions between seedling species, soil source, and *Streptomyces* (total densities and pathogen-suppressive capacities) on seedling biomass. Slopes of relationships between

seedling biomass and densities of pathogen-suppressive *Streptomyces* were significantly different than zero in roughly one fourth of seedling x soil combinations. We cannot determine causality from significant associations between *Streptomyces* densities and biomass, but we discuss several possibilities. One explanation for our results is that *Streptomyces* communities differentially affect seedling biomass depending on the species and soil source. Alternatively, seedling size could influence the densities of *Streptomyces* associated with plant roots (e.g. larger root systems, greater carbon inputs, higher *Streptomyces* densities and more antagonistic interactions).

Although *Streptomyces* could influence seedlings directly, for example through plant growth-promoting hormones or signaling molecules that promote seedling growth or activate defense pathways, our results suggest that *Streptomyces* effects on biomass are related to antibiotic-mediated interactions with other soil microbes. Relationships between seedling biomass and the pathogen-suppressive capacities of *Streptomyces* sometimes differed between the two pathogens used in this study, suggesting specificity in the effects of antibiotics in soil. Furthermore, *Streptomyces* had both positive and negative correlations with seedling biomass, which is consistent with the possibility of antibiotic-mediated inhibition of pathogens and mutualists, respectively (see Box 3.2). Negative relationships with biomass (i.e. smaller seedlings associated with more pathogen-suppressive *Streptomyces*) could also reflect direct effects of pathogenic *Streptomyces* on seedlings, or seedlings recruiting antagonists of pathogens after being infected (Dudenhoffer et al. 2016). Variation in responses of seedling species to pathogen-suppression of the two indicator strains (*S. scabies* and *F. oxysporum*) could reflect differences in the importance of antibiotic-mediated suppression of bacteria vs.

fungi, or relate to the suppression of indigenous *F. oxysporum* isolates in these soils. Pathogens in the genus *Fusarium* have been implicated in plant-soil feedbacks and patterns of negative density dependence in tropical forests (Gallery et al. 2007, McCarthy-Neumann Kobe 2008), and *F. oxysporum* in particular has been shown to promote distance-dependent seedling mortality and Janzen-Connell spatial distributions in soils from a congener of the *Ormosia* species used here (Liu et al. 2012, 2015, 2016). We did not measure the abundance or composition of pathogens in soil, however we used a rolled-towel (RT) bioassay developed in agricultural systems to estimate soil disease potential, which should capture variation in the potential effects of pathogens in different soils.

We found substantial variation in the net effects of soils associated with individual focal trees and tree species on the development of seedling disease (SI Fig. 3.2). Although estimates of soil disease potential were influenced most strongly by size-related variation in the susceptibility of seedling species to disease (Fig. 3.2a), the significant interactive effect of seedling species x soil source on disease severity (Fig. 3.2b) suggests that soils associated with some tree species have the potential to influence the earliest stages of seedling recruitment, when seedlings may be most vulnerable to soil pathogens and/or most reliant on soil mutualists. In soils from *Ormosia* and *Tabebuia* trees, levels of disease against the three seedling species deviated from expectations based on seed size alone (Fig. 3.2b). There were also significant relationships between soil disease potential and pathogen suppressive capacities in these soils (Fig. 3.3a,c), which suggests that the net effect of *Ormosia* and *Tabebuia* soils on seedling performance may result from microbial interactions. In addition to influencing disease

and survival of recently germinated seedlings, results from the shadehouse experiment suggest that pathogen-*Streptomyces* interactions in these soils may continue to play a role in performance of seedlings once they have successfully established.

Streptomyces densities (total and inhibitors only) were positively correlated with *Ormosia* and *Tabebuia* biomass in *Tabebuia* soils (Fig. 3.6b,c), which is consistent with the reduction in disease severity in *Tabebuia* soils in the RTs (Fig. 3.2b, soil x seedling interaction). By contrast, responses of *Apeiba* seedlings to *Ormosia* soils were not consistent between the RTs and shadehouse experiment, suggesting that the net effect of microbial interactions on seedlings may change over time (Kardol et al. 2013). *Apeiba* seedlings suffered significantly more disease in *Ormosia* soils than in *Apeiba* soils in the RTs (positive feedback, home soil advantage; SI Fig. 3.3), but in the shadehouse biomass of *Apeiba* seedlings grown in these soils was similar after six months (Table 3.5), suggesting that the effect of *Ormosia* soils shifted from a negative effect on survival of early *Apeiba* germinants (≈ 2 weeks old) to a neutral or weakly positive effect on young, recently established *Apeiba* seedlings (≈ 7 months old). Because the same soils were used in both the RTs and the shadehouse experiment, this suggests that shifts in feedback strength and direction resulted from changes in how seedlings responded to the same microbial community at different life stages. It is also possible that *Apeiba* seedlings influenced pathogen-suppressive capacities of their *Streptomyces* communities over the course of the six-month experiment, which was not measured in the current study.

These results suggest the importance of considering seed size and seedling growth rate in feedback experiments. In the shadehouse experiment, the smallest-seeded species (*Tabebuia*) experienced negative feedback and the largest-seeded species (*Virola*)

experienced positive feedback, which is consistent with the negative correlation between seed size and disease observed in the RTs (higher disease severity in smaller seeded species; Fig. 3.2a). Large-seeded species may be more tolerant to stressful conditions (fecundity-stress tolerance tradeoff; Muller-Landau 2010), better defended against soil pathogens, and less affected by nearby conspecifics as young seedlings (Lebrija-Trejos et al. 2016). Tradeoffs related to seed size and initial seedling biomass may become less important over time (Dalling and Hubbell 2002), which could explain why recently germinated *Apeiba* seedlings responded differently to *Ormosia* soils than six month old *Apeiba* seedlings exposed to the same soil communities. Large-seeded species tend to have greater seed reserves and larger initial biomass, potentially making them better able to tolerate or recover from interactions with plant pests. We found that belowground disease symptoms were most common among *Virola* seedlings at harvest (necrotic root tissue observed in 17/27 seedlings scored), therefore it is possible that compensatory growth in response to infection contributed to the positive feedback for this species.

DO *STREPTOMYCES* EFFECTS ON SEEDLING BIOMASS HAVE THE POTENTIAL TO MEDIATE PLANT-SOIL FEEDBACKS? Traditional feedback models and the Janzen-Connell hypothesis assume that specificity in soil effects on seedlings is driven by the effects of specialized pathogens in home soils and escape from those pathogens in soils from other tree species (Box 3.1a). If *Streptomyces* were highly coevolved with plant hosts, they might also have significant effects on seedling biomass in home soils, potentially generating plant-soil feedbacks independently of other microbes in soil (as in Box 3.1b). All but one of the instances in which *Streptomyces* densities were significantly related to seedling

biomass occurred in soils from heterospecific tree species ("away" soils). Thus, by promoting variation in seedling responses to heterospecific soils (see Box 3.2*d-f*), *Streptomyces* may effectively dilute the importance of specialized microbes in home soil and in doing so influence the net effect of feedbacks on plant communities.

We did not experimentally modify *Streptomyces* communities and therefore we cannot directly compare how net feedbacks experienced by seedlings would change with and without the effects of *Streptomyces* (as in Box 3.2). However, we can speculate on possible outcomes based on the strength of associations observed between *Streptomyces* characteristics and seedling biomass combined with predictions about how different types of *Streptomyces*-seedling interactions would affect feedbacks. The direction (positive or negative) and location (home soil, away soil, or both) of these relationships provide insight into possible effects of *Streptomyces* on feedback strength (Box 3.2), and the specificity of *Streptomyces* effects – which seedling species are affected and to what degree – will determine the implications for plant community composition (Box 3.3). Collectively, the significant relationships between *Streptomyces* and seedling biomass in Figure 3.6 suggest that without *Streptomyces*, the positive feedback for *Virola* and the negative feedback for *Tabebuia* might be stronger and more species-specific than those observed in this study.

OPENING THE SOIL BLACK BOX IN TROPICAL FORESTS: IMPLICATIONS FOR FEEDBACK EXPERIMENTS: Analyses of home vs. away feedbacks may be limiting if the goal is to open the microbial black box and identify mechanisms underlying plant-soil feedbacks. Many feedback calculations in the literature pool seedling responses in multiple

heterospecific soils into one category and focus only on the comparison of growth in home soils to average growth in all other soils. Home vs. away approaches ignore or obscure variation in soils conditioned by different heterospecific tree species and the potential for unique responses of seedlings to such variation. We found that biomass of individual seedling species often varied substantially when grown in heterospecific soils from different species, but the associated variability in feedbacks was masked by averaging across heterospecific soil sources (Table 3.5), consistent with a recent feedback meta-analysis (Kulmatiski et al. 2016).

Soils beneath individual trees of the same species also varied in their effects on seedlings, which suggests the importance of incorporating host-associated variation in soils and plant-microbe interactions into feedback experiments (McCarthy-Neumann et al. 2008, Liu et al. 2015). Soils are very heterogeneous (Ettema 2002) and variation in biotic and abiotic soil characteristics can affect plant competitive abilities, disease, and the outcome of plant-soil feedbacks (Abbott et al. 2015, Hendriks et al. 2015, Wubbs and Bezemer 2016). Our results emphasize that variation in soil effects on biomass also occurs within individual tree species (Fig. 3.7 a vs. b), which can result from site differences from tree to tree (Kuebbing et al. 2015) or intraspecific variation in host-microbe interactions (McCarthy-Neumann and Kobe 2008, Wagg and Van der Heidjen et al. 2014, Liu et al. 2015). Nevertheless, many feedback experiments conducted in the greenhouse use soils that have been pooled by plant species, thus homogenizing microbial variation associated with individual trees. This can result in artificially low variability in biomass among seedlings grown in soils from the same host species, leading

to unrealistically precise estimates of feedbacks that have a higher likelihood of being detected as significant (Reinhart and Rinella 2016).

The positive feedback for *Virola* contrasts with Mangan et al. (2010a), who demonstrated species-specific negative plant-soil feedback for *Virola surinamensis* at this field site. However, Mangan et al. used an inoculation approach, which controls for differences in abiotic properties among soils but can dilute the effects of soil microbes. Approaches that use soil inoculum rely on microbial recolonization of sterilized treatment soils from a very small volume of live, field soil, which biases communities towards fast-growing microbes and likely results in soils that are not representative of microbial densities and relative abundances in the field (Brinkmann et al. 2010, Van der Voorde et al. 2012). The potential effects of *Streptomyces* on plants and pathogens are highly density dependent (Kinkel et al. 2011), both in terms of total *Streptomyces* densities as well as total bacterial and fungal abundance. Therefore, to more closely replicate the microbial communities experienced by seedlings in the field we used live soil mixed with sand. As a result, we cannot isolate abiotic and biotic effects of soil on seedling performance. Isolating independent contributions of biotic and abiotic soil components was a necessary step toward establishing a role for soil microbes in diversity-promoting plant-soil feedbacks, but the next challenge is to parse out the microbial interactions and environmental influences that interactively shape the net effect of soil on seedlings that is measured in feedback experiments.

Our findings suggest that effects of *Streptomyces* on plant-soil feedbacks at this site are likely to be context dependent rather than strictly host specific. Soil heterogeneity in antibiotic-producing *Streptomyces* suggests that *Streptomyces*

communities are locally adapted but that individual plant hosts are not necessarily the strongest factor structuring inhibitory phenotypes. This is consistent with previous work in a tropical dry forest in which we found that site differences in soil nutrient availability tended to have greater effects on *Streptomyces* community function than individual tree species (Becklund et al. 2016). Results of the current study emphasize the potential importance of biotic-abiotic interactions in generating net plant-soil feedback effects, consistent with other studies (Perkins and Nowak 2013, Revillini et al. 2016). Soil C and nutrients were not significantly different among tree species and were not directly related to seedling biomass, which suggests that they do not directly affect plant-soil feedbacks. However, soil resource availability could influence plants indirectly through plant-microbe and microbe-microbe interactions. Soil disease potential was higher in more fertile soils (C and N in particular) and inhibitor densities were higher in soils with more available K, both of which could contribute to variation in the strength of relationships between pathogen suppression, disease potential, and seedling biomass among soils.

We found that *Streptomyces* are unlikely to have predictable, directional effects on tropical seedlings, however they have the potential to influence community composition by mediating interactions between plants and soil-borne pathogens in ways that promote variability in pairwise feedbacks between tree species. This is consistent with complex plant-soil feedbacks, in which microbes may influence plant diversity by increasing variability in recruitment success and thereby delaying community convergence (Fukami et al. 2013). Manipulative experiments that alter various components of the soil community are needed to tease apart microbial interactions underlying plant-soil feedback effects, but our results provide proof of concept that

pathogen-suppressive *Streptomyces* have the potential to influence plant-soil feedbacks in this tropical forest.

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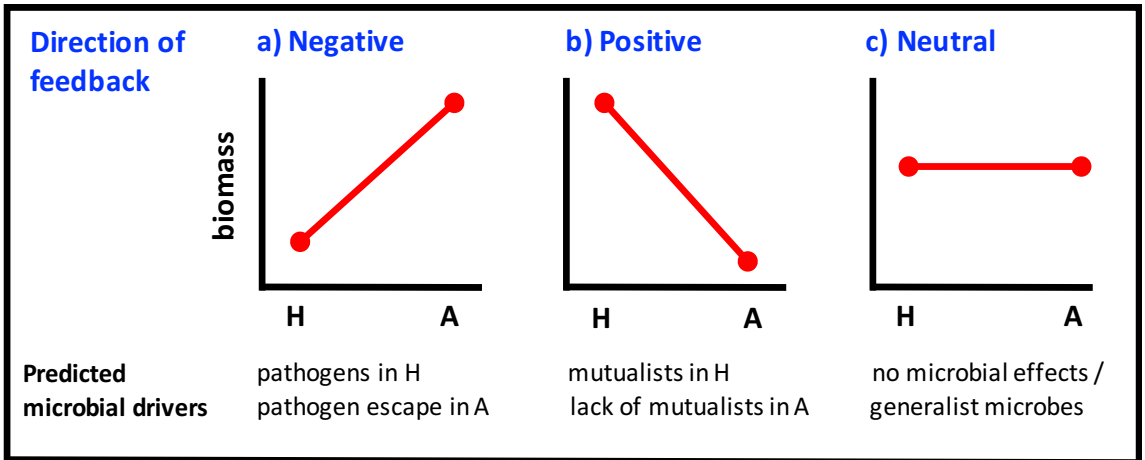
BOX 3.1. 1:1 PLANT-SOIL FEEDBACKS AND TRADITIONAL ASSUMPTIONS

Plant-soil feedbacks develop when plants influence microbial community composition and/or activity, and these changes positively or negatively affect performance of the host plant or its offspring. Feedbacks are typically measured by comparing seedling performance (usually biomass or mortality) in home and away soils (also referred to as "self" and "other"). 1:1 refers to the assumption that feedbacks result from interactions between plant hosts and a singular microbial functional group (i.e. pathogens, mutualists), in which microbial interactions are generally not considered. The Janzen Connell hypothesis and 1:1 feedback models typically assume that the net effect of soils on seedlings is due to the presence or absence of specialized soil microbes that accumulate beneath parent trees. The identity of the predicted microbial drivers of feedback depends on the direction of feedback observed (positive [a], negative [b], or neutral/no feedback [c]).

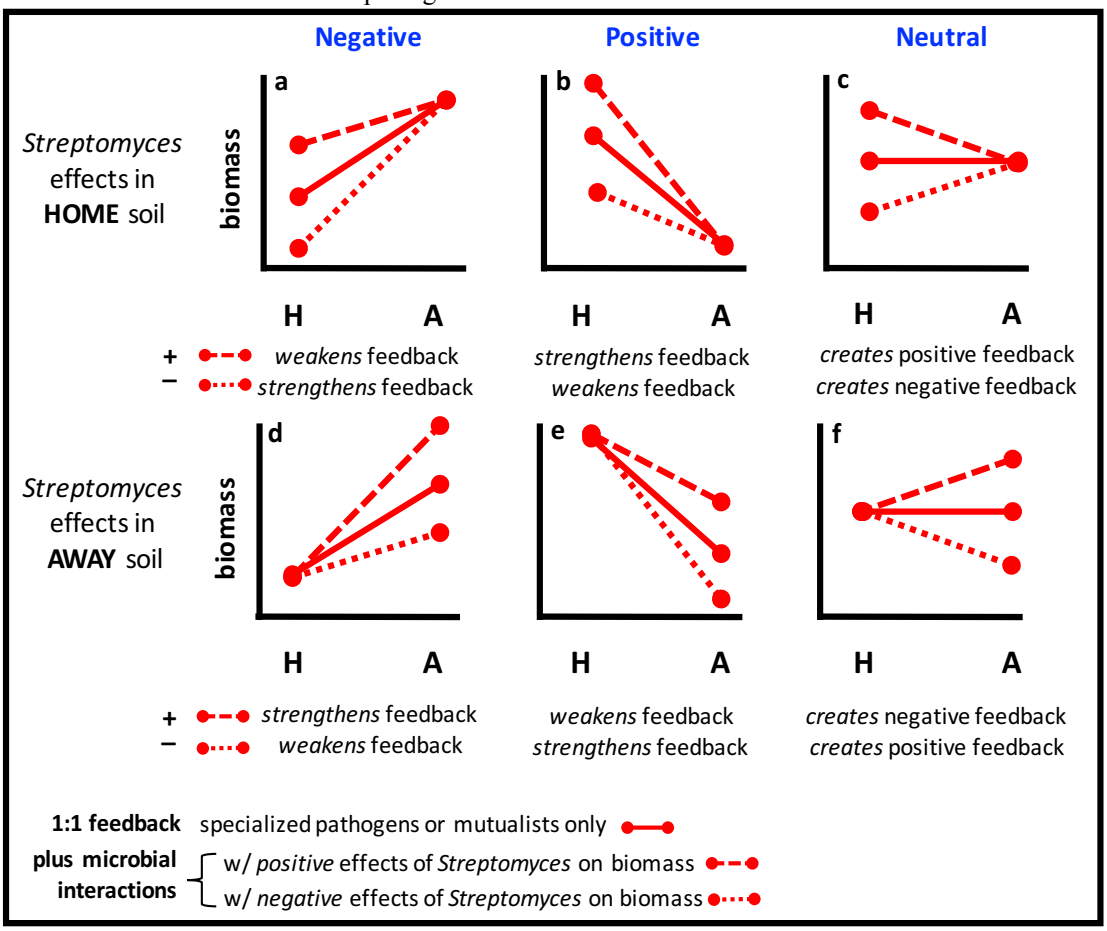


1:1 plant-soil feedbacks and assumed microbial drivers. Seedling biomass is compared between home soil ("H"; beneath conspecific adults) and away soil ("A"; beneath heterospecific adults). The null expectation is that feedbacks will be similar regardless of the heterospecific soil used for comparison. This is not expected to be the case, however, if the effects of specialized pathogens (a) or mutualists (b) are moderated by interactions with other soil microbes that are spatially heterogeneous but are not necessarily under strong selection by plant hosts. In such cases, microbial interactions may promote variation in plant-soil feedbacks by affecting the difference in seedling biomass between home and away soils. See Box 2 for examples.

There is a rich body of theory linking home and away feedbacks and predicted microbial drivers to mechanisms of species coexistence, however soil is often still viewed as a black box. The patterns of seedling biomass shown above could also be generated by biotic or abiotic interactions that do not involve the biological process of plant-soil feedbacks. For example, panel a) could result from interactions between plants and beneficial microbes in away soils and be unrelated to the microbes associated with home soils, creating the appearance of feedback when none exists.

BOX 3.2. HYPOTHESIZED MECHANISMS BY WHICH ANTIBIOTIC-PRODUCING *STREPTOMYCES* COULD PROMOTE VARIATION IN PLANT-SOIL FEEDBACKS

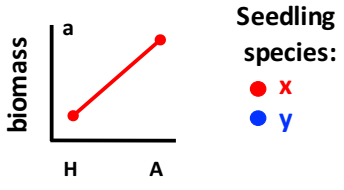
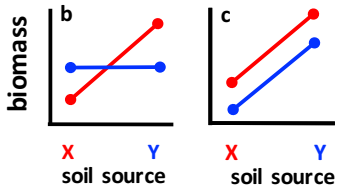
Possible effects of antibiotic-producing *Streptomyces* on plant-soil feedbacks are shown relative to 1:1 feedbacks which assume no microbial interactions. *Streptomyces* have the potential to exacerbate or ameliorate existing feedbacks (panels *a,b,d,f*) or create the appearance of feedback when none exists (panels *c,f*). These outcomes depend on the direction and location of *Streptomyces* effects on seedling biomass. *Streptomyces* could increase seedling biomass (positive effects; dashed lines), which could happen through pathogen suppression and reduced seedling disease, or through direct effects of plant growth-promoting hormones produced by *Streptomyces*. *Streptomyces* could also have negative effects on seedling biomass (dotted lines), for example if antibiotics suppress mutualistic microbes instead of pathogens.



Potential effects of *Streptomyces* on plant-soil feedbacks: Solid lines reflect 1:1 feedbacks resulting from effects of specialized microbes alone (as in Box 3.1), and dashed lines illustrate how *Streptomyces* could influence feedback strength and direction through positive or negative effects on seedling biomass (dashed vs. dotted lines, respectively). Columns indicate the direction of the 1:1 feedback (negative, positive, or neutral/no feedback). Rows show *Streptomyces* effects on seedling biomass in home soil ("H"; *a-c*) and away soils ("A"; *d-f*). Text beneath each panel indicates the net effect on feedback. *Streptomyces* effects on biomass that are equivalent in magnitude have contrasting effects on feedbacks depending on whether effects are positive or negative, whether they occur in home or away soils, and whether the underlying feedback is positive, negative, or neutral. *Streptomyces* could also have simultaneous effects on biomass in both home and away soils (not shown), further complicating the potential mechanisms by which *Streptomyces* could modify the net feedback effect experienced by seedlings.

BOX 3.3. COMPARISON OF TWO FEEDBACK METHODS USED IN THIS STUDY

Direct feedbacks measure the difference in seedling biomass between home and away soils for one focal species. *Pairwise feedbacks* compare relative differences in biomass between soils for two seedling species and thus determine whether the net feedback is species-specific. The specificity of feedbacks refers to whether the net effects of soils are similar for different seedling species. In a species-specific feedback, soils have a disproportionately stronger effect on con- or heterospecific seedlings (slopes of red and blue lines differ, *panel b*). Species-specific positive feedbacks are predicted to promote species dominance and species-specific negative feedbacks are predicted to promote diversity. In a non-specific feedback, the direct feedbacks for seedlings of each species have similar slopes (*panel c*), thus soils have no net effect on the relative performance of different species and feedbacks are not predicted to influence species coexistence.

Type of Feedback	# seedling species	Definition	Example
Direct	1	difference in biomass between home and away soils	
Pairwise	2	*relative* difference in biomass in home and away soils	

POTENTIAL FOR *STREPTOMYCES* TO MEDIATE FEEDBACK STRENGTH AND SPECIFICITY:

Streptomyces could affect the *strength* of feedbacks by changing the difference in seedling biomass between home and away soils (see Box 3.2). *Streptomyces* could influence the *specificity* of feedbacks by changing the strength of direct feedbacks for one or both species (i.e. by changing the difference in slopes of red and blue lines). *Streptomyces* effects that increase the difference in slopes would make feedbacks more species-specific, and effects that reduce the difference in slopes would reduce specificity and thus ameliorate pairwise feedback strength.

FIGURES:

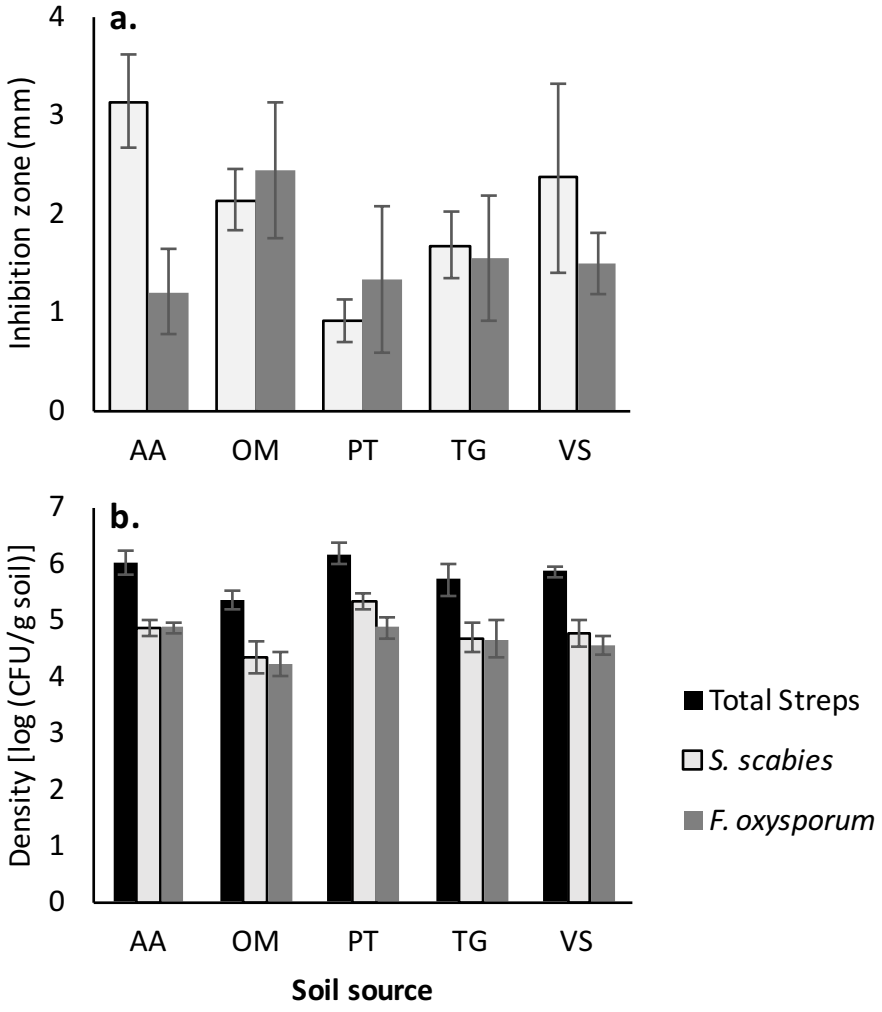


Figure 3.1. Variation in pathogen-suppressive capacities of *Streptomyces* communities among soil source species. Inhibition zone sizes (mm) **(a)** and inhibitor densities (log CFU/g soil) **(b)** against bacterial plant pathogen *S. scabies* (light grey) and fungal plant pathogen *F. oxysporum* (dark grey) in soils from AA (*Apeiba aspera*), OM (*Ormosia macrocalyx*), PT (*Protium tenuifolium*), TG (*Tabebuia guayacan*), and VS (*Virola surinamensis*). Densities of total *Streptomyces* ("Total Streps") are also shown (black). Bars are means and standard errors of five trees per soil source species. Trees without any inhibitors of a given pathogen were excluded from mean inhibition zone sizes.

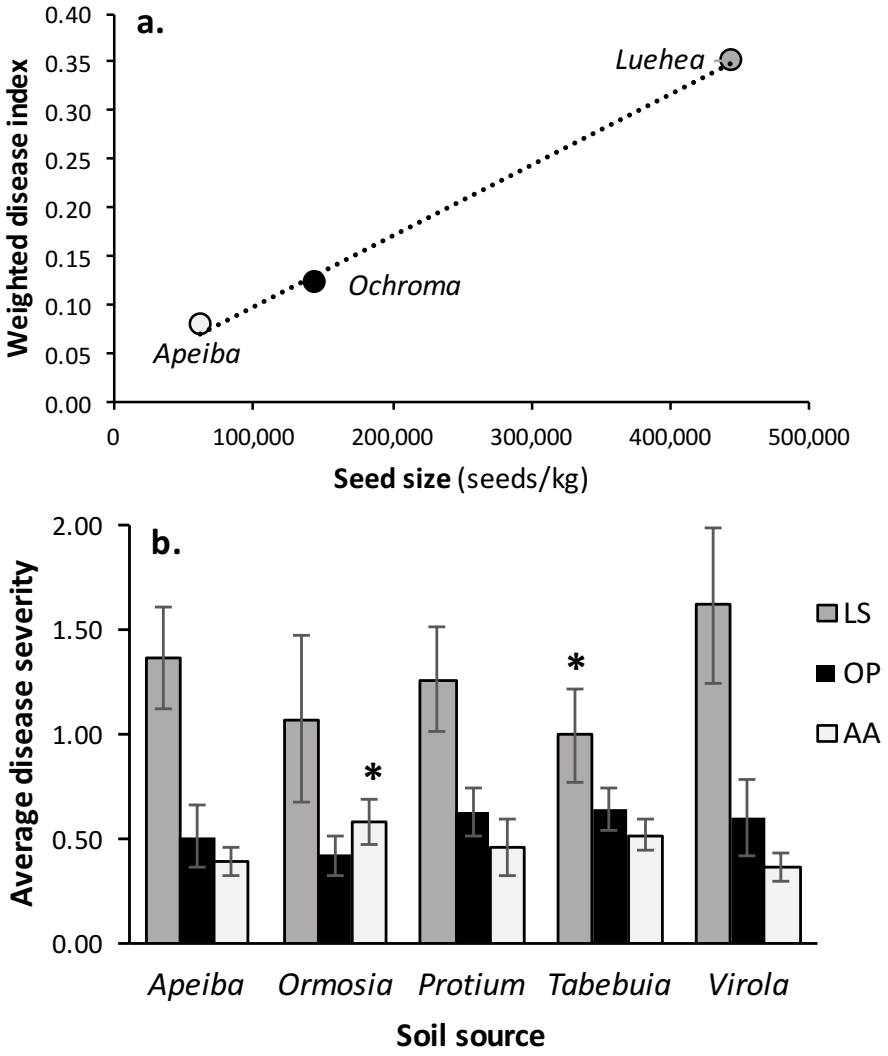


Figure 3.2. Soil disease potential measured with rolled-towel (RT) bioassays against three seed indicator species: *Luehea seemanii* (LS), *Ochroma pyramidale* (OP), and *Apeiba aspera* (AA). **a)** Seed size was inversely related to an index of seedling disease (values range from 0 to 1 and incorporate disease severity and incidence). Larger values of seeds/kg reflect smaller seed size (i.e. LS < OP < AA). (**b)** The effect of seedling species on disease severity was influenced by soil source (two-way ANOVA, species x soil source interaction: $F_{8, 322} = 3.95$, $p = 0.0002$). The species x soil interaction reduced *Luehea* disease severity in *Tabebuia* soils (parameter estimate = -0.22 ± 0.06; significantly different from 0) and increased *Apeiba* disease severity in *Ormosia* soils (parameter estimate = 0.19 ± 0.06; significantly different from 0). Values are means (and standard errors) per soil source averaged across five trees per species. Disease severity was based on a four-category scoring system: 0 = no disease, 3 = dead seedling or > 75% necrotic tissue). Results were similar when disease incidence (the proportion of diseased seedlings per RT) was used as a response instead of disease severity (data not shown).

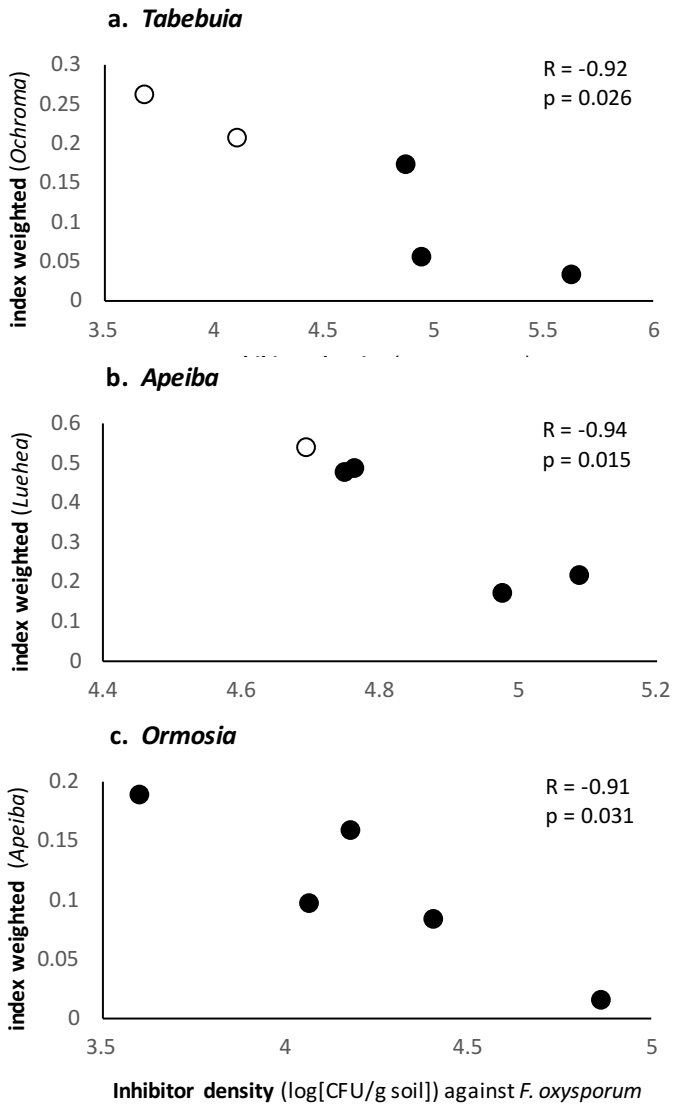


Figure 3.3. Soil disease potential decreased as inhibitors of the fungal plant pathogen *F. oxysporum* increased in soils from *Tabebuia*, *Apeiba*, and *Ormosia* trees. Pearson correlation coefficients and p-values are shown. Soil disease potential was measured with rolled-towel (RT) bioassays against three seed indicator species: OP = *Ochroma pyramidale*, AA = *Apeiba aspera*, and LS = *Luehea seemanii*. Each point represents soil from one tree. Inhibitor densities (log CFU/g soil) were calculated from the number of inhibitors per plate plus 1 to allow for log transformation. When no inhibitors were observed on a plate (open symbols), the expected density of inhibitors per gram soil reflects the limit of detection. **a)** In *Tabebuia* soils, inhibitor densities (and inhibition zone sizes, not shown) were negatively correlated with *Ochroma* disease, **b)** In *Apeiba* soils, inhibitor densities were negatively correlated with disease among *Luehea* seedlings, **c)** In *Ormosia* soils, inhibitor densities were negatively correlated with *Apeiba* disease.

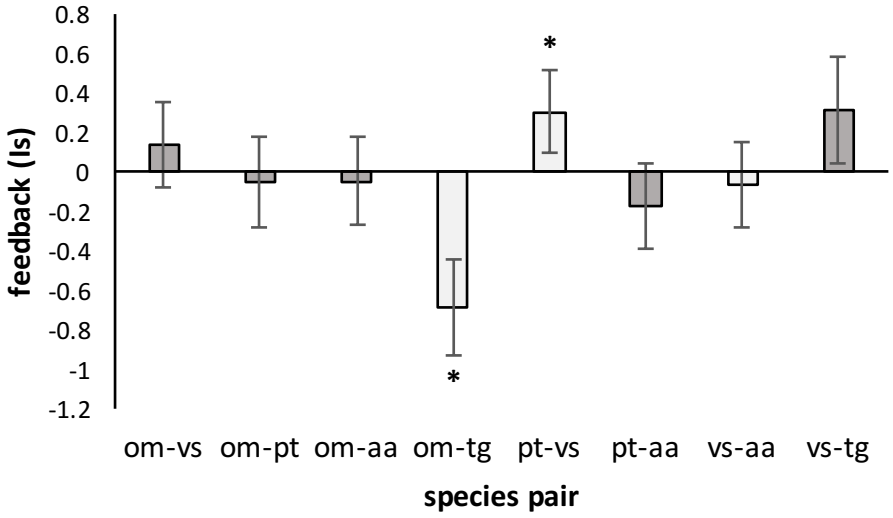


Figure 3.4. Net pairwise feedbacks varied in strength and direction among species pairs in the plant-soil feedback experiment. Pairwise feedbacks (represented by the Interaction coefficient I_s) were calculated using *apriori* contrasts within the soil source x seedling species interaction term of the mixed effects model of seedling biomass (following Bever et al. 1997). Pairings in which either species was represented by fewer than three replicate pots were excluded. Negative feedback between *Ormosia* and *Tabebuia* (om-tg) and positive feedback between *Protium* and *Virola* (pt-vs) were significantly different from zero (denoted by asterisks). Light grey filled bars (om-tg, pt-vs, and vs-aa) denote pairwise feedbacks for which corresponding interaction plots are included in Fig 3.5.

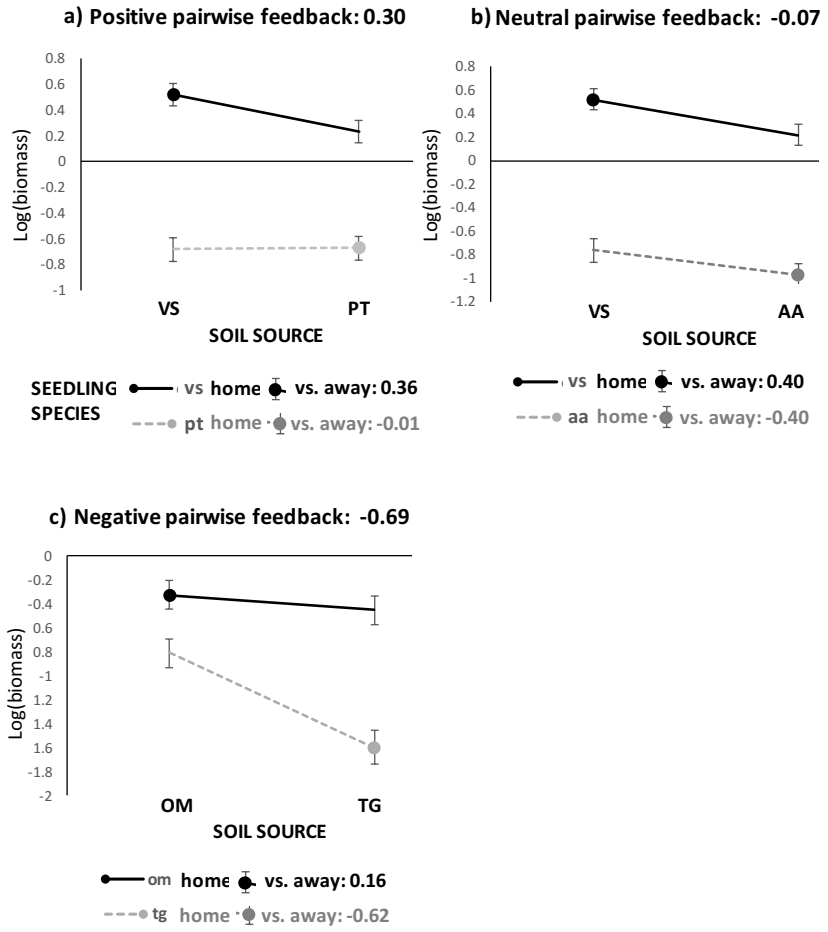


Figure 3.5. Comparison of net pairwise (interaction plots) vs. direct feedbacks for **a)** *Virola* (vs) and *Protium* (pt) seedlings, and **b)** *Virola* (vs) and *Apeiba* (aa) seedlings, and **c)** *Ormosia* (om) and *Tabebuia* (tg) seedlings. The direct feedback values for each component species (Table 3.5) are listed beneath each panel. **a)** Positive pairwise feedback between *Virola* and *Protium* (pairwise feedback = 0.30, p = 0.07). **b)** By contrast, *Virola* and *Apeiba* seedlings experience the same proportional increase in biomass in *Virola* soils relative to biomass in *Apeiba* soils (i.e. direct positive feedback for *Virola*, direct negative feedback for *Apeiba*). Thus, despite two-fold differences in biomass between home soil (indicated by filled circles) and away soil for each species, the net pairwise feedback is neutral (i.e. near 0, non-significant) because direct feedbacks of equivalent magnitude and opposite sign cancel each other out. **c)** Although both *Ormosia* and *Tabebuia* seedlings have higher biomass in *Ormosia* soils than *Tabebuia* soils, there is a disproportionate advantage to *Tabebuia* seedlings, hence the negative pairwise feedback between these two species (pairwise feedback = -0.69, p = 0.0043).

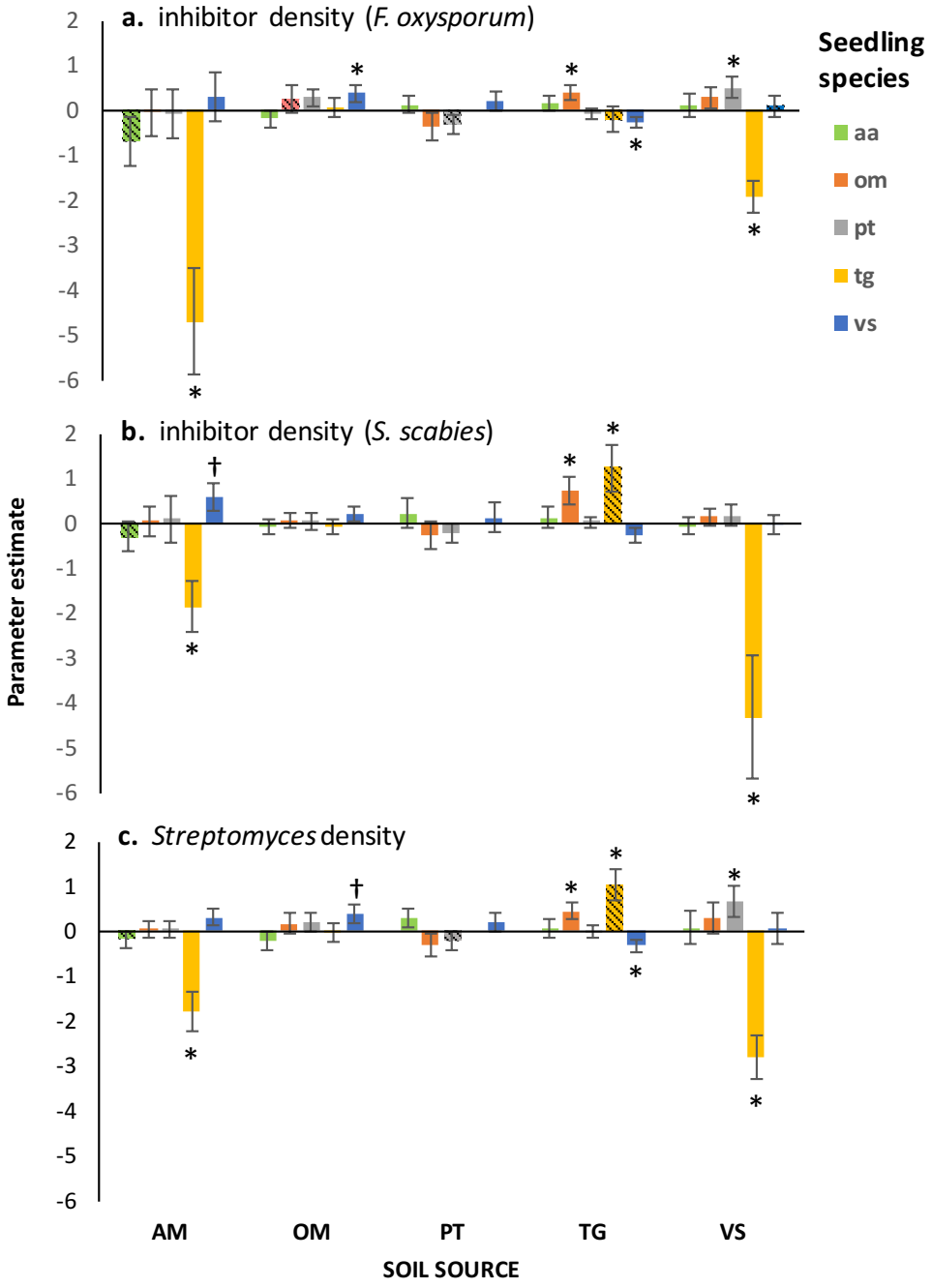


Figure 3.6. Parameter estimates relating seedling biomass to *Streptomyces* densities for each soil source x seedling species combination. Values reflect the slopes of relationships between *Streptomyces* densities and plant biomass for each species in each soil source. * and † indicate slopes that were significantly different from zero ($p < 0.05$ and $p < 0.1$, respectively). The height of bars shows how quickly biomass changed with density (steepness of slope). Positive values indicate larger seedlings in soils with higher densities. Panels show associations between seedling biomass and a) inhibitor density against *F. oxysporum*, b) inhibitor density against *S. scabies*, and c) Total *Streptomyces* density. Hatched lines denote seedlings grown in soils from conspecific adults.

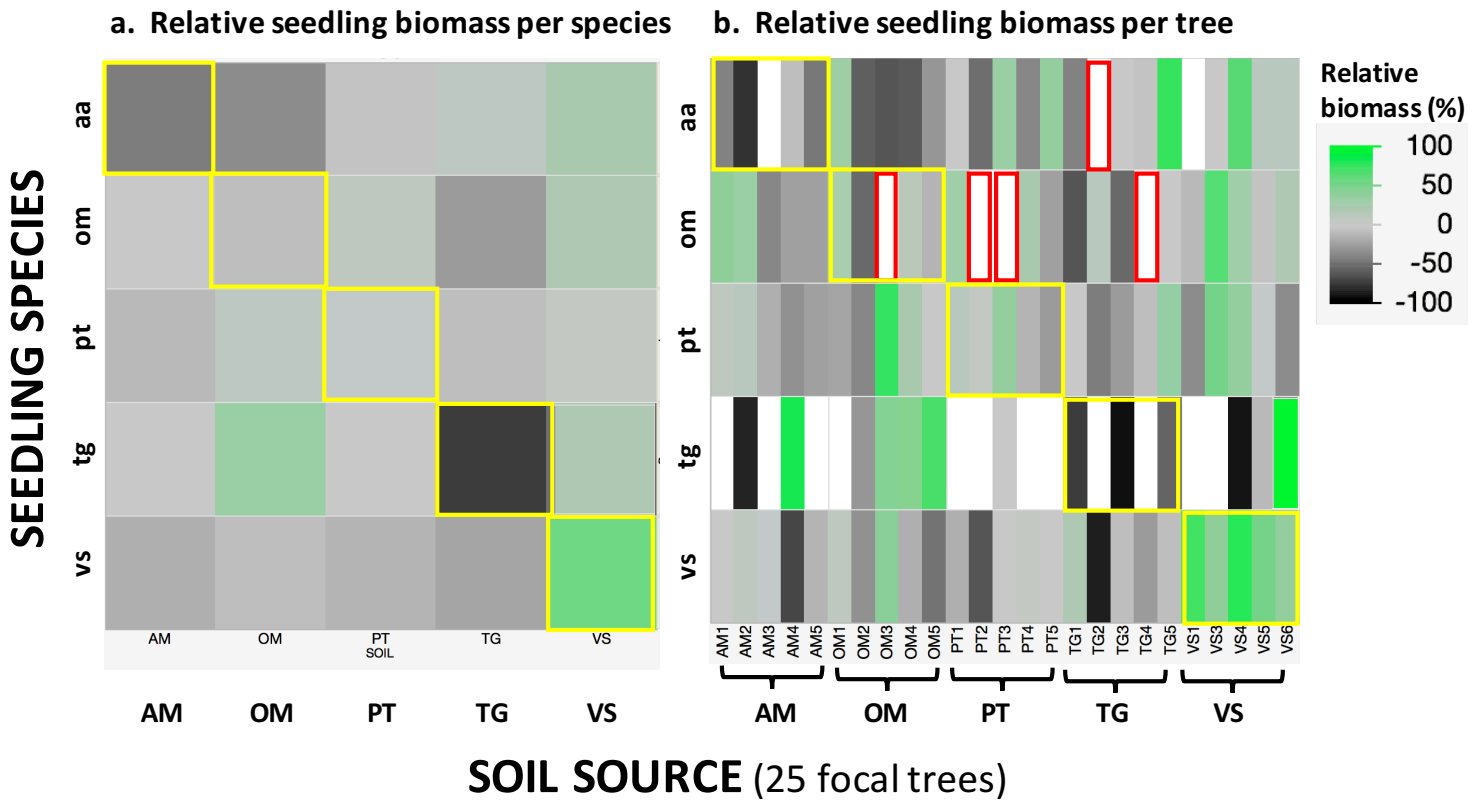


Figure 3.7. Heat maps of relative seedling biomass in soil from **a)** each tree species (mean of n = 5 trees/species), and **b)** each of the 25 focal trees. Rows denote seedling species (lower case letters) and columns indicate the species of the soil in which they were grown (capital letters). Each cell shows biomass of an individual seedling relative to the average biomass of that species across all soil sources (e.g. relative biomass for seedling species A, individual i = $[(A_i - A_{avg})/A_{avg}]*100$). White indicates a seedling that died prior to harvest (red outline) or was never planted due to limited seed availability. Light grey (0%) indicates no difference in biomass

relative to the species average. Brighter colors indicate seedlings with higher than average relative biomass, darker colors indicate lower than average relative biomass. Bright green indicates seedlings with 100% more biomass (2x bigger) than the species average. Black indicates seedlings that are half the size of the species average (relative biomass = -100%). Conspecific seedling x soil source combinations are highlighted in yellow.

TABLES:

Table 3.1. Species codes, functional characteristics, and germination treatments for the seedling species used in this work. Seeds/kg were taken from Sautu et al. 2006; smaller values indicate larger average seed mass. "Shade index" represents an index of shade tolerance taken from Comita et al. 2010. More positive values indicate higher shade tolerance. References for seed germination treatments are included when necessary.

	Species	Code	Shade index	seeds/kg	Dispersal	Treatment
Shadehouse experiment	*Apeiba aspera*	AA	-1.00	62,097	mammal/bird	70 C for 2 mins
	Ormosia macrocalyx	OM	1.53	2096	mammal/bird	soak for 12-24 hours
	Protium tenufolium	PT	0.63	4719	mammal/bird	n/a
	Tabebuia guayacan	TG	1.04	51,743	wind	n/a
	Virola surinamensis	VS	0.04	346	bird/mammal	n/a
RT bioassays	*Apeiba aspera*	AA	-1.00	62,097	mammal/bird	70 C for 2 mins
	Luehea seemanii	LS	-1.14	445,186	wind	70 C for 2 mins*
	Ochroma pyramidale	OP	-1.07	145,985	wind	30 s hot water (80 C), 30 s cold water**

*Pearson et al. 2002
**STRI Vivero de Plantas Nativas

†*Apeiba aspera* (AA) was the only species used as both a seedling species and soil source in the rolled-towel (RT) bioassays and the shadehouse experiment, therefore we were able to calculate feedbacks based on **disease** among ≈2 week old *Apeiba* germinants (SI Figure 3.3) in addition to feedbacks based on **biomass** among ≈7 month old *Apeiba* seedlings

Table 3.2. Proportions of *Streptomyces* capable of inhibiting bacterial (*S. scabies*) and fungal (*F. oxysporum*) plant pathogens. Values are means (and ranges) of average proportions in soils from each tree species (inhibitor density/total *Streptomyces* density).

	Proportion of inhibitors	
	S. scabies	*F. oxysporum*
Apeiba	0.08 (0.04-0.10)	0.11 (0.02-0.24)
Ormosia	0.12 (0.04-0.24)	0.08 (0.05-0.13)
Protium	0.14 (0.09-0.20)	0.07 (0.02-0.13)
Tabebuia	0.10 (0.07-0.13)	0.17 (0.02-0.51)
Virola	0.11 (0.03-0.29)	0.06 (0.03-0.08)

Table 3.3. Average final biomass (g), height (cm), leaf number and leaf increase differed among seedling species after 25 weeks of growth (1-way ANOVAs, $p < 0.0001$). Different letters indicate significant differences in growth characteristics among seedling species (Tukey HSD, $p < 0.05$). *Virola* seedlings had significantly higher biomass than the other species and were on average 4x larger than the average size of the next biggest seedling species (*Ormosia*) and 20x larger than the average biomass of the smallest species (*Tabebuia*). Values represent means (averaged across all seedlings alive at harvest) and ranges (smallest seedling - largest seedling) for each species.

Seedling species	**Final biomass** (g)	**Height** (cm)	**Leaf number**	**Leaf increase**
Apeiba	0.13 (0.03-0.25) D	5.32 C	9.0 A	7.4 A
Ormosia	0.54 (0.19-0.89) B	18.82 A	4.6 B	2.6 C
Protium	0.21 (0.12-0.37) C	15.49 B	2.6 C	1.6 C
Tabebuia	0.10 (0.01-0.23) D	6.48 C	8.9 A	6.9 A
Virola	2.11 (0.32-3.81) A	20.31 A	5.1 B	4.7 B

Table 3.4. Results of mixed effects models of log-transformed seedling biomass (response) with and without inclusion of *Streptomyces* covariates (either total *Streptomyces* density or Inhibitor density against the plant pathogen *F. oxysporum*). All models include soil source species ("Soil") and seedling species ("Seedling") as main effects as well as the Soil x Seedling interaction. Tree was included as a random effect. *Streptomyces* covariates were nested within the random effect of tree. Values are Chi-square estimates and likelihood ratio tests. All five seedling species and soil sources are included in the analysis shown here. Results were similar when *Tabebuia* seedlings were excluded and initial seedling biomass was included as an additive covariate.

	Base model		***Streptomyces* density**		**Inhibitor density**	
Effect(df)	Chi sqr	Pr>(Chisq)	Chi sqr	Pr>(Chisq)	Chi sqr	Pr>(Chisq)
Soil	10.82	**0.0286**	20.37	**0.0004**	15.103	**0.0044**
Seedling	486.31	**<0.0001**	923.68	**<0.0001**	856.52	**<0.0001**
Strep	-	-	0.46	ns	0.00	ns
Soil*Seedling	27.224	**0.039**	47.78	**<0.0001**	41.032	**0.0005**
Soil*Strep	-	-	2.71	ns	6.217	ns
Seedling*Strep	-	-	8.53	**0.0738**	19.05	**0.0007**
Soil*Seedling*Strep	-	-	84.71	**<0.0001**	60.596	**<0.0001**

Table 3.5. Direct feedbacks for individual seedling species in each heterospecific soil source. For example, feedback for seedling species A in soil source $_B$ is calculated as $BiomassA_A$-$BiomassA_B$/$Max(A_A,A_B)$. Positive values indicate higher seedling biomass in soils from conspecific trees ("home soils"), negative values indicate higher seedling biomass in soils from the heterospecific tree species. **Home vs. Away** is the average of direct feedback values across all heterospecific soil sources. A value of zero denotes the home soil used as reference for direct feedback calculations. Note limited sample sizes for *Tabebuia* seedlings (n = 1 for PT, data not shown)

	Soil source					
Seedling species	AA	OM	PT	TG	VS	**Home vs. Away**
Apeiba (AA)	0	-0.05	-0.29	-0.29	-0.40	**-0.26**
Ormosia (OM)	-0.03	0	-0.12	0.16	-0.17	**-0.04**
Protium (PT)	0.10	-0.03	0	0.09	-0.01	**0.04**
Tabebuia (TG)	-0.40	-0.62	-	0	-0.27	**-0.43**
Virola (VS)	0.40	0.36	0.36	0.43	0	**0.39**

Supplementary Information for this Chapter can be found in Appendix 3.

CONCLUSION CHAPTER:

Chapters 1-3 addressed the following questions to explore the potential for antibiotic-producing soil bacteria to influence plant diversity and community composition in tropical forests: 1) Do pathogen-suppressive capacities of *Streptomyces* communities vary across tropical forest soils? 2) What are the biotic and abiotic correlates of variation in pathogen-suppressive capacities? In particular, does the frequency or intensity of pathogen suppression vary with respect to soil resources, tropical tree species, or individual host trees? 3) Is functional variation among *Streptomyces* communities related to the performance of tropical seedlings? If so, do *Streptomyces* have similar effects on seedlings of different species or is there evidence of specificity in plant-pathogen-*Streptomyces* interactions?

Pathogen-suppressive capacities of *Streptomyces* communities were positively correlated among tropical forest soils in all three Chapters, regardless of the specific indicator strains used. The positive correlations in inhibitor densities among soils are consistent with evidence from temperate agricultural systems in which *Streptomyces* communities identified as suppressive against one type of plant pathogen also tend to be suppressive of other pathogens in soil (Wiggins and Kinkel 2005). My results show that *Streptomyces* communities in tropical forest soils capable of inhibiting growth of one strain were also likely to inhibit another strain, which validates our approach and provides support for the underlying assumption that suppression of indicator strains would be predictive of inhibitory capacities against indigenous microbes in tropical forest soils.

Total *Streptomyces* densities and inhibitor densities in the tropical forest soils studied here were within the range of densities that have been documented previously in temperate agricultural systems, grasslands, and a limited number of other tropical ecosystems (Table 1.1 in Ch1; Becklund et al. 2014). This further suggests that tropical *Streptomyces* communities have the potential to inhibit indigenous plant pathogens *in situ* and create locally disease-suppressive soils, as has been demonstrated in temperate systems with similar inhibitor densities.

There was extensive variation in pathogen-suppressive capacities of *Streptomyces* communities in both tropical dry forests (Costa Rica) and tropical moist forest (Panama), which is a prerequisite for *Streptomyces* to influence community composition. We also demonstrated substantial variation in soil disease potential in these tropical forest soils (Ch1; Supplemental Text SI 1.1, SI Fig. 1.2; and Ch3; Fig. 3.2 and SI Fig. 3.2). Relationships among pathogen suppression, soil disease potential, and nutrient availability varied among soils and sites.

Antibiotic production is a form of interference competition which provides an advantage to the producing strain through enhanced resource acquisition. Resource competition within *Streptomyces* populations and between *Streptomyces* and other bacterial and fungal soil saprotrophs can exert strong selection for antibiotic production as well as diversity of inhibitory phenotypes (Kinkel et al. 2011). Thus, antagonism is expected in competitive environments, such as soils with high microbial densities and/or low resource availability. We found significant relationships among inhibitory capacities of *Streptomyces* communities and soil nutrient availability in all three studies. However, the strength and direction of relationships varied between sites. At the landscape scale

(Ch1), inhibitor densities were negatively correlated with soil P and K but were unrelated to soil C. By contrast, inhibition zone sizes, which reflect the strength of antibiotic-mediated inhibition per isolate, increased with available P, K, and %C and %N in tropical dry forest soils. These trends were stronger in Santa Rosa than Palo Verde. In a tropical moist forest in Panama (Ch3), on the other hand, we found the opposite pattern against the same overlay strain (*S. scabies)*; Inhibitor densities were positively correlated with soil K, while inhibition zone size tended to decrease with soil K.

Collectively, my findings suggest that individual host trees, tree species, and site characteristics may have interacting effects on the potential for pathogen suppression among soil *Streptomyces* communities. Santa Rosa soils consistently harbored significantly greater densities and proportions of pathogen-suppressive *Streptomyces* than soils in Palo Verde, which was likely related to differences in soil fertility between the sites. Furthermore, Chapters 2 and 3 suggest that the impact of tree species on *Streptomyces* inhibitory phenotypes also varied within and among sites.

Similarly, results suggest that the effects of *Streptomyces* on tropical plant communities are also likely to be context dependent rather than strictly host specific. In particular, heterogeneity in associations between plants and antagonistic *Streptomyces* in soil has potential to influence seedling biomass and community composition by increasing variation in the performance or survival of seedling species.

Concluding statements:

The next challenges in plant-soil feedback studies include understanding how plant-microbe interactions vary across plant species, functional groups, and ecosystems

as well as how they will change in response to altered climate and anthropogenic disturbances (Van der Putten et al. 2013, Bardgett et al. 2014, Powers et al. 2015). My findings emphasize that studies should consider microbial interactions as well as site- and host-associated heterogeneity in plant-microbe-soil interactions, which are likely to be highly adapted to local conditions.

Manipulative experiments that alter various components of the soil community can help tease apart the microbial interactions underlying plant-soil feedback effects. Numerous tropical forest studies have employed fungicide in attempts to isolate the effects of pathogens on seedling performance (Bell et al. 2009, Bagchi et al. 2014, Liu et al. 2015). This approach should be coupled with experimental inoculation of soil-borne pathogens and antibiotic-producing soil bacteria in fully factorial design in order to explore how microbial interactions shape variation in plant-soil feedbacks and their consequences in tropical forest ecosystems.

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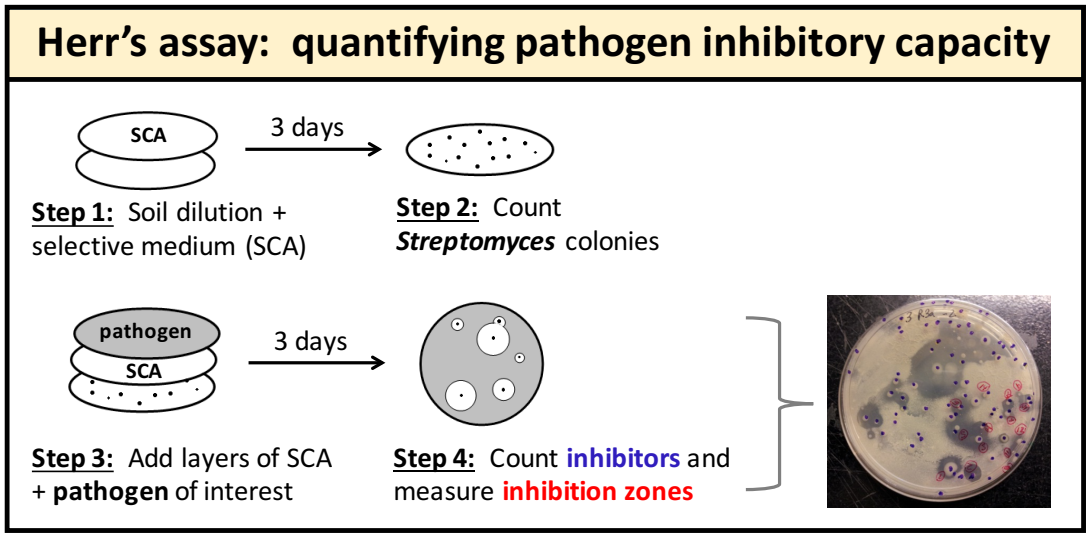
APPENDIX 0 – Methods and overlay strains used in modified Herr's assays

SI Figure 0.1. Methods diagram for modified Herr's assay for estimating pathogen-suppressive capacities of *Streptomyces* communities in soil. Pathogens or non-pathogenic overlay strains used in Chapters 1-3 are listed in SI Table 0.1.



SI Table 0.1. Overlay strains used in modified Herr's assays to estimate pathogen-suppressive capacities of *Streptomyces* communities in tropical forest soils. Densities of *Streptomyces* capable of suppressing distinct plant pathogens or non-pathogenic *Streptomyces* indicator strains were significantly positively correlated among soils in all three studies. BCI stands for Barro Colorado Island (field site for Chapter 3).

Chapter	Country	Site	Overlay strains used	Pathogenic?
1	Costa Rica	Santa Rosa	*Streptomyces scabies*[a]	Y
		Palo Verde	*Fusarium oxysporum*[b]	Y
			Rhizoctonia solani[c]	Y
2	Costa Rica	Santa Rosa	*S. scabies*	Y
		Palo Verde	*Streptomyces* strain 4-21[d]	N
			Streptomyces strain 1324[d]	N
3	Panama	BCI	*S. scabies*	Y
			F. oxysporum	Y

[a] Strain 87; Isolated from potato; Liu et al. 1995
[b] Isolate 08-78
[c] Isolate 48 (Ag-2-1)
[d] Davelos 2004c

SI Table 0.2 Soil samples from Chapter 3 that were processed using a modification of the Herr's assay. Chloroform was used to reduce fast-growing non-*Streptomyces* bacteria that consistently outcompeted *Streptomyces* on plates. Chloroform was used to kill all colonies prior to the overlay procedure (see SI Fig. 0.1), based on the method described by Otto-Hanson and colleagues (2013). Results from chloroform-modified assays should be comparable to samples overlaid with the standard Herr's method because chloroform does not affect antibiotics already excreted from colonies (L. Kinkel, personal communication).

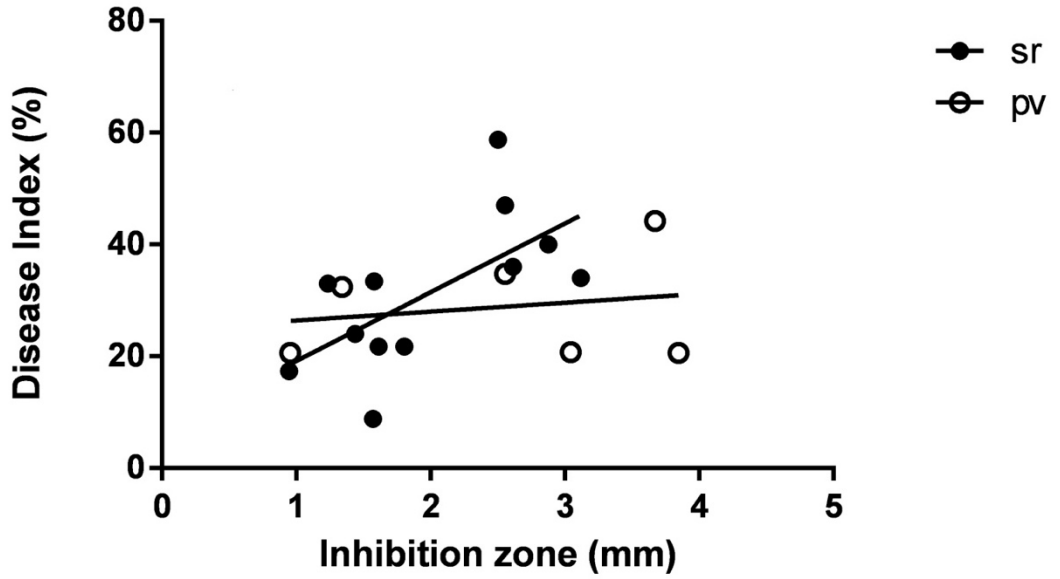
Soil source (tree species)	**Individual host tree**
Apeiba	AM5
Ormosia	OM5
Protium	PT5
Tabebuia	TG5
Virola	VS4, VS6

APPENDIX 1: Supplementary Information for rolled-towel (RT) bioassays

Supplemental Text S1.1 – Rolled-towel (RT) bioassays in TDF

I used a modified rolled-towel (RT) bioassay to create an index of soil disease potential across the 18 tropical dry forest plots. This method quantifies soil inoculum potential (expressed as the % of seedlings with disease), which is an integrated measure of disease potential that is sensitive to factors such as the spatial distribution of pathogens in soil, pathogen inhibition by *Streptomyces*, and plant susceptibility to disease.

Soil samples used in this assay were collected from the plots in June 2011 (6 subsamples pooled per plot). Pre-germinated surface-sterilized seeds of *Gliricidia sepium* were rolled in a damp paper towel with or without soil and stored in the dark for one week. Sterile water was added every other day to maintain moist, humid conditions which are optimal for pathogen growth. Disease incidence and severity was scored for each seedling on a 0-4 scale according to the extent and severity of symptoms. Disease index values were calculated from severity scores according to the following formula: Disease index (%) = [(∑scale x number of infected plants) / (highest scale x total number of plants)] x 100. Soil disease potential (disease index) varied significantly among the 18 plots (1-way ANOVA: $R^2 = 0.54$, $p < 0.0001$).



SI Figure S1.1. Mean inhibition zone vs. soil disease index across 18 tropical dry forest plots in Costa Rica. Closed circles denote plots located in Santa Rosa, open circles denote plots located in Palo Verde. In Santa Rosa (but not Palo Verde), inhibition zones increased significantly with soil disease potential ($R^2 = 0.41$, $p = 0.0256$). This suggests that conditions that promote the buildup of pathogen inoculum in soil may also select for more intense antibiotic-mediated pathogen-suppression among *Streptomyces* communities (reflected by larger zone sizes).

Supplemental Text SI 1.2 – RT bioassays in tropical moist forest (BCI)

To create an index of soil disease potential for each of the 25 soils used in the feedback experiment, we used three seedling indicator species: *Luehea speciosa* (LS), *Ochroma pyramidale* (OP), and *Apeiba aspera* (AA). Seeds were provided by Vivero de Plantas Nativas de STRI and Camilo Zalamea. Studies have demonstrated a phylogenetic signal in host range among soil-borne plant pathogens (Gilbert et al. 2015, Parker et al. 2015), meaning that closely related plants are more likely to overlap in their susceptibility to different pathogens. However, in this study, average disease severity scores and index values were not correlated for seed indicator species from the same family (*Apeiba* and *Luehea*). Instead, we found that estimates of soil disease potential against *Luehea* and *Ochroma* were significantly positively correlated across soil sources, potentially due to the smaller seed size or lower shade tolerance of these species.

For all three species, average severity of disease symptoms was significantly higher in RTs conducted with field soil than in sterile soil-free controls (t-tests; $p < 0.05$). Furthermore, disease severity scores varied significantly among soil samples from the 25 focal trees, both when averaged across the three seedling species ($df = 24$, $F = 3.51$, $p < 0.0001$) and for each species individually (*Apeiba*: $F = 1.57$, $p = 0.064$; *Ochroma*: $F = 6.17$, $p = < 0.0001$; *Luehea*: $F = 12.95$, $p < 0.0001$). This suggests that this method is reproducible and successful in differentiating between samples.

APPENDIX 2: Supplementary Information for Chapter 2

SI Table 2.1 Loading factors for PCA axes show the relative contributions of individual (a) soil and (b) litter elements to each principal components axis from Figure 2.1. Concentrations of soil Mo (a) and litter Ni (b) were below detection limits and were therefore excluded

a.

	PC1	PC2
Al soil	0.50837	-0.4108
B soil	0.87104	-0.32242
Ca soil	0.58773	0.69137
Cr soil	0.81948	0.09567
Cu soil	0.6003	-0.3545
Fe soil	0.70885	-0.60295
K soil	0.6794	0.06698
Mg soil	0.84898	0.13224
Mn soil	0.2372	-0.56554
Na soil	0.14715	0.72912
Ni soil	0.76824	0.04488
P soil	0.62003	0.57662
Zn soil	0.5038	0.24263

b.

	PC1	PC2
Al litter	0.70413	0.40325
B litter	-0.53065	0.17958
Ca litter	-0.31001	-0.13387
Cr litter	-0.1441	0.79692
Cu litter	0.16129	0.68881
Fe litter	0.62408	0.11506
K litter	0.36308	-0.15327
Mg litter	-0.51827	0.46598
Mn litter	0.74748	-0.1112
Mo litter	-0.53139	0.40375
Na litter	-0.26031	-0.23928
P litter	-0.17169	0.27053
Zn litter	0.40304	0.69663

APPENDIX 3 – Supplementary Information for Chapter 3

Supplemental Text SI 3.1 - Estimating initial seedling biomass

In order to account for any variation in initial seedling biomass that could affect final plant biomass at harvest, we estimated initial biomass of all experimental seedlings and included this term as a covariate in analyses. Seedling height, leaf number, and leaf length and width were measured for every experimental seedling within the first week of planting and used to estimate initial seedling biomass. Allometric equations used to estimate initial biomass of experimental seedlings are shown in SI Table 3.1. Total biomass was based on dry weights taken after a minimum of one week of oven drying at 70° C.

Initial biomass estimates were correlated with final biomass after six months of growth for *Protium* (Pairwise correlation coefficient: $R = 0.66$; $p = 0.0003$) and *Apeiba* (Pairwise correlation coefficient: $R = 0.62$, $p = 0.0027$) seedlings. However, models of log-transformed seedling biomass that included initial biomass as a covariate were never significantly different from models without this term (see SI Table 3.2). Therefore, in the main text we present analyses without this covariate.

SI Table 3.1. Extra seedlings of similar age as those transplanted into the feedback experiment were harvested in order to predict total initial biomass of experimental seedlings based on measurements that could be taken non-destructively in the shadehouse. Species-specific regression equations that explained the most variation in total biomass of extra seedlings are shown, as well as R^2 values, specific predictor variables, and sample sizes for each species. Initial biomass was not estimated for *Tabebuia* seedlings due to insufficient availability of extra seedlings.

Species	**Equation**	**R^2**	**predictors:**	**N**
Protium	biomass = 0.0035*AreaLvs – 0.0029	0.56 (0.002)	Estimated leaf area	14
Virola	biomass = 0.040*height+0.044	0.80(<0.0001)	height	12
Ormosia	biomass = 0.037*height-0.22	0.87(<0.0001)	height	10
Apeiba	biomass = 0.0023*no_lvs+0.0043	0.83 (0.0006)	Number leaves	9

SI Table 3.2. Comparison of ANCOVA models examining predictors of log-transformed seedling biomass (response) with and without covariates to control for initial seedling size ("Biomass0"), *Streptomyces* density ("Strep"), or both. The base model is specified as: (LogBiomass)~Soil+Seedling+Soil*Seedling with "tree" included as a random effect. "+" indicates the addition of *Streptomyces* to the model as an additive covariate and "x" indicates *Streptomyces* as an interactive covariate. *Streptomyces* density was nested within the random effect of tree. Dashes (--) indicate effects that were not included in the specified model. Only *Virola, Protium,* and *Ormosia* are included in the analysis shown here.

Effect (d.f.)	**Base model**	**+Biomass0**	**+Strep**	**+Both**	**xStrep**	**xStrep,+Biomass0**
Seedling (2)	516.11(<0.0001)	125.03(<0.0001)	526.01(<0.0001)	129.99 (<0.0001)	669.15 (<0.0001)	176.47 (<0.0001)
Soil (2)	3.13 (ns)	2.72 (ns)	5.22 (0.074)	4.56 (ns)	6.33 (0.042)	5.23 (0.073)
Seedling*Soil(4)	9.81 (0.044)	10.98 (0.027)	9.87 (0.043)	10.96 (0.027)	14.82 (0.0051)	17.72 (0.0014)
Initial biomass	--	0.65 (ns)	--	0.60 (ns)	--	1.25 (ns)
Streptomyces	--	--	3.74 (0.053)	3.44 (0.063)	4.52 (0.034)	3.99 (0.045)
Soil*Strep	--	--	--	--	2.77 (ns)	2.57 (ns)
Seedling*Strep	--	--	--	--	3.55 (ns)	3.53 (ns)
Soil*Seedling*Strep		--	--	--	9.79 (0.044)	10.8 (0.029)

Supplemental Text SI 3.2 – Comparison of seedling biomass in treatment soils vs. a sterile, "control" soil

Average seedling biomass at harvest was typically higher in field soils than in the control soil, both overall and for individual seedling species in each soil source (SI Table 3.3). *Apeiba* seedlings in control soils had significantly fewer leaves than seedlings in treatment soils (t-test, $p < 0.05$), due to leaf senescence prior to harvest that resulted in a net reduction in total leaf number in four out of five control pots. The number of new leaves produced by *Virola* over the course of the experiment also varied by soil source ($df = 4$, $F = 3.76$, $p = 0.017$), and was significantly highest in the control soil (mean = 5.8; Tukey's HSD, $p < 0.05$) and lowest in soils from *Apeiba* and *Ormosia* trees (Table 3.3). There were no significant differences in leaf number across soil sources for the other seedling species.

SI Table 3.3. Seedling growth in treatment soils relative to control (sterile) soils. Control values are average seedling biomass per species (N=5) when grown in a common, sterile soil source. Seedling responses in live soil sources (PT, VS, OM, AA, TG) relative to controls are presented in two ways. Arrows indicate whether mean seedling biomass in each live soil source is greater (↑) or less (↓) than the average biomass in control soil. For each soil source, the number of seedlings with biomass greater than the average in control soil is also reported.

	Control Mean (min-max)	**PT** Mean / #	**VS** Mean / #	**OM** Mean / #	**AA** Mean / #	**TG** Mean/#	Total*
pt	0.165 (0.11-0.26)	↑ (4/5)	↑ (3/5)	↑ (4/5)	↑ (4/5)	↑ (3/5)	5 (18/25)
vs	1.727 (1.31-2.63)	↑ (4/5)	↑ (5/5)	↑ (3/5)	↑ (4/5)	↓ (3/5)	4 (19/25)
om	0.543 (0.41-0.66)	↑ (2/3)	↑ (3/5)	↓ (2/4)	↓ (2/5)	↓ (1/4)	2 (10/21)
aa	0.106 (0.069-0.21)	↑ (3/5)	↑ (4/4)	↓ (1/5)	↑ (2/5)	↑ (3/4)	4 (13/23)
Total**		4 (13/18)	4 (15/19)	2 (10/19)	3 (12/20)	2 (10/18)	

*** seedling biomass per species relative to control (across 5 soil sources):**

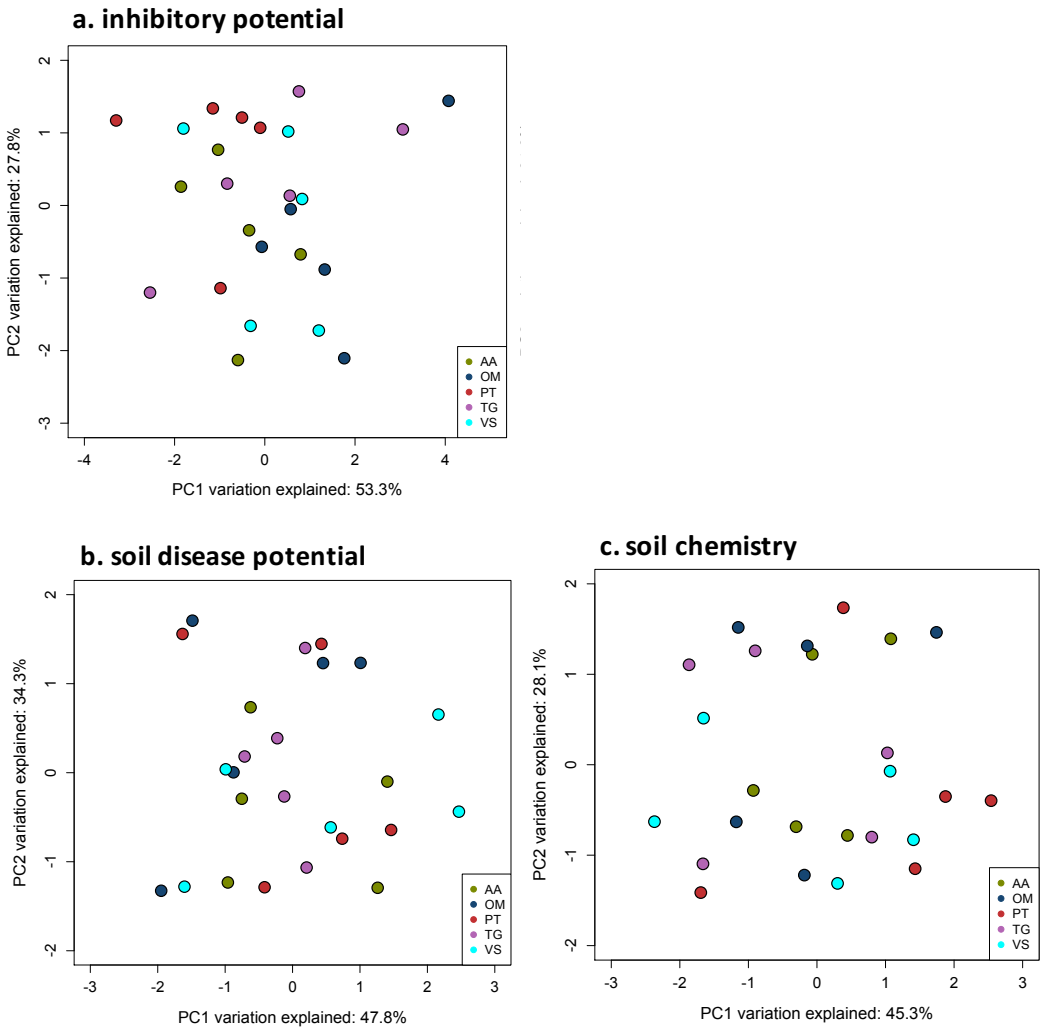
in 5/5 soil sources, *Protium* seedlings in treatment soils have higher average biomass than in control soil

-18/25 *Protium* seedlings do better in treatment soils than average in controls

**** seedling biomass per soil source relative to control (across 4 seedling species):**

4/4 seedling species always have higher average biomass in *Protium* soils than in control soil

-13/18 seedlings grown in *Protium* soils had higher biomass than in control soil



SI Figure 3.1. Principal components analyses of **a)** *Streptomyces* community characteristics, **b)** soil disease potential, and **c)** soil C, N, P, and K in soils from the 25 focal trees. Each point represents soil from one tree and color denotes tree species. **a)** Strep PC1 (53.3%) describes variation in *Streptomyces* densities and densities of bacterial and fungal inhibitors, and Strep PC2 (27.8%) describes variation in inhibition zone sizes. **b)** Soil disease potential was measured with Rolled-towel (RT) bioassays against three seedling indicator species: OP = *Ochroma pyramidale*, AA = *Apeiba aspera*, and LS = *Luehea seemanii*. RT PC1 (47.8%) describes variation in disease severity against OP and LS, whereas PC2 (34.3%) represents disease severity against AA. **c)** soil PC1 (45.3%) describes variation in C and N and soil PC2 (28.1%) describes variation in P and K. Loading factors are shown in SI Table 3.4.

SI Table 3.4. Loading factors for the principal components ordinations shown in SI Fig. 3.1. Bolded values show the variables that contribute most to each principal component. **a)** The first two principal components explained over 81% of variation in total *Streptomyces* densities, inhibitor densities, and zone sizes against bacterial and fungal pathogens (five variables in total). Strep PC1 (53%) was associated with total *Streptomyces* and inhibitor densities, whereas Strep PC2 (27.8% variation) described variation in inhibition zone sizes. **b)** RT PC1 (47.8%) and PC2 (34.3%) collectively explained 82% of variation in disease severity against the three seedling indicator species. Ordinations based on disease index scores were similar but explained slightly less variation overall (data not shown). **c)** Soil PC1 (45.3%) represents an index of soil C and N and soil PC2 (28.1%) is representative of P and K availability (collectively 73.4% of total variation explained).

a. inhibitory potential (Strep PCs)

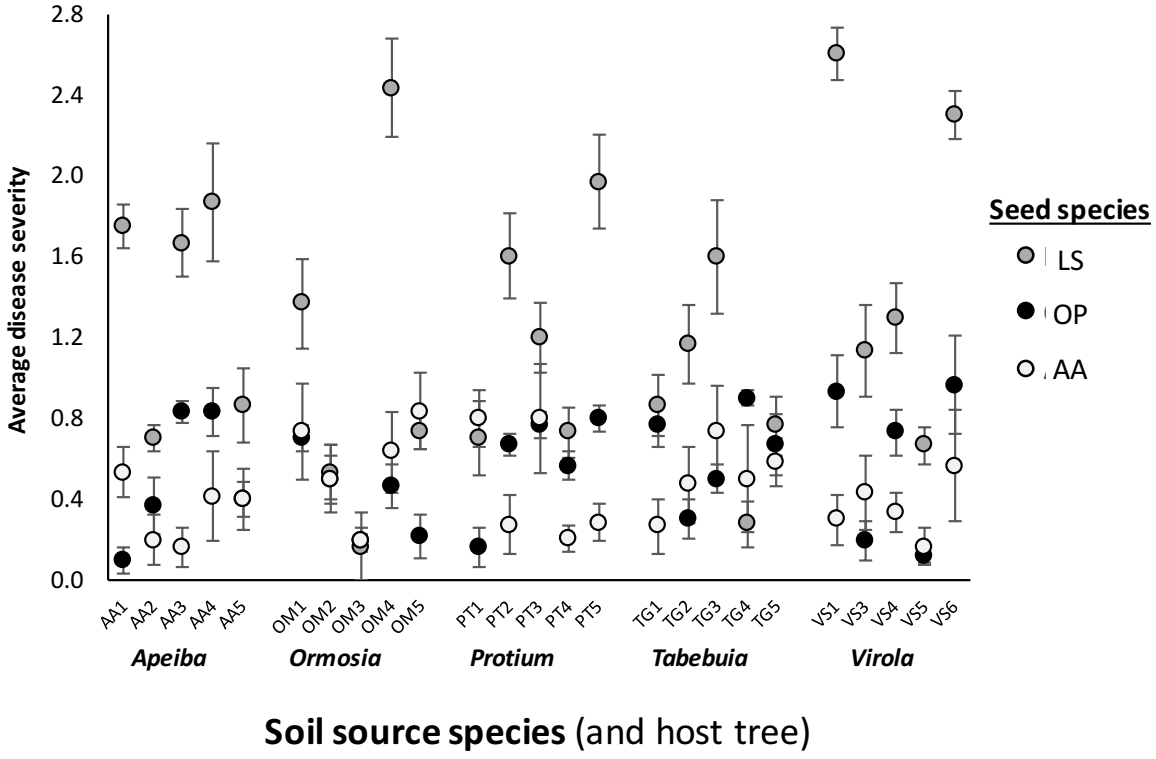
	PC1	**PC2**
Streptomyces	**-0.59**	0.04
Inhibitors (Ss)	**-0.58**	0.05
Inhibitors (Fo)	**-0.56**	-0.14
Inhibition zones (Ss)	-0.04	**-0.71**
Inhibition zones (Fo)	0.07	**-0.69**

b. soil disease potential (RT PCs)

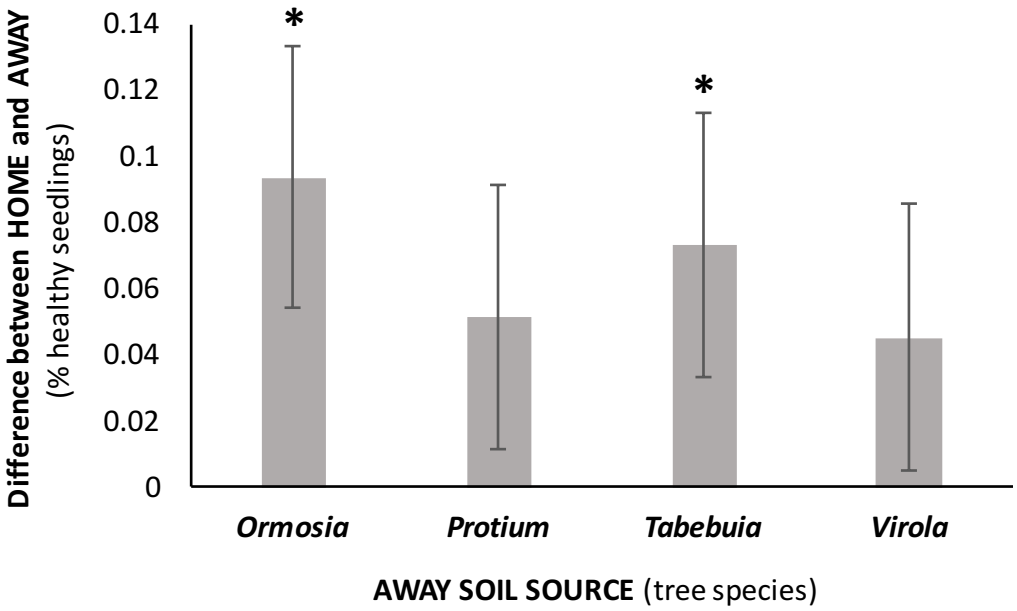
	PC1	**PC2**
severity (AA)	-0.06	**0.97**
severity (LS)	**0.70**	0.21
severity (OP)	**0.71**	-0.13

c. soil chemistry (soil PCs)

	PC1	**PC2**
Bray P (ppm)	0.00	**0.70**
K (ppm)	0.05	**0.71**
% C	**0.71**	-0.01
% N	**0.70**	-0.04



SI Figure 3.2. Average disease severity in soil sources used in the shadehouse experiment. Soil sources were collected from the following tree species (N=5 trees/species): AA = *Apeiba aspera*, OM = *Ormosia macrocalyx*, PT = *Protium tenuifolium*, TG = *Tabebuia guayacan*, VS= *Virola surinamensis*. Soil disease severity was measured with rolled-towel (RT) bioassays against three seedling indicator species: *Luehea speciosa* (LS), *Ochroma pyramidale* (OP), and *Apeiba aspera* (AA). Values are means of five replicates per tree x seed species combination. Each replicate (one RT unit) consisted of six seedlings. Severity = average disease severity score based on a four-category scoring system: 0 = no disease, 3 = dead seedling or > 75% necrotic tissue.



SI Figure 3.3. *Apeiba aspera* was used in both the rolled-towel (RT) tests and the shadehouse experiment, therefore we were able to calculate feedbacks based on disease among ≈2 week old *Apeiba* germinants as well as feedbacks based on *Apeiba* biomass after six months of growth in the same soil sources. *Apeiba* experienced significantly less disease in home soils than in away soils (positive feedback in RTs), which contrasts with the negative feedback observed based on biomass among older seedlings. Feedback values are based on the difference in the average proportion of healthy *Apeiba* seedlings in *Apeiba* soil (home soil) vs. soils from other tree species (away soils). Healthy seedlings were those with no or very little disease (categories 0 and 1). Positive values indicate that the % healthy *Apeiba* seedlings was consistently higher in home soils than away soils (positive feedback; average difference is 6.6 ± 3.1%). Asterisks indicate home vs. away comparisons in which the difference in % healthy seedlings between the specified away soil and *Apeiba* soil was significantly different from zero.

SI Table 3.5. Soil disease indices of 25 focal trees measured with rolled-towel (RT) bioassays against three seed indicator species: *Luehea seemanii* (LS), *Ochroma pyramidale* (OP), and *Apeiba aspera* (AA). Values are means of five replicates per tree x seed species combination. Each replicate consisted of six seedlings within one RT unit. Proportion = average proportion of infected seedlings (proportion incidence). Severity = average disease severity score (based on a four-category scoring system: 0 = no disease, 3 = dead seedling or > 75% necrotic tissue). % Healthy = average proportion of seedlings with no or very little disease (categories 0 and 1).

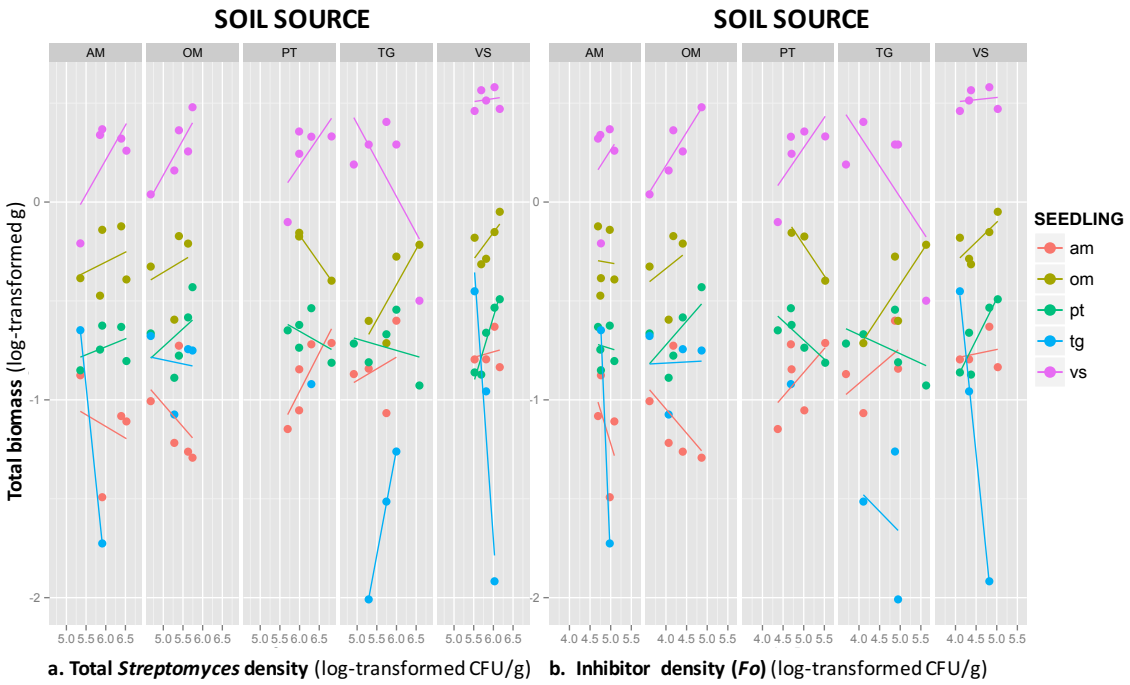
		Proportion			**Severity**			**% Healthy**		
Species	**Tree #**	LS	OP	AA	LS	OP	AA	LS	OP	AA
Apeiba	AA1	0.92	0.10	0.47	1.75	0.10	0.53	0.38	1.00	0.97
	AA2	0.70	0.30	0.20	0.70	0.37	0.20	1.00	0.97	1.00
	AA3	0.83	0.80	0.17	1.67	0.83	0.17	0.50	0.97	1.00
	AA4	0.73	0.70	0.29	1.87	0.83	0.42	0.40	0.87	0.88
	AA5	0.67	0.30	0.40	0.87	0.40	0.40	0.80	0.90	1.00
Ormosia	OM1	0.87	0.70	0.47	1.37	0.70	0.73	0.60	1.00	0.83
	OM2	0.47	0.43	0.40	0.53	0.50	0.50	0.93	0.93	0.90
	OM3	0.17	0.20	0.20	0.17	0.20	0.20	1.00	1.00	1.00
	OM4	1.00	0.33	0.33	2.43	0.47	0.63	0.39	0.90	0.83
	OM5	0.63	0.21	0.60	0.73	0.21	0.83	0.93	1.00	0.80
Protium	PT1	0.52	0.17	0.60	0.70	0.17	0.80	0.87	1.00	0.84
	PT2	0.87	0.67	0.21	1.60	0.67	0.28	0.57	1.00	0.94
	PT3	0.80	0.77	0.60	1.20	0.77	0.80	0.73	1.00	0.87
	PT4	0.73	0.57	0.14	0.73	0.57	0.21	1.00	1.00	0.93
	PT5	0.90	0.77	0.29	1.97	0.80	0.29	0.37	0.97	1.00
Tabebuia	TG1	0.73	0.76	0.20	0.87	0.76	0.27	0.90	1.00	0.97
	TG2	0.67	0.23	0.34	1.17	0.30	0.47	0.73	0.93	0.90
	TG3	0.80	0.29	0.53	1.60	0.50	0.73	0.50	0.83	0.87
	TG4	0.28	0.87	0.33	0.28	0.90	0.50	1.00	0.97	0.88
	TG5	0.70	0.67	0.42	0.77	0.67	0.59	0.97	1.00	0.87
Virola	VS1	1.00	0.73	0.30	2.60	0.93	0.30	0.22	0.80	1.00
	VS3	0.80	0.13	0.28	1.13	0.20	0.43	0.70	0.97	0.89
	VS4	0.92	0.73	0.22	1.29	0.73	0.33	0.75	1.00	0.89
	VS5	0.67	0.13	0.13	0.67	0.13	0.17	1.00	1.00	0.97
	VS6	0.97	0.57	0.33	2.30	0.97	0.57	0.29	0.67	0.87

SI Table 3.6. Means (± SE) of total soil C, total N, Bray P, exchangeable K, C/N ratio, and soil moisture (volumetric water content) for soils from focal tree species (N = 5 trees/species). Tree species codes are as follows: AA = *Apeiba aspera*, PT = *Protium tenuifolium*, OM = *Ormosia macrocalyx*, TG = *Tabebuia guayacan*, VS = *Virola surinamensis*. There were no significant differences in any of the abiotic characteristics among soils from the five tree species (1-way ANOVAs, $p > 0.05$)

	SOIL SOURCE SPECIES				
	AA	**PT**	**OM**	**TG**	**VS**
Total C (%)	4.95(0.23)	5.25(0.38)	4.78(0.33)	4.52(0.32)	4.78(0.38)
Total N (%)	0.44(0.022)	0.50(0.036)	0.44(0.02)	0.43(0.031)	0.43(0.042)
Bray P (ppm)	6.2(1.6)	3.4(0.5)	6.4(2.0)	6.0(1.1)	3.8(1.2)
K (ppm)	161.2(32.6)	182.2(52.2)	192.0(50.5)	159.0(34.1)	149.6(20.5)
C/N ratio	11.27(0.73)	10.58(0.25)	10.96(0.42)	10.56(0.302)	11.26(0.81)
Moisture (%)	57.42 (0.074)	66.28(0.071)	68.70(0.029)	50.1(0.014)	59.60(0.066)

SI Table 3.7. Maximum Likelihood ratio tests comparing models with the same random effect (Tree) but different fixed effects (with and without *Streptomyces* covariates). All models include soil source species and seedling species as main effects. The three models in which *Streptomyces* variables were included as interactive covariates (nested within the random effect of Tree) were significant improvements over the base model. Models were named for the specific *Streptomyces* variable that was incorporated into the model: Total Strep density = Total *Streptomyces* density (both inhibitors and non-inhibitors); Inhibitor density (Fo) = Densities of inhibitors of the plant pathogen *F. oxysporum*; Inhibition size (Fo) = Inhibition zone sizes against *F. oxysporum*. LogLik is the log likelihood score.

Model Comparison	**df**	**AIC**	**logLik**	**deviance**	**Chisq (Chi df)**	**Pr(>Chisq)**
Base model	27	22.433	15.783	-31.56		
Total Strep density	51	-33.512	67.756	-135.51	103.95 24	**<0.0001**
Base model	27	22.433	15.783	-31.56		
Inhibitor density (*Fo*)	51	-24.452	63.226	-126.45	94.88 24	**<0.0001**
Base model	27	22.433	15.783	-31.56		
Inhibition size (*Fo*)	51	-1.504	51.752	-103.504	71.93 24	**<0.0001**



SI Figure 3.4 Relationships between seedling biomass and **a)** *Streptomyces* densities and **b)** inhibitor densities against the plant pathogen *F. oxysporum* for each species in the five soil sources. *Streptomyces* and inhibitor densities are log-transformed colony-forming units (CFUs) per g soil. There were significant three-way interactions between seedling species, soil source, and total *Streptomyces* density and inhibitor density on log-transformed seedling biomass (Table 3.4), which suggests that *Streptomyces* communities have differential effects on seedling biomass depending on the species by soil source combination. Slopes that are significantly different from zero are indicated in SI Table 3.8.

SI Table 3.8. Soil source by seedling species combinations in which total *Streptomyces* density, inhibitor density (against *F. oxysporum*), or inhibition zone size (against *F. oxysporum*) were significantly related to seedling biomass based on parameter estimates within mixed effects models. A significant parameter estimate means that the slope of the coefficient for the three-way interaction was significantly different from zero for the specified soil source x seedling species combination (see Figure 3.6). Parameter estimates reflect the slope of the relationship between the specified *Streptomyces* variable and seedling biomass (SI Figure 3.4), arrows indicate whether correlations were positive or negative. In most cases, total *Streptomyces* density, inhibitor density, and zone sizes had similar effects on biomass of seedlings for a particular soil source. *Tabebuia* seedlings in soils from conspecifics and *Virola* trees were the exceptions: in *Tabebuia* soils, seedlings were positively affected by total *Streptomyces* density but negatively affected by zone size, and the reverse was true in *Virola* soils.

		***Streptomyces* density**		**Inhibitor density**		**Inhibition zone size**	
Soil	**Seedling**	Effect	Estimate	Effect	Estimate	Effect	Estimate
Tabebuia	*Ormosia*	↑	0.44	↑	0.39	-	-
	Tabebuia	↑	1.04	-	-	↓	-0.4
	Virola	↓	-0.33	↓	-0.28	↓	-0.27
Virola	*Protium*	↑	0.67	↑	0.49	-	-
	Tabebuia	↓	-2.79	↓	-1.94	↑	3.2
Apeiba	*Tabebuia*	↓	-1.80	↓	-4.69	↓	-0.82
Ormosia	*Virola*	↑	0.37	↑	0.38	-	-