FEEDBACK DYNAMICS BETWEEN PLANTS AND SOIL MICROORGANISMS IN A FRAGMENTED LANDSCAPE IN THE TROPICAL ANDES

By

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FEEDBACK DYNAMICS BETWEEN PLANTS AND SOIL MICROORGANISMS IN A FRAGMENTED LANDSCAPE IN THE TROPICAL ANDES

By

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Studies addressing soil microbial communities (SMC) have mostly emphasized particular soil organisms such as arbuscular mycorrhizal fungi (AMF) or soil pathogens within a single habitat. The main goals of my dissertation were to better understand how the interaction between plants and SMC vary across habitats of contrasting abiotic and biotic conditions, and address the role of whole SMC as well as distinct components of SMC. My study took place in the Central Cordillera in Colombia, which is a heterogeneous agricultural landscape comprised of tropical pre-montane forest fragments embedded in highly fertilized, agricultural monocultures, mainly sun-grown coffee plantations and pastures. I worked with SMC, and plants that are common in three contrasting habitats: pastures (Brachiaria grass), coffee plantations (coffee), and forest fragments (forest tree species).

I first tested for the effects of whole-SMC (i.e. all microorganisms in the soil) on plant growth, and found that SMC from the three contrasting habitats had differential and substantial effects on plant growth both in the greenhouse and in the field. Furthermore, fast-growing plant species (Brachiaria grass and pioneer forest trees)

benefited from “away” (habitats where plant species rarely occur or do not occur at all) compared to “home” (habitats where plant species typically occur) SMC, while slow-growing shade tolerant forest tree species benefited the most from home SMC. I then evaluated how plants were affected by two main components of SMC: potentially mutualistic AMF, and likely antagonistic non-AMF soil organisms. I found that most plants grew significantly better with non-AMF microbes from away, compared to home habitats, while showing limited response to AMF from different habitats. Finally, I tested for plant-soil feedback for both AMF and for non-AMF soil microbes and found that feedbacks driven by AMF were weak, while feedbacks driven by non-AMF soil microbes were significantly negative. Furthermore, feedbacks were only significant for non-native species Brachiaria grass and coffee, while being weak for forest tree species.

Together, these results suggest that plant-soil dynamics have been severely disrupted with the replacement of tropical forest for agriculture, and advocate for future studies on non-AMF soil microbes, which have significant impacts on plant communities but remain largely understudied.

CHAPTER 1
INTRODUCTION

"The lush green vegetation of moist tropical forest is not what it appears. Dissolve away all the plant matter from the dense foliage, giant buttressed trunks, tangled lianas, and sinuous roots, and a ghostly fungal shadow of the forest will remain."

—Gregory S. Gilbert, and Donald R. Strong, 2007

Plant communities consist of populations of different species that colonize a specific site where they persist until becoming locally extinct (Hubell 2001, Van Andel 2005). Accordingly, the presence and abundance of a species in a plant community depend on the availability of propagules and safe sites, and on the abiotic resources (nutrients, water, light) and conditions (climate, soil, pH, human impact) that allow their growth and survival (Van Andel 2005). Plant abundance is also modified by a variety of interspecific interactions that structure communities in space and time (Looijen and Van Andel 1999). Competition, facilitation, allelopathy, predation, parasitism, and mutualistic interactions interactively play a role in maintaining and excluding plants from communities at small spatial scales (alpha or local diversity) and may potentially contribute to larger scale distribution patterns (beta diversity or species turn-over). Thus, the interplay of these biotic interactions with abiotic factors affects species presence and abundance in a particular plant community. The sum of such interactions are thought to maintain the great diversity of plant communities in tropical forests, where local and regional plant diversity is greater than any other vegetation type (Gentry 1988, Kraft et al. 2011), and hundreds of species can coexist within a single hectare (Wright 2002).

Understanding the mechanisms that determine plant community composition in tropical forests has been, and continues to be, a major challenge for ecologists. Myriads of hypotheses have been proposed, most of them acknowledging the importance of biotic interactions in providing mechanisms that allow for plant coexistence and maintain diversity (Dobzhansky 1950, Corner 1954, Ashton 1969, Givnish 1999, Wright 2002, Leigh et al. 2004, Schemske et al. 2009). Yet, most of the attention of tropical ecologists has been given to aboveground, rather than belowground, biotic interactions. While there are numerous studies addressing the importance of pollination (e.g., Kiester et al. 1984, Kay et al. 2005), seed dispersal (e.g., Levin et al. 2003, Link and Fiore 2006), and herbivory (Janzen 1970, Connell 1978, Coley 1983, Fine et al. 2004) on maintaining and excluding plants from communities at small spatial scales, research on the interaction between plants and belowground soil organisms remains uncommon. However, recent studies have shown that the same plant-soil dynamics that determine plant community composition in the temperate region (Van der Heijden et al. 1998, Klironomos 2002) operate in tropical forests (Mangan et al. 2010a, Mangan et al. 2010b). Moreover, advanced molecular techniques have revealed that plants in tropical forests host highly diverse communities of microbial symbionts both below (Aldrich-Wolfe 2007), and aboveground (e.g., Arnold et al. 2000, Gilbert and Strong 2007, Gilbert et al. 2007). In my dissertation, I intended to advance understanding of the role of plant-soil microbe interactions in driving local dynamics in tropical plant communities across natural and modified habitats that have contrasting abundance of native and non-native plant species, and soil fertility in a fragmented landscape in the tropical Andes.

Soil microbial communities are comprised of diverse organisms that establish direct and indirect relationships with plants along continuums of mutualistic to antagonistic and non-specific to highly specific interactions (Kuyper and Goede 2004). For example, arbuscular mycorrhizal fungi (AMF) are thought to establish mutualistic relationships with plants by improving nutrient acquisition in exchange of sugars, but the benefits that these fungi confer to plants vary depending on host genotype and soil conditions (Johnson et al. 1997). Furthermore, it is now well known that the effects of different species of AMF vary across plant species (Munkvold et al. 2004), and that plant species show association with different strains of AMF, even though host specificity is generally weak (Bever 2002a, Klironomos 2003, Jansa et al. 2008, Mangan et al. 2010a). Similarly, plant pathogens are detrimental organisms that consist of highly diverse arrays of organisms ranging from opportunistic endophytes apparently harmless to non-stressed plants (e.g. fungal endophytes that cause disease when plants are stressed), to extremely aggressive and destructive generalist pathogens that can wipe out entire plant communities (Gilbert 2005). Other soil microorganisms such as root fungal endophytes (Rodriguez et al. 2009) and soil bacteria (Huguet and Rudgers 2010) can also have significant effects on plants, but their role in plant communities have been less studied.

Given that particular soil organisms and mixtures of soil organisms can have substantial and differential effects across plant species, the composition of communities of soil organisms may determine plant community composition (Grime et al. 1987, Bever 1994, VanderPutten and Peters 1997, Hartnett and Wilson 2002, Klironomos 2002, Reynolds et al. 2003, Van der Heijden 2003, Mangan et al. 2010b). Two feedback

mechanisms, negative and positive, have been proposed by which soil organisms and plants interact, contributing to determine the diversity of plants in communities (Bever 1994, Bever et al. 1997, Bever 2002a). Negative feedbacks occur when plants associate with a rhizosphere that is less beneficial or more detrimental to themselves than to neighboring plants of different species (heterospecific neighbors). As a consequence of such build up of detrimental organisms, plant species may indirectly enhance the establishment of other plant species that are less susceptible to those soil organisms. Such negative feedback, if common, should limit growth and local dominance of each plant species, and contribute to the maintenance of plant diversity within the local community. Negative density-dependency due to host-specialized natural enemies has been shown, in temperate (Packer and Clay 2000, Packer and Clay 2003, Casper et al. 2008, Petermann et al. 2008, Harrison and Bardgett 2010) and tropical forests (Augspurger 1983, Wright 2002, Hood et al. 2004, Bell et al. 2006, Webb et al. 2006, Comita et al. 2010, Mangan et al. 2010b). Even organisms expected to be mutualists such as AMF, may act as parasites and also generate negative plant-soil feedbacks (Bever 2002b).

Positive feedbacks occur when plants accumulate a rhizosphere microbial community that promotes their growth more than that of other plant species, thereby locally outperforming other species. As a result, increases in abundance of such plant species should further promote their abundance and suppress neighbor abundance, leading to a decrease in local plant community diversity. For example, some non-native species have been shown to have escaped their soil enemies in their native ranges ("enemy-release hypothesis"), and consequently positive feedback in their new ranges

promote their invasive behaviors (e.g., Klironomos 2002, Reinhart et al. 2003, Callaway et al. 2004, Wolfe and Klironomos 2005, Reinhart and Callaway 2006, Van Grunsven et al. 2007,Vogelsang and Bever 2009). At the same time, positive feedbacks can also promote habitat partitioning of ecologically similar species across environmental gradients, contributing to beta diversity at larger spatial scales. For example, Pizano et al. (2010) showed that positive feedback of two cryptic plant species and their associated soil microbial communities was mediating habitat segregation and incipient speciation of these two tree species in Panama.

Plant-soil dynamics have been widely studied in natural habitats and for particular types of ecosystems such as temperate grasslands (e.g., Casper et al. 2008, Petermann et al. 2008, Harrison and Bardgett 2010, Wagg et al. 2011) and old fields (e.g., Klironomos 2002, Kardol et al. 2007, Schnitzer et al. 2011, de Voorde et al. 2011). Yet, we still have a poor understanding on how plant-soil dynamics vary with variations in abiotic conditions within biological communities, and across heterogeneous habitat types of human-modified ecosystems. This is particularly true in the tropics, where we are just starting to uncover the role of SMC in natural habitats, but not in human-modified landscapes. Thus, the purpose of my dissertation is to better understand how plant-soil interactions vary across natural habitats of high plant diversity (forest fragments) and managed habitats with high soil fertilization and low plant diversity (pastures and coffee plantations) in a fragmented landscape in the tropical Andes. In addition, I want to address the role of distinct components of SMC, namely AMF vs. non-AMF soil microbes, in driving plant-soil dynamics across these habitats.

CHAPTER 2
GREENHOUSE AND FIELD EVIDENCE FOR THE ROLE OF PLANT-SOIL INTERACTIONS IN DRIVING PLANT AND SOIL MICROBIAL COMMUNITY COMPOSITION ACROSS CONTRASTING HABITATS

Summary

Soil microbial communities (SMC) have been shown to influence plant community composition and drive key ecosystem processes in a wide range of environments, but we still have a poor understanding of how plant-soil dynamics vary across abiotic and biotic environmental heterogeneity. In this study we examined the prediction that SMC would differ across habitats with contrasting soil fertility and plant community composition. Our general expectation was that soil microbes that have negative effects accumulate in each community depending on the different plant species that are abundant in each habitat. In particular, we predicted that plants should benefit more from SMC from "away" habitats (where the plant species rarely occur or don't occur at all) compared to "home" habitats (where species typically occur and thus accumulate detrimental soil microorganisms). To test these predictions, we compared the effects of whole-soil microbial communities from highly fertilized, low diversity agricultural monocultures, and unmanaged, highly diverse pre-montane tropical forest fragments on growth of plant species abundant in each of these habitat types both in the greenhouse and in the field. In addition, the impacts of SMC were also assessed relative to complete elimination (soil sterilization in the greenhouse) and reduction (fungicide application in the field) of SMC on plant performance. We found that SMC from contrasting habitats had differential and significant effects on plant growth both in the greenhouse and in the field (confirmed by a significant effect of soil sterilization and fungicide addition), and different plant groups varied in their response to these SMC. Fast growing species

(Brachiaria grass and forest tree pioneers) benefited from away compared to home SMC, while shade tolerant forest tree species and coffee benefited the most from home SMC. Combined, these results suggest that SMC is significantly modified in agricultural monocultures with low species diversity, and these different SMC have substantial negative impacts on both crop and forest plant species, providing further evidence for the complexity and importance of plant-soil interactions across heterogeneous habitats.

Background

Symbiotic associations are important drivers of many ecological and evolutionary processes. A central question in community ecology is how these interactions vary across heterogeneous environments (Thrall et al. 2007), as symbiotic organisms must adapt to one another and to diverse abiotic environmental conditions (Johnson et al. 2010). For example, it might be crucial to understand how key symbiotic interactions are impacted in ecosystems that are modified by humans at unprecedented rates and scales (Bascompte 2009, Kiers et al. 2010).

Plants are constantly interacting with, and being influenced by, highly diverse soil microbial communities (SMC) comprised of neutral, mutualistic and antagonistic soil microbes. Theoretical models predict that increasing resource supply promotes parasitic or pathogenic microbes over mutualistic ones (Thrall et al. 2007). For instance, nutrient enrichment may cause host plants to decrease resource allocation to their rhizosphere partners, shifting the competitive balance among microbes to favor antagonistic microbes (Thrall et al. 2007). Previous studies on how AMF mutualism is affected by soil fertilization have shown that AMF in fertilized soils can become parasitic (i.e. acquire plant resources without providing any benefit to the host) compared to those present in non fertilized soils (Graham and Eissenstat 1998, Graham and Abbott 2000,

Treseder 2004, Kiers and Van der Heijden 2006). In addition, fertilization may act directly or indirectly on both plants and parasitic pathogens, influencing the outcome of their interactions (reviewed in Ghorbani et al. 2008). For example, Davies et al. (1997) showed that excessive fertilization made plants more susceptible to disease, although other studies have shown conflicting results on the effects of soil fertility on disease development (Ghorbani et al. 2008).

In addition to soil nutrient availability, plant diversity also affects the presence of particular microbes in the soil, as there are reciprocal interactions between plants and soil microbes (i.e. plant species composition affects the relative abundance of organisms in the soil, and soil microbial communities shape plant community composition) (e.g., Mangan et al. 2010a). For example, there is a lower diversity of AMF in agricultural monocultures than in natural ecosystems with high plant diversity (e.g., Tchabi et al. 2008, Verbruggen et al. 2010), and SMC including more diverse AMF species can also maintain high plant diversity (Van der Heijden et al. 1998, Van der Heijden et al. 2008). At the local level, plants host unique arrays of mutualistic and antagonistic soil microorganisms that can either promote or limit the establishment of other plants (Bever et al. 1997). For instance, local abundance of a particular plant species may increase as a result of greater benefits from soil organisms more beneficial to itself than to heterospecific hosts (e.g., Klironomos 2002, Callaway et al. 2004). On the other hand, soil organisms that are particularly detrimental to specific plant species can keep the abundance of that species in check and thus contribute to the maintenance of local plant species diversity (Nijjer et al. 2007, Mangan et al. 2010b).

Evidence on the crucial roles that soil microbial communities play in determining plant community composition (Van der Heijden et al. 1998, Klironomos 2002, Mangan et al. 2010b) and ecosystem processes (Van der Heijden et al. 2008) has increased in recent years, although most studies (done along abiotic and biotic gradients) have focused on either mutualists or antagonists, and either in natural or managed habitats. Furthermore, laboratory, experimental and molecular techniques have restricted our ability to study the myriad diversity of soil organisms (calculated to be 10,000 to 50,000 species per gram of soil; Roesch et al. 2007), constraining studies to the few organisms that we can isolate. For example, there are numerous studies on arbuscular mycorrhizal fungi (AMF) (e.g., Van der Heijden et al. 1998, Bever 2002b, Vogelsang et al. 2006, Mangan et al. 2010a) and soil microbial pathogens (e.g., Zhu et al. 2000, Mitchell 2003). But few studies have taken both into account simultaneously, examining the net effects of whole communities of soil microorganisms as they occur naturally (e.g., Mangan et al 2010b). In addition, most studies comprising SMC are done in greenhouses, therefore the extent to which the results can be extrapolated to field conditions is generally uncertain (but see Pringle and Bever 2008, Mangan et al. 2010). In this study we intended to better understand plant-soil interactions of whole soil communities from natural and managed habitats with experiments both in a greenhouse and in the field. To do this, we compared interactions between plants and SMC from habitats with contrasting soil fertility and plant community composition: highly fertilized, poorly diverse agricultural monocultures, and unmanaged, highly diverse pre-montane tropical forest fragments in the tropical Andes.

We intended to test 1) if plants respond differently to SMC coming from contrasting habitats, 2) whether plant species grow better with SMC from “away” (habitats where the plant species rarely occur or don’t occur at all) than with SMC from “home” (habitats where species typically occur), and 3) the effects of elimination or reduction of SMC by soil sterilization (greenhouse) or fungicide addition (field) to test if SMC from these different habitats had a net positive (driven by mutualistic soil organisms) or negative (driven by antagonistic soil organisms) effect on plants. The fungicide benomyl was used for SMC reduction in the field experiment, to compare plant growth and survival under normal field conditions (all soil organisms) and when treated with the fungicide (reduced soil fungi).

Methods

Study Site and Species

This study was conducted in the agricultural region of the Central Cordillera of Colombia between 1200 and 2000 m.s.l. (4°6′N 75°4′ W). The climate is tropical humid, with an average annual temperature of 21ºC, and an annual rainfall of 2550 mm concentrated in two wet seasons (March to July and September to December) (Guzmán-Martínez et al. 2006). Soils are Udands (Andisols) (Ortíz-Escobar et al. 2004). The landscape is dominated by three contrasting habitat types: heavily fertilized, monoculture of sun-grown coffee plantations, occasionally fertilized pastures with low species diversity (mainly African grasses *Pennisetum clandestinum, Melinis minutiflora* and *Brachiaria spp.*), and unmanaged fragments of pre-montane tropical forest (Orrego et al. 2004a). The forest fragments are usually small (1-30 ha), biologically more diverse than the other two habitats, mostly dominated by early and mid-successional plant species with high liana densities. Seedling recruitment of the native species appeared

poor in the understory, where seedlings of exotic species including *Coffea arabica* are common (C. Pizano pers. obs.).

Forest tree species for the experiments were selected from those abundant in forest fragments in the region (Orrego et al. 2004b), encompassing a wide range of seed size and life histories (Table 2-1, Appendix A). *Brachiaria brizantha* and *Coffea arabica* were chosen because they dominate pastures and coffee plantations, respectively in the study region. To sample the high heterogeneity in altitude and climatic conditions in the study region, five farms with similar conditions each containing a pasture, a coffee plantation, and a forest fragment (Table 2-2) were chosen to collect soil for the greenhouse experiment. These farms were treated statistically as blocks in the field experiment. At each of the 15 sites, five samples of 200 g of mineral soil (5-15 cm in depth) each were taken and pooled in a composite sample from which a subsample was analyzed. Soils across the three habitats had similar levels of pH, organic matter content, and some nutrients (N, K, Mg). But forest fragment soils contained only 10-12 % of P in the pastures and coffee plantations, and significantly greater Ca content than the latter (Table 2-3). In addition, light levels in pastures and coffee plantations were approximately 30 times higher than in forest fragments (Table 2-3).

Greenhouse Experiment: Response of Pasture Grass, Coffee, and Forest Trees to SMC From Home and Away Habitats

In this factorial experiment we compared seedling growth of pasture grass, coffee, and eight forest tree species (Table 2-1) inoculated with fresh soil inoculum (i.e. fine roots, rhizosphere soil, and associated biota) sampled from pastures, coffee plantations, and forest fragments. In order to test our hypotheses, we grew plants with the same

common sterilized soil (controlling for soil nutrients) in a single green house environment such that the only varying factor in the experiment was the origin of the SMC inoculum. We compared the growth of each species with SMC from home vs. away habitats, in order to test the effects of whole SMC including beneficial and antagonistic soil microbes. In addition, half of the plants were randomly selected to receive sterilized inoculum, to 1) account for potential abiotic differences between inocula from different habitats, and 2) compare the growth of each plant species with live vs. sterilized inocula to assess if SMC from the different habitats had overall beneficial (i.e. driven by mutualists) or detrimental (i.e. driven by antagonists) effects on plant growth.

Initial growth conditions. Seeds of the ten plant species (Table 2-1, individually identified hereafter by abbreviations) were surface sterilized (0.6 % sodium hypochlorite for 15 minutes) and germinated in trays containing steam-sterilized (for 2hrs) soil 3:2 soil and river sand mixture. The soil (5.4 pH, 0.1 % N, 1.9 % OM-Walkley-Black colorimetry, 7 mg kg^{-1} P-Bray II Bray Kurtz colorimetry, 0.37 mg kg^{-1} K-Ammonium acetate 1N, 3.4 mg kg^{-1} Ca-Ammonium acetate 1N, and 1.1 cmol kg^{-1} Mg-Ammonium acetate 1N) was collected from an open area near the greenhouses in Cenicafé (Colombian National Research Center for Coffee) (Chinchiná, Caldas, Colombia), and was classified as an acrudoxic melanudand from Chinchiná unit (Ortíz-Escobar et al. 2004).

Inoculum preparation. We collected whole-soil inoculum that included mineral soil, roots, rhizosphere soil, and associated organisms from 3 habitat types (pastures, coffee plantations, forest fragments) at each of five farms (blocks), for a total of 15

sampling sites (Table 2-2). At each site, roots from randomly selected plants and rhizosphere soil (5-15 cm in depth) were collected from five random locations and were pooled together to use as inoculum in the greenhouse. We combined and homogenized the inocula from the five sites for each habitat type. Half of the inoculum was used live (i.e. as brought from the farm), while half of it was sterilized in the autoclave (for 2 hrs) to control for possible abiotic differences across inocula (McCarthy-Neumann and Kobe 2010).

Growth conditions and treatments. The same sterilized soil and sand mixture used for seed germination was used to fill 70 % of the 1-L volume pots to which an equal quantity (100 mL) of soil inoculum from one of the three habitats either live or sterilized was added. Each treatment combination of inoculum source habitat (forest fragments, coffee plantations, pastures) $\times$ plant species (2 crop plant species, 8 forest tree species) $\times$ sterilization (live, sterile) was replicated in 10 pots. At the start of the experiment, 25 seedlings per species were harvested to estimate initial biomass for each species. Plants in the greenhouse were grown under 20 % light for 130 days and then harvested. All harvested plants were dried at 60°C for three days. Relative growth rate (RGR) was calculated based on initial and total final biomass of seedlings; RGR=[Ln(final biomass)-Ln(initial biomass)]/(# of days). We examined roots from a subsample of 3 plants from each treatment for the presence of AMF. No plants grown with sterilized inocula had signs of AMF colonization or other soil fungi.

Statistical analyses. The effects of inoculum source, plant species, and inoculum sterilization (and their interactions) on relative growth rate (RGR) were analyzed using a fixed effect three-way ANOVA. We then used *a priori* contrasts to compare seedling

growth among four species groups (Brachiaria grass, coffee, pioneer, and shade tolerant; Table 2-1) when grown with inoculum from their "home" habitat (e.g., coffee with inoculum from coffee plantations) versus inoculum from "away" habitats (e.g., coffee with inoculum from forest fragments) separately for live inoculum and sterile inoculum treatment. We also used *a priori* contrasts to test for the effects of soil sterilization on the response of each species group to home vs. away inoculum, and to compare the growth of each species group with live vs. sterilized SMC from the three habitat types. Statistical analyses were done with JMP®, version 8.0 (SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513). We used the Dunn-Sidak correction to adjust the significance levels of contrasts.

Field experiment: Response of pasture grass, coffee, and forest trees to fungicide treatment in home and away habitats

In this experiment we intended to test for the effects of SMC on plant growth under field conditions, where the effects of SMC may be masked by large variations in environmental factors including light, soil resource availability, and competition. We selected a subset of the species used in the greenhouse experiment (Table 2-1, including the two crop species, two pioneer species, and two shade tolerant forest tree species) and transplanted their seedlings to agricultural lands and forest fragments, treating half of them with the fungicide benomyl, which reduces the activity of soil fungi. In order to test our hypotheses, we 1) compared the growth of each species in home vs away habitat types, and 2) compared the growth of each species between control and fungicide treatments to test if SMC from these habitats had overall beneficial, detrimental, or neutral effects on plant growth.

Initial growth conditions. Seeds of the six plant species were surface sterilized (0.6 % sodium hypochlorite for 15 minutes) and germinated in trays containing the same steam-sterilized soil 3:2 soil and river sand mixture that we used for the greenhouse experiment. Prior to transplanting to the field, seedlings had been grown for two months (Cof and forest shade tolerant), one month (forest pioneer), or two weeks (Brachiaria grass) to allow small-seeded seedlings to acquire a similar size to that of other species at the time of transplanting.

Field surveys and site preparation. We selected four of the five farms (blocks) previously used for soil sampling and inoculum collection for the greenhouse experiment (Table 2-2, Table 2-3). In each of the twelve sites (3 habitat types $\times$ 4 blocks), we randomly marked ninety 1 $\times$ 1 m plots where seedlings were to be transplanted (one seedling per plot). One month before transplanting the seedlings into the field, we recorded proportion vegetation ground cover and plant species richness (total number of species) for each seedling plot (neighbor species richness), then cleared the area with a machete (only for plots with ground cover > 60 %), and opened a 20 $\times$ 20 cm and 30 cm deep hole in the soil. Neighbor species richness was measured because plant neighbors strongly influence the composition of SMC and its impacts on the focal plant (e.g., Mummey et al. 2005). Plots were randomly assigned to either control (C) or fungicide (F) treatment, and the holes of the F plots received 1 L of the fungicide benomyl (1-[(Butylamino) carbonyl]-1H-benzimidazol-2-yl] carbamic acid methyl ester) at a concentration of 1.125 g L^{-1} (Helgason et al. 2007). We chose this fungicide because it reduces a wide range of soil fungi including many AMF species and pathogenic asco- and basidiomycete fungi (Helgason et al. 2007, Nijjer et al. 2007).

Experiment monitoring and harvest. Every month throughout the three-month experiment fungicide was re-applied (same rate as mentioned above), and survival of each seedling was recorded. The amount of light reaching each seedling was calculated as the proportion of photosynthetic active radiation (PAR) measured above each seedling compared to that of an open site, taken twice during the experiment at different times of the day using a light meter (LI-250, Lincoln, NE, USA). After three months all seedlings were harvested (with roots), dried for 3 days at 60°C, and weighed (total dry weight).

Statistical analyses. The effects of species type (light-demanding, shade tolerant), plant species (nested within species type), habitat type, and fungicide on seedling survival were analyzed using a proportional hazards model with block, light, $\log_{10}$-transformed initial leaf area, neighborhood species richness, and proportion vegetation cover as covariates. In addition, we compared seedling survival curves for each species in each habitat using the Kaplan-Meier method (Fox 2001). A split-plot mixed effect three-way ANCOVA was used to examine the response of log-transformed final biomass to block and block $\times$ habitat type as random factors, and species, habitat type, and fungicide (and their interactions) as fixed factors. In addition, light, $\log_{10}$-transformed initial leaf area, neighborhood species richness, and vegetation cover were included as covariates. We then used *a priori* contrasts to compare the growth of the four species types (Brachiaria grass, coffee, forest pioneer, and forest shade tolerant; Table 2-1) in their “home” habitat (e.g., coffee in coffee plantations) versus “away” habitats (e.g., coffee in forest fragments) separately for control and fungicide treatments. In addition, we also used contrasts to compare the effect of fungicide on the

response of each species group to home vs. away habitat types, and to compare the growth of each species under the control and the fungicide treatment in each of the three habitats. Because Sol, a pioneer species, had very high mortality across all habitat types, this species was excluded from the growth analyses. Statistical analyses were done with JMP, version 8.0 (SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513). We used the Dunn-Sidak correction to adjust the significance levels of contrasts.

Results

Greenhouse experiment: Response of pasture grass, coffee, and forest trees to SMC from home and away habitats

Inoculum source, plant species, and inoculum sterilization all significantly affected the growth of seedlings (Table 2-4). In addition, species growth differed across different combinations of inoculum source and sterilization as indicated by significant two-way and three-way interactions (Table 2-4).

When inoculated with live soil organisms, Brachiaria grass grew marginally less with inoculum from home (pastures) than with inoculum from away habitats (coffee plantations and forest fragments) ($F_{1,526}$ = 5.8; P = 0.017; Fig. 2-1A), however growth of this species was similar between home and away habitats for sterilized inoculum ($F_{1,526}$ = 1.8; P = 0.18; Fig. 2-1A). Thus, soil sterilization eliminated the response of Brachiaria grass to SMC from home compared to away habitats, indicating a strong home-disadvantage with live inoculum relative to sterile inoculum ($F_{1,526}$ = 7.0; P = 0.0015) (Fig. 2-2A). There was no difference between the two “away” habitats (coffee plantations and forest fragments) for either live ($F_{1,526}$ = 3.2; P = 0.074), or sterilized ($F_{1,526}$ = 0.006; P = 0.94) inocula. Growth was significantly better with sterile than with

live inoculum from pastures ($F_{1,526}$ = 28.3; P < 0.001) and forest fragments ($F_{1,526}$ = 9.1; P = 0.003) (Fig. 2-3A), indicating that this species encounters antagonistic soil organisms in the SMC from these habitats. Growth of coffee did not differ significantly between home and away habitats whether inoculum was sterilized or not (P > 0.2 for all contrasts; Fig. 2-1B, 2-2A), and between live and sterilized inocula (P > 0.12 for all contrasts) (Fig. 2-3B).

Forest woody pioneer species grew significantly less with live inoculum from home (forest fragments) compared to inoculum from away (pastures and coffee plantations) habitats ($F_{1,526}$ = 14.3; P < 0.001), but did not differ when the inocula were sterilized ($F_{1,526}$ = 0.6; P = 0.46) (Fig. 2-1C). Thus, similar to Brachiaria grass, difference between home vs. away SMC was significantly stronger with live than with sterile inoculum as indicated by the negative *a priori* contrast ($F_{1,526}$ = 10.2; P = 0.0015) (Fig. 2-2A). Notably, growth woody pioneer species did not differ between the two away habitats (pastures and coffee plantations) for live ($F_{1,526}$ = 0.01; P = 0.84), or sterilized ($F_{1,526}$ = 0.4; P = 0.52) inocula. In addition, these species grew significantly better with sterilized, compared to live inoculum from pastures ($F_{1,526}$ = 12.4; P < 0.001), coffee plantations ($F_{1,526}$ = 7.6; P = 0.006), and forest fragments ($F_{1,526}$ = 51.1; P <0.001) (Fig. 2-3C), suggesting that SMC from all three habitat types have overall detrimental effects on this group of species.

Finally, shade tolerant species grew better with home (forest fragments) compared to away (pastures and coffee plantations) SMC with both live ($F_{1,526}$ = 17.2; P < 0.001) and sterilized (marginally significant; $F_{1,526}$ = 4.9; P = 0.03) inocula (Fig. 2-1D). Growth of these species was best with both live and sterilized inocula from forest

fragments compared to SMC from the other two habitats ($F_{1,526}$ = 2.0; P = 0.16), and soil sterilization only marginally decreased the home advantage (Fig. 2-2A). This suggests that shade tolerant species found both biotic, and abiotic benefits from SMC from home compared to that of away habitats. In terms of the effects of inocula from the two away habitats, they grew marginally better with live ($F_{1,526}$ = 5.2; P = 0.023) inocula from coffee plantations compared to that from pastures, but there was no significant difference with sterilized inocula ($F_{1,526}$ = 0.02; P = 0.89) (Fig.2- 3D). Lastly, these species grew significantly better with sterilized than with live inoculum from pastures ($F_{1,526}$ = 20.5; P < 0.001), but had similar growth with sterilized and live inoculum from coffee plantations ($F_{1,526}$ = 5.5; P = 0.02) and forest fragments ($F_{1,526}$ = 2.9; P = 0.09) (Fig. 2-3D). These results suggest that shade tolerant species encounter more antagonistic soil organisms in pastures and coffee plantations than in forest fragments.

Field experiment: Response of pasture grass, coffee, and forest trees to fungicide treatment in home and away habitats

Seedling survival was higher for shade tolerant species (Cof*,* Gar*,* Ret*)* than for the fast growing Brachiaria grass and the two woody pioneer plant species (CecA*,* Sol) across different habitat types and treatments (Tables 2-5, 2-6). In fact, only 7 seedlings of shade tolerant species died during the experiment. Survival of fast growing species varied across habitat types, as indicated by a significant habitat type $\times$ species type [species] interaction (Table 2-6). For instance 80 % ($\pm$ 5.7) of Brachiaria grass seedlings survived in pastures, while 53.4 % ($\pm$ 13.4) and 31.6 % ($\pm$ 9.4) survived in coffee plantations and in forest fragments, respectively (Wilcoxon χ^2 = 23.7, P < 0.001). Similarly, 83 % ($\pm$ 4.7) of CecA seedlings survived in pastures, while 59.6 % ($\pm$ 10.9) and 59.6 % ($\pm$ 14.2) survived in coffee plantations and forest fragments, respectively

(Wilcoxon χ^2 =12.0, P = 0.0025). Sol had very high mortality across habitats, with only 34.6 % (± 7.3) seedlings surviving in pastures, 9.5 % (± 5.2) in forest fragments, and 4.7 % (± 2.5) in coffee plantations (Wilcoxon χ^2 = 8.4, P = 0.015). Fungicide had a marginal but significant effect on seedling survival, affecting fast growing and pioneer species more than shade tolerant species, as indicated by a significant species type × fungicide interaction (Tables 2-5, 2-6). In fact, seedlings treated with fungicide had marginally lower survival for CecA (C: 67.9 % ± 9.3; F: 66.9 % ± 9.0), but higher survival for Sol (C: 13.8 % ± 4.8; F: 20 ± 6.4) across the three habitat types.

Seedling biomass was affected by species, habitat type, and fungicide (Table 2-7). Furthermore, seedling growth differed across different habitat types, and across different combinations of fungicide treatment and habitat type, indicated by significant two- and three-way interactions (Table 2-7).

Both control ($F_{1,33}$ = 72.5; P < 0.001) and fungicide-treated ($F_{1,35}$ = 99.1; P < 0.001) seedlings of Brachiaria grass grew almost 200-fold more in their home habitat (pastures) compared to away (coffee plantations and forest fragments) habitats (Figs. 2-1E, 2-4A), most likely driven by the much higher light availability in pastures (64 ± 1.4 %) and coffee plantations (50.4 ± 1.7 %) compared to forest fragments (1.6 ± 0.1 %). Growth did not differ across habitats with similar light conditions (pasture and coffee plantations) for control ($F_{1,29}$ = 3.5; P = 0.072) and fungicide-treated seedlings ($F_{1,33}$ = 0.95; P = 0.95), suggesting the overwhelming importance of light on growth rates. Nevertheless, fungicide marginally enhanced the growth increase of this grass in pastures compared to other habitats ($F_{1,672}$ = 2.2; P = 0.14; Figs. 2-1E, 2- 2B), compatible with the pattern expected from the dominance of antagonistic organisms in

its home habitat compared to that of other habitats. Finally, control and fungicide-treated seedlings of Brachiaria grass grew similarly in coffee plantations and forest fragments ($P > 0.23$ for all contrasts) (Fig. 2-4A).

Consistent with the greenhouse experiment, growth of coffee did not differ between home and away habitats across control and fungicide-treated seedlings ($P > 0.5$ for all contrasts; Fig. 2-1F), and between control and fungicide-treated seedlings across habitat types ($P > 0.38$ for all contrasts) (Fig. 2-2B, 2-4B). Both control ($F_{1,39} = 58.9$; $P < 0.001$) and fungicide-treated seedlings ($F_{1,25} = 24.9$; $P < 0.001$) of pioneer species CecA grew significantly less in home (forest fragments) than in away habitats (pastures and coffee plantations) (Fig. 2-1G), and this response was reduced by the fungicide ($F_{1,672} = 9.5$; $P = 0.0021$; Fig.2- 2B). Between the two high-light habitats (pastures and coffee plantations), it grew significantly better in coffee plantations than in pastures ($F_{1,23} = 18.5$; $P < 0.001$) (Fig. 2-4C). In addition, control seedlings grew significantly better than fungicide-treated seedlings in coffee plantations ($F_{1,672} = 29.6$; $P < 0.001$), suggesting that CecA not only benefits from the higher light levels, but also from more beneficial SMC in coffee plantations compared to forest fragments. Growth of this species did not differ between control and fungicide-treated seedlings in pastures ($F_{1,671} = 0.1$; $P = 0.74$) and forests ($F_{1,672} = 0.7$; $P = 0.42$) (Fig. 2-4C). In case of the two shade tolerant forest tree species (Gar and Ret), control ($F_{1,16} = 0.5$; $P = 0.5$) and fungicide-treated seedlings ($F_{1,17} = 0.001$; $P = 0.97$) grew similarly in home and away habitats (Fig. 2-1H), regardless of fungicide treatments, across habitat types ($P > 0.2$ for all contrasts) (Fig. 2-2B, 2-4D).

Discussion

In this study we found strong evidence both in the greenhouse and the field that SMC from contrasting habitats had differential effects on plant growth. Only live (and not sterilized) inocula brought from agricultural lands and forest fragments significantly differed in their effects on plant growth of most plant species in the greenhouse (Fig. 2-3 A-D), and fungicide had a significant effect on both survival and growth of these species in the field (Table 2-5, 2-6, 2-7). Plants' response to SMC from different habitats varied among species both in the greenhouse and in the field, suggesting that the composition of SMC differed across habitat types, which had differential effects on plant hosts. Furthermore, we found that fast-growing species (Brachiaria grass and forest pioneer species) were negatively impacted by SMC from home compared to that of away habitats, while shade tolerant forest species benefited from SMC from home compared to that of away habitats (greenhouse experiment) (Fig. 2-1).

Comparison of the effects of soil sterilization in the greenhouse and fungicide addition in the field revealed that suppression of SMC had consistent directional effects on performance of each species across the three different habitats (Fig.2-2). Additionally, we also found that while most species grew better with sterilized than with live soil inoculum in the greenhouse (Fig. 2-3), fungicide did not have a consistent effect on plant growth across different habitats in the field (Fig. 2-4). In fact, fungicide actually decreased the growth of pioneer forest species in coffee plantations. Thus, results from the greenhouse experiment suggest that SMC from agricultural lands and forest fragments can exert net negative effects, while results from the field experiment suggest that SMC in at least some of these habitats are dominated by mutualistic, and not antagonistic, soil microorganisms. Combined, these results provide further evidence for

the complexity and importance of plant-soil interactions across habitats with contrasting abiotic characteristics and different plant community composition.

Abiotic vs. biotic variation across different habitats and the response of plants to soil microbial communities from these habitats

Previous studies have shown that SMC in managed habitats with low plant diversity and chemical fertilization (i.e. agricultural lands) contain AMF communities that are less diverse (e.g. Bradley et al. 2006, Verbruggen et al. 2010) and beneficial (e.g. Graham and Abbott 2000) to plants than those present in natural habitats with higher plant diversity and no fertilization (e.g. Neuhauser and Fargione 2004, Kiers and Denison 2008, Verbruggen and Kiers 2010). Furthermore, it is widely known from agricultural settings that species-specific pathogens accumulate in low diversity monocultures, and that environmental factors such as light and nutrient availability determine how resistant or susceptible plants are to antagonistic soil organisms (Zhu et al. 2000, Reynolds et al. 2003). Thus, we expected that SMC from habitats with fertilized, species-poor plant communities (i.e. agricultural lands) should have an overall negative effect on plant growth compared to SMC from unmanaged, highly diverse natural habitats (i.e. forest fragments). We contrasted this prediction with the hypothesis that plants would benefit from SMC from “away” compared to “home” habitats. Implicit in these predictions is the likelihood of SMC being dominated by either generalist (first prediction) or specialist (second prediction) soil microorganisms across different habitats.

The results from the greenhouse show that SMC from pastures and coffee plantations were indeed different from those from forest fragments, as there was no common pattern in the response of plants species across inocula sources (Table 2-4).

However, we found no evidence suggesting that SMC from pastures and coffee plantations were less beneficial or more detrimental than those present in natural forests regardless of the host plant species (Table 2- 4). In fact, the net effect of SMC on plant performance was mainly determined by the habitat of origin of both plants and SMC (home vs. away), supporting our second prediction that host-specialized microbes had a significant ecological role. Furthermore, the response of plants to different SMC was mainly determined by their life history. Fast growing species (Brachiaria grass and forest pioneer species CecT*,* Ochr*,* and Sol) grew significantly better with whole-soil inoculum from away habitats compared to their respective home habitats (Fig. 2-1A, 2-1C), while having similar growth across inocula from away habitats. Moreover, sterilization eliminated the effect of home vs. away inocula on the growth of these species (i.e. no growth difference across sterilized inocula) (Fig. 2-2A), verifying the role of soil organisms in driving their response to soils from different habitats.

In contrast to the results for fast-growing species, forest shade tolerant plant species (Sip*,* Gar*,* Ret*,* Gus*,* and Jug) grew significantly better with home, rather than with away SMC (Fig. 2-1D). These results support previous studies showing that the susceptibility of plant species to soil microorganisms is inversely proportional to seedling shade tolerance (e.g. Augspurger 1983, Zangaro et al. 2003, Kardol et al. 2006, Kardol et al. 2007, McCarthy-Neumann and Kobe 2008); fast-growing species, which are expected to invest less to defensive traits are likely to be susceptible to antagonistic soil organisms in their home habitat that are absent in other habitat types. Conversely, shade tolerant species, investing heavily in defense with support from large seed reserves, may be less susceptible to antagonistic soil pathogens common in their

home habitat (Kitajima 2002). Finally, shade tolerant species grew unexpectedly better with the sterilized inocula from home compared to away habitats, even though forest soils had almost ten-fold lower P than coffee plantations and pastures (Table 2- 3). These species could have benefited from higher organic matter content of forest soils and associated organic N availability (Table 2-3), as nutrients other than P might be limiting to seedling growth (Fetcher et al. 1996).

In the greenhouse we compared the effects of SMC under the same abiotic environmental conditions, standardizing environmental factors associated with different habitat types in the field (e.g. soil nutrients and light levels). In the field we intended to test whether modifying SMC (by adding fungicide) would have significant effects in the light of variability of other environmental factors among habitats. Our habitats had marked differences in soil nutrient levels and light levels; forest fragments had an almost five-fold lower soil P content, and 50-fold lower light level than pastures and coffee plantations (Table 2-3). Despite these large differences in abiotic environmental variables that could overwhelm the potential effects of SMC, we detected a significant effect of fungicide on both plant survival (Table 2- 6) and growth (Table 2-7). Furthermore, the effect of fungicide varied across habitats and species, showing the same directional interactive effects as in the greenhouse, suggesting that 1) SMC differed across different habitat types, and 2) SMC had differential host-specific effects on plant species.

Fast-growing species (Brachiaria grass and pioneer tree CecA) responded strongly to different light levels between habitats, therefore grew significantly better in open pastures and coffee plantations than in shaded forest fragments (Fig. 2-1E, 2-1G).

Nevertheless, the effect of fungicide on these species suggests that SMC play a significant role in determining their performance in the field. For instance, Brachiaria grass grew similarly better across pastures and coffee plantations than in the shade of forest fragments (Fig. 2-1E). Yet, consistent with the greenhouse experiment, fungicide marginally increased its growth in pastures (Fig. 2-1E,2- 2B), indicating that this grass encounters more antagonistic or less mutualistic soil organisms in its home habitat of pasture. Likewise, the pioneer species CecA also grew better in pastures and coffee plantations compared to its home habitat (forest fragments), however fungicide significantly reduced the away advantage (Fig. 2-1F, 2-2B), largely because beneficial SMC in coffee plantations apparently were suppressed (Fig. 2-4D).

Despite showing differences in growth across inocula from contrasting habitat types in the greenhouse (Fig. 2-3D), shade tolerant species Gar and Ret showed no response to different habitat types and fungicide addition (Fig. 2-1H, 2- 2B). One possible explanation for this lack of response is that the field experiment was too short (three months) to detect growth responses in these slow growing species. Likewise, growth of coffee did not differ across treatments in either of the two experiments (Fig. 2-1B, 2-1F, 2-2A,2- 2B). Furthermore, these seedlings had large seed reserves whose effects persisted throughout the experiment.

Net effects of soil microbial communities on plant performance: mutualist vs. antagonistic soil microbes

Although there are numerous studies on the impacts of SMC on plant performance, few studies have included appropriate control treatments in both the greenhouse and the field to address if the overall net effect of SMC on plant performance is driven by mutualistic (e.g. AMF) or antagonistic (e.g. soil microbial

pathogens) soil microorganisms (but see McCarthy-Neumann and Kobe 2008, Mangan et al. 2010b). Previous studies with proper control treatments have found contrasting results. For example, Van der Putten and Peters (1997) found the competitive outcome between two successional plant species was driven by parasitic nematodes and pathogenic fungi in an experiment comparing sterilized and not-sterilized soils from coastal sand dunes. Similarly, Reinhart et al. 2005 showed that *Prunus serotina* grew better with sterilized and fungicide-treated soils compared to untreated soils, confirming a predominant effect of soil antagonists over mutualists. In contrast, Mangan et al. (2010b) found that six shade tolerant tree species grew better with live, than with sterilized, whole-soil microbial communities collected from around parent trees of these species in the field in Panama, suggesting an overall positive effect of these SMC on plant growth.

In our study, both types of net effects of whole SMC on plant performance occurred in the greenhouse and in the field. In the greenhouse, most plant species grew better with sterilized than with live inoculum from different habitats (indicated by a significant effect of inoculum sterilization; Table 2-4, Fig. 2-3), suggesting that SMC from pastures, coffee plantations and forest fragments are dominated by antagonistic soil microorganisms over mutualistic ones. This indicates that most plants species encounter generalist antagonistic soil microorganisms across habitat types. At the same time, the response of plant species to SMC from home and away habitats suggests that plants also encounter specialist antagonistic soil microorganisms in their respective habitats. Seedlings treated with fungicide in the field had marginally higher survival than those not treated with fungicide (Table 2-5, 2-6), although fungicide marginally

decreased growth (Table 2-7). In fact, the only species that showed a significant response to fungicide treatment was the pioneer *C. angustifolia*, which grew better without than with fungicide in coffee plantations (Fig. 2-4). This suggests that SMC from coffee plantations might host mutualistic soil organisms that are particularly beneficial for some species such as forest pioneer species.

The differences in results from the greenhouse and the field indicate that soil sterilization and fungicide addition are not analogous treatments. Soil sterilization eliminates all soil biota (and this was evident in the roots from sterile treatments in the greenhouse), while the fungicide benomyl reduces the abundance of some, and increases the abundance of other, soil fungi. For example, Helgason et al. 2007 showed that plants treated with benomyl in the field hosted AMF communities with the same number of AMF species as control plants, however the AMF species composition shifted in the fungicide-treated plants. In addition, more generalist AMF (those occurring in a wide range of host plants) were more resilient to the fungicide treatment. Thus, the lack of a strong response to fungicide in the field may also reflect that 1) plants show a limited response to changes in soil fungal species abundance in SMC in the field, and 2) agricultural lands and forest fragments in our study region are dominated by generalist soil fungi that were not greatly affected by the addition of fungicide. Results from the greenhouse experiment strongly suggest the first explanation is the most plausible one.

Ecological implications

Conversion of natural ecosystems to agricultural lands decreases both plant diversity aboveground, and the diversity of soil microbial communities belowground (e.g. Hooper et al. 2000, Postma-Blaauw et al. 2010, Verbrugeen et al. 2010). Studies have shown that in addition to low plant diversity, agricultural practices such as tillage

(reviewed by Gosling et al. 2006), and fertilization (e.g., Bradley et al. 2006, Rasmann et al. 2009) further decreases the diversity of belowground communities. However, fewer studies have addressed the potentially important effects of these losses on ecosystem services and plant community composition (e.g., Strickland et al. 2009). We found marked differences in the SMC between managed and natural systems, and significant impacts of soil microorganisms on the performance of all plant species except for coffee. Thus, differences in SMC might significantly impact forest restoration (Allen et al. 2005), and agricultural production (Banwart 2011). Our results suggest that while forest pioneer tree species encounter less antagonistic soil organisms in agricultural lands than in forest fragments (i.e. SMC from agricultural lands, in particular coffee plantations, benefit these species), forest shade tolerant tree species encounter more antagonistic soil organisms in agricultural lands, which can potentially hinder their regeneration in these habitats. Furthermore, we found that pastures accumulate antagonistic soil organisms with negative impact on the economically important grass *B. brizantha*. In contrast, coffee was not responsive to different SMC although coffee plantations host soil organisms that are particularly beneficial for other plant species. Collectively, these findings add substantiate on the still poorly understood great complexity and significance of plant-soil interactions in both natural and agricultural settings.

Table 2-1. General characteristics of the eleven plant species used in the greenhouse and field experiments. Seed mass (mean ± S.D) was measured from 25-50 seeds/species dried for 3 days at 60 °C.

Species	Family	Species group	Typical habitat ("home")	Seed dry mass (g)	Abbreviation
Brachiaria brizantha[+]	Poaceae	Brachiaria grass (crop)	P[1]	0.0077 ± 0.0013	Bra
Coffea arabica[+]	Rubiaceae	Coffee (crop)	C[2]	0.22 ± 0.023	Cof
*Cecropia angustifolia**	Cecropiaceae	Pioneer	F[3]	0.0011 ± 0.00028	CecA
Cecropia telealba	Cecropiaceae	Pioneer	F[3]	0.00039 ± 0.00012	CecT
Ochroma pyramidale	Bombacaceae	Pioneer	F[3]	0.0037 ± 0.0012	Ochr
Solanum aphynodendrum[+]	Solanaceae	Pioneer	F[3]	0.0015 ± 0.00053	Sol
Garcinia madrunno[+]	Clusiaceae	Shade tolerant	F[3]	2.92 ± 0.77	Gar
Gustavia superba	Lecythidaceae	Shade tolerant	F[3]	10.55 ± 2.78	Gus
Juglans neotropica	Juglandaceae	Shade tolerant	F[3]	26.04 ± 14.49	Jug
Retrophyllum rospigliosii[+]	Podocarpaceae	Shade tolerant	F[3]	0.86 ± 0.18	Ret
Siparuna aspera	Monimiaceae	Shade tolerant	F[3]	0.014 ± 0.025	Sip

*Used in the field but not in the greenhouse experiment.
[+]Used in both the greenhouse and field experiment
[1]Pastures
[2]Coffee plantations
[3]Forest fragments

Table 2-2. Characteristics of the farms (blocks) and sites where the soil inocula for the greenhouse experiment were collected (Guzmán-Martinez et al 2006), and where the field experiment was set up. At each farm there were three habitats: a pasture (P), a sun-exposed coffee plantation (C), and a forest fragment (F).

Farm (block)	Geographic coordenates	Altitude (m.s.l)	Mean annual precipitation (mm yr^{-1})	Mean annual temperature (ºC)	Habitat (site)	Habitat patch size (ha)
Cenicafé	05º00′N 75º36′W	1380	2733	20.9	P	0.5
					C	0.5
					F	40.0
Playa rica	05º00′N 75º36’W	1290	2750	20.7	P	10.0
					C	60.0
					F	30.0
Alto español*	04º56′N 75º42′W	1720	3140	18.8	P	1.0
					C	2.0
					F	0.3
Naranjal	04º59′N 75º39′W	1400	3137	21.4	P	22.0
					C	38.0
					F	27.0
La Argentina	05º02′N 75º41′W	1354	2935	19.9	P	0.5
					C	100.0
					F	1.5

* This block was not used in the field experiment.

Table 2-3. Mean (± SE) soil pH, organic matter (OM) content, nutrient content, and light level of pastures (P), coffee plantations (C), and forest fragments (F) where soil inocula were collected.

Habitat	pH[1]	OM[3] (%)	N[2] (%)	P[4] (mg/kg)*	K[5] (cmol/kg)	Ca[5] (cmol/kg)*	Mg[5] (cmol/kg)	Light (%)*
P	5.6 (± 0.1)	7.2 (± 1.4)	0.3 (± 0.04)	54.4 (± 25.3)	0.6 (± 0.2)	5.6 (± 0.7)	2.1 (± 0.4)	64.1 (±1.4)
C	5.0 (±0.3)	9.6 (± 1.7)	0.4 (± 0.06)	42.0 (± 16.7)	0.3 (± 0.1)	4.4 (± 1.1)	1.7 (± 0.6)	50.4 (± 1.7)
F	5.5 (± 0.2)	12.4 (± 2.1)	0.5 (± 0.06)	5.6 (± 0.4)	0.4 (± 0.05)	8.3 (± 2.3)	2.4 (± 0.7)	1.7 (± 0.1)

Notes: Log-transformed data was analyzed with one-way ANCOVA including block as a random factor and habitat as a fixed factor. Light was measured as the percentage of photosynthetic active radiation (PAR) above each of the 1080 seedlings transplanted to the field (field experiment) compared to that of an open site.

[1]pH: Potentiometer soil: water 1:1

[2]N (total): Calculated

[3]OM: Walkley-Black – colorimetry

[4]P: Bray II - colorimetry Bray Kurtz

[5]K, Ca, Mg: Ammonium acetate 1N

* Significant differences between habitat types $P < 0.05$

Table 2-4. ANOVA results for relative growth rate (RGR) in the greenhouse of two crop plant species and eight forest tree species (4 pioneer and 4 shade tolerant species) (Table 2-1) across different inoculum sources (forest fragments, coffee plantations, pastures), species, and inoculum sterilization (no sterilization-live; sterilized), and their interactions.

		Total biomass (Error df = 526)	
Source	**df**	***F***	***P***
Inoculum source	2	2.7	0.066
Species	9	1730.3	<0.001
Sterilization	1	100.5	<0.001
Inoculum source × Species	18	1.9	0.012
Inoculum source × Sterilization	2	3.6	0.029
Species × Sterilization	9	11.9	<0.001
Species × Sterilization × Inoculum source	18	2.8	<0.001

Notes: ANOVA was used to analyze RGR (RGR=[Ln(final biomass)-Ln(initial biomass)]/(# of days)]. Initial biomass was calculated as the average of 25 seedlings/species harvested just before the experiment was set up. Contrasts within the three-way interaction tested the differences between RGR of plants grown with inoculum coming from their corresponding habitat ("home") and inoculum coming from "away" habitats.

Table 2-5. Percentage survival (± SE) in the field of seedlings of two crop plant species (Bra and Cof) and four forest tree species (CecA, Sol, Gar, and Ret) (species abbreviations in Table 2-1) in each of 12 sites (3 sites-one per habitat type per block). Sixteen seedlings from each species (except Ret, for which there were 10 seedlings) were transplanted to each site; half served as control and half were treated once a month with fungicide (benomyl).

		Plant species					
		Fast-growing and pioneer			Shade tolerant species		
Habitat	Treatment	Bra	CecA	Sol	Cof	Gar	Ret
Pastures	Control	78.5 (± 9.3)	87.8 (± 5.1)	31.5 (± 8.0)	97 (± 3.0)	97 (± 3.0)	100 (± 0.0)
	Fungicide	81.5 (± 8.0)	81.5 (± 7.8)	37.8 (± 13.4)	100 (± 0.0)	94 (± 3.5)	100 (± 0.0)
Coffee p.	Control	53.3 (± 19.4)	62.8 (± 16.9)	9.8 (± 3.3)	100 (± 0.0)	100 (± 0.0)	100 (± 0.0)
	Fungicide	53.5 (± 21.4)	56.5 (± 16.5)	3.3 (± 3.3)	97 (± 3.0)	100 (± 0.0)	100 (± 0.0)
Forests	Control	31.5 (± 16.5)	53.3 (± 20.7)	0.0 (± 0.0)	100 (± 0.0)	100 (± 0.0)	100 (± 0.0)
	Fungicide	31.8 (± 12.0)	66.0 (± 22.0)	19.0 (± 8.1)	100 (± 0.0)	100 (± 0.0)	90 (± 5.8)

Table 2-6. Seedling survival in the field of two crop plant species (Bra and Cof), and four forest tree species (CecA, Sol, Gar, and Ret) (species abbreviations in Table 2-1) as affected by plant species (nested within species type), species type (light demanding, shade tolerant), habitat type (pasture, coffee plantation, forest fragment) and fungicide (control, benomyl application).

Source	**Df**	***L-R Chisquare***	***P***
Species type	1	99.7	<0.001
Species type [Species][1]	4	70.2	<0.001
Habitat type	2	0.0	1.000
Fungicide	1	4.0	0.046
Species type*Habitat type	2	0.0	1.000
Habitat type* Species type [Species][1]	8	22.5	0.004
Species type*Fungicide	1	4.2	0.041
Fungicide*Species type [Species][1]	4	6.0	0.200
Habitat type* Fungicide	2	4.1	0.130
Habitat type* Fungicide*Species type	2	3.7	0.160
Block	3	12.6	0.006
Light	1	0.0	0.860
Initial leaf area	1	40.7	<0.001
Neighborhood species richness	1	0.1	0.780
Proportion vegetation cover	1	1.0	0.320

[1] Species nested within species type.

Notes: A nested proportional hazard model was used to analyze seedling survival in the field, with block (4 farms), light (proportion of light on each seedling), $\log_{10}$-transformed initial leaf area, neighborhood species richness (quantified in an 1×1 m area around each seedling transplanted to the field), and proportion vegetation cover (quantified in an 1×1 m area around each seedling transplanted to the field) as covariates.

Table 2-7. Seedling growth in the field of two crop plant species, and three forest tree species (CecA, Gar, and Ret) (species abbreviations in Table 2-1) as affected by plant species, habitat type (pasture, coffee plantation, forest fragment), and fungicide (control, benomyl application).

		Total biomass	
Source	**Df**	***F***	***P***
Species	4	160.6	<0.001
Habitat type	2	26.9	<0.001
Fungicide	1	5.4	0.021
Species *Habitat type	8	108.3	<0.001
Species*Fungicide	4	1.5	0.220
Habitat type *Fungicide	2	3.3	0.036
Species*Habitat type*Fungicide	8	3.1	0.002
Initial leaf area	1	433.6	<0.001
Light	1	5.9	0.015
Neighborhood species richness	1	1.7	0.190
Proportion vegetation cover	1	<0.001	0.990

Notes: ANCOVA was used to analyze $\log_{10}$-transformed final biomass, with block (4 farms where the experiment was set up) as a random factor, and species, habitat type, and fungicide as fixed factors. Block*habitat was also included as a random factor to partition the variance although not shown in the table. Light (proportion of PAR above each seedling), $\log_{10}$-transformed initial leaf area, neighborhood species richness (quantified in an 1×1 m area around each seedling transplanted to the field), and proportion vegetation cover (quantified in an 1×1 m area around each seedling transplanted to the field) were included as covariates. The species Sol was excluded from the growth analyses of the field experiment due to its high mortality.

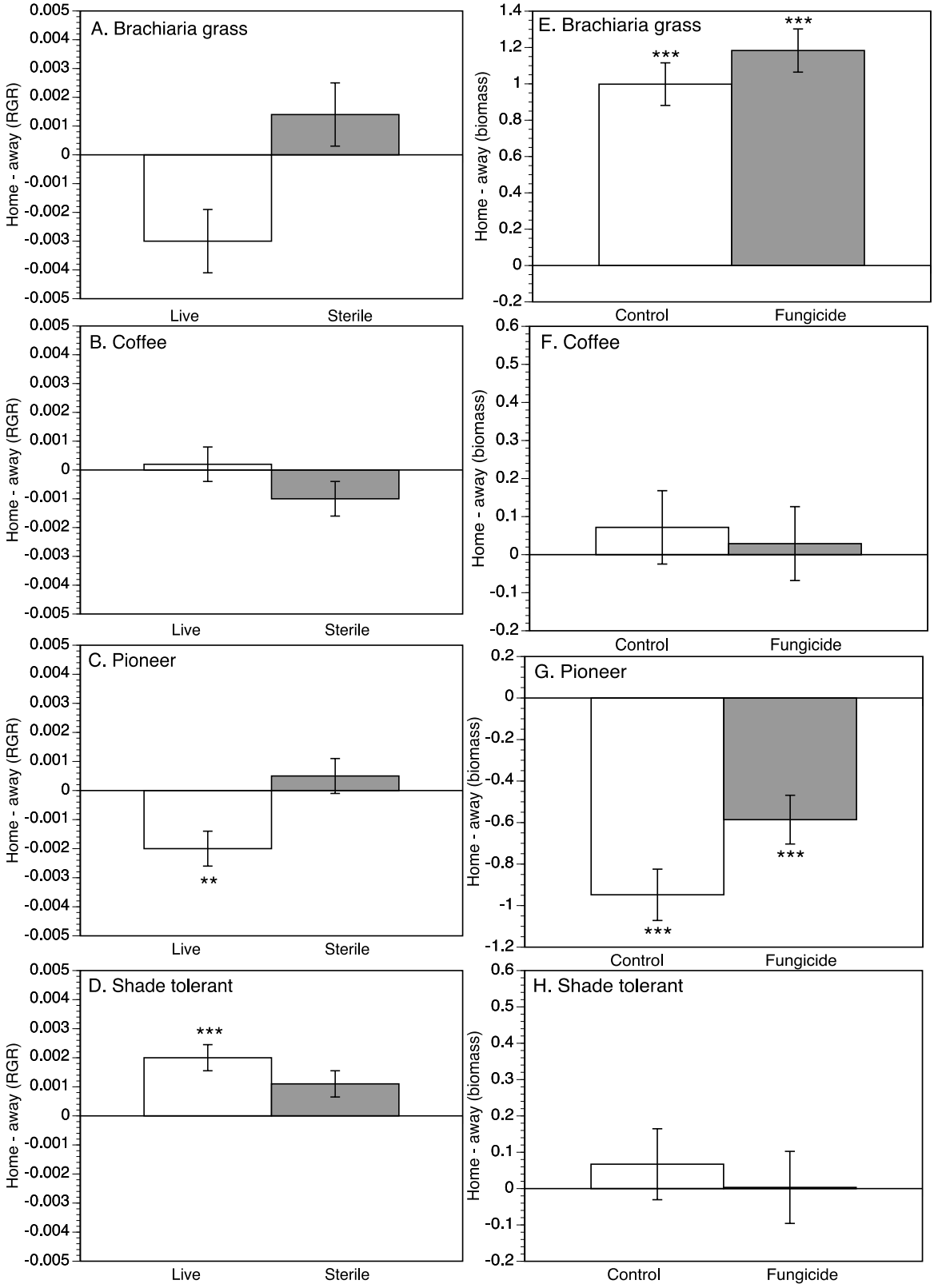

Figure 2-1. Contrasts of seedling growth when inoculated with soil microbial communities (SMC) in the greenhouse experiment (A-D) and when grown in the field (E-H) quantifying the effects of “home” (species typical habitat)

versus “away” (habitats where species rarely occur or don not occur at all) for each experimental treatment. The results were analyzed separately for the four species groups (Table 2-1). Bars indicate the value (± SE) of *a priori* contrasts examining the growth of each plant species group when grown with SMC from their home habitat compared to when grown with SMC from away habitats. Positive values indicate better performance with SMC from home habitat, while negative values indicate better performance with SMC from away habitats. Note: in the field experiment we used only pioneer species CecA and shade tolerant species Gar and Ret (species abbreviations in Table 1). ** $P < 0.01$; *** $P < 0.001$

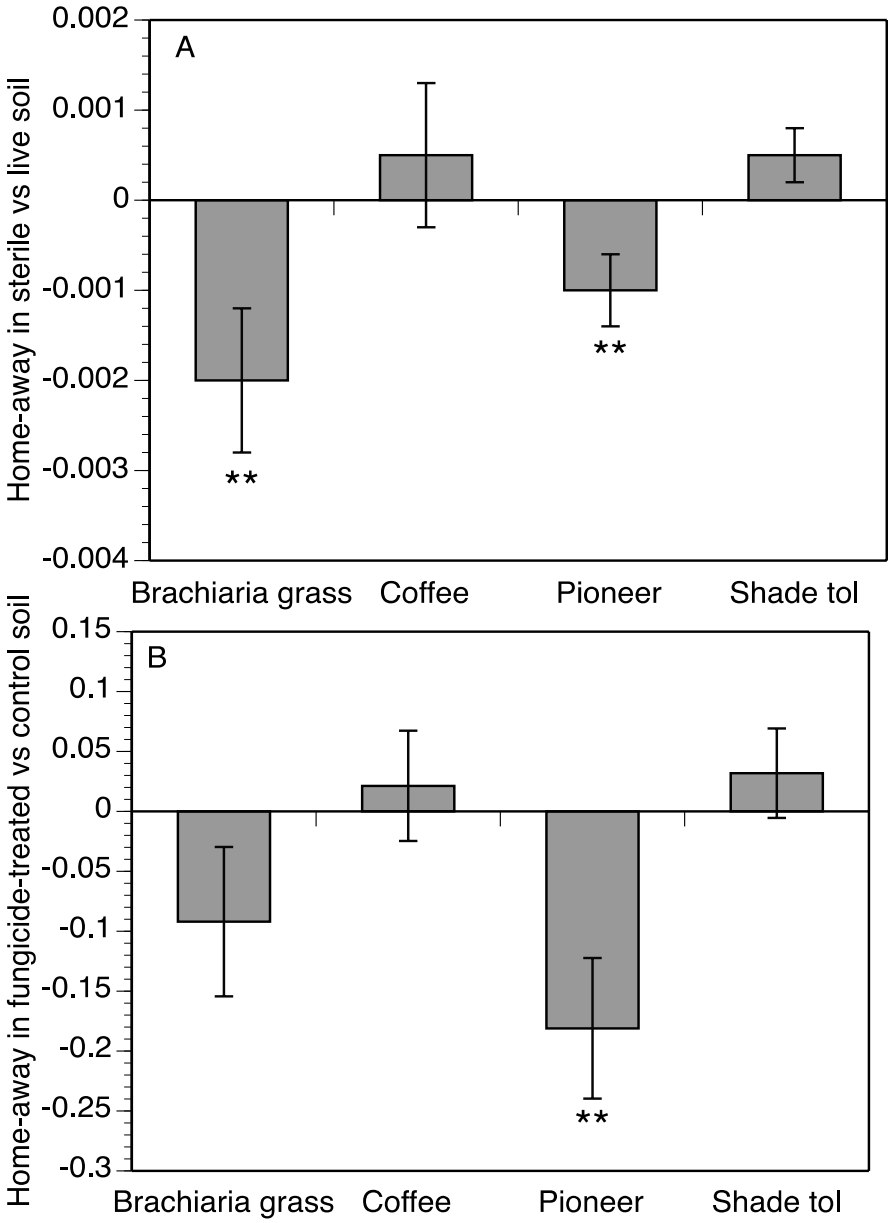

Figure 2-2. Effect of soil sterilization in the greenhouse experiment (A) and fungicide treatment in the field experiment (B) on the growth of seedlings grown with SMC from "home" (species typical habitat) versus "away" (habitats where species rarely occur or don't occur at all) (species groups in Table 2-1). Bars indicate the value (± SE) of *a priori* contrasts comparing the home vs. away contrast with live inocula (greenhouse exp) or control seedlings (field exp) vs. that for sterilized inocula or fungicide-treated seedlings. Positive values indicate that away advantage was greater for sterile (or fungicide-treated) than for live (or control) soil, or that home advantage was greater for live (or control) than for sterilized (or fungicide-treated) soil. Negative values indicate that away advantage was greater for live (or control) than for sterilized (or fungicide-treated) soil, or that home advantage was greater for sterilized (or fungicide-treated) than for live (or control) soil. Note: in the field experiment we used only pioneer species CecA and shade tolerant species Gar and Ret. ** $P < 0.01$; *** $P < 0.001$

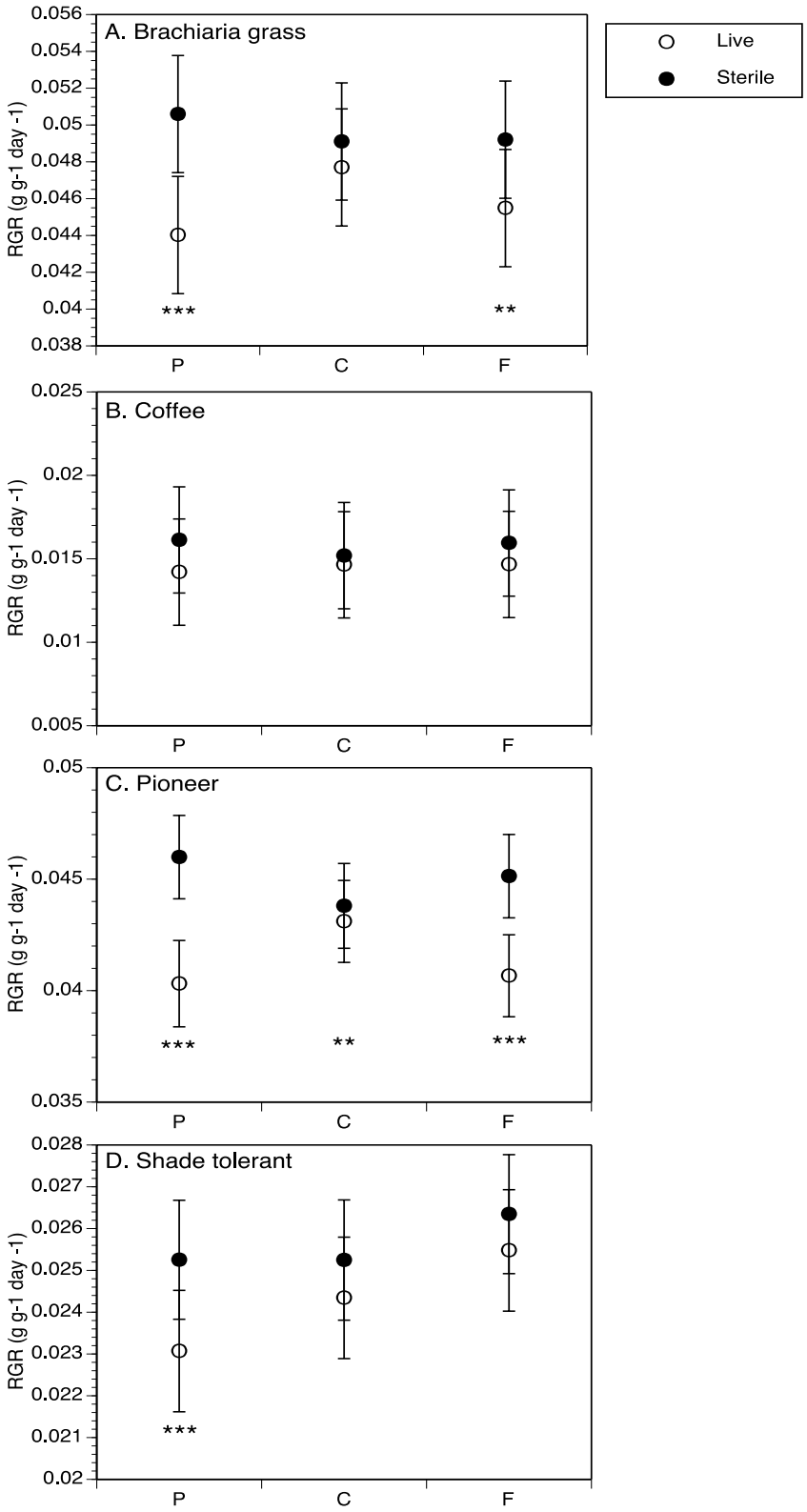

Figure 2-3. Relative growth rate (RGR) (least square means ± SE) in the greenhouse of four species groups (Table 2-1) when inoculated with soil microbial communities (SMC) from pastures (P), coffee plantations (C), and forest fragments (F). Plants were inoculated with an either live (open circles) or sterilized (closed circles) mixture of roots and rhizosphere soil collecte from 5 sites per habitat type. ** P < 0.01; *** P < 0.001

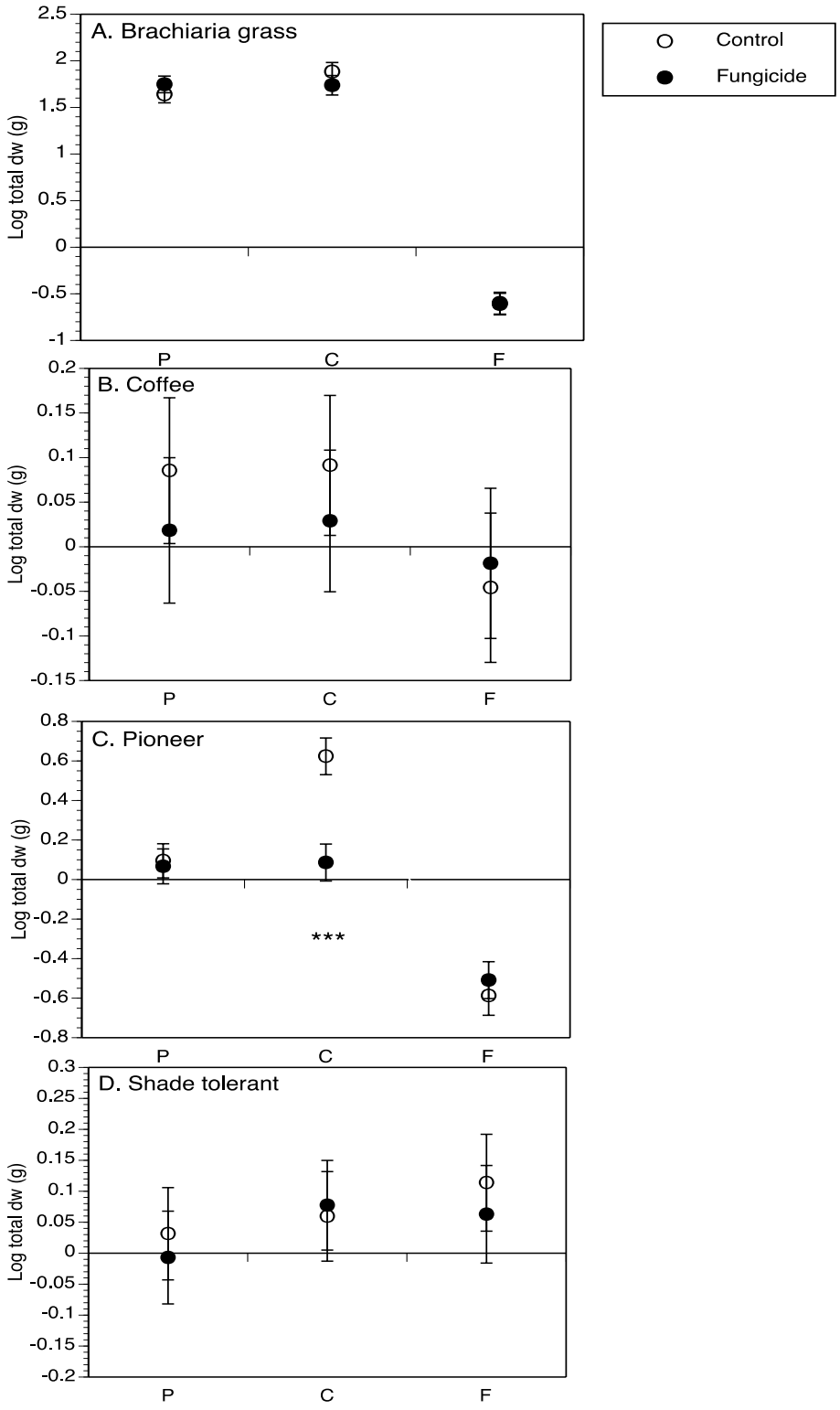

Figure 2-4. Growth (log total dry weight leas square means ± SE) in the field of four species groups (Table 2-1) in pastures (P), coffee plantations (C), and forest fragments (F). Seedlings were grown in 12 sites (4 sites per habitat type) for 3 months during which plants were either treated with water (control) (open circles) or with the fungicide benomyl (closed circles) at the beginning of the experiment and then every month. *** $P < 0.001$

CHAPTER 3
IS SOIL AT HOME MORE BITTER THAN SOIL FROM AWAY? HOST-SPECIFIC EFFECTS OF SOIL MICROBIAL COMMUNITIES FROM AGRICULTURAL AND NATURAL HABITATS

Summary

Symbiotic interactions are vital for the maintenance of biodiversity. Thus, it is crucial that we have a better understanding of how these interactions vary across heterogeneous human-modified landscapes. We examined how plant interactions with beneficial and detrimental components of soil microbial communities vary across three types of habitats, highly fertilized, low diversity agricultural monocultures (pastures and coffee plantations), and unmanaged, highly diverse pre-montane tropical forest fragments. To do this, we set up a greenhouse experiment in which arbuscular mycorrhizal fungal (AMF) (likely mutualists) and non-AMF soil microbial communities (containing antagonistic soil symbionts) were isolated and amplified on common host plant species in the three habitats, and examined their effects on growth of 11 host plant species with reciprocal inoculation. We tested two alternative, non-mutually exclusive hypotheses. The first was that plants would grow better with both the AMF and non-AMF inocula from an “away” habitat (where a plant species rarely or never occurs) relative to a “home” habitat (where a plant species typically occurs). The second was that soils from agricultural monocultures had less beneficial AMF (due to chemical fertilization) and/or more detrimental non-AMF symbionts (due to an accumulation of species-specific antagonistic soil microbes). The results showed that plants differentially interact with diverse arrays of AMF and non-AMF soil microbes from contrasting habitats. Overall, different plant species benefited more from away compared to home non-AMF inocula, suggesting that host-specific antagonistic soil symbionts accumulate

where their hosts were abundant. In contrast, most plants responded similarly to AMF from different habitats, with the exception of forest pioneer trees, which benefited more from AMF in coffee plantations compared to AMF in forest fragments. Combined, these results indicate that host prevalence has a great effect on shaping soil microbial communities, which have significant effects on plant communities. Thus, the replacement of native forests by agricultural monocultures may impact aboveground plant biodiversity through plant-soil dynamics.

Background

Despite the key role of mutualistic and antagonistic symbiotic associations in driving ecological and evolutionary processes, we still have a poor understanding of how symbiotic associations vary across environmental gradients and community complexity (Thrall et al. 2007). Notably, understanding how symbiotic interactions are changing in altered ecosystems might be crucial for conservation of ecosystem services (Flynn et al. 2009) and biological diversity (Kiers et al. 2010), as organisms adapt to one another and to abiotic environmental heterogeneity (Johnson et al. 2010). For example, much research is needed to fully understand how different factors interact to cause the loss of endosymbiotic microalgae in corals (coral bleaching), and how corals cope with stressful environmental conditions in different locations (Hughes et al. 2003). Similarly, little is known about how the interactions of plants and soil symbionts are altered in human modified ecosystems, even though their importance is recognized for plant fintess (Van der Heijden et al. 2006), diversity and distributions (e.g., Mangan et al. 2010b, Pizano et al. 2011).

Soils contain extremely diverse microbial communities that interact with plant communities as mutualistic, pathogenic, or neutral agents. For example, some

arbuscular mycorrhizal fungi (AMF) benefit plants with acquisition of mineral nutrients (especially phosphorus) and water, and protect against pathogens, in exchange for sugars from host plants (reviewed in Koide and Mosse 2004). However, other AMF, as well as many non-AMF soil microbes, may act as plant pathogens or parasites. Both beneficial and antagonistic soil organisms interactively influence plant populations (Rodriguez et al. 2009, Sanon et al. 2009), shaping competitive interactions, distribution and abundance of plant species in natural (e.g., Mitchell 2003) and agricultural settings (e.g., Zhu et al. 2000). Thus, both types of microbes must be considered simultaneously to test for the positive or negative effects of the soil microbial community on host plant species. In most studies, however, the effects of whole-soil microbial communities on plant performance are often attributed to either AMF or antagonistic soil organisms without proper identification of these different components of soil microbial communities. Most studies pertaining to plant-soil interactions along abiotic and biotic gradients have focused on either mutualistic or antagonistic soil microbes, and in just one type of habitat (e.g. natural or managed setting). The goal of this study was to improve the understanding of how abiotic and biotic factors shape plant-soil interactions by experimentally separating the effects of AMF and non-AMF soil microorganisms from three habitats contrasting in soil fertility and plant community diversity.

Soil microbial communities are shaped by both soil nutrient conditions and plant community composition. Increasing soil fertility is predicted to promote non-mutualistic exploitation by groups of microbes that are usually considered to be mutualists, favoring the increased abundance of symbionts that act as functional parasites (Thrall et al. 2007). The nutritional benefits of AMF associations are only evident in low-fertility soils

(e.g., Reynolds et al. 2006), and chemical fertilization has been shown to reduce resource allocation to plant roots, decreasing mycorrhizal infection, and possibly selecting for parasitic AMF strains that exploit host-derived resources while providing limited benefit to the host (Johnson et al. 1997, Graham and Abbott 2000, Treseder 2004, Kiers and Van der Heijden 2006). Likewise, chemical fertilizers may also act directly on both plants and pathogens, influencing the outcome of their interactions (reviewed in Ghorbani et al. 2008). On the other hand, there is a clear interdependence between plant community composition and soil microorganism community composition, as plant species influence which organisms occur in the soil, and soil microbes shape plant community composition (Van der Heijden et al. 1998, Mangan et al. 2010b). For example, there is evidence for lower diversity of AMF in agricultural monocultures than in natural and more plant-diverse ecosystems (e.g., Verbruggen et al. 2010).Similarly, the diversity-disease hypothesis (Elton 1958) states that the spread of host-specific pathogens depends on the abundance of their host species, therefore low plant diversity (e.g. monoculture) results in an increased incidence of specialist antagonistic soil pathogens (e.g., Mitchell 2003, Schnitzer et al. 2011). In summary, abundance of host-specific vs. generalist soil microorganisms, whether beneficial or antagonistic, is interactively determined by plant species composition and diversity in natural communities.

In this study we explored interactions between individual plant species and soil microbial communities from pre-montane tropical forest fragments, coffee plantations and pastures in the tropical Andes. These habitats contrast in application of chemical fertilizers (none in forest fragments, high in coffee plantations and pastures) and in plant

species richness. Whether the whole soil community from “home” soil (habitat where a plant species commonly occurs) relative to “away” soil (habitat where a plant species rarely or never occurs) has positive, neutral, or negative effects depends on the balance between beneficial and antagonistic soil microbes. We experimentally differentiated the effects of AMF (which are largely believed to be mutualists of plants) and non-AMF soil microbes on growth of 11 plant species, by using AMF inocula (prepared from AMF spores) and non-AMF inocula (microbial filtrate excluding large AMF spores). The specific objectives were to test for 1) overall net effects of AMF and the microbial filtrate from contrasting habitats on growth of plant species common in these habitats, and 2) response of plants to AMF and the microbial filtrate from their home and away habitats. We tested two alternative, non-mutually exclusive hypotheses. The first was that the “home” soil contained less effective mutualists to host plants (Bever 2002b) as well as host-specific pathogens. The second was that AMF communities in the fertilized soils of pastures and coffee plantations were likely to be dominated by less mutualistic AMF.

Methods

Study site and species

This study was conducted in the agricultural region of the Central Cordillera of Colombia between 1200 and 2000 m.s.l. (4°6′N 75°4′ W). The climate is tropical humid, with an average annual temperature of 21ºC, and an annual rainfall of 2550 mm concentrated in two wet seasons (March to July and September to December) (Guzmán-Martínez et al. 2006). Soils are Udands (Andisols) (Ortíz-Escobar et al. 2004). The landscape is dominated by three contrasting habitat types: occasionally fertilized pastures with low species diversity (mainly African grasses *Pennisetum clandestinum*, *Melinis minutiflora* and *Brachiaria* spp.), heavily fertilized monocultures of sun-grown

coffee plantations, and unmanaged fragments of pre-montane tropical forest (Orrego et al. 2004a). The forest fragments are usually small (1-30 ha), biologically more diverse than the other two habitats, and mostly dominated by early and mid-successional plant species, with high liana densities. Seedling recruitment of native species appeared poor in the understory, where seedlings of non-native species, including *Coffea arabica* (coffee) are common (C. Pizano pers. obs.).

Forest tree species were selected from those abundant in forest fragments in the region (Orrego et al. 2004b), encompassing a wide range of seed size and life histories (Table 3-1, Appendix A). *Brachiaria brizantha* and *Coffea arabica* were chosen because they dominate pastures and coffee plantations, respectively in the study region (Table 3-1). To sample across heterogeneous altitude and climatic conditions in the study area, five private farms with similar conditions each containing a pasture, a coffee plantation, and a forest fragment (Table 3-2) were chosen to collect soil for the greenhouse experiment. At each of the 15 site-habitat combinations, five samples of 200 g of mineral soil (5-15 cm in depth) each were taken and pooled in a composite sample from which a subsample was analyzed. Soils across the three habitats had similar levels of pH, organic matter content, and some nutrients (N, K, Mg), but forest fragment soils contained only 10-12 of P, and significantly greater Ca content than pasture and coffee plantation soils (Table 3-3). In addition, light levels in pastures and coffee plantations were approximately 30 times higher than in forest fragments (Table 3-3).

Seed preparation and soil medium for growing seedlings

Seeds of Brachiaria grass and coffee were provided by Cenicafé, while seeds of the nine forest plant species (Table 3-1) were collected from at least three parent trees in forest fragments in the study region. Seeds were surface sterilized with 0.6 % sodium

hypochlorite for 15 minutes and germinated in trays containing a steam-sterilized mixture of soil and sand river (3:2) soil. The soil was collected from an open area near the greenhouses at Cenicafé (Colombian National Research Center for Coffee, in Chinchiná, Caldas, Colombia), and it was classified as an acrudoxic melanudand from the Chinchiná unit (Ortiz-Escobar et al. 2004), with moderately fertile chemical characteristics (5.4 pH, 0.1 % N calculated, 1.9 % OM-Walkley-Black colorimetry, 7 mg kg^{-1} P-Bray II Bray Kurtz colorimetry, 0.37 mg kg^{-1} K-Ammonium acetate 1N, 3.4 mg kg^{-1} Ca-Ammonium acetate 1N, and 1.1 cmol kg^{-1} Mg-Ammonium acetate 1N).

Initial inoculum collection

Whole soil inocula (i.e. roots, rhizosphere soil, and associated organisms) were collected from pastures, coffee plantations, and forest fragments at five farm sites each having these three habitats (total of 15 site-habitat combinations) (Table 3-2). Roots and rhizosphere soil (5-15 cm in depth) were collected from five random locations within each habitat at each site and pooled together. We then pooled, and homogenized the inoculum from the five sites for each habitat type.

AMF inoculum amplification

Inoculum amplification was done in two steps. In the first step, individually potted plants of each of the 11 species (Table 3-1) were initially inoculated with soils coming from their respective habitat types (i.e. *B. brizantha* with the soil from pastures, *C. arabica* with the soil from coffee plantations, and forest species with the soil from forest fragments) (Fig. 3-1.1). The same sterilized soil and sand mixture used to germinate seeds filled 20 % of 1-L pots, to which an equal quantity (100 mL) of the whole-soil inoculum from a particular habitat was added. Ten seedlings per species were grown for 5 months (Fig. 3-1.2) in the greenhouse after which they were harvested and their roots

and soil were mixed to provide 8 subsamples of 20 g of soil from each plant-inoculum combination (Fig. 3-1.3). Each soil subsample was washed with water through a series of sieves, and the fraction that was retained on the 45 μm-diameter sieve was collected (mostly AMF spores) (Klironomos 2002) (Fig. 3-1.4a). In order to clean the AMF spores and separate them from debris and other possible contaminants, the spore fraction was concentrated by sucrose density gradient centrifugation (Daniels and Skipper 1982) and then individual spores were separated using micro-tweezers. Once all clean spores were obtained, they were used to inoculate previously germinated seedlings of *Brachiaria decumbens* and *Cecropia angustifolia* (6 seedlings per species) in sterilized soil. To inoculate seedlings, the spores were pipetted directly onto the plant root system (Fig. 3-1.5a). The same process was repeated for the inoculant from the 11 host species, therefore we had a total of 66 seedlings of *Brachiaria decumbens* and *Cecropia angustifolia* each inoculated with 11 different AMF communities. These hosts were selected to amplify the AMF inoculum because they grow fast and produce high amounts of AMF spores in a relatively short time (Mangan et al. 2010a). In addition, this allowed for standardization of the amount of spores, given that AMF sporulate at different rates on different hosts.

Inoculated seedlings grew for five months in the greenhouse (Fig. 3-1.6) after which they were harvested and their soil and roots were used as inoculum in the greenhouse experiment (Fig 3-1.7). For each inoculum source the seedlings of *B. decumbens* and *C. angustifolia* were harvested, and their roots and soil were pooled together. This was done for the “coffee plantation” and “pasture” inocula. For the “forest” inoculum, the roots and soil from the 9 forest species was pooled together. Thus, while

the AMF inocula from coffee plantation and pasture were initially amplified on a single species (*Coffea arabica* and *Brachiaria brizantha* respectively), the forest inoculum was amplified on 9 forest plant species. Therefore, AMF inocula isolated from coffee plantations and pastures were likely dominated by AMF that colonize the roots of the respective crop species, while the forest inoculum probably included a greater diversity of AMF species from the different host plants.

Filtrate inocula

These inocula were produced by amplifying soil microbes collected in a filtrate from the 25 μm-diameter sieve that excludes large AMF spores (Klironomos 2002). Other studies (e.g., Klironomos 2002, McCarthy-Neumann and Kobe 2008) have used the same method to isolate a representative community of non-AMF soil organisms including microbial pathogens like bacteria and non-AMF fungi. The filtrate was used to inoculate seedlings of the respective species used in the initial amplification (Fig.3-1.1). For example, we inoculated *Juglans neotropica* seedlings with filtrate obtained from pots with *Juglans neotropica* seedlings (Fig. 3-1.4b, 3-1.5b). These seedlings were also grown for 5 months in the greenhouse after which they were harvested and their roots and soil were collected and pooled together to use as “forest” inoculum in the greenhouse experiment (Fig. 3-1.6, Fig. 3-1.7).

Greenhouse experiment- growth conditions and treatments

Pots of 1-L volume were 70 % filled with a steam-sterilized (for 2 hrs) mixture of soil and river sand (3:2). The soil was collected from an open area at CIAT (International Center for Tropical Agriculture) in Palmira, Valle, Colombia, and had intermediate fertility (6.8 pH, 0.05 % N, 0.6 % OM-Walkley-Black colorimetry, 42 mg kg^{-1} P-Bray II Bray Kurtz colorimetry, 0.35 mg kg^{-1} K-Ammonium acetate 1N, 3.7 mg kg^{-1} Ca-

Ammonium acetate 1N, and 2.8 cmol kg^{-1} Mg-Ammonium acetate 1N). Then, we added an equal amount (100 mL) of AMF or filtrate inoculum. Each treatment combination of inoculum source (forest fragments, coffee plantations, pastures) × inoculum type (AMF, filtrate) × plant species (11 plant species) was replicated in 8 pots and plants were equally distributed on 4 benches (blocks) in the shade house. Plants were grown under 20 % light for 130 days. We measured the leaf area of each plant at the beginning of the experiment to use as a covariate with final total dry mass at harvest in the statistical analyses. Also at harvest, AMF root colonization was quantified for a subsample of 3 plants from each treatment. For this assessment, a 1 g aliquot of fine roots was washed with running water, cleared with 3 % KOH and 3 % H_2O_2, acidified with 3 % HCl, washed with running water, and stained with 0.05 % tryplan blue in lactoglycerol solution (Zangaro et al. 2000). Root segments were then mounted on slides (2 slides/seedling) and presence or absence of AMF was recorded at 200 intersect points for each slide (for a total of 400 intersect points for each individual).

Statistical analyses

Two sets of analyses were performed to examine 1) the effects of the six types of soil inocula (three habitats × two microbes-types) relative to the control (sterile soil; no inoculation), and 2) the effects of AMF and filtrate components of “home” soil microbial community relative to “away” soil microbial community on the 11 plant species, which were classified to four plant species groups: Brachiaria grass, coffee (a non-native shade tolerant species), forest pioneer trees, and forest shade tolerant trees. First, we used analysis of covariance (ANCOVA) to examine the response of the final seedling biomass to two fixed main factors including inoculum type (7 types, including sterile,

AMF and filtrate from each of the three habitat types pastures, coffee plantations, and forest fragments) and plant species (and their interactions), farm site (block) as a random effect, and initial leaf area, and “days in experiment” as covariates. Inclusion of the latter covariate accounted seedling age heterogeneity introduced by the replacement of dead seedlings during the first month. We then used *a priori* contrasts to compare the growth of each species group when inoculated with either AMF or filtrate from different habitat types relative to the control (Fig. 3-2).

For the second ANCOVA the sterile treatment was excluded to examine the response of final plant mass to three fixed-effect factors including inoculum source (pastures, coffee plantations, forest fragments), inoculum type (AMF, filtrate), and plant species and two-way and three-way interactions among them, as well as block as a random factor, and initial leaf area and days in experiment as covariates. Then, *a priori* contrasts were used to compare seedling growth of each species group when grown with AMF and filtrate inoculum from their home habitat relative to that from away habitats separately (Fig. 3-3). Statistical analyses were done with JMP®, version 8.0 (SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513). We used the Dunn-Sidak correction to adjust the significance levels of contrasts.

To compare AMF colonization (%) across the different soil inocula, the statistical tests were conducted for a subset of randomly-selected plants for which roots were processed (3 replicates per treatment). This model included inoculum source (forest fragments, coffee plantations, and pastures), inoculum type (AMF, filtrate), and plant species (and their interactions) as fixed effects, and block, initial leaf area, and final

biomass as covariates. Plants in the sterile treatment were not included in this analysis as none of the examined plant roots showed evidence of AMF colonization.

Results

In the first analysis we compared seedling growth for the six types of soil inocula (3 habitats × two microbes-types) relative to the control (sterile soil) and found that biomass was significantly affected by soil inocula and plant species (Table 3-4). In the second analysis we contrasted seedling growth across inoculum types from the three habitats (excluding the control treatment), and found that inoculum source (3 habitats), inoculum type (AMF and filtrate) and plant species had significant effects on seedling final biomass (Table 3-5). In addition, significant two- and three-way interactions indicated that plant species differed in their response to different inocula (Table 3-4), and to different combinations of inoculum source habitat and inoculum types (Table 3-5).

Inoculation had significant negative effects on Brachiaria grass, which grew slightly better with sterile soil than with AMF inocula from pastures ($F_{1,498} = 5.3$; $P = 0.021$) and coffee plantations ($F_{1,498} = 4.1$; $P = 0.043$; not significant with the Sunn-Sidak correction for multiple contrasts) and better with sterile soil than with the microbial filtrate (from pastures: $F_{1,498} = 61.4$; $P < 0.001$; Table 3-6, Fig.3- 2A). *A priori* contrast tests (Fig. 3-3) showed that AMF from different habitats had similar negative effect on the growth of this grass ($P > 0.1$ for all contrasts), while the microbial filtrate from its home habitat was significantly more detrimental than that of coffee plantations ($F_{1,428} = 55.7$; $P < 0.001$) and forests ($F_{1,428} = 59.5$; $P < 0.001$; Table 3-7, Fig. 3-3A). Growth of coffee was improved by the inoculation of AMF from all three habitats (pastures: $F_{1,428} =$

16.4; P < 0.001, coffee plantations: $F_{1,428}$ = 10.7; P = 0.0012, and forests: $F_{1,428}$ = 6.2; P = 0.013), and also by the filtrate from pastures ($F_{1,428}$ = 7.1; P = 0.008) and forests ($F_{1,428}$ = 22.1; P < 0.001) compared to the sterile treatment (Table 3-6, Fig. 3-2B). AMF from different habitats had the same positive effect on the growth of this species (P > 0.4 for both types of "home" vs. "away" contrasts), but the filtrate from its home habitat was the least beneficial compared to that of pastures ($F_{1,428}$ = 4.0; P = 0.046; not significant with the Sunn-Sidak correction for multiple contrasts) and forests ($F_{1,428}$ = 17.1; P < 0.001; Table 3-7, Fig. 3-3B).

Pioneer tree species (CecA, CecT, Sol, Ochr, and Sip) grew significantly better when inoculated with AMF from the three habitat types (pastures: $F_{1,498}$ = 33.9; P < 0.001; coffee plantations: $F_{1,498}$ = 75.9; P < 0.001, forests: $F_{1,498}$ = 37.7; P < 0.001), and with filtrate from pastures ($F_{1,498}$ = 32.3; P < 0.001) compared to the sterile treatment (Table 3-6, Fig. 3-2C). In contrast, their growth did not significantly differ between sterile soil and filtrates from coffee plantations ($F_{1,498}$ = 2.5; P = 0.11) and forests ($F_{1,498}$ = 0.04; P = 0.85; Table 3-7, Fig. 3-2C). When comparing the growth of these pioneer species with AMF from "home" vs. "away" habitats, AMF from pastures had a similarly positive effect ($F_{1,427}$ = 0.4; P = 0.51), while AMF from coffee plantations were more beneficial ($F_{1,427}$ = 6.2; P = 0.013) compared to AMF from the "home" habitat (Table 3-7, Fig. 3-3C). Pioneer species grew better with the filtrate from pastures ($F_{1,427}$ = 23.6; P < 0.001), but similarly with the filtrate from coffee plantations ($F_{1,427}$ = 3.2; P = 0.077) compared to that of their home habitat (Table 3-7, Fig. 3-3C).

Growth of shade tolerant species (Gar, Gus, Jug, and Ret) was only marginally increased by AMF from coffee plantations ($F_{1,498}$ = 4.1; P = 0.044; not significant with

the Sunn-Sidak correction for multiple contrasts) compared to the sterile treatment, while being similar across all other AMF and filtrate treatments ($P > 0.1$ for all contrasts; Table 3-6, Fig. 3-2D). Similarly, these species produced comparable growth with AMF and the microbial filtrate from their home habitat compared to AMF and filtrate from pastures and coffee plantations ($P > 0.2$ for all contrasts; Table 3-7, Fig. 3-3D).

AMF colonization was significantly higher in plants that received AMF inocula (61.6 %) than those inoculated with microbial filtrate (29.1 %). Thus, although AMF inoculum potential was greatly reduced in the filtrate, AMF hyphae in the microbial filtrate probably colonized the roots (Table 3-8). AMF root colonization in filtrate-treated plants was highest in seedlings treated with the microbial filtrate from pastures (39.6 %), intermediate in seedlings treated with the microbial filtrate from forests (33.7 %), and lowest in plants inoculated with the microbial filtrate from coffee plantations (13.9 %) (Fig. 3-4).

Discussion

In this study, communities of AMF and other soil microorganisms (microbial filtrate) from habitats with contrasting soil fertility and plant diversity had differential effects on growth of plant species. Hence, 1) soil microbial communities differed across the habitats, and 2) different host species associated with particular subsets of beneficial and antagonistic microorganisms. When testing for the overall net effects of AMF and the microbial filtrate from different habitats, AMF communities from heavily fertilized pastures and coffee plantations had a net negative effect on growth of Brachiaria grass (Fig. 3-2A), but improved the growth of coffee (Fig. 3-2B) and pioneer forest species (Fig. 3-2C) in comparison the sterilized soil control (Table 3-6). The microbial filtrate from pastures also decreased the growth of Brachiaria grass (Fig. 3-2A), but had a net

positive effect on coffee (Fig. 3-2B) and pioneer forest species (Fig. 3-2C) relative to the sterile control (Table 3-6). These positive effects can be attributed to the incomplete elimination of AMF from the filtrate, although plants inoculated with the filtrate had significantly lower AMF colonization rates (Table 3-8, Fig.3-4). Finally, all plants grew similarly with sterile soil and with the microbial filtrate from coffee plantations and forest fragments, except coffee, which benefited from the filtrate from forests (Table 3-6, Fig. 3-2B).

When comparing seedling growth with “home” and “away” soils, most plant species grew better with soils from away habitats, but this response was driven by the filtrate inocula, and not by AMF. In fact, the only species group that showed a significant response to AMF from different habitats were forest pioneer trees, which grew significantly better with AMF from coffee plantations compared to AMF from their home habitat (Fig. 3-3C). In contrast, Brachiaria grass (Fig. 3-3A), coffee (Fig. 3-3B), and pioneer forest trees (Fig. 3-3C) grew significantly better with the filtrate from away compared to that from their home habitats (Table 3-7). These results suggest that forest fragments and pastures AMF communities have a similar impact on plants, although fertilized coffee plantations seem to be dominated by AMF that are more beneficial to forest pioneer species than those present in forests. Furthermore, agricultural lands and forests seem to have contrasting communities of non-AMF soil microorganisms that differ in beneficial and detrimental effects on different host species.

AMF from contrasting habitats have similar effects on most plant species

Numerous studies have reported lower AMF diversity in managed agricultural lands than in unmanaged natural habitats (e.g., Picone 2000, Oehl et al. 2003), but few have actually tested for the effects of these AMF on plant performance. Here, we

attempted to separate the effects by inoculating plants with AMF and microbial filtrates from highly fertilized agricultural lands and natural forests. Chemical fertilization and other agricultural practices (e.g. tillage) have been predicted to select for AMF that act as parasites without offering any benefit to their host (Graham and Eissenstat 1998, Kiers and Van der Heijden 2006, Verbruggen and Kiers 2010). In support of this idea, AMF communities from managed, fertilized habitats may be less diverse and less beneficial than those from unfertilized, natural habitats (e.g., Neuhauser and Fargione 2004, Kiers and Denison 2008, Rasmann et al. 2009, Verbruggen and Kiers 2010). Graham and Abbott (2000) showed that AMF from fertilized agricultural lands increased plant P in exchange for sucrose status in the roots of wheat, but did not increase plant biomass, suggesting that these AMF were acting as parasites. In natural grasslands, Johnson et al. (2010) showed that two ecopytes of *Andropogon gerardii* adapted to high- and low-nutrient soils obtained the most benefit from AMF present in nutrient-poor soils, and the less benefit from AMF common in nutrient-rich soils, demonstrating again that the latter were less mutualistic.

The effects of AMF from contrasting habitats on plant performance might not only vary according to different soil nutrient status, but also based on host identity. For instance, AMF from fertilized pastures and coffee plantations had a marginally net negative effect, i.e., a pathogenic effect, on the growth of the Brachiaria grass, but AMF from pastures were beneficial to coffee and forest pioneer species (Fig. 3-2). In addition, AMF from coffee plantations had a net positive effect on coffee*,* forest pioneer species, and forest shade tolerant species (Fig. 3-2) compared to the sterile control. Furthermore, AMF from coffee plantations were significantly more beneficial to forest

pioneer species than AMF from their home habitat (Fig. 3-3C), while all other species grew similarly well with AMF from the three habitat types (Fig. 3-3). These results contradict other studies suggesting that AMF from highly fertilized agricultural lands are less beneficial than those present natural habitats (Neuhauser and Fargione 2004, Kiers and Denison 2008, Verbruggen and Kiers 2010), and only partially support our prediction that plants would grow better with home than with away AMF. Moreover, the fact that most species grew similarly well with AMF from home and away habitats suggests that plants either find similar AMF genotypes across habitat types, or that different AMF communities from contrasting habitats have the same effect across plant species. One potential factor that influenced the results was that the method used to isolate AMF (picking individual AMF spores) biased our representation of real AMF communities from the field, as we probably selected from all habitat types AMF that produced copious spores (i.e. “r-strategist” AMF; Verbruggen and Kiers 2010), perhaps representing only one of many AMF functional types (Munkvold et al. 2004).

Non-AMF soil microorganisms (microbial filtrate) from contrasting habitats had different effects across plant species

The diversity-disease hypothesis (Elton 1958) states that there is an inverse correlation between the incidence of specialist pathogens and plant diversity, but few studies have actually tested this hypothesis. Schnitzer et al. (2010) showed that there is indeed a higher incidence of soil pathogens in grasslands of low plant diversity compared to grasslands with higher plant diversity, and that host-specific disease and root infection by pathogens decrease with an increase in plant diversity. In this study we tested the diversity-disease hypothesis by inoculating different plant hosts with microbial filtrates (containing antagonistic soil organisms) from habitats with contrasting plant

diversity, to evaluate whether 1) microbial filtrates from species-poor habitats were more detrimental to plants, and 2) microbial filtrate from each plant species' home habitat was more detrimental than filtrate from away habitats.

We found no pattern showing that microbial filtrates from species-poor pastures and coffee plantations were more detrimental than the filtrate from species-rich forest fragments. Filtrate from pastures had a negative effect on Brachiaria grass (Fig. 3-2A), but benefited growth of coffee (Fig. 3-2B) and pioneer forest species (Fig. 3-2C), while growth did not differ significantly when inoculated with the filtrate from coffee plantations compared to growth on sterile soil (Fig. 3-2). In addition, filtrate from forest fragments had a positive effect on coffee (Fig. 3-2B), but no effect on other plants relative to the sterile control (Fig. 3-2). These results suggest that the incidence of antagonistic soil microbes did not differ consistently between agricultural monocultures and natural forests, but it is also possible that the results reflected the net balance between negative and positive microbes. We did not completely exclude AMF from the filtrate inocula (Table 3-8, Fig. 3-4), and thus, the positive effect of filtrate from pastures and forests on some plant species could be explained by a relatively high AMF root colonization in seedlings under these treatments (Fig. 3-4) probably due to fragments of hyphae passing through the 25 μm-diameter sieve. In addition, other beneficial soil microorganisms such as bacteria would have been present in our microbial filtrate inocula (Sanon et al. 2009).

Although no common pattern of plant species-specific response to microbial filtrate inocula from different habitats was found, filtrate from plants' away habitats positively influenced the growth of most plant species. Brachiaria grass (Fig. 3-3A),

coffee (Fig. 3-3B), and forest pioneer species (Fig. 3-3C) all grew better with the filtrate from away than with filtrate from their home habitats. In the case of the grass, away advantage was due to less detrimental effect of away filtrates compared to a very negative effect of the filtrate from pastures (Fig. 3-2A), suggesting that pastures accumulate antagonistic soil organisms that are detrimental for *B. brizantha.* In the case of coffee (Fig. 3-3B) and pioneer species (Fig. 3-3C), away advantage was due to an increased benefit of the away microbial filtrate (Fig. 3-2B, 3-2C), which further suggests that AMF in the microbial filtrate inocula may have had a significant effect on seedling growth. Moreover, it indicates that the away vs. home advantage for most of our species was driven by the presence of mutualistic, and not by the absence of antagonistic soil organisms in the away, compared to home habitats. For example, it is possible that forest fragments host soil organisms beneficial to coffee that are absent in coffee plantations. Alternatively, AMF and other mutualists may be less effective in home, compared to away soils (Bever 2002b). Together, these results only partially support the diversity-disease hypothesis; incidence of antagonistic soil microbes was indeed higher in pastures compared to more diverse forest fragments, however these microbes only negatively affected Brachiaria grass, the dominant species in pastures. Following this same line of evidence, coffee and forest species appeared to encounter AMF and other mutualistic soil organisms in coffee plantations and forest fragments (correspondingly) that were less beneficial than those present in away habitats. Thus, habitats with contrasting plant diversity seem to similarly accumulate antagonistic non-AMF microbes, as well as less effective mutualists that negatively impact the most abundant plant species in each habitat type.

Applied ecological implications

Environmental degradation represents a great threat to symbiotic interactions that are key for the maintenance of biodiversity and ecosystem services (e.g., Bascompte 2009, Kiers et al. 2010). For instance, it is now clear that there is a world-wide pollination crisis that is affecting both natural and agricultural systems (Steffan-Dewenter et al. 2005, Biesmeijer et al. 2006). Similarly, numerous studies have addressed the consequences of the loss of seed dispersers on plant populations due to forest fragmentation (Cordeiro and Howe 2003, Tscharntke et al. 2008). However, compared to aboveground symbiotic interactions, our understanding of belowground symbionts remains poor and limited to the few soil organisms that we can isolate (e.g. earth worms, AMF). Most studies have only focused on comparing the diversity of a relatively small number of soil organisms that are easy to isolate across natural and degraded ecosystems (but see Postma-Blaauw et al. 2010). Here we tested for the effects of communities of belowground symbionts from natural and degraded ecosystems on plant performance, with potentially important implications for agricultural production and natural forest regeneration. Our results show that forest fragments and agricultural lands in the tropical Andean region share functionally similar communities of AMF, with coffee plantations hosting particularly beneficial mycorrhizae for forest pioneer tree species. In contrast, pastures, coffee plantations and forest fragments seem to have contrasting communities of non-AMF soil organisms. In particular, pastures accumulate antagonistic soil microbes, but also AMF that are detrimental to the widely planted grass *B. brizantha* but beneficial to coffee and pioneer forest species. Forest fragments, on the other hand, apparently have non-AMF, beneficial microbes that improved the growth of coffee. Combined, these results not only provide further

evidence for how the establishment of agricultural monocultures has modified belowground microbial communities, but also reveal that differential mutualistic and antagonistic soil symbionts from modified and natural ecosystems can significantly shape plant communities in these environments.

Table 3-1. General characteristics of the eleven plant species used in the greenhouse experiment. Seed dry mass (mean ± S.D) was measured from 25-50 seeds/species dried for 3 days at 60 °C.

Species	Family	Species group	Typical habitat ("home")	Seed dry mass (g)	Abbreviation
Brachiaria brizantha	Poaceae	Brachiaria grass (crop)	P[1]	0.0077 ± 0.0013	Bra
Coffea arabica	Rubiaceae	Coffee (crop)	C[2]	0.22 ± 0.023	Cof
Cecropia angustifolia	Cecropiaceae	Pioneer	F[3]	0.0011 ± 0.00028	CecA
Cecropia telealba	Cecropiaceae	Pioneer	F[3]	0.00039 ± 0.00012	CecT
Ochroma pyramidale	Bombacaceae	Pioneer	F[3]	0.0037 ± 0.0012	Ochr
Siparuna aspera	Monimiaceae	Pioneer	F[3]	0.014 ± 0.025	Sip
Solanum aphynodendrum	Solanaceae	Pioneer	F[3]	0.0015 ± 0.00053	Sol
Garcinia madrunno	Clusiaceae	Shade tolerant	F[3]	2.92 ± 0.77	Gar
Gustavia superba	Lecythidaceae	Shade tolerant	F[3]	10.55 ± 2.78	Gus
Juglans neotropica	Juglandaceae	Shade tolerant	F[3]	26.04 ± 14.49	Jug
Retrophyllum rospigliosii	Podocarpaceae	Shade tolerant	F[3]	0.86 ± 0.18	Ret

[1]Pastures
[2]Coffee plantations
[3]Forest fragments

Table 3-2. Characteristics of the farms where the initial soil inocula were were collected (Guzmán-Martinez et al 2006). At each farm there were three habitats: a pasture (P), a coffee plantation (C), and a forest fragment (F).

Farm (block)	Geographic coordenates	Altitude (m.s.l)	Mean annual precipitation (mm yr^{-1})	Mean annual temperature (ºC)	Habitat	Habitat patch size (ha)
Cenicafé	05º00′N 75º36′W	1380	2733	20.9	P	0.5
					C	0.5
					F	40
Playa rica	05º00′N 75º36’W	1290	2750	20.7	P	10
					C	60
					F	30
Alto español*	04º56′N 75º42′W	1720	3140	18.8	P	1.0
					C	2.0
					F	0.3
Naranjal	04º59′N 75º39′W	1400	3137	21.4	P	22
					C	38
					F	27
La Argentina	05º02′N 75º41′W	1354	2935	19.9	P	0.5
					C	100
					F	1.5

Table 3-3. Mean (± SE) soil pH, organic matter (OM) content, and nutrient content of pastures (P), coffee plantations (C), and forest fragments (F) where soil inocula were collected.

Habitat	pH[1]	OM[3] (%)	N[2] (%)	P[4] (mg/kg)*	K[5] (cmol/kg)	Ca[5] (cmol/kg)*	Mg[5] (cmol/kg)
P	5.6 (± 0.1)	7.2 (± 1.4)	0.3 (± 0.04)	54.4 (± 25.3)	0.6 (± 0.2)	5.6 (± 0.7)	2.1 (± 0.4)
C	5.0 (±0.3)	9.6 (± 1.7)	0.4 (± 0.06)	42.0 (± 16.7)	0.3 (± 0.1)	4.4 (± 1.1)	1.7 (± 0.6)
F	5.5 (± 0.2)	12.4 (± 2.1)	0.5 (± 0.06)	5.6 (± 0.4)	0.4 (± 0.05)	8.3 (± 2.3)	2.4 (± 0.7)

Notes: Log-transformed data was analyzed with one-way ANCOVA including block as a random factor and habitat as a fixed factor.

[1]pH: Potentiometer soil: water 1:1

[2]N (total): Calculated

[3]OM: Walkley-Black – colorimetry

[4]P: Bray II - colorimetry Bray Kurtz

[5]K, Ca, Mg: Ammonium acetate 1N

* Significant differences between habitat types $P < 0.05$

Table 3-4. Growth response of 11 plant species (Brachiaria grass, coffee, and nine forest tree species; Table 3-1) to seven inoculum types (sterile, AMF and filtrate from each of the three habitat types, pastures, coffee plantations, and forest fragments) in the greenhouse.

	Total biomass (error df = 498)		
Source	**Df**	***F***	***P***
Inoculum	6	18.5	<0.001
Species	10	493.1	<0.001
Inoculum × Species	60	5.3	<0.001
Block	3	2.6	0.054
Initial leaf area	1	388.2	<0.001
Days in experiment	1	168.6	<0.001

Notes: ANCOVA was used to analyze $\log_{10}$-transformed final biomass, with block (4 benches in the greenhouse), days in experiment (dead seedlings were replaced during the first month of the experiment), and $\log_{10}$-transformed initial leaf area as covariates.

Table 3-5. Growth response of 11 plant species (Brachiaria grass, coffee, and nine forest tree species; Table 3-1) to inoculum source (pastures, coffee plantations, and forest fragments), inoculum type (AMF, Fil), and plant species (and interactions) in the greenhouse.

	Total biomass (error df = 428)		
Source	**Df**	***F***	***P***
Inoculum source	2	0.3	0.760
Inoculum type	1	75.9	<0.001
Species	10	431.5	<0.001
Inoculum source × Inoculum type	2	8.3	<0.001
Inoculum source × Species	20	5.0	<0.001
Inoculum type × Species	10	6.9	<0.001
Species × Inoculum type × Inoculum source	20	5.6	<0.001
Block	3	2.8	0.040
Initial leaf area	1	339.4	<0.001
Days in experiment	1	168.8	<0.001

Notes: ANCOVA was used to analyze $\log_{10}$-transformed final biomass, with block (4 benches in the greenhouse), days in experiment (dead seedlings were replaced during the first month of the experiment), and $\log_{10}$-transformed initial leaf area as covariates. All contrasts within the three-way interaction were done to examine the differences between biomass of plants grown with inoculum coming from their home habitat compared to that of away habitats.

Table 3-6. F and P (in parenthesis) values of *a priori* contrasts examining growth of four plant species groups across the 6 inocula used in the experiment with respect to the control treatment (sterilized soil).

	Plant group			
Inoculum contrasted vs. control	Brachiaria grass	Coffee	Forest pioneer	Forest shade-tolerant
AMF from pastures	5.3 (0.021)	16.4 (< 0.001)	33.9 (< 0.001)	1.6 (0.20)
AMF from coffee plantations	4.1 (0.043)	10.7 (0.0012)	75.9 (< 0.001)	4.1 (0.044)
AMF from forest fragments	0.4 (0.520)	6.2 (0.013)	37.7 (< 0.001)	1.5 (0.22)
Filtrate from pastures	61.4 (< 0.001)	7.1 (0.008)	32.3 (< 0.001)	0.2 (0.69)
Filtrate from coffee plantations	0.3 (0.59)	0.5 (0.48)	2.5 (0.11)	1.1 (0.29)
Filtrate from forest fragments	0.1 (0.78)	22.1 (< 0.001)	0.04 (0.85)	2.7 (0.1)

Table 3-7. F and P (in parenthesis) values of *a priori* contrasts examining growth of four plant species groups with either AMF or a microbial filtrate from their home compared to that of away habitats (P = pastures, C = coffee plantations, and F = forest fragments).

	AMF			Filtrate		
Plant group	Home vs. C	Home vs. F	Home vs. P	Home vs. C	Home vs. F	Home vs. P
Brachiaria grass (Home: P)	0.08 (0.78)	2.8 (0.1)	-	55.7 (< 0.001)	59.5 (< 0.001)	-
Coffee (Home: C)	-	0.6 (0.42)	0.6 (0.43)	-	17.1 (< 0.001)	4.0 (0.046)
Forest pioneer trees (Home: F)	6.2 (0.013)	-	0.4 (0.51)	3.2 (0.077)	-	23.6 (< 0.001)
Forest shade tolerant trees (Home: F)	0.7 (0.41)	-	< 0.001 (0.99)	0.3 (0.59)	-	1.7 (0.2)

Table 3-8. AMF proportion root colonization of 11 plant species (Brachiaria grass, coffee, and nine forest tree species; Table 3-1) as affected by inoculum source (pastures, coffee plantations, and forest fragments), inoculum type (AMF, Filtrate), and plant species (and interactions) in the greenhouse.

	Total biomass (error df = 195)		
Source	**Df**	***F***	***P***
Inoculum source	2	21.4	<0.001
Inoculum type	1	202.7	<0.001
Species	10	13.4	<0.001
Inoculum source × Inoculum type	2	8.5	<0.001
Inoculum source × Species	20	3.1	<0.001
Inoculum type × Species	10	2.9	0.003
Species × Inoculum type × Inoculum source	20	2.4	0.0015
Block	1	0.4	0.51
Initial leaf area	1	0.4	0.52
Final biomass	1	0.95	0.33

Notes: ANCOVA was used to analyze AMF proportion root colonization, with block (4 benches in the greenhouse), $\log_{10}$-transformed initial leaf area, and $\log_{10}$-transformed final biomass as covariates.

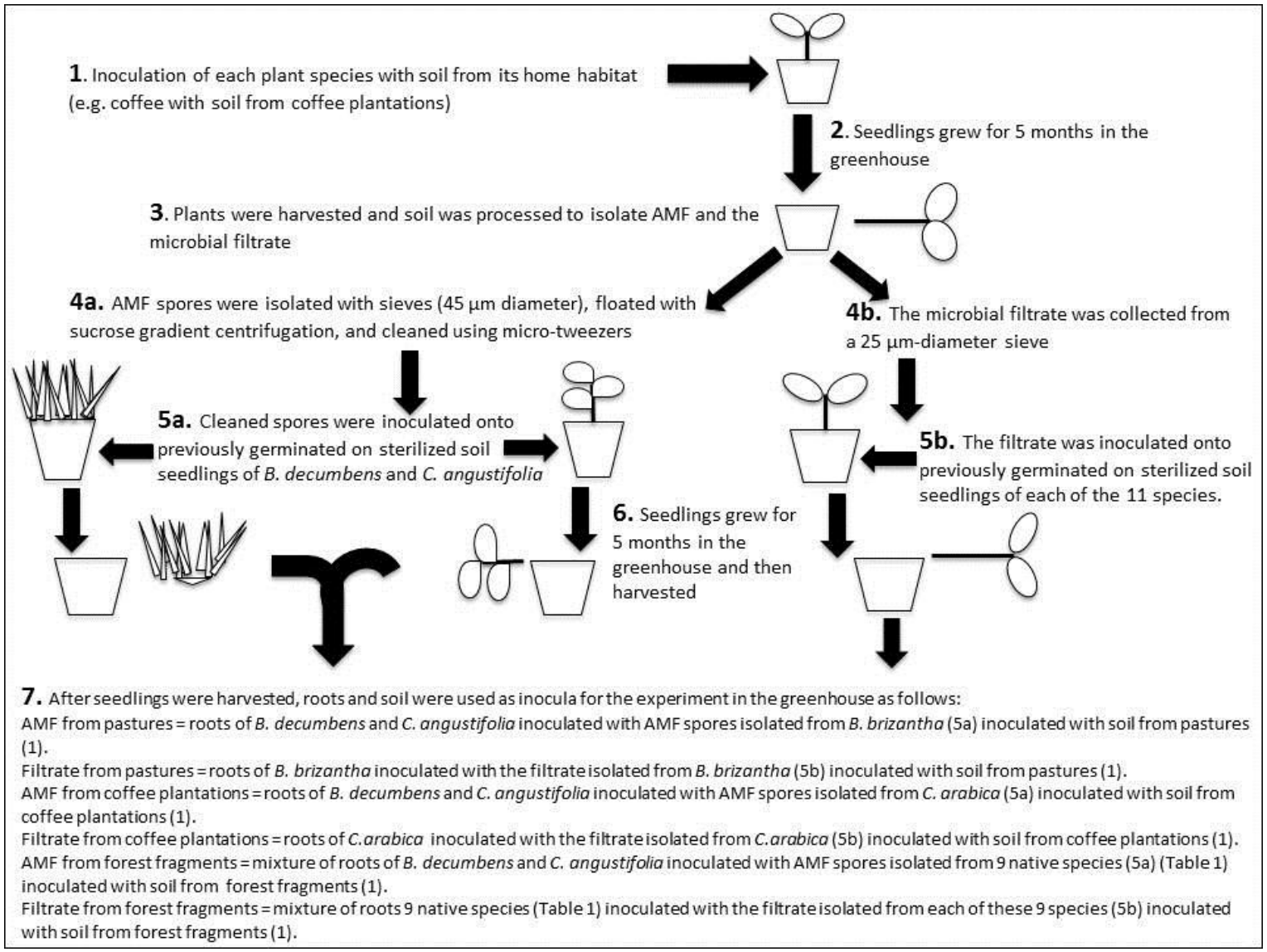

Figure 3-1. Flow diagram showing the procedure that we used to produce the inocula for setting up the experiment in the greenhouse.

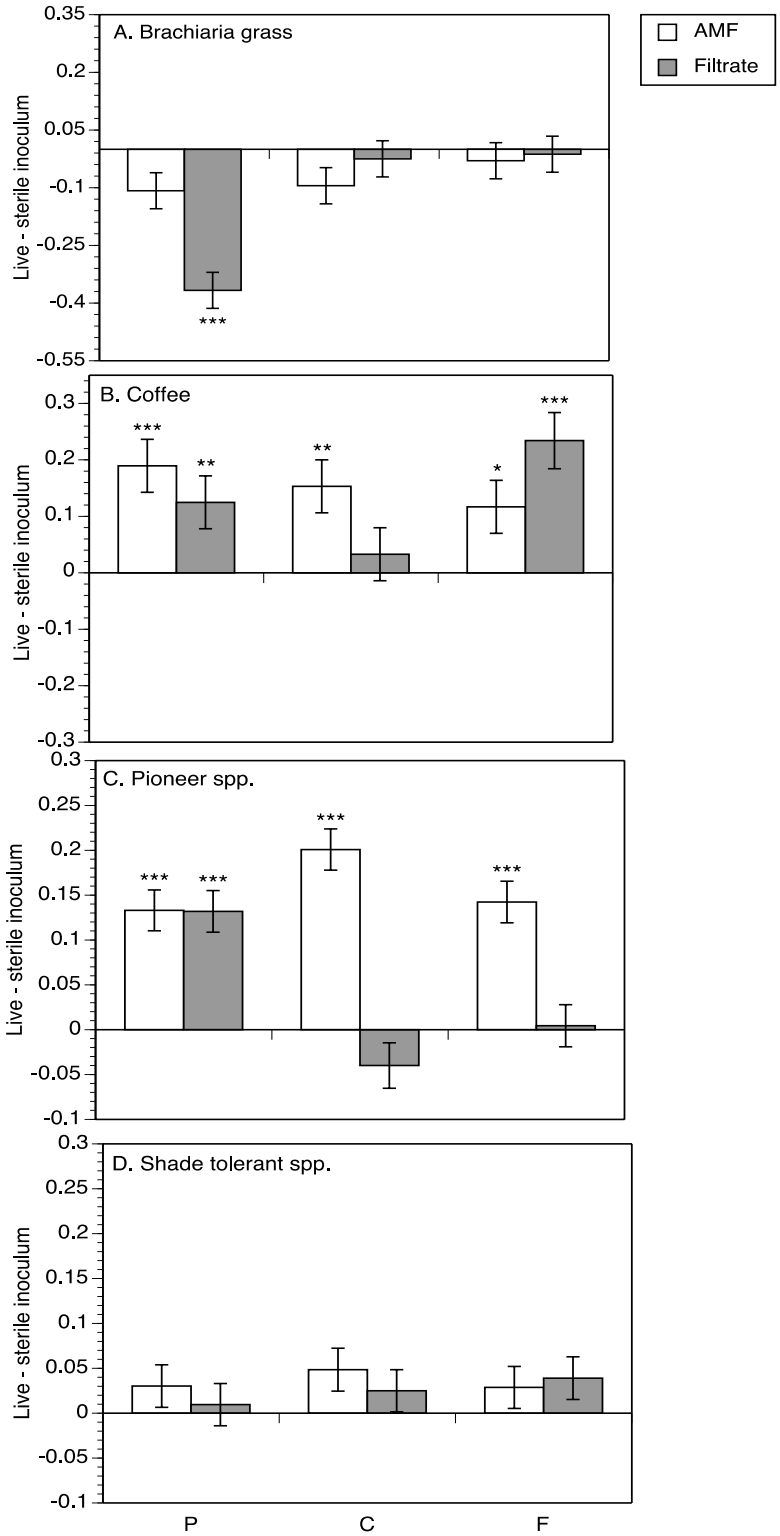

Figure 3-2. Effects of soil microbial presence (AMF or filtrate) relative to sterilized soil for growth of Brachiaria grass (A), coffee (B), forest pioneer trees (5 species) (C), and forest shade tolerant species (4 species) (D) (Table 3-1). Sol microbial inocula (AMF spore isolates: open bars, soil filtrate: closed bars) were prepared from soils collected from pastures (P; habitat for Brachiaria grass), coffee plantations (C), and forest fragments (F, habitat for forest tree species). The values indicate the value (± SE) of *a priori* contrasts calculated from all replicates, and comparing the growth of each species group when inoculated with AMF or filtrate from each habitat compared to that with sterilized soil. Positive values indicate growth enhanced by the microbial inoculum, while negative values indicate growth suppressed by the microbial inoculum. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

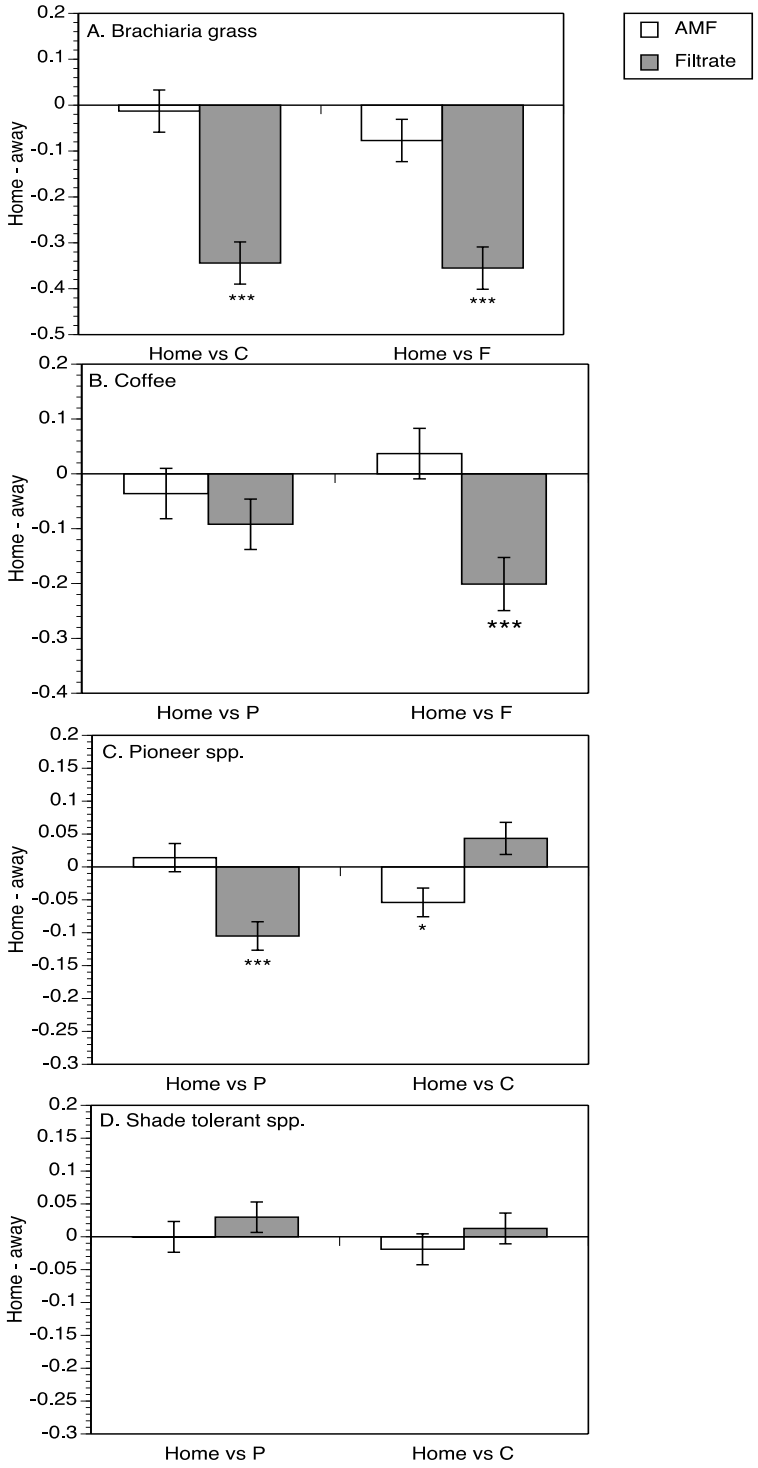

Figure 3-3. Effects of inoculation with AMF (open bars) or a microbial filtrate (closed bars) from "home" (species typical habitat) relative to "away" (habitats where species rarely occur or don't occur at all) habitats for growth of Brachiaria grass (A), coffee (B), forest pioneer trees (5 species) (C), and forest shade tolerant species (4 species) (D) (Table 3-1). Sol microbial inocula (AMF spore isolates: open bars, soil filtrate: closed bars) were prepared from soils collected from pastures (P; habitat for Brachiaria grass), coffee plantations (C), and forest fragments (F, habitat for forest tree species). Bars indicate the value (± SE) of *a priori* contrasts calculated from all replicates, and comparing the growth of each species group when inoculated with AMF or filtrate from their home habitat compared to that with away habitats. Positive values indicate better performance with soil microbial inocula from home habitat ("home advantage), while negative values indicate better performance with soil microbes from away habitats ("home disadvantage"). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

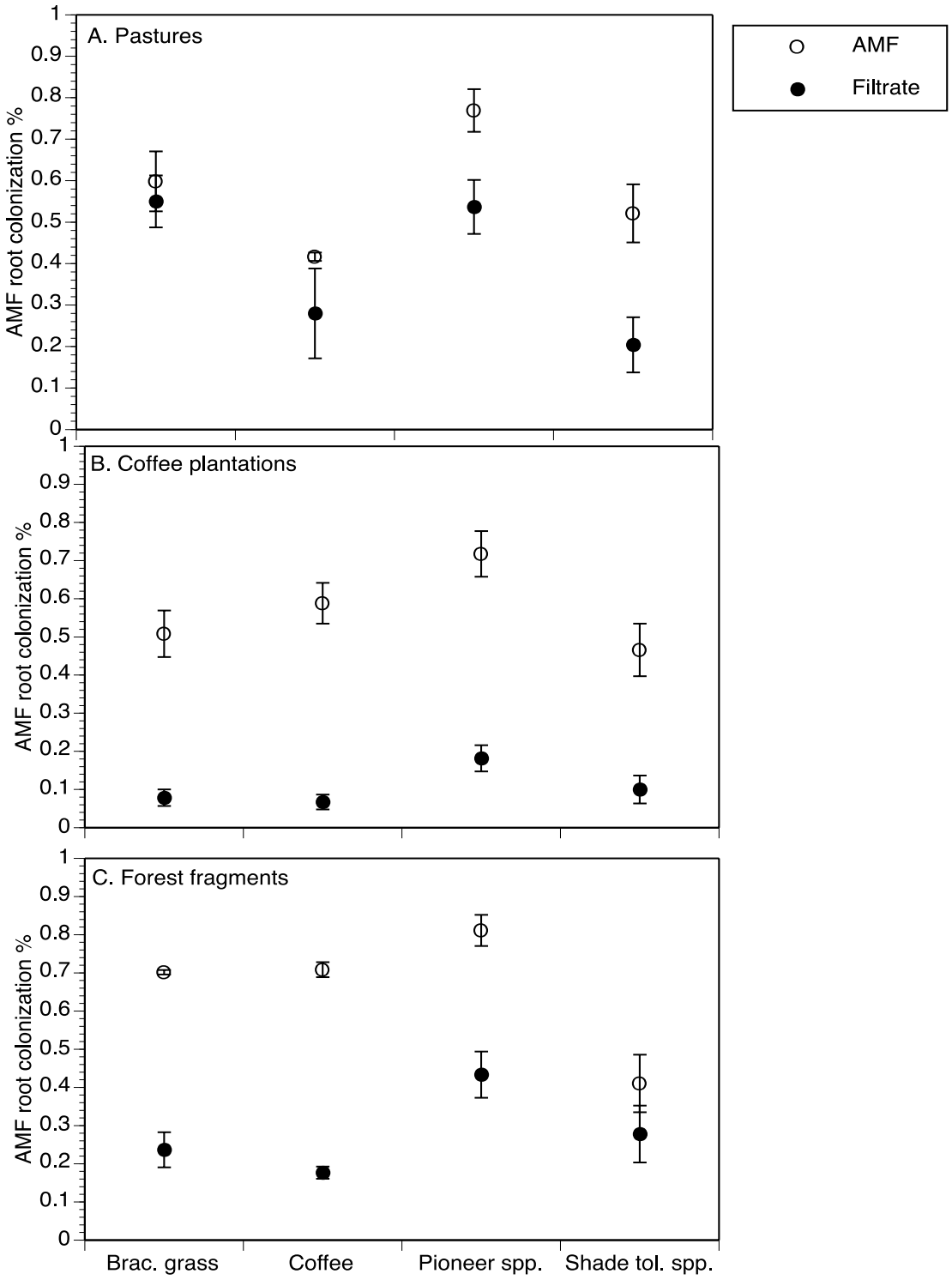

Figure 3-4. Proportion AMF root colonization in seedlings of Brachiaria grass, coffee, forest pioneer trees (5 species), and forest shade tolerant species (4 species) (Table 3-1) when inoculated with AMF (open circles) or a microbial filtrate (closed circles) from pastures (A), coffee plantations (B), or forest fragments (C) in the greenhouse. Circles indicate the mean proportion (± SE) AMF root colonization quantified in a subset of 3 replicates from each treatment and averaged for each species group.

CHAPTER 4
NEGATIVE FEEDBACK DOMINATES FOREST FRAGMENTS AND AGRICULTURAL LANDS IN THE TROPICAL ANDES.

Summary

The interactions between plants and soil organisms, plant-soil feedbacks, have increasingly been shown to determine plant community composition. In both temperate grassland and species-rich tropical forests, native species tend to experience negative feedback (rhizosphere soil microbes are more detrimental to conspecifics than to heterospecifics, indirectly promoting heterospecific neighbors to the host). In contrast, positive feedback (root soil microbes of plant species are more detrimental to heterospecifics than to conspecifics) has been reported from non-native invasive species, and interpreted as a factor that promotes dominance by these non-native species. However, it remains unknown how these interactions might change across heterogeneous habitats differing in the abundance of native, non-native, invasive, and non-invasive plant species. We used a factorial greenhouse experiment to test plant-soil feedback for 2 common non-native crop species and 8 native forest plant species in a montane region of Colombia to address: 1) How does feedback differ between arbuscular mycorrhizal fungi (AMF) compared to non-AMF soil microbes? And 2) What type of feedback occurs in agricultural monocultures of non-native species vs. forest fragments? We inoculated each plant species with either AMF spore-cultures or a microbial filtrate derived from soil microbial communities (SMC) associated with each of the 10 species. Doing so, growth of each host plant species could be examined in response to inoculation with conspecific AMF and filtrate, compared to inoculation with AMF and filtrate culture obtained from nine heterospecific host species. Overall, feedback mediated by AMF was much weaker than feedback mediated by the microbial

filtrate. Surprisingly, *Brachiaria brizantha*, a non-native invasive grass that dominates pastures, and *Coffea arabica*, a non-native not shrub that is naturalized in forest fragments, both experienced strong negative feedbacks for the microbial filtrate that contained soil pathogens. In contrast, most forest tree species showed neutral to slightly negative feedback, suggesting that the negative feedback shown for natural forests may be disrupted with fragmentation. These results suggest that antagonistic species-specific soil microbes that mediate negative plant-soil feedback accumulate in proportion when a single host species dominates, as is the case in agricultural habitats. Forest fragments, on the other hand, may have lost the diversity of soil microbial communities that drive negative plant-soil feedbacks in undisturbed tropical forests.

Background

Biotic interactions (e.g. competition, predation, disease) mediate plant coexistence at the local level and have proved to be vital for the maintenance of diversity in natural communities (Schemske et al. 2009). Recent studies have demonstrated the importance of the interaction between plants and soil organisms, “plant-soil feedbacks”, for local plant biodiversity (Bever 1994, Van der Putten and Peters 1997, Hartnett and Wilson 2002, Klironomos 2002, Bever 2003, Reynolds et al. 2003, Mangan et al. 2010b) and plant productivity (Van der Heijden et al. 1998, Van der Heijden et al. 2006, Van der Heijden et al. 2008, Vogelsang et al. 2006). For instance, negative plant-soil feedback, resulting from soil microbes that are more detrimental to conspecific than to heterospecific plant species, seems to dominate both temperate grasslands (Casper et al. 2008, Petermann et al. 2008, Harrison and Bardgett 2010) and species rich tropical forests (Mangan et al. 2010b). Such negative feedbacks limit dominance of a single species and are expected to contribute to species coexistence and local plant diversity.

Positive feedback, on the other hand, can reduce local plant diversity, as plants experiencing positive feedback should become dominant. Examples of such positive feedbacks have been reported for many non-native species that have escaped their natural enemies in their original distribution ranges (enemy-release hypothesis), and exhibit positive feedback in their new ranges (Klironomos 2002, Reinhart et al. 2003, Callaway et al. 2004, Wolfe and Klironomos 2005, Reinhart and Callaway 2006, Van Grunsven et al. 2007, Vogelsang and Bever 2009).

Plant-soil feedbacks are mediated by soil microbial communities (SMC) comprised of diverse arrays of organisms that interact both directly and indirectly with plants, varying in the type of interactions from mutualistic to antagonistic, and from non-specific to highly specific interactions (Klironomos 2003, Kuyper and Goede 2004). Because of difficulties of identifying and isolating the different components of SMC, many of the studies addressing the interaction between plants and soil microbes have focused on a small number of microbes that can be isolated with several culture techniques. For example, it is now well established that different plant species host, and respond differently to, diverse arrays of arbuscular mycorrhizal fungi (AMF) (Bever 2002a, Castelli and Casper 2003, Mangan et al. 2010a) that can be easily isolated from soils as spores. In addition, plant-AMF feedbacks can be either positive (Mangan et al. 2010a) or negative (Bever 2002b). In contrast, other soil organisms are more difficult to study and it is a challenge to untangle their role in driving plant-soil feedbacks. For example, soil bacteria, fungal endophytes, fungal pathogens, and virus are far more difficult to isolate and culture for experimental purposes. These constraints limited the number of

studies that have examined the role of different SMC constituents in driving plant-soil feedbacks (Klironomos 2002).

The role of plant-soil feedbacks in determining plant species relative abundance has been mainly tested within particular natural habitats (e.g., a prairie grassland by Klironomos 2002, and a moist tropical forest by Mangan et al. 2010b). But, we have a limited understanding on how these interactions vary across the landscape consisting of habitats that differ in the abundance of native, and non-native, and of invasive, and not invasive, plant species. Although many studies report that non-native invasive species become dominant because they experience positive feedback (e.g., Klironomos 2002, Reinhart et al. 2003, Agrawal et al. 2005, Wolfe and Klironomos 2005, van Grunsven et al. 2007), there is also evidence that the direction and strength of plant-soil feedbacks are not simply determined by where plant hosts originated from (Mackay and Kotanen 2008, Beest et al. 2009). In fact it is well established that agricultural monocultures of non-native plant species accumulate heavy loads of pathogens that limit their productivity (Ghorbani et al. 2008). Thus, as non-native plants become “resident” and increase their abundance in the novel ranges for a prolonged time (Lankau et al. 2010), they may accumulate species-specific soil antagonistic organisms and experience negative, instead of positive feedbacks (Beest et al. 2009). Besides, plant-soil feedbacks are not the only mechanism that leads to dominance of invasive species, as particular physiological, reproductive, defense, etc., characteristics may also give them a competitive advantage over other plant species (Van Kleunen et al. 2010). Here we intended to test how plant-soil feedbacks vary across native, non-native, invasive, and not-invasive plants by comparing plant-soil interactions across habitats dominated by

single resident invasive and not invasive non-native plant species vs. habitats containing species-rich arrays of native plant species.

Our aims were to compare plant-soil feedbacks driven by 1) AMF vs. non-AMF soil microbes from these contrasting habitats, and 2) non-native plants that dominate agricultural lands (pasture grass and coffee) vs. native tree species common in species rich pre-montane tropical forest fragments. To do this, we inoculated Brachiaria grass (dominating species in pastures), coffee (dominating species in coffee monocultures), and 8 native forest tree species with either AMF spore-cultures or a microbial filtrate amplified on each of the 10 species inoculated with SMC from their respective habitats (e.g. coffee with SMC from coffee plantations, native plants with SMC from forests). Brachiaria grass has been widely planted in the area of study for cattle pastures and is a common and very noxious weed in coffee and other plantations. Therefore it is considered an invasive species. Coffee is extensively planted in the area of study. Although it is not considered a weed nor an invasive species, it successfully colonizes the understory of forest fragments (C. Pizano pers. obs.). We then estimated feedback for AMF and non-AMF separately between the individual species with other species (e.g. Brachiaria grass with coffee, a native pioneer species with Brachiaria grass, etc.) or species groups (e.g., Brachiaria grass with native pioneer species). We expected plant-soil feedback driven by non-AMF soil microbes would be stronger compared to feedback caused by AMF. In addition, we predicted that Brachiaria grass (a non-native invasive grass) would experience positive feedbacks, while non-invasive or native species, such as coffee and forest tree species, would experience negative feedbacks.

Methods

Study site and species

This study was conducted in the agricultural region of the Central Cordillera of Colombia between 1200 and 2000 m.s.l. (4°6′N 75°4′ W). The climate is tropical humid, with an average annual temperature of 21ºC, and an annual rainfall of 2550 mm concentrated in two wet seasons (March to July and September to December) (Guzmán-Martínez et al. 2006). Soils are Udands (Andisols) (Ortíz-Escobar et al. 2004). The landscape is dominated by three contrasting habitat types: occasionally fertilized pastures of low diversity (mainly African grasses *Pennisetum clandestinum*, *Melinis minutiflora* and *Brachiaria* spp.), highly fertilized, low diversity sun-grown coffee plantations (coffee monocultures), and unmanaged fragments of pre-montane tropical forest (Orrego et al. 2004a). The forest fragments are usually small (1-30 ha), are mostly dominated by early and mid-successional plant species and have high liana densities. Recruitment of native species in the understory appears to be low, where seedlings of non-native species such as *Coffea arabica* (coffee) and *Musa veluntina* (pink banana) are common (C. Pizano pers. obs.).

Native plant species were selected based on the their abundance in the region (Orrego et al. 2004b), sampling a wide range of seed size and life histories (Table 4-1, Appendix A). *Brachiaria brizantha* (Brachiaria grass) and *Coffea arabica* (coffee) were chosen because they are the dominating species in the two most extensive human-modified habitat types in the region (Table 4-1). Pastures were never treated with fungicide, while coffee plantations are treated with fungicide only when soil pathogens such as *Rhizoctonia solani* (Gaitán 2003), *Rosellinia bunodes* (Castro-Caicedo 2003), *Rosellinia pepo* (Castro-Caicedo 2003), and *Ceratocystis fimbriata* (Castro Caicedo-

2003a) become prominent in an area. To sample across heterogenous altitude and climatic conditions in the study area, five private farms with similar conditions each containing a pasture, a coffee plantation, and a forest fragment (Table 4-2) were chosen to collect soil for the greenhouse experiment. At each of the 15 site-habitat combinations, five samples of 200 g of mineral soil (5-15 cm in depth) each were taken and pooled in a composite sample from which a subsample was analyzed. Soils across the three habitats had similar levels of pH, organic matter content, and some nutrients (N, K, Mg), but forest fragment soils contained only 10-12 % of P, and significantly greater Ca content than pasture and coffee plantation soils (Table 4-3). In addition, light levels in pastures and coffee plantations were approximately 30 times higher than in forest fragments (Table 4-3).

Seed preparation and soil medium for growing seedlings

Seeds of Brachiaria grass and coffee were provided by Cenicafé, while seeds of the eight native plant species (Table 4-1) were collected from at least 3 parent trees in forest fragments. Seeds were surface sterilized (0.6 % sodium hypochlorite for 15 minutes) and germinated in trays containing steam-sterilized soil 3:2 soil and river sand mixture. The soil was collected from an open area near the greenhouses in Cenicafé (Colombian National Research Center for Coffee, Chinchiná, Caldas, Colombia), and it was classified as an acrudoxic melanudand from Chinchiná unit (Ortíz-Escobar et al. 2004) with moderately fertile chemical characteristics (5.4 pH, 0.1 % N calculated, 1.9 % OM-Walkley-Black colorimetry, 7 mg kg^{-1} P-Bray II Bray Kurtz colorimetry, 0.37 mg kg^{-1} K-Ammonium acetate 1N, 3.4 mg kg^{-1} Ca-Ammonium acetate 1N, and 1.1 cmol kg^{-1} Mg-Ammonium acetate 1N).

Initial inoculum collection

We collected whole-soil inocula (i.e. roots, rizhosphere soil, and associated organisms) from pastures, coffee plantations, and forest fragments at 5 farm sites each having these three habitats (total of 15 site-habitat combinations) (Table 4-2). Roots and rhizosphere soil (5-15 cm in depth) were collected from 5 random locations within each habitat at each site and pooled together. We then combined and homogenized the sampled soil from the 5 sites for each habitat type.

AMF inoculum amplification

Each inoculum was amplified in two steps. In the first stage, individually potted plants of the 10 species (Table 4-1) were inoculated with soils collected in their respective habitat types as above (i.e. Brachiaria grass with the soil from pastures, coffee with the soil from coffee plantations, and native species with the soil from forest fragments) (Fig.4-1, step 1). The same sterilized soil and sand mixture as used to germinate the seeds filled 70 % of 1-L pots to where an equal quantity (100 mL) of the whole-soil inoculum from a particular habitat, was added. Ten seedlings per species were grown for 5 months in the greenhouse (Fig.4-1, step 2) after which we harvested and mixed their roots and pot soil to process 8 subsamples of 20 g of soil from each plant-inoculum combination (Fig. 4-1, step 3). Each soil subsample was washed with water through a series of sieves, and we collected what remained in the 45 μm-diameter sieve (mostly AMF spores) (Klironomos 2002) (Fig.4-1, step 4a). In order to clean the AMF spores and separate them from other structures and possible contaminants, we used sucrose density gradient centrifugation (Daniels and Skipper 1982) and then isolated individual spores using micro-tweezers. Once all clean spores were obtained, they were used to inoculate seedlings previously germinated on sterilized soil of

Brachiaria decumbens and *Cecropia angustifolia* (6 seedlings per species) (Fig.4-1, step 5a). To do this, we suspended the clean AMF spores in distilled water and added this solution directly onto the plant root system. The same process was repeated for each inoculum obtained from the 10 host species, therefore we had a total of 60 seedlings of *Brachiaria decumbens* and *Cecropia angustifolia* each inoculated with 10 different AMF communities. These species were selected to amplify the AMF inoculum because they grow fast and produce high amounts of AMF spores in a relatively short time (Mangan et al. 2010a). In addition, because AMF sporulate at different rates on different host species (Jansa et al. 2008), it allowed us to standardize the amount of spores produced in each species AMF inoculum.

Inoculated seedlings grew for 5 months in the greenhouse (Fig.4-1, step 6) after which they were harvested and their soil and roots were used as inoculum in the greenhouse experiment (Fig. 4-1, step 7). For each inoculum source the seedlings of *B. decumbens* and *C. angustifolia* were harvested, and their roots and soil were pooled together (Fig.4-1, step 7).

Filtrate inocula

These inocula were produced by amplifying soil microbes collected in a filtrate from the 25 μm-diameter sieve that excluded large AMF spores (Klironomos 2002). The scope was to create non-AMF inocula. Although AMF hyphal contamination was possible, this was the practical method used in other studies (e.g., Klironomos 2002, McCarthy-Neumann and Kobe 2008) to isolate a representative community of non-AMF soil organisms including microbial pathogens like bacteria and non-AMF fungi. The filtrate was used to inoculate seedlings of the respective species used in the initial

amplification (Fig. 4-1, step 1). For example, we inoculated *Juglans neotropica* seedlings with filtrate obtained from pots with *Juglans neotropica* seedlings. These seedlings were also grown for 5 months in the greenhouse after which they were harvested and their roots and soil were collected to use as inoculum in the greenhouse experiment.

Greenhouse experiment- growth conditions and treatments

Each 1-L pot was filled with 70 % steam-sterilized (for 2 hrs) soil 3:2 soil and river sand mixture. The soil was collected from an open area at CIAT (International Center for Tropical Agriculture) in Palmira, Valle, Colombia, and was shown to have moderately fertile chemical characteristics (6.8 pH, 0.05 % N, 0.6 % OM-Walkley-Black colorimetry, 42 mg kg^{-1} P-Bray II Bray Kurtz colorimetry, 0.35 mg kg^{-1} K-Ammonium acetate 1N, 3.7 mg kg^{-1} Ca-Ammonium acetate 1N, and 2.8 cmol kg^{-1} Mg-Ammonium acetate 1N). Then we added an equal amount (100 mL) of AMF or filtrate inoculum consisting of a mixture of roots and soil previously produced in the greenhouse (Fig. 4-1, step 7). Each treatment combination from the combination of inoculum species (10 species) × inoculum type (AMF, filtrate) × plant species (10 plant species) was replicated in 4 pots and plants were randomly distributed in the growing house. We additionally grew 4 seedlings of each of the 10 plant species as non-inoculated sterile plants. Thus, we had a total of 840 plants in the experiment. Plants were under 20 % light for 75 days. We measured the leaf area of each plant at the beginning of the experiment to use it as covariate of final total dry weight in the statistical analyses. In addition, we also quantified AMF root colonization in the roots of half of the seedlings; 1 g of fine roots were washed with running water, cleared with 3 % KOH and 3 % H_2O_2, acidified with 3

% HCl, washed with running water, and stained with 0.05 % tryplan blue in lactoglycerol solution (Zangaro et al. 2000). Root segments were then mounted on slides (2 slides/seedling) and presence or absence of AMF and non-AMF soil organisms was recorded at 200 intersect points for each slide (for a total of 400 intersect points for each individual).

Statistical analyses

The did an initial analysis to estimate the net effects of AMF and the microbial filtrate inocula relative to the non-inoculated sterile (control) on plant growth. More specifically, we ran an analysis of covariance (ANCOVA) on final seedling biomass that included inoculum type (sterile, AMF and filtrate) and plant species (and their interaction) as fixed effects, and initial leaf area and days in experiment as covariates. This facilitated the analysis in seedling age heterogeneity as dead seedlings were replaced during the first month of the experiment.

We then did two sets of analyses to examine the effects and feedback of 1) AMF inocula, and 2) filtrate inocula on growth of the 10 plant species. For both of these analyses we did an initial analysis of covariance (ANCOVA) on final seedling biomass that included inoculum species (10 species) and plant species (10 species) and their interaction as fixed effects, and initial leaf area and days in the experiment as covariates (non-inoculated sterile plants were not included). We then calculated feedback for AMF and the filtrate separately by using *a priori* contrasts within the "inoculum species $\times$ plant species" (Mangan et al. 2010b). These contrasts not only compare growth of each plant species with conspecific vs. heterospecific inoculum, but also growth of other plant species with conspecific vs. heterospecific inculum (two-way analysis). Given that

Brachiaria grass is the dominating species in pastures in the region of study, pairwise feedback between this grass and all other nine plant species was calculated as a proxy of feedback in pastures (Bever 2003, Mangan et al. 2010b). Similarly, pairwise feedback between coffee and all other species was estimated as a proxy of feedback in coffee plantations (Bever 2003, Mangan et al. 2010b). In contrast, feedback in forest fragments had to be estimated considering all possible combinations between all ten plant species, as there are no dominating plant species in these habitats. Accordingly, feedback in forest fragments was calculated by pairwise feedback for each possible pair of species and then averaging all pairwise feedbacks involving each species (Bever 2003, Mangan et al. 2010b).

Finally, to compare AMF colonization proportion across the different inocula types, an analysis of variance (ANOVA) was conducted for half of plants for which we measured AMF colonization (2 replicates per treatment). This model included inoculum species (10 species), inoculum type (AMF, filtrate), and plant species (and their interactions) as fixed effects. AMF colonization proportion was transformed with arcsine-square root to improve normality. Plants in the sterile treatment were not included in this analysis as none of the examined plant roots showed evidence of AMF colonization. Statistical analyses were done with JMP®, version 8.0 (SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513).

Results

Overall, plants grew better when inoculated with AMF than when grown on sterile soil, but better with sterile soil than when inoculated with the microbial filtrate (Table 4-4, Figure 4-2). Next, we compared plant growth across AMF and microbial filtrate inocula

obtained from different source species (= inoculum species). Seedling biomass significantly differed among inoculum species, plant species, and the combination of inoculum species and plant species (indicated by a significant interaction) for AMF inocula (Table 4-5). Similarly, for the microbial filtrate inocula, plant biomass significantly differed among plant species, as well as across different inoculum species and plant species (indicated by a significant interaction) (Table 4-6). These significant interactions meant that plant species responded differently to soil inocula from conspecific and heterospecific sources. These often reflected significantly negative feedbacks that could be attributed to sufficiently strong negative effects of conspecific inocula compared to heterospecific inocula in both (Fig. 4-3 A, B) or one in the pair-wise comparisons (Fig. 4-3C).

Feedbacks for AMF inocula were mostly neutral and non-significant in pair-wise tests involving Brachiaria grass (Table 4-7, Fig. 4-4A), tests involving coffee (Table 4-7, Fig. 4-4B), and tests involving forest tree species as a group (Table 4-7, Fig. 4-4C). The only exception was the weak negative feedback between coffee and *Ochroma pyramidale*, reflecting a positive heterospecific inoculum effect on *Ochroma* and coffee growth compared to conspecific AMF (Fig. 4-4C).

In contrast, feedbacks with the microbial filtrate were often negative and significant. Growth of Brachiaria grass, in particular, was negatively impacted by conspecific soil filtrate inocula more strongly than by heterospecific inocula obtained from other species (Fig. 4-3A, B, C). Conversely, other species tended to be more strongly impacted by conspecific soil filtrate inocula than by filtrate inocula obtained from Brachiaria grass. As a result, eight of the nine pair-wise feedback response tests

involving Brachiaria grass were significantly negative (Table 4-8, Fig. 4-4A). Pair-wise feedback tests for soil filtrate involving coffee also showed negative feedbacks for several species pairs: coffee-Brachiaria grass, coffee-CecA, coffee-CecT, and coffee-Ochr (Table 4-8, Fig. 4-4B), in which non-coffee species were a grass or native pioneer trees. Pair-wise feedback tests for microbial soil filtrate involving native tree species collectively as heterospecific partners (Fig. 4-4C) showed significant negative feedbacks in two cases (Table 4-8). A strong negative feedback was found between Brachiaria grass and native trees, owing largely to a reduced growth of Brachiaria grass with conspecific filtrate inoculum compared to growth with heterospecific inocula. Also strong negative effects of conspecific filtrate inoculum on growth of *Cecropia angustifolia* compared to heterospecific inocula from eight other species resulted in significant negative feedback (Fig. 4-4C).

AMF root colonization varied greatly across inoculum species, inoculum type, plant species and different combinations of inoculum species, plant species and inoculum type (Table 4-9, Fig. 4-5). As expected, AMF colonization was higher for the AMF inocula than for the microbial filtrate inocula for nine out of 10 plant species (Fig. 4-5A). Similarly, proportion root colonization by non-AMF soil microbes also varied significantly across inoculum species, inoculum type, plant species, and two-way interactions between inoculum species and inoculum type, and between inoculum species and plant species (Table 4-10). Root colonization by non-AMF soil microbes was higher in plants treated with the microbial filtrate than in those treated with the AMF inocula (Fig. 4-5B).

Discussion

The two key results of this study were that 1) plant-soil feedbacks driven by non-AMF soil microbes were much stronger than feedbacks driven by AMF (Fig. 4-4), and 2) both non-native plant species that dominate agricultural lands, and native species common in natural forests, experience negative plant-soil feedbacks (Fig. 4-4). Surprisingly, Brachiaria grass, an invasive non-native grass that dominates pastures and colonizes other habitats (e.g. coffee plantations), experienced significant negative feedbacks for the microbial filtrate (Fig. 4-4A), instead of positive feedbacks shown for temperate invasive exotics in other studies (e.g., Klironomos 2002, Reinhart et al. 2003, Van Grunsven et al. 2007) . Similarly, feedback between coffee, a non-native species, and Brachiaria grass, and between coffee and forest pioneer tree species was negative for the microbial filtrate, but neutral or slightly positive for forest shade tolerant tree species (Fig. 4-4B). Finally, feedback of forest tree species was mostly negative with the microbial filtrate (Fig. 4-4C). Overall, plants grew better with sterile soil than when inoculated with the soil microbial filtrate (Fig. 4-2), and that root colonization by non-AMF soil organisms was higher for the microbial filtrate than for the AMF inocula (Fig. 4-5). These results suggest that the microbial filtrate included antagonistic soil microbes. Pasture grass dominates the species-poor habitats despite accumulation of species-specialized antagonistic soil microbes. Native tree species did slightly better with these detrimental soil microbes from pastures than with those they encounter in forest fragments. Thus, negative plant-soil feedback dynamics occur both in species-poor agricultural lands, as well as in more species-rich forest fragments. In other words, soil antagonistic microbes specialized in common plant species accumulate regardless of habitat type with contrasting plant diversity and management regimes.

AMF vs. non-AMF soil organisms in driving feedback

Feedbacks driven by AMF have been well studied because these fungi are relatively easy to isolate and experiment with. Previous studies have shown that although AMF are not strictly species-specific, their effects on plant hosts vary significantly across plant species (Castelli and Casper 2003, Moora et al. 2004, Munkvold et al. 2004, Mangan et al. 2010a), with significant implications for plant community composition (Bever 2002a, Klironomos 2002, Van der Heijden 2004, Vogelsang et al. 2006). Similarly, when the effects of non-AMF organisms were examined with microbial filtrate, species-specific antagonistic soil microbes that mediate significant plant-soil feedbacks tend to accumulate with each host plant (e.g., Klironomos 2002, McCarthy-Neumann and Kobe 2008, McCarthy-Neumann and Kobe 2010). We found that plant-soil feedbacks via AMF were neutral and mostly insignificant across plant species from contrasting habitats (Table 4-7, Fig. 4-4). The only significant plant-AMF feedback was between coffee and the pioneer native tree *Ochroma pyramidale* (balsa), indicating that both species benefit more from each other's AMF compared to their own AMF. Thus, it seems like forest fragments and agricultural lands in the study region share similar communities of mycorrhizal fungi, with similar, positive effects across different plant species (Table 4-4, Fig. 4-2). An alternative explanation for the general neutrality of AMF plant-soil feedbacks, is that the AMF inocula represented a biased subset of AMF community in the soil. The AMF inocula were prepared from individually picked AMF, which probably favored AMF strains with copious spore production ("r-strategy" AMF; Verbruggen and Kiers 2010) selecting for only one of many AMF functional types from the three sampled habitats (Munkvold et al. 2004).

Opposite to AMF plant-soil feedbacks, feedback mediated by the soil microbial filtrate was significantly negative for most plant species. This was particularly true for Brachiaria grass, which showed strong negative feedback with the filtrate for 7 out of 9 plant species (all but *Solanum aphynodendrum* and *Garcinia madrunno*) (Table 4-8, Fig. 4-4A), and for coffee, which had significant negative feedback for four out of nine species (Table 4-8, Fig. 4-4B). Similarly, although average filtrate feedback between Brachiaria grass, coffee and native tree species was only slightly negative for most species, it was significantly negative for the grass and for the pioneer tree *Cecropia angustifolia* (Table 4-8, Fig. 4-4C). The fact that plants grew overall better with sterile soil than with the microbial filtrate (Table 4-4, Fig. 4-2) indicates the presence of antagonistic soil microbes. Furthermore, we also found that AMF colonization was lower, and non-AMF colonization higher, in plants treated with the filtrate than in those inoculated with AMF (Table 4-9, Table 4-10, Fig. 4-5), indicating that the microbial filtrate effectively contained more antagonistic soil microorganisms than the AMF inocula. These results suggest that while pastures and coffee plantations accumulate species-specific antagonistic soil microbes that mediate negative plant-soil feedbacks, forest fragments seem to hold more generalist soil microbes that mediate only weak plant-soil feedbacks. Perhaps forest fragmentation has resulted in a loss of the high biodiversity of species-specific soil microbes (Gilbert and Hubbell 1996) that mediate negative feedback in undisturbed tropical forests (Mangan et al. 2010b).

Feedback across habitats of contrasting diversity and abundance of native, non-native, invasive, and not invasive plant species

Studies in both temperate (Klironomos 2002, Casper et al. 2008, Petermann et al. 2008, Harrison and Bardgett 2010) and species-rich plant communities in the tropics

(Mangan et al. 2010b) species rich plant communities have shown that negative feedback between plants and soil organisms is weaker for more abundant plant species. By a similar logic, plants introduced to new ranges may become invasive by escaping their home soil enemies (enemy-release hypothesis). For example, *Centaurea melitensis* and *Centaurea malucosa*, two European species invasive in North America, experience negative feedback in their native range but show positive feedback in their new territory (Callaway et al. 2004). Similarly, the invasion of *Prunus serotina* (black cherry) in north-western Europe is facilitated by soil microbes in positive feedbacks, while growth of this species is limited by the soil community that develops in the roots of conspecific in its native range in USA (Reinhart et al. 2003). However, non-native species may accumulate antagonistic soil organisms and may experience negative feedback (Nijjer et al. 2007) after being in their new ranges for a prolonged time, and becoming "resident". For instance, it is well known that non-native plant species grown as agricultural crops may accumulate heavy loads of soil pathogens and therefore experience negative feedback. Thus, plant-soil feedbacks may vary across plants irrespective to their classification as native, non-native, and invasive and not invasive.

Our results show that plant-soil feedbacks were largely negative regardless of whether the species were exotic invasive, exotic non-invasive, or native. Brachiaira grass, the dominating species in pastures and a highly invasive weed in fruit orchads (e.g., citrus and coffee) and open natural habitats (Almeida-Neto et al. 2010), showed significant negative feedback with coffee and 6 out of 8 native plant species (Table 4-7, Table 4-8, Fig. 4-4A). This goes against one of our *a priori* expectation that the invasiveness of this grass was related to positive plant-soil feedbacks, and contradicts

studies showing this type of feedback for non-native invasive species (Callaway et al. 2003, Reinhart et al. 2003, Wolfe and Klironomos 2005, van Grunsven et al. 2007). However, this African grass was introduced to the study region at least 50 years ago (Parsons 1972), since then it may have accumulated species-specific antagonistic soil microbes and experiences negative feedback (Nijjer et al. 2007). Continued dominance of this grass in pastures and invasion in plantations may instead be related to other traits such as physiology, leaf-area allocation, shoot allocation, growth rate, size or fitness (Van Kleunen et al. 2010). Alternatively, Brachiaria grass may produce a root exhudate chemical that inhibits nitrification similar to that one recently found in *Brachiaria humidicola* (Subbarao et al. 2009), therefore having a competitive advantage over other plant species.

Coffee, the most abundant plant in coffee plantations, also showed negative feedback with Brachiaria grass and three forest tree species (Table 4-7, Table 4-8, Fig. 4-3B, Fig. 4-4B). This non-native shrub was introduced in the study region at least 150 years ago, and plantations are often dimmed by root fungal pathogens such as *Rhizoctonia solani* (Gaitán 2003)*, Rosellinia bunodes* (Castro-Caicedo 2003)*, Rosellinia pepo* (Castro-Caicedo 2003)*,* and *Ceratocystis fimbriata* (Castro Caicedo-2003a). Thus, it is not surprising that coffee appears to have accumulated species-specific antagonistic soil organisms and exhibits negative feedback. Interestingly, with native tree species, coffee only showed negative feedback with pioneer, and not with shade tolerant tree species. This suggests that coffee and shade tolerant forest tree species share similar soil enemies, therefore no feedback dynamics arise between these species (Fig. 4-3D). For example, coffee plantations increase the abundance of

Rosellinia bunodes in Puerto Rico and the fungus colonizes other Rubiaceae native plants (Lodge 2001). In contrast, coffee appears to accumulate a different array of soil microbes than those present in native pioneer trees, therefore negative feedback takes place among these species.

Finally, there was a strong negative feedback between native tree species and Brachiaria grass, and between *Cecropia angustifolia* and other native trees due mostly to a reduced growth of the grass and CecA with conspecific filtrate inoculum compared to growth with heterospecific inoculum (Table 4-7, Table 4-8, Fig. 4-4C). However, feedback between coffee and native trees, and among the other seven native tree species was mostly neutral, and only slightly negative. Thus, the negative feedback shown for undisturbed tropical forest (Mangan et al. 2010b) may have been disrupted with forest fragmentation and land use change. For instance, antagonistic soil organisms that become common in agricultural lands may invade forest fragments, displacing other native soil organisms (Gilbert and Hubbell 1996) responsible for driving negative feedbacks. Alternatively, the low signal of feedback for most native species may be due to the low replication, or short running of the experiment.

Applied ecological implications:

Biological interactions that play a key role in maintaining plant diversity can become disrupted when natural ecosystems are fragmented and replaced by agriculture (Bascompte 2009, Kiers et al. 2010). For example, previous studies have found that seed dispersal declines (Wijdeven and Kuzee 2000, Hooper et al. 2005), while seed predation intensifies (Holl and Lulow 1997, Holl et al. 2000) in fragmented, compared to undisturbed tropical forests. Likewise, our results indicate that plant-soil feedbacks are substantially altered by forest fragmentation by land conversion to agriculture. First,

although the importance of plant-AMF feedbacks for plant community composition have been demonstrated both in the greenhouse (Klironomos 2002, Mangan et al. 2010a) and in the field (Pringle and Bever 2008), we found little evidence that suggest similar feedbacks with AMF. This may indicate that AMF communities in the study region are impoverished probably due to the cultivation of single non-native plant species in monoculture (Kabir et al. 1997, Menendez et al. 2001, Oehl et al. 2003), and agricultural practices such as tillage and soil fertilization (Helgason et al. 1998, Menendez et al. 2001). Additionally, AMF community composition may be significantly altered by forest fragmentation (Mangan et al. 2004).

In contrast to plant-AMF dynamics, we found strong negative feedbacks driven by a microbial filtrate comprising antagonistic soil microbes. This was mainly true for Brachiaria grass and coffee, showing there is an accumulation of species-specific soil pathogens in pastures and coffee plantations that hinder agricultural production, but may enhance the regeneration of forest trees. In contrast, feedbacks mediated by the filtrate were only slightly negative for native tree species from forest fragments, implying that SMC in these degraded forests may have lost key drivers of plant-soil feedbacks that are present in undisturbed forests (Mangan et al. 2010b). Together, these results provide strong evidence that parallel to other biotic interactions, plant-soil dynamics are also severely disrupted with habitat alteration. Further studies are needed to effectively compare the composition and role of SMC and their different components across heterogeneous landscapes, including human-modified landscapes.

Table 4-1. General characteristics of the ten plant species used in the greenhouse experiment. Seed dry mass (mean ± S.D) was measured from 25-50 seeds/species dried for 3 days at 60 °C.

Species	Family	Plant form	Habitat	Seed dry mass (g)	Abbreviation
Brachiaria brizantha	Poaceae	Brachiaria grass (crop)	P[1]	0.0077 ± 0.0013	Bra
Coffea arabica	Rubiaceae	Coffee (crop)	C[2]	0.22 ± 0.023	Cof
Cecropia angustifolia	Cecropiaceae	Pioneer	F[3]	0.0011 ± 0.00028	CecA
Cecropia telealba	Cecropiaceae	Pioneer	F[3]	0.00039 ± 0.00012	CecT
Ochroma pyramidale	Bombacaceae	Pioneer	F[3]	0.0037 ± 0.0012	Ochr
Solanum aphynodendrum	Solanaceae	Pioneer	F[3]	0.0015 ± 0.00053	Sol
Garcinia madrunno	Clusiaceae	Shade tolerant	F[3]	2.92 ± 0.77	Gar
Gustavia superba	Lecythidaceae	Shade tolerant	F[3]	10.55 ± 2.78	Gus
Juglans neotropica	Juglandaceae	Shade tolerant	F[3]	26.04 ± 14.49	Jug
Retrophyllum rospigliosii	Podocarpaceae	Shade tolerant	F[3]	0.86 ± 0.18	Ret

[1]Pastures
[2]Coffee plantations
[3]Forest fragments

Table 4-2. Characteristics of the farms where the initial soil inocula were collected (Guzmán-Martinez et al 2006). At each farm there were three habitats: a pasture (P), a coffee plantation (C), and a forest fragment (F).

Farm (block)	Geographic coordenates	Altitude (m.s.l)	Mean annual precipitation (mm yr^{-1})	Mean annual temperature (ºC)	Habitat	Habitat patch size (ha)
Cenicafé	05º00′N 75º36′W	1380	2733	20.9	P	0.5
					C	0.5
					F	40
Playa rica	05º00′N 75º36’W	1290	2750	20.7	P	10
					C	60
					F	30
Alto español*	04º56′N 75º42′W	1720	3140	18.8	P	1.0
					C	2.0
					F	0.3
Naranjal	04º59′N 75º39′W	1400	3137	21.4	P	22
					C	38
					F	27
La Argentina	05º02′N 75º41′W	1354	2935	19.9	P	0.5
					C	100
					F	1.5

Table 4-3. Mean (± SE) soil pH, organic matter (OM) content, and nutrient content of the three habitats where soil inocula were collected.

Habitat	pH[1]	OM[3] (%)	N[2] (%)	P[4] (mg/kg)*	K[5] (cmol/kg)	Ca[5] (cmol/kg)*	Mg[5] (cmol/kg)
Pastures	5.6 (± 0.1)	7.2 (± 1.4)	0.3 (± 0.04)	54.4 (± 25.3)	0.6 (± 0.2)	5.6 (± 0.7)	2.1 (± 0.4)
Coffee plantations	5.0 (±0.3)	9.6 (± 1.7)	0.4 (± 0.06)	42.0 (± 16.7)	0.3 (± 0.1)	4.4 (± 1.1)	1.7 (± 0.6)
Forest fragments	5.5 (± 0.2)	12.4 (± 2.1)	0.5 (± 0.06)	5.6 (± 0.4)	0.4 (± 0.05)	8.3 (± 2.3)	2.4 (± 0.7)

Notes: Log-transformed data was analyzed with one-way ANCOVA including block as a random factor and habitat as a fixed factor.
[1]pH: Potentiometer soil: water 1:1
[2]N (total): Calculated
[3]OM: Walkley-Black – colorimetry
[4]P: Bray II - colorimetry Bray Kurtz
[5]K, Ca, Mg: Ammonium acetate 1N
* Significant differences between habitat types $P < 0.05$

Table 4-4. Growth response of 10 plant species (Brachiaria grass, coffee, and 8 forest tree species; Table 4-1) to sterile (non-inoculated; control), AMF (isolated from the rhizosphere of these 10 plant species), and microbial filtrate (isolated from the rhizosphere of these 10 plant species) inocula in the greenhouse.

		Total biomass(error df = 766)	
Source	**Df**	***F***	***P***
Inoculum type	2	5.0	0.007
Plant species	9	94.0	<0.001
Inoculum type × Plant species	18	2.3	0.0015
Initial leaf area	1	540.0	<0.001
Days in experiment	1	305.1	<0.001

Notes: ANCOVA was used to analyze $\log_{10}$-transformed final biomass, with $\log_{10}$-transformed initial leaf area and days in experiment (dead seedlings were replaced during the first month of the experiment) as covariates.

Table 4-5. Growth response of 10 plant species (Brachiaria grass, coffee, and eight native tree species; Table 4-1) to AMF isolated from the rhizosphere of these 10 plant species ("inoculum species") in the greenhouse.

		Total biomass (error df = 285)	
Source	**Df**	***F***	***P***
Inoculum species	9	2.4	0.0113
Plant species	9	156.2	<0.001
Inoculum species × Plant species	81	1.4	0.0314
Initial leaf area	1	171.7	<0.001
Days in experiment	1	12.9	<0.001

Notes: ANCOVA was used to analyze $\log_{10}$-transformed final biomass, with $\log_{10}$-transformed initial leaf area and days in experiment (dead seedlings were replaced during the first month of the experiment) as covariates.

Table 4-6. Growth response of 10 plant species (Brachiaria grass, coffee, and eight native tree species; Table 4-1) to a microbial filtrate isolated from the rhizosphere of these 10 plant species ("inoculum species") in the greenhouse.

		Total biomass (error df = 285)	
Source	**Df**	***F***	***P***
Inoculum species	9	1.2	0.3015
Plant species	9	240.7	<0.001
Inoculum species × Plant species	81	1.7	<0.001
Initial leaf area	1	220.0	<0.001
Days in experiment	1	24.2	<0.001

Notes: ANCOVA was used to analyze $\log_{10}$-transformed final biomass, with $\log_{10}$-transformed initial leaf area and days in experiment (dead seedlings were replaced during the first month of the experiment) as covariates.

Table 4-7. Statistical summary of the strength of *a priori* contrasts (F-values followed by P-values in parenthesis) testing AMF feedback between Brachiaria grass and other 9 plant species (coffee, and 8 forest tree species; Table 4-1), coffee and other 9 plant species, and average feedback between 8 forest species, Brachiaria grass, and coffee.

	Heterospecific partner species for a-priori tests for AMF inocula		
	Brachiaria grass	Coffee	8 native trees (or 7 heterospecific native trees)
Brachiaria grass	NA	0.31 (0.58)	0.045 (0.83)
Coffee	0.31 (0.58)	NA	0.15 (0.69)
CecA	1.52 (0.22)	0.28 (0.60)	<0.001 (0.98)
CecT	0.15 (0.70)	1.18 (0.28)	0.12 (0.73)
Ochr	0.73 (0.40)	5.05 (0.025)	0.003 (0.96)
Sol	0.26 (0.61)	0.006 (0.94)	1.3 (0.26)
Ret	0.17 (0.68)	<0.001 (0.99)	0.37 (0.55)
Gar	<0.001 (0.98)	0.009 (0.93)	0.1 (0.75)
Gus	0.66 (0.42)	1.43 (0.23)	0.16 (0.69)
Jug	0.53 (0.47)	0.075 (0.79)	0.054 (0.82)

Table 4-8. Statistical summary of the strength of *a priori* contrasts (F-values followed by P-values in parenthesis) testing filtrate feedback between Brachiaria grass and other 9 plant species (coffee, and 8 forest tree species; Table 4-1), coffee and other 9 plant species, and average feedback between 8 forest species, Brachiaria grass, and coffee.

	Heterospecific partner species for a-priori tests for filtrate inocula		
	Brachiaria grass	Coffee	8 native trees (or 7 heterospecific native trees)
Brachiaria grass	NA	11.73 (<0.001)	17.6 (<0.001)
Coffee	11.73 (<0.001)	NA	1.61 (0.21)
CecA	18.30 (<0.001)	4.53 (0.034)	2.87 (0.009)
CecT	6.34 (0.012)	3.63 (0.058)	1.82 (0.18)
Ochr	8.90 (0.003)	3.31 (0.07)	1.59 (0.21)
Sol	0.60 (0.44)	0.094 (0.76)	1.44 (0.23)
Ret	6.9 (0.009)	0.14 (0.70)	0.39 (0.53)
Gar	3.76 (0.054)	0.74 (0.39)	1.63 (0.2)
Gus	4.64 (0.032)	2.46 (0.12)	0.08 (0.78)
Jug	5.45 (0.02)	0.23 (0.64)	1.22 (0.27)

Table 4-9. Proportion of roots colonized by AMF for 10 host plant species (Brachiaria grass, coffee, and 8 forest tree species; Table 4-1) as affected by inoculum species (10 plan species), inoculum type (AMF, filtrate), and plant species (and interactions) in the greenhouse.

	Total biomass (error df = 199)		
Source	**Df**	***F***	***P***
Inoculum species	9	11.6	<0.001
Inoculum type	1	162.8	<0.001
Plant species	9	53.2	<0.001
Inoculum species × Inoculum type	9	3.9	<0.001
Inoculum species × Plant species	81	1.9	<0.001
Inoculum type × Plant species	9	2.4	0.015
Inoculum species × Inoculum type × Plant species	81	1.9	<0.001

Table 4-10. Proportion of roots colonized by of non-AMF for 10 host plant species (Brachiaria grass, coffee, and 8 forest tree species; Table 4-1) as affected by inoculum species (10 plan species), inoculum type (AMF, filtrate), and plant species (and interactions) in the greenhouse.

	Total biomass (error df = 199)		
Source	**Df**	***F***	***P***
Inoculum species	9	10.5	<0.001
Inoculum type	1	4.9	0.028
Plant species	9	5.4	<0.001
Inoculum species × Inoculum type	9	3.1	0.002
Inoculum species × Plant species	81	1.8	<0.001
Inoculum type × Plant species	9	1.5	0.150
Inoculum species × Inoculum type × Plant species	81	1.1	0.390

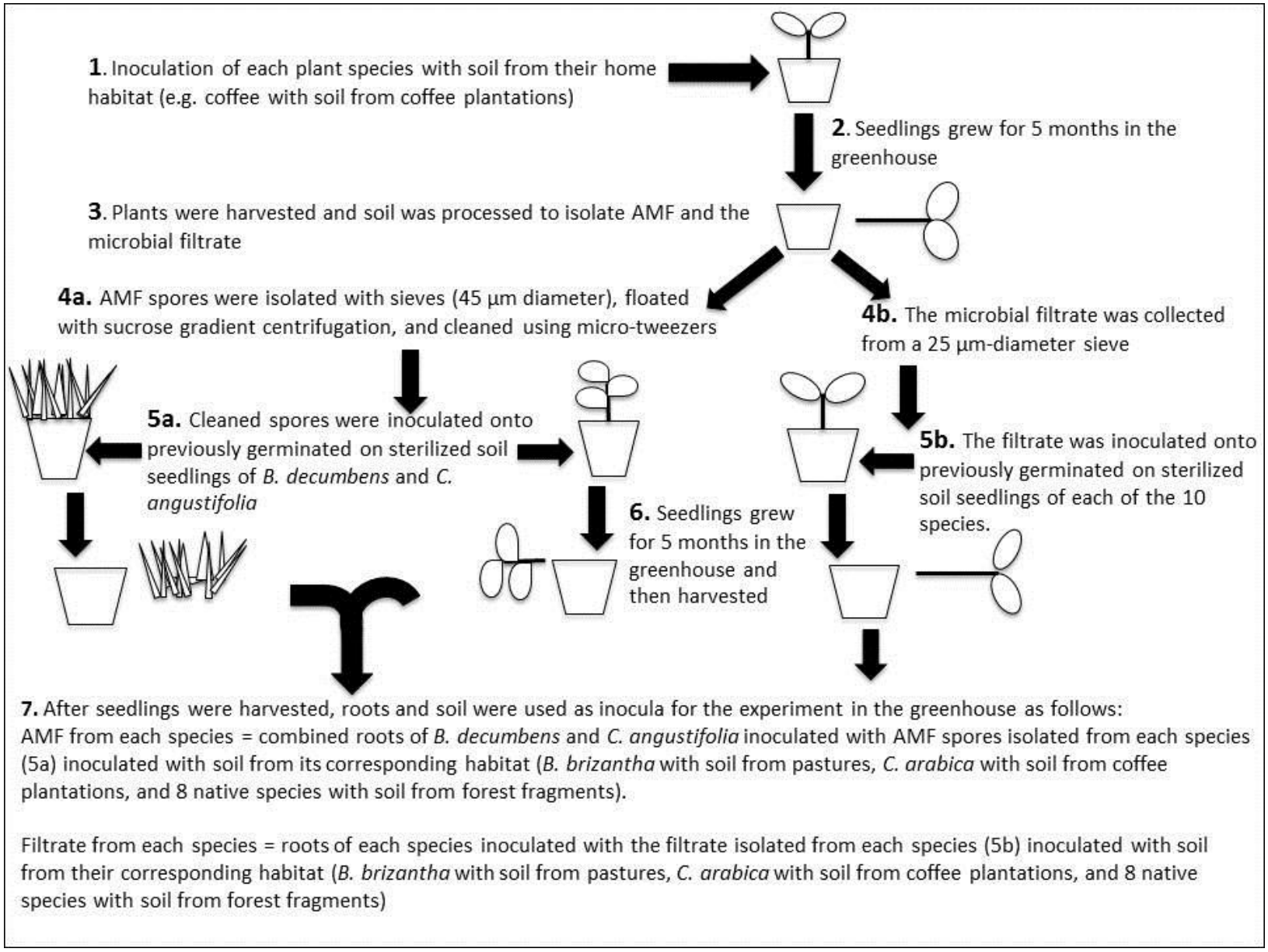

Figure 4-1. Flow diagram showing the procedure that we used to produce the inocula for setting up the experiment in the greenhouse.

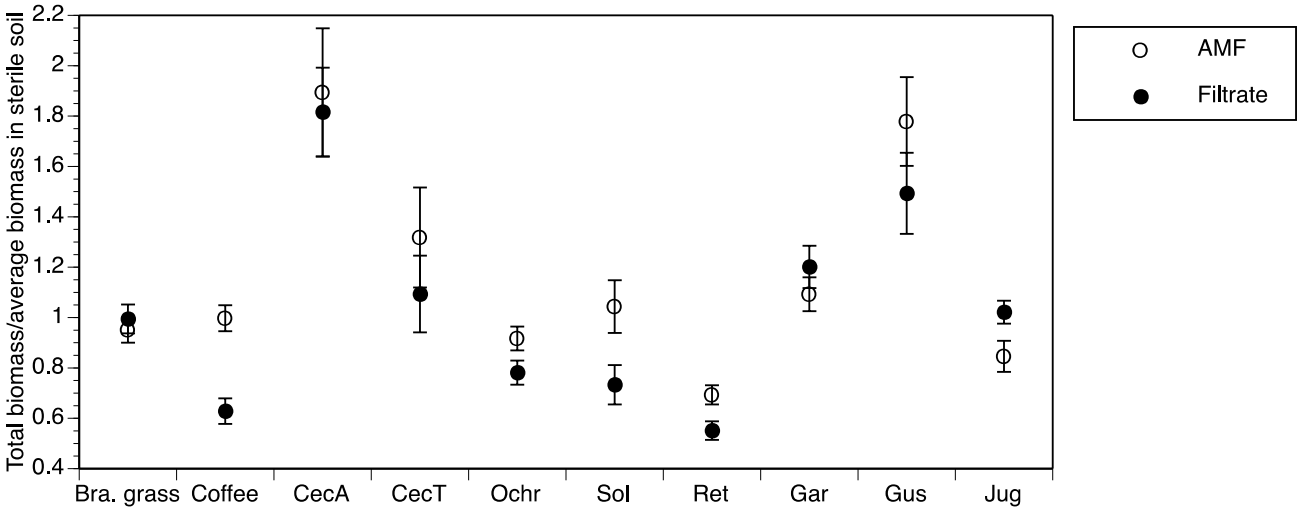

Figure 4-2. Proportion of seedling biomass when inoculated with AMF (open circles) or a microbial filtrate (closed circles) in comparison to biomass when grown on sterile soil for Brachiaria grass, coffee, and 8 forest tree species (Table 4-1). Circles indicate the mean (± SE) total seedling biomass with AMF or the filtrate divided by seedling biomass with sterile soil.

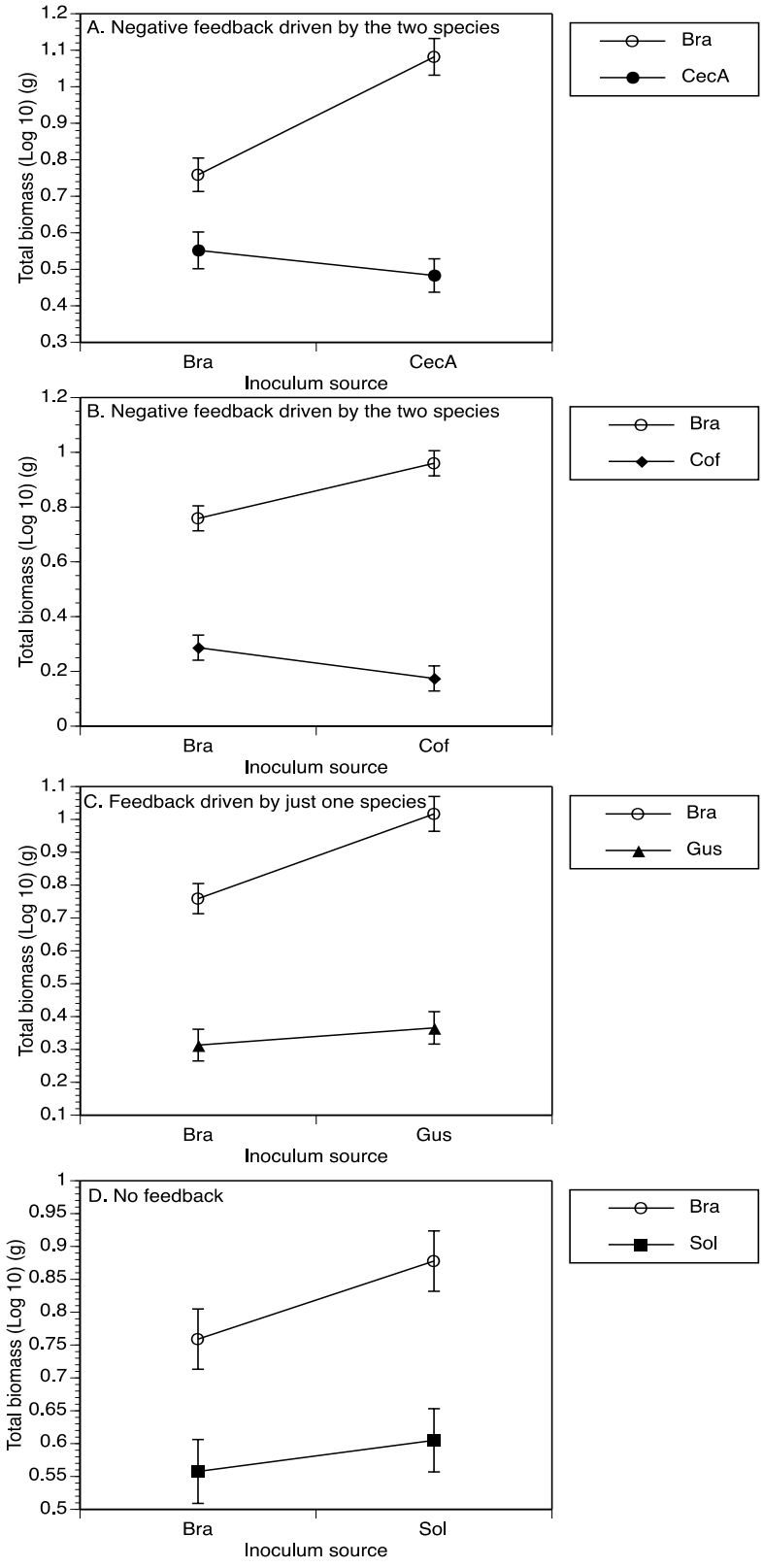

Figure 4-3. Examples of significant negative feedback (A, B, C) and no feedback (C) between seedlings of different species. The graphs represent growth of two

species with a microbial filtrate collected from conspecific versus heterospecific seedlings. The resulting interaction between seedling species and inoculum source species was used to define the strength and direction of feedback. For example, in graph A, both Brachiaria grass and *Cecropia angustifolia* grew better with the microbial filtrate from each other than with the microbial filtrate from their own roots (same than in graph B), therefore there is significant negative feedback between these two species. In graph C, feedback is significant, but is driven by Brachiaria grass growing better with the microbial filtrate from *Gustavia superba* compared to conspecific filtrate; growth of *Gustavia superba* was similar across conspecific and heterospecific microbial filtrate. In graph D, both Brachiaria grass and *Solanum aphynodendrum* grew better with the microbial filtrate isolated from Brachiaria grass roots than with the filtrate from *S. aphynodendrum* roots, therefore there is no feedback.

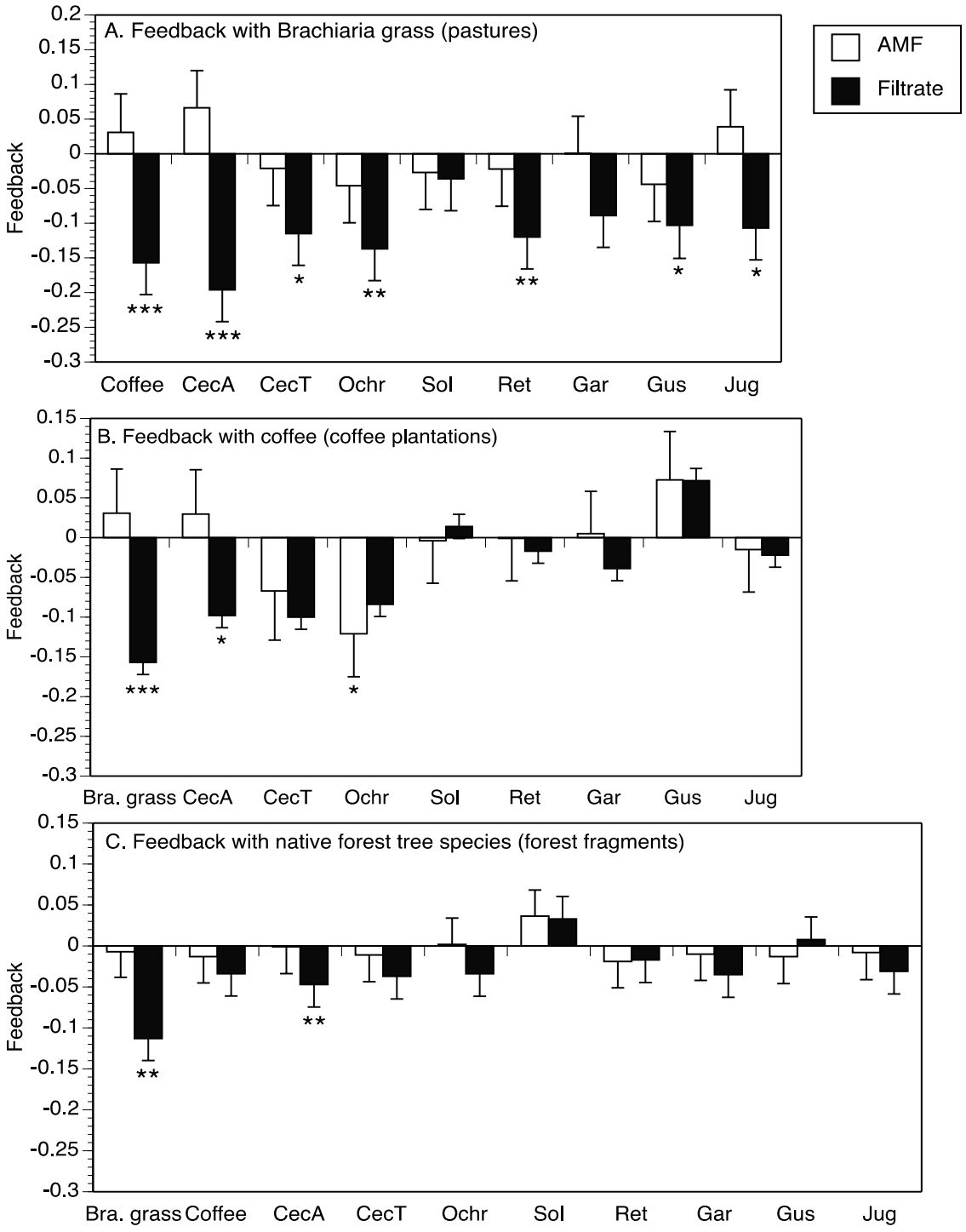

Figure 4-4. Feedback mediated by AMF (open bars) and a microbial filtrate (closed bars) between (A) the pasture grass *Brachiaria brizantha* and nine other plant species (coffee and 8 forest tree species; Table 4-1) (pairwise feedback), B) coffee and nine other plant species (Brachiaria grass and 8 forest tree species) (pairwise feedback), and C) 8 forest tree species and Brachiaria grass, or coffee, or other seven heterospecific forest species (average feedback per species). Bars indicate the value (± SE) of *a priori* contrasts calculated from all replicates, comparing the growth of each species when inoculated with AMF or filtrate from each other species ("heterospecific") compared to their own ("conspecific"), as well as how other species grew with heterospecific vs. conspecific AMF and filtrate. Means that differ from zero are indicated by asterisks (* $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$).

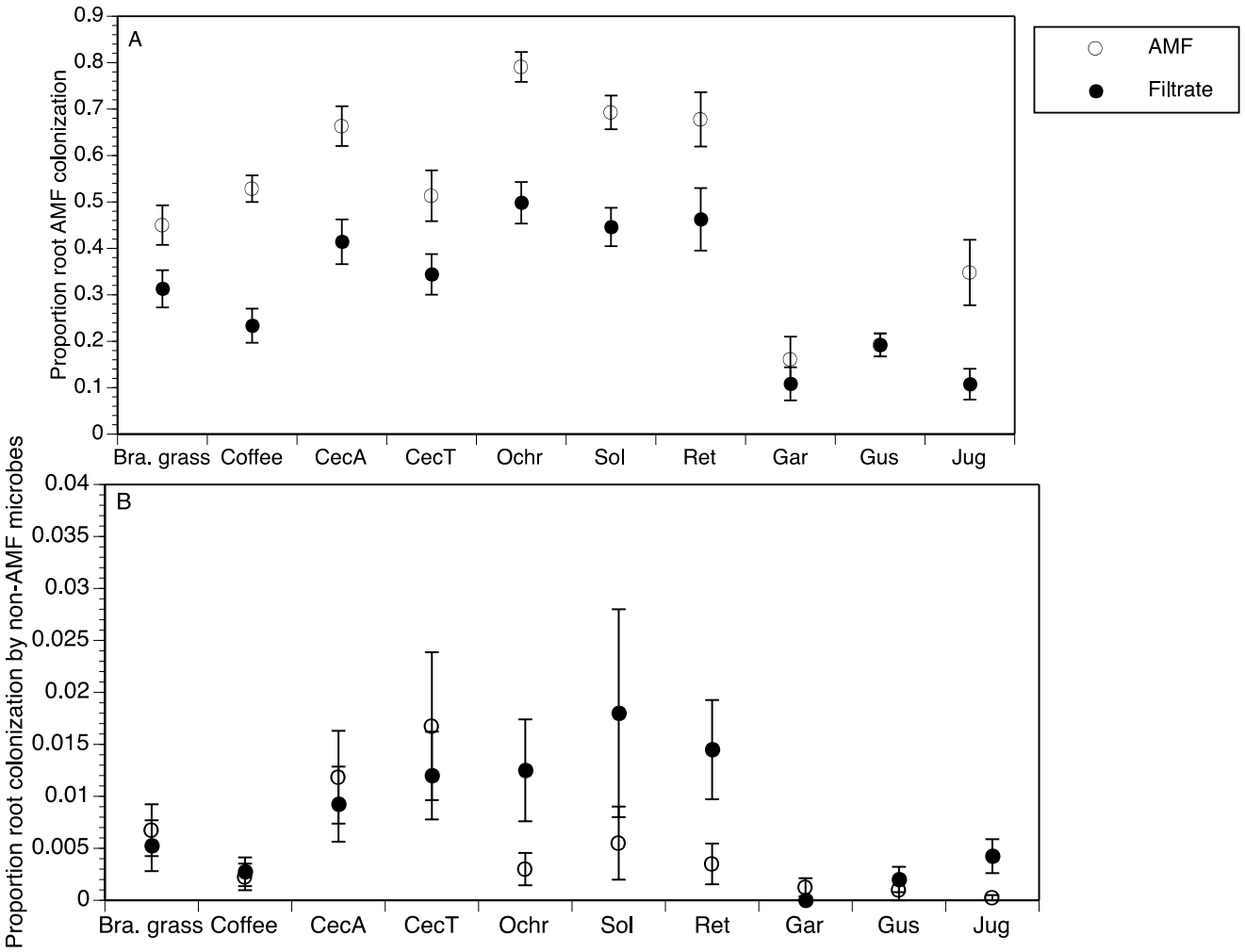

Figure 4-5. Proportion root colonization of A) AMF and B) non-AMF soil microbes in seedlings of Brachiaria grass, coffee, and 8 forest tree species (Table 4-1) when inoculated with AMF (open circles) or a microbial filtrate (closed circles) isolated from the roots of these 10 plant species. Circles indicate the mean (± SE) AMF root colonization proportion quantified for half of the seedlings in the experiment and averaged for each inoculum type (AMF and filtrate).

CHAPTER 5
CONCLUSION

My research was motivated by a desire to understand how the replacement of tropical forests for agriculture has modified the composition of microbial soil communities, and how these "novel" soil communities may limit the regeneration of pre-montane native forest species and agricultural production. I worked in the Central Andes of Colombia, where I studied the dynamics between soil organisms and plant species from the three dominating habitats in my study region: pastures (Brachiaria grass), sun-grown coffee plantations (coffee), and forest fragments (forest tree species). In addition, I addressed the effects of two main components of soil microbial communities (SMC), mutualistic arbuscular mycorrhizal fungi (AMF), and antagonistic non-AMF soil organisms, on plant growth.

My expectation was to find that SMC from forest fragments and agricultural lands would be significantly different, having differential effects across plant species, as these habitats not only have contrasting abiotic soil conditions, but also differ greatly in the composition of the plant communities they hold. On one hand, pastures and coffee plantations are heavily fertilized (Farfán-Valencia and Mestre-Mestre 2004, Farfán-Valencia and Mestre-Mestre 2005) while forest fragments are unmanaged. These management practices affect SMC; it has been shown that fertilization reduces SMC diversity in general (Giller 1996, Giller et al. 1997) and mycorrhizal inoculum potential (Janos 1980, Fischer et al. 1994, Picone 2000, Zangaro et al. 2000). On the other hand, agricultural monocultures are dominated by single, non-native plant species, while forest fragments are comprised of highly diverse plant communities. Thus, given that plant identity determines the composition of AMF communities and the presence of

particular soil organisms in the soil (Johnson et al. 1992, Bever et al. 1996, Westover et al. 1997, Eom et al. 2000, Lovelock et al. 2003, Lovelock and Ewel 2005), soil organisms that colonize crop species are likely to dominate SMC in agricultural lands, while forests could hold more diverse SMC.

In my dissertation I explored three main hypotheses: 1) SMC from pastures, coffee plantations, and forest fragments have a differential and significant effect on growth and survival of plants from these contrasting habitats, 2) plants would grow better with both AMF and non-AMF soil organisms from “away” (habitats where species rarely occur or don’t occur at all) compared to “home” (habitats where species normally occurs) SMC, and 3) plant-soil feedbacks with AMF and non-AMF soil organisms would be positive for non-native plants from agricultural monocultures, while being negative for native forest species. To test these hypotheses, I set up three greenhouse experiments, and one field experiment in which I exposed plants from pastures, coffee plantations, and forest fragments to SCM from these contrasting habitats.

SMC from pastures, coffee plantations, and forest fragments had significantly different effects on plants both in the greenhouse and in the field, suggesting that SMC diverge among habitats. Furthermore, fast-growing plant species (Brachiaria grass and pioneer forest trees) benefited from away compared to home SMC, while slow-growing shade tolerant forest tree species benefited the most from home SMC. When testing for the effects of communities of AMF and non-AMF microbes on plants from the three studied habitats, I found that most plant species grew significantly better with non-AMF microbes from away, compared to home habitats, while showing limited response to AMF from different habitats. Similarly, plant-soil feedbacks were strong for non-AMF

organisms, but mostly insignificant for AMF. Finally, feedbacks with non-AMF soil organisms were significantly negative for non-native species Brachiaria grass and coffe, but overall neutral for native tree species.

These results suggest that species composition of soil microbial communities greatly differs between fertilized agricultural monocultures and diverse native forest fragments in the Central Cordillera coffee-grown region of Colombia. Overall, it seems like these contrasting habitats hold similar communities of AMF. In contrast, antagonistic non-AMF soil microbes appear to be widespread across contrasting habitat types, limiting the growth of their host plants where these are more abundant. Thus, this study urges for further research on SMC, and in particular on non-AMF soil microbes, a very poorly studied group of microbes that yet have shown to have major impacts on plants.

APPENDIX
ILLUSTRATIONS OF STUDY SPECIES

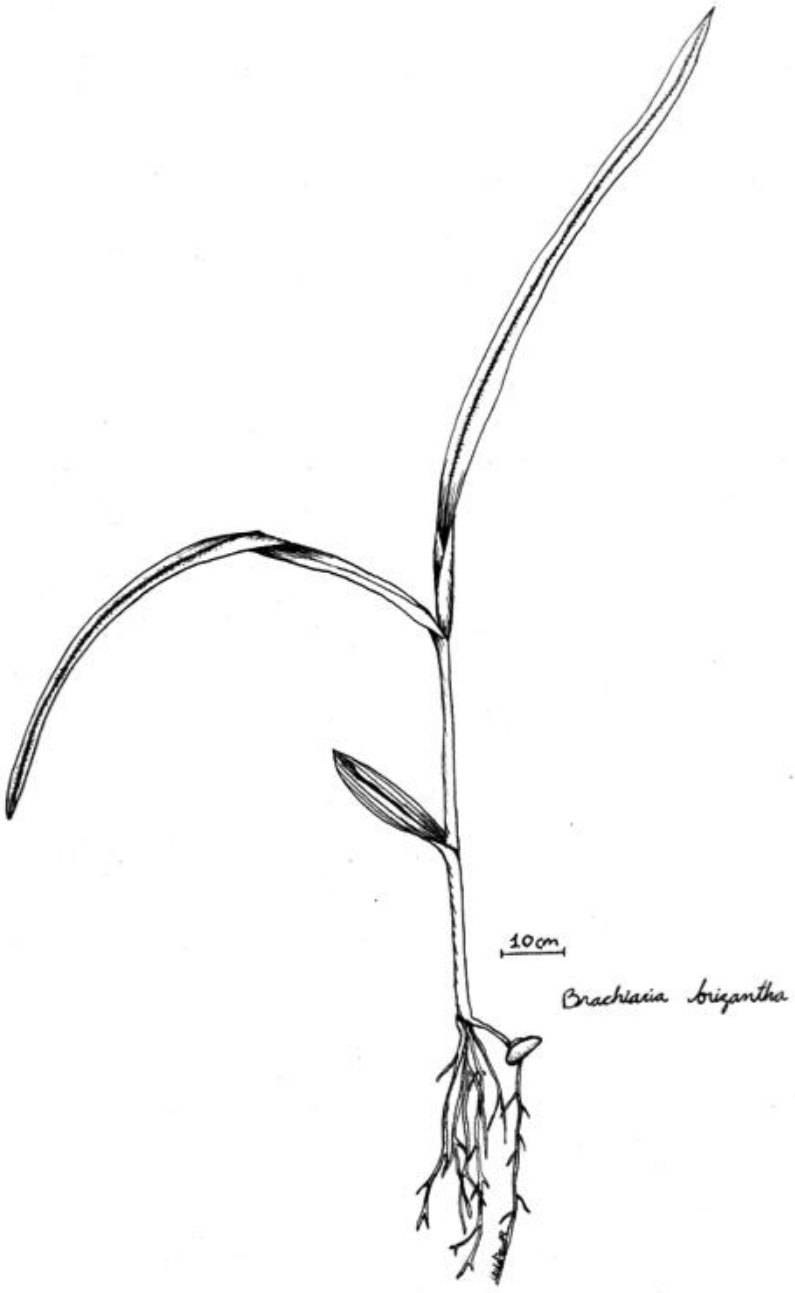

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Figure A-1. Illustration of a *Brachiaria brizantha* seedling by Camila Pizano.

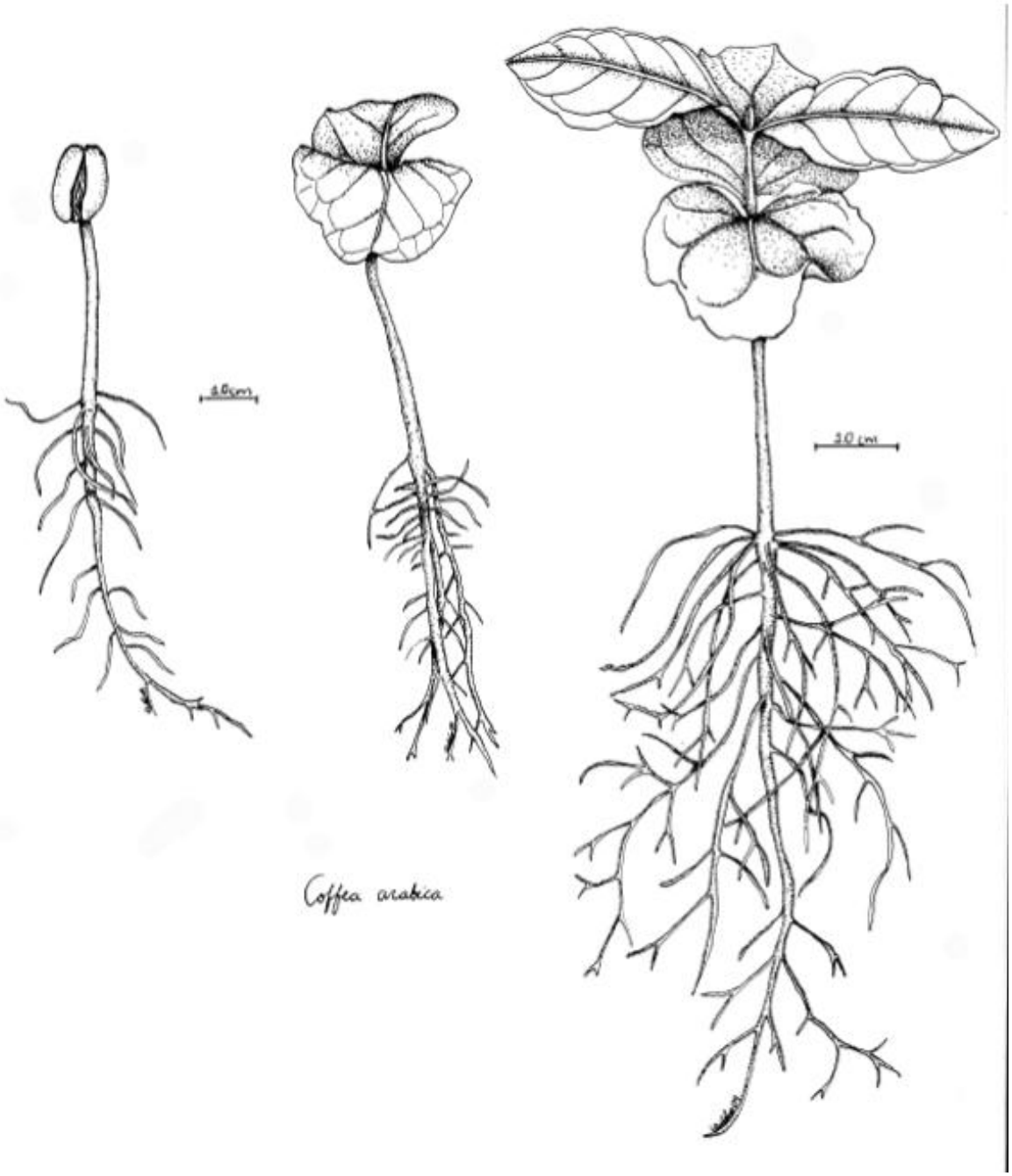

Figure A-2. Illustration of a Coffea arabica seedling by Camila Pizano.

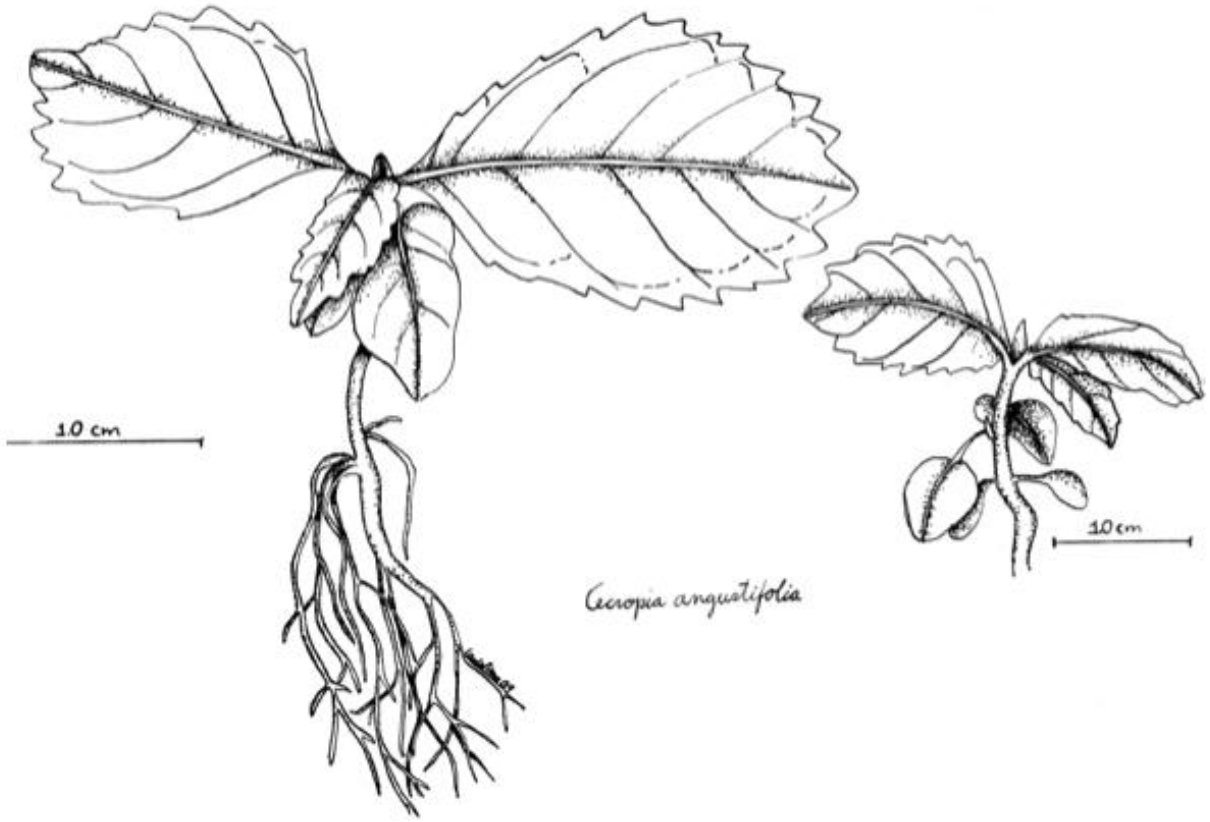

Figure A-3. Illustration of a *Cecropia angustifolia* seedling by Camila Pizano.

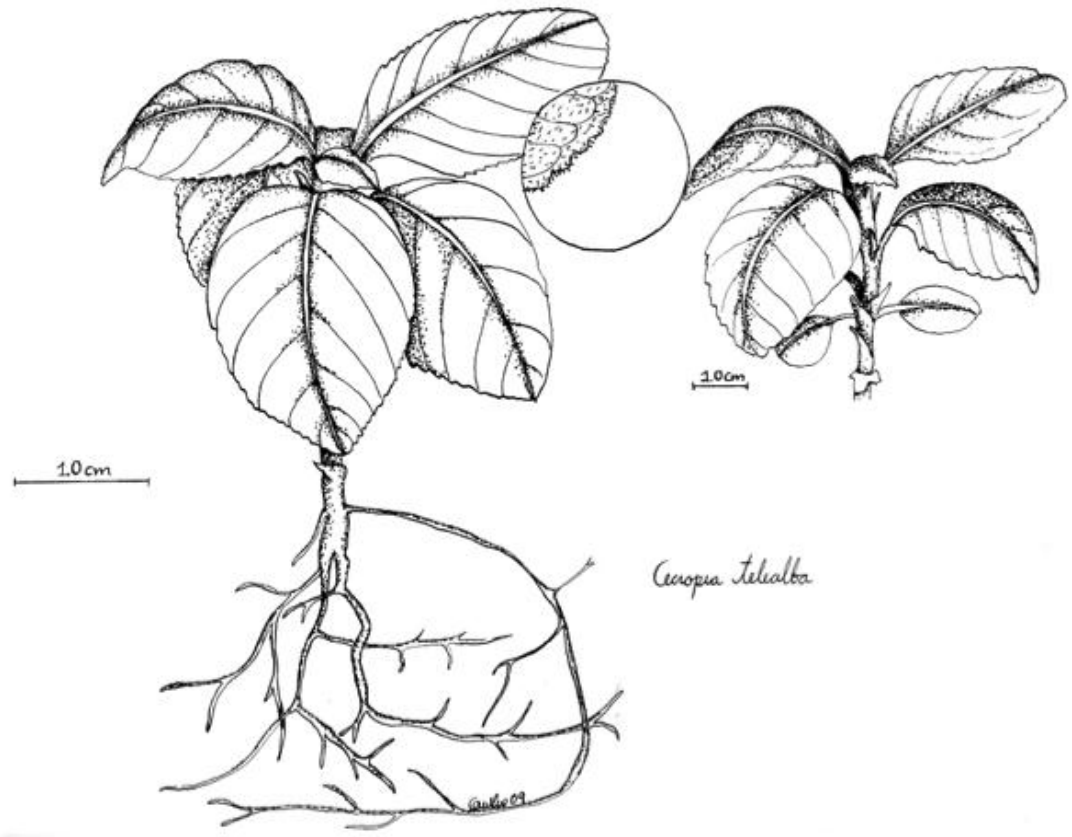

Figure A-4. Illustration of a *Cecropia telealba* seedling by Camila Pizano.

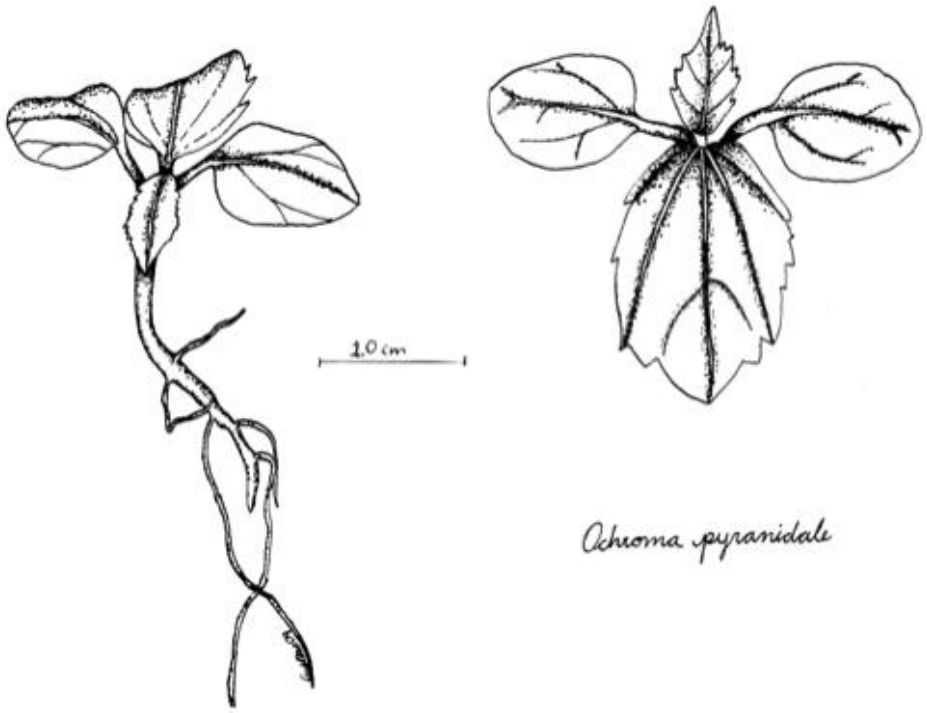

Figure A-5. Illustration of a *Ochroma pyramidale* seedling by Camila Pizano.

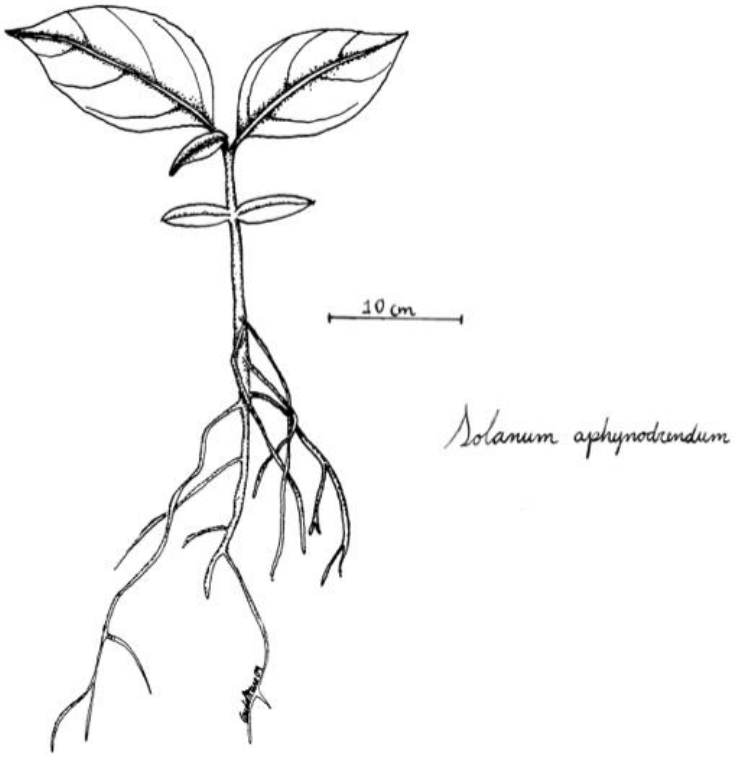

Figure A-6. Illustration of a *Solanum aphynodendrum* seedling by Camila Pizano.

Figure A-7. Illustration of a *Siparuna aspera* seedling by Camila Pizano.

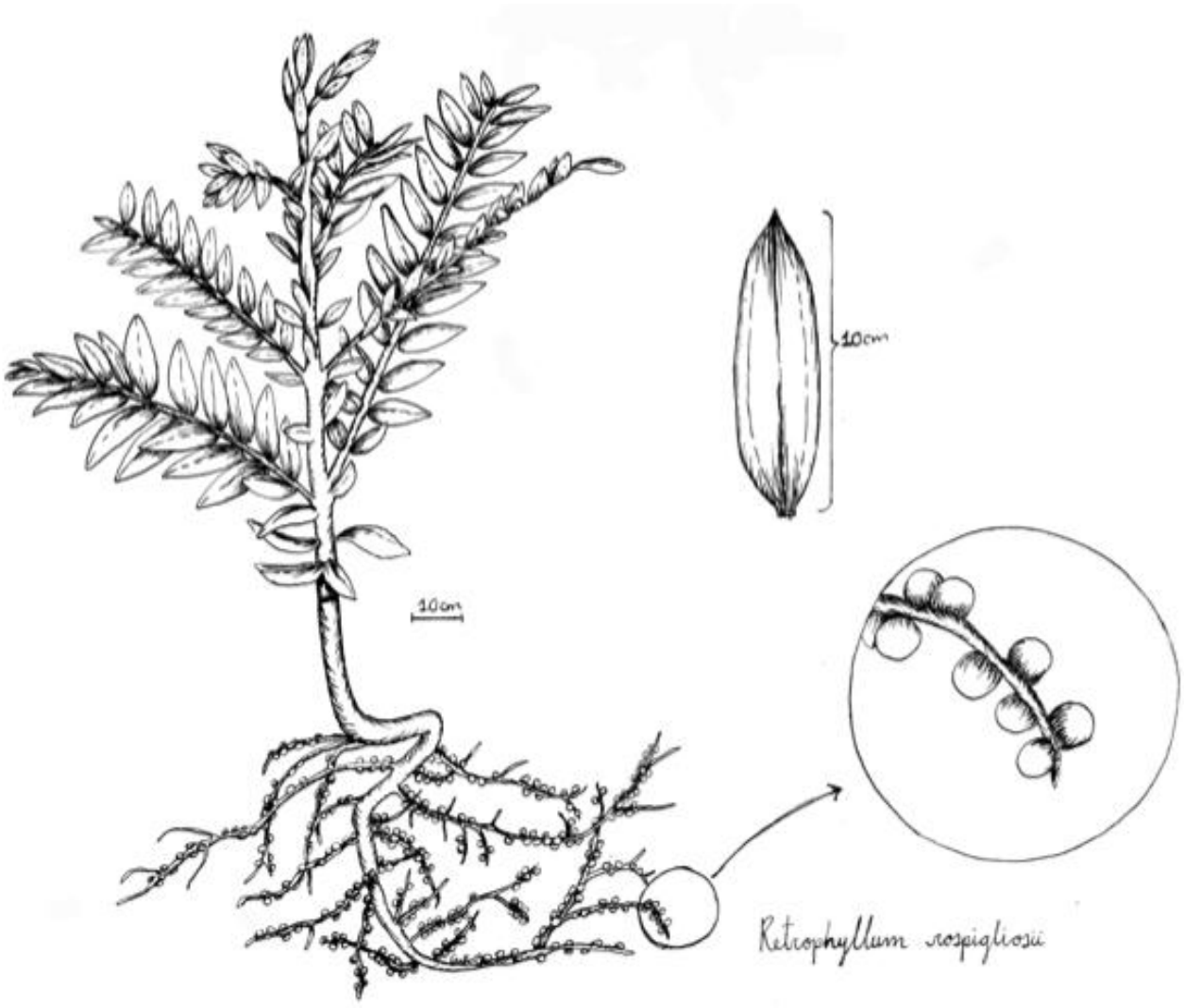

Figure A-8. Illustration of a *Retrophyllum rospigliossii* seedling by Camila Pizano.

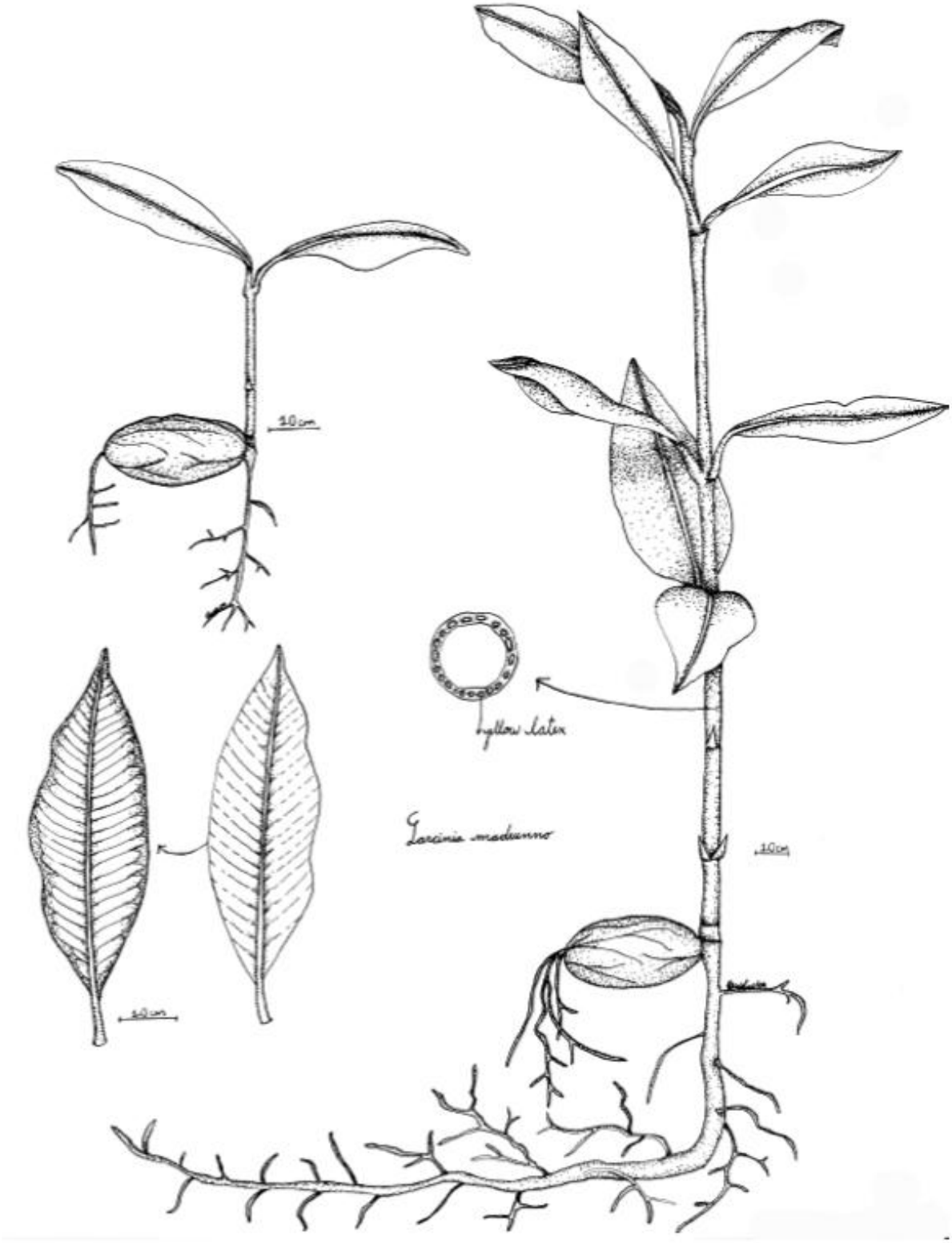

Figure A-9. Illustration of a *Garcinia madrunno* seedling by Camila Pizano.

Figure A-10. Illustration of a *Gustavia superba* seedling by Camila Pizano.

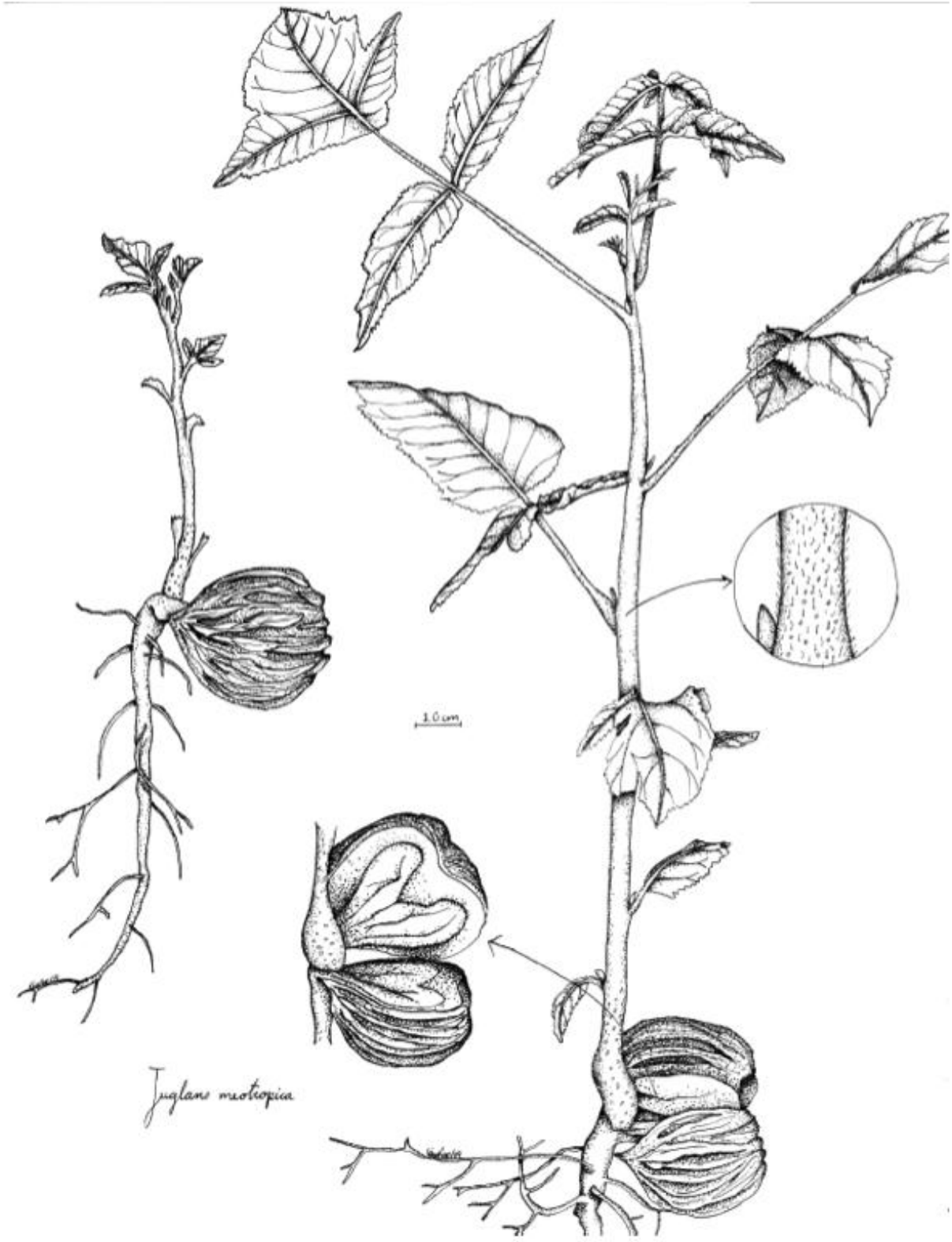

Figure A-11. Illustration of a *Juglans neotropica* seedling by Camila Pizano.

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BIOGRAPHICAL SKETCH

Camila Pizano was born in Bogotá, Colombia, South America. She grew up between the massive city of Bogotá, and numerous farms around her country where she was always outside enjoying nature. She had the great privilege to attend one of the best schools in Bogotá, Colegio Los Nogales, where she was exposed to a solid discipline, a robust moral system, and really good science and math foundations for her later career. In addition, she started developing her art skills guided by her mom, Maria Lucia Gómez, who is a professional artist.

A year before graduating from high school, Camila spent a summer at Cornell University, where she took a botany class and learned how to do scientific illustration; the two professions of her actual life. She then had the privilege of attending one of the best Colombian universities, Universidad de los Andes, where she studied biology. While studying at los Andes, she spent three months as a volunteer at the Galápagos National Park. There she worked in the tortoise project at the Charles Darwin Research Station and travelled through the Southern Galápagos islands. In addition, she did an internship at the Smithsonian Tropical Research Institute (STRI) in Panama, where she spent 11 months illustrating plants for the “Panama Watershed Tree Atlas” (ctfs.si.edu/webatlas/maintreeatlas.html). She then received a STRI Short-term fellowship to work on her undergrad thesis project examining the role of soil microbes in driving incipient speciation between two cryptic species of a tropical pioneer tree. This project extended for three years during which Camila lived on Barro Colorado Island (STRI) and worked with Scott Mangan, James Dalling, and Allen Herre (Pizano et al. 2011).

Camila was awarded with a Lewis Anthony Dexter Fellowship and a Grinter fellowship in 2004 at University of Florida when she started her PhD. While doing her PhD, she enjoyed a great privilege of travelling to wonderful places such as various National Natural parks in Florida, the Amazon region in Brazil, the Yucatán peninsula in Mexico, Toolik research station in Alaska, and Austin, Texas. Parallel to her scientific career, she continued with her art career as a scientific illustrator. She also greatly enjoys travelling with her dad, Pablo Pizano who is a pilot, and her mom, to their beautiful farms in Colombia and some of the most stunning natural areas in her country: the Chocó, the Tayrona National Park, the páramos of the Andes, the Vichada river in the Llanos, the Magdalena valley, the Guajira region, and the Casanare region in the Llanos. Camila owes to her parents' adventurous personality that she studied Biology and has dedicated her life to scientific research and scientific illustration.