INFLUENCES ON FUNGAL COMMUNITY COMPOSITION IN ROOTS AND SOIL OF COFFEE AND OTHER RUBIACEAE IN COSTA RICA

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Elizabeth Christine Sternhagen

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Environmental and Conservation Sciences

December 2018

Fargo, North Dakota

North Dakota State University
Graduate School

Title

INFLUENCES ON FUNGAL COMMUNITY COMPOSITION IN ROOTS AND SOIL OF COFFEE AND OTHER RUBIACEAE IN COSTA RICA

By

Elizabeth Christine Sternhagen

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Laura Aldrich-Wolfe
Chair

Dr. Jon Sweetman

Dr. Berlin Nelson

Approved:

7 December 2018
Date

Craig Stockwell
Department Chair

ABSTRACT

Belowground fungi interact with plants directly as pathogens and mutualists, and indirectly as nutrient cyclers, yet the factors governing fungal community composition are poorly understood. Here I examined root and soil fungi of coffee (*Coffea arabica*) and eight native species in the coffee family to determine 1.) whether coffee management affects guild structure of fungal communities in coffee roots and 2.) relative importance of microclimate and plant host relatedness in structuring belowground fungal communities of coffee and forest Rubiaceae. Coffee management resulted in differences in the soil environment that were associated with the richness and abundance of several fungal guilds. Soil environment differed between coffee field and forest habitats. Light availability differed by tree species, and the effect of light niche on fungal community composition was indifferentiable from a host effect. These studies elucidate patterns of belowground fungal community composition related to environment and host and could guide coffee management.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

COAR .....*Coffea arabica*

DNA.....Deoxyribonucleic acid

HAPA.....*Hamelia patens*

ITS2.....Internal transcribed spacer 2

OTU .....Operational taxonomic unit

PCR.....Polymerase chain reaction

RAGR .....*Randia grandifolia*

RAMA.....*Randia matudae*

PAPU .....*Palicourea pubescens*

PAVA.....*Palicourea valerioana*

PSMO.....*Psychotria monteverdensis*

PSPA.....*Psychotria panamensis*

PSQU .....*Psychotria quinqueradiata*

LIST OF APPENDIX FIGURES

CHAPTER ONE: MANAGEMENT OF COFFEE (*COFFEA ARABICA*) CONTRIBUTES TO DIFFERENCES IN COMPOSITION AND GUILD STRUCTURE OF FUNGAL COMMUNITIES IN COFFEE ROOTS[1]

Introduction

Land plants have diverse interactions with belowground fungal communities, and these relationships are understudied compared to their ecological importance (Jeffries et al. 2003; Tedersoo et al. 2014; Dassen et al. 2017). Belowground plant-fungal relationships span the range of symbioses and include fungal pathogens that attack plant roots, mycorrhizal fungi that improve plant nutrition and pathogen resistance, and commensal endophytes that live inside plant tissues usually asymptomatically (Tedersoo et al. 2014, 2016; Delgado-Baquerizo et al. 2016; Schulz and Boyle 2005).

Guild assessments of fungal communities provide valuable information on possible plant-fungal functional relationships and therefore complement taxonomic studies (Nguyen et al. 2016). A guild is a group of species employing similar strategies to exploit the same type of environmental resource (Root 1967). Guild composition gives insight into functionality of a system in terms of interspecific competition for similar resources, interactions between trophic levels of a community, and nutrient cycling (Nguyen et al. 2016; Fitzgerald et al. 2017). Additionally, a fungal species' guild can change based on environmental conditions, such as plant pathogens that become saprotrophic in the absence of a host, or saprotrophs that occur in

[1]The material in this chapter was co-authored by Elizabeth Sternhagen, Stefanie Vink and Laura Aldrich-Wolfe. Elizabeth Sternhagen was the primary developer of the conclusions that are advanced here. Elizabeth Sternhagen also drafted and revised all versions of this chapter. Stefanie Vink conducted the bioinformatic processing for this chapter. Laura Aldrich-Wolfe had primary responsibility for collecting samples and taking measurements in the field and for carrying out the subsequent lab work. Laura Aldrich-Wolfe also served as proofreader and checked the math in the statistical analyses conducted by Elizabeth Sternhagen.

plants as asymptomatic endophytes until the plant begins to senesce (Selosse, Schneider-Maunoury, and Martos 2018; Agtmaal et al. 2017; Kuo et al. 2015; Promputtha et al. 2007; Schulz and Boyle 2005).

Environment can change to create filters which affect fungal communities by benefiting or harming particular species. Edaphic and biotic properties can support or suppress guilds of belowground fungi depending on nutritional needs and competitiveness of the fungi in different environments (Liu et al. 2017; Högberg et al. 2003; Xiao et al. 2018; Suding et al. 2005; Voříšková and Baldrian 2013; Prescott and Grayston 2013). For example, when a nutrient or resource increases in availability, the fungi that exploit it may become more abundant and more competitive, leading to less fungal diversity when the favored fungal species outcompetes other species in the community. Also, changes in the soil environment can lead to unfavorable conditions for certain species. For example, soil acidification filters out fungi that are intolerant to highly acidic conditions (Grinhut, Hadar, and Chen 2007).

The influence of anthropogenic environmental filters should be clearly detectable in agricultural systems. In this study we used fields of coffee (*Coffea arabica* L.), as our focal system. Coffee is a crop grown across the tropics (Janzen 1983; Padovan et al. 2015) which has a wide range of management strategies aimed at maximizing the shrub's health and productivity (Lyngbæk, Muschler, and Sinclair 2001; De Beenhouwer, Van Geel, et al. 2015; Jezeer et al. 2018). In conventional management, this is generally achieved through the application of synthetic chemicals such as fertilizers and fungicides, while organic methods forego these synthetic products and instead benefit the crop through the use of natural ecological properties of soil, as well as natural inputs such as compost or biocontrol fungi (Jezeer et al. 2018; Bagyaraj et al. 2015; De Beenhouwer, Muleta, et al. 2015; Lichtfouse 2011).

Management strategy can affect soil properties and create environmental filters that change fungal communities (Bagyaraj et al. 2015). For example, one conventional coffee management technique is to apply synthetic nitrogen (N), which, in other agricultural systems, has been observed to acidify soils and decrease fungal diversity (López-Rodríguez et al. 2015; Barak et al. 1997; Xiao et al. 2018). Herbicides and fungicides are also staples of conventional coffee management (Jezeer et al. 2018). In other agricultural systems, these chemicals have been observed to alter guild composition of root and soil fungal communities, for example by favoring pathogens and suppressing mutualists (Edwards et al. 2015; Taheri, Hamel, and Gan 2015). Since coffee in its natural habitat grows in the shady understory of a forest, incorporation of shade trees is another common strategy in coffee management (Jezeer et al. 2018). The litter, exudates and roots of these shade trees create diverse niches which influence the composition and diversity of fungal communities (Jezeer et al., 2018; Wartenberg et al., 2017; López-Rodríguez et al., 2015; Ayres et al., 2009; Wardle, 2004).

Previous research has assessed fungal responses to different environmental factors, provided support for the use of guilds in assessing fungal communities, and shown strong environmental disparities between organic and conventional agricultural systems (Andrade et al. 2009; Kamiyama et al. 2011; Lammel et al. 2015). However, the relationship between agricultural management and guild composition of root fungi remains to be clarified. In this study, we used high throughput sequencing of the ITS2 region of fungal DNA to elucidate how coffee rhizosphere fungal communities in conventional coffee fields differ from those under organic management. We also hypothesized that differences in nutrient and substrate availability would lead to differences in richness and abundance of fungal species within guilds. We hypothesized that conventional management would create environmental filters on rhizosphere

fungi that would lead to lower diversity overall than in fungal communities on organic. Through this study of root fungal community composition, we improve the current understanding of the effects of agricultural management on the structure and function of belowground fungal communities.

Methods

Site Description and Experimental Design

Two coffee-growing regions of Costa Rica, Monteverde (10º 19'27.8" N, 084º 50'30.1" W) and San Vito (08º52'41.1" N, 082º 57'03.1" W), were chosen as study sites. Both regions have a premontane wetforest climate (Janzen 1983), but Monteverde experiences slightly lower rainfall on average (300 cm/year vs. 400 cm/year in San Vito; http://www.monteverdeinfo.com/facts.htm, www.ots.ac.cr, Janzen 1983). Soils in both Monteverde and San Vito are Andisols, a volcanic soil type with high organic matter, high leaching capacity and a natural pH of 5.6-5.8 (Dixon et al. 2011, Van Wambeke 1992).

Thirteen fields in Monteverde (six fields in 2011, seven in 2012) and 12 in San Vito (six in 2011, six in 2012) were sampled. At each site, the farmer was interviewed to determine type of herbicides, pesticides, fungicides, and fertilizers used on the field and the frequency of their application as well as the age of the field, prior land use and pruning regimen. At each field, environmental data were collected, including coffee density, percent canopy cover, slope, aspect, and shade tree richness.

One coffee plant every five meters was sampled along a 20m transect. At each plant, leaf litter depth was measured at the dripline and a soil core 2cm in diameter and 15-25cm deep as taken. Fine roots were excavated at three points from a depth of 0-15 cm from every other sampled plant, combining these subsamples within each plant. Five rows were sampled in this

way for a total of 20 soil samples and 10 root samples per field. Each field's soil samples were pooled, mixed thoroughly and air dried for nutrient analysis. In the lab, each root sample was rinsed with tap water and divided in two. One subsample was placed in 1-2% KOH at 4˚C, transported to the United States at room temperature, and then stored at 4°C. The other subsample was stored with desiccant at room temperature.

Root Staining and Scoring

Root samples in 1% KOH were transferred to 10% KOH (*w/v)* at approximately 95°C for 10 min; rinsed under tap water for 10 min, acidified in 2% HCl (*w/v*) for 15 min at room temperature and stained with 0.05% trypan blue (dH_2O, 85% lactic acid and glycerol (1:1:1) with 0.05% (*w/v*) trypan blue) for 5 min. Samples were then rinsed in tap water > 1 h to remove excess stain. Twenty root sections per plant were mounted on a microscope slide in polyvinyl-lacto-glycerol and scored at a minimum of 100 intersections for the presence of AM fungal structures (arbuscules, vesicles, hyphae) at 200 × magnification (technique modified from McGonigle *et al.* 1990) using a Nikon Eclipse 80*i* microscope with DIC (Nikon Instruments, Inc., Melville, New York, USA).

DNA Extraction

Dried root samples were pulverized using six 2.33-mm diameter chrome-steel beads (Biospec Products, Bartlesville, Oklahoma, USA) in a vortex adapter (Mo Bio Laboratories, Carlsbad, California, USA) on a Vortex-Genie® 2 Mixer for 1 h (Scientific Industries, Inc., Bohemia, New York, USA). DNA was extracted from 20 mg of each sample for 8-10 root samples per field using the Qiagen DNAasy Plant Mini Kit (Qiagen, Germantown, Maryland, USA), following the manufacturer's protocol with a final elution volume of 100 µL and stored at -80 °C.

Amplification, Purification and Sequencing of Fungal DNA

The internal transcribed spacer region 2 (ITS2) was amplified by polymerase chain reaction (PCR) for each DNA extract using 12.5 μL of 2× Kapa HiFi Hotstart Ready Mix (Kapa Biosystems, Wilmington, Massachusetts), 10 μL nuclease-free water, 0.8 μL each of 10 mM fungal-specific HPLC-purified primers 5.8SR and ITS4 (White et al., 1990), and 1 μL of DNA template for a total reaction volume of 25.1 μL. Each extract was amplified in triplicate using an Eppendorf Mastercycler (Hamburg, Germany) with 3 min activation at 95 °C, 30 cycles of denaturing at 98 °C for 20 s, annealing at 65.7 °C for 15 s and elongation at 72 °C for 45 s, and a final elongation at 72 °C for 5 min. PCR products were confirmed by electrophoresis in 1% agarose and 0.5× TBE followed by staining with ethidium bromide. Extracts which failed to produce PCR products were diluted tenfold and amplified using the above reaction conditions with an annealing temperature of 64.4 °C. PCR products were stored overnight at 4 °C and for longer periods at -20 °C.

Triplicate PCR products were pooled and purified using the Agencourt® Ampure® XP system (Beckman Coulter, Indianapolis, Indiana, USA) following the manufacturer's protocol, with two washes with ethanol and elution in 10 mM Tris. Concentration of dsDNA in each sample was measured using a Qubit 2.0 fluorimeter (Invitrogen, Carlsbad, California, USA). Eight (2011) or ten (2012) samples per field were pooled at equal DNA concentration in 10 mM Tris, and 3-5 ng of DNA per field was submitted for sequencing at the University of Minnesota Genomics Center (UMGC, St. Paul, Minnesota, USA).

PCR products from each field were amplified using Nextera™ indexing primers (Illumina, San Diego, California, USA) and 10 cycles of denaturation at 98 °C for 20 s, annealing at 55 °C for 15 s, and elongation at 72 °C for 1 min. Indexed PCR products were denatured with 8 pM

NaOH in Illumina HTI buffer (20% PhiX) at 96 °C for 2 min prior to loading and sequencing on an Illumina Miseq® using Reagent Kit v3 with separate index reads. Preliminary quality control (QC) and demultiplexing were conducted by the UMGC.

Data Analysis

Sequences were de-multiplexed at the University of Minnesota Genomics Center (UMGC) and subsequently processed with the PIPITS 1.4.0 pipeline (Gweon et al. 2015), which employs a number of different software packages, using the standard settings. Briefly, forward and reverse reads were merged using PEAR 0.9.8 (http://www.exelixis-lab.org/pear), followed by quality filtering using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), and extraction of the fungal-specific ITS2 region using ITSx 1.0.11(Bengtsson-Palme et al. 2013). Dereplication, removal of singleton sequences or those less than 100bp, clustering to 97% sequence identity and subsequent chimera detection, using the UNITE Uchime 7.1 dataset (Nilsson et al., 2015) as reference, were conducted with VSEARCH 2.3.0 (Rognes et al. 2016). Representative sequences were taxonomically assigned using the Warcup_retrained V2 ITS training set (Deshpande et al. 2016) with RDP Classifier 2.11 (Q. Wang et al. 2007) to a taxonomic confidence level of 50% to retain a greater level of taxonomic resolution in the downstream analyses. The final step produced an OTU table based on the 97% clustering step. Samples from the OTU table were rarefied to 132460 sequences per sample (the minimum in a single sample) in QIIME 1.9.1 (Caporaso et al. 2010), to take any difference in sequencing depth into account. The rarefied OTU table was used in statistical analysis and to assign OTUs to guilds using FUNGuild (Nguyen et al. 2016).

Abundance tables were Hellinger-transformed in R Version 3.4.1 (R-project.craran) using the vegan package (Oksanen et al. 2017) to weight low-abundance OTUs lower than OTUs with

high abundance (Buttigieg and Ramette 2014). We used response screening in JMP Pro (JMP®, Version <*13.0*>. SAS Institute Inc., Cary, NC, 1989-2007) to determine which of the 25 OTUs with the highest relative abundances after rarefaction and Hellinger transformation differed by year, region or field type. Our model also included all possible interactions of these three factors.

OTUs were assigned to a functional guild using FUNGuild, currently the largest database of fungal guilds (Nguyen et al. 2016; Kivlin and Hawkes 2016). Guilds given by FUNGuild were pooled into a simplified guild that reflected a similar function (eg. "wood saprotroph" and "leaf saprotroph" were both considered "saprotroph"). We used a three-factor, full-factorial ANOVA in JMP Pro (JMP®, Version <*13.0*>. SAS Institute Inc., Cary, NC, 1989-2007) to assess the effects of management type, region and year on fungal richness and abundance, and on environmental characteristics. Dependent variables were transformed to meet model assumptions. Saprotroph richness showed no effect of year, so a reduced model of management and region was used. As a conservative estimate of the true species diversity in organic vs. conventional coffee, the Chao 1 estimator was calculated for each field type-by-region combination using EstimateS version 8.0 (Colwell 2006).

A non-metric multidimensional scaling (NMS) ordination was carried out on the rarefied, Hellinger-transformed OTU table using the Sorenson distance measure in PC-ORD Version 7.02 (MjM Software, Gleneden Beach, Oregon, U.S.A.) to visualize fungal communities based on field type. We used two-way PERMANOVA (Anderson 2001) to assess fungal community composition in organic and conventional fields as a function of each possible two-factor combination of year, region and field type. We excluded minimal conventional fields due to small sample size. Additionally, four conventional fields were excluded at random to ensure a balanced design. A randomized Monte Carlo was run to ensure that the result of the ordination

was different from one generated by chance. Environmental characteristics were transformed when necessary and overlaid as a biplot onto the ordination plot to visualize the correlation of these characteristics with fungal community composition.

Results

Fungal Diversity Overall

After rarefaction, 6668 fungal OTUs were detected in coffee roots from all field types. Using FUNGuild, 52% of OTUs (41% of sequences, Fig. 1.1) could not be assigned to a guild. Identifiable fungi were distributed among many guilds, including saprotrophs, arbuscular mycorrhizal fungi, plant pathogens and fungi that were classified as both saprotrophs and plant pathogens, hereafter referred to as "saprotroph-plant pathogens." Fungi that could be assigned to more than two of these simplified guilds were referred to as "multiple." The remaining OTUs belonged to several guilds, each of which represented no more than 1% of the total richness. In the full dataset, saprotrophs made up the largest portion of the OTU richness, and saprotroph-plant pathogens had the highest sequence abundance (Fig. 1.1).

Of the 25 most abundant OTUs by Hellinger value, 44% were unknown, 24% were saprotroph-plant pathogens, 16% were plant pathogens, and the remaining 16% came from various guilds (Table 1.1). All were either ascomycetes (72%) or basidiomycetes (28%, Table 1.1). Of the 25 most abundant OTUs, only an OTU related to *Knufia* differed in abundance between organic and conventional fields ($F_{1,21}$ = 46.28, P = 0.0015, Table 1.1). The guild composition by abundance of the most abundant OTUs mirrored that of the total dataset in that the percentages of the most abundant guilds were nearly identical in the full dataset and the subset of most abundant OTUs (Fig. 1.1). There was an effect of field type ($F_{1,15}$ = 6.97, P = 0.0194) and of the interaction of field type and region ($F_{1,15}$ = 5.01, P = 0.0419) on OTU

richness. Estimated richness was greater in organic fields than in conventional fields in Monteverde but did not differ between conventionally-managed and organic fields in San Vito (Fig. 1.2). Community composition of root fungi also differed by region ($F_{1,15} = 2.78$, $P = 0.0002$) and by field type ($F_{1,15} = 1.92$, $P = 0.0066$). The effect of the interaction between region and field type was marginally significant ($F_{1,15} = 1.48$, $P = 0.0574$).

Environmental Differences Between Coffee Field Types

Some environmental characteristics differed between organic and conventional coffee fields. Shade and shade tree richness were higher in organic than in conventional fields in Monteverde but did not differ between field types in San Vito. Organic fields in both sites had 6-7 times more shade than conventional fields in Monteverde, which had the lowest amount of shade, and about twice as much as conventional fields in San Vito, which had statistically intermediate levels (Table 1.2). Conventional coffee fields in Monteverde had the lowest shade tree species richness, and were shaded by 0-1 species on average, while organic fields in Monteverde and both field types in San Vito were most often shaded by four tree species (Table 1.2). Soils of coffee fields under conventional management were more acidic and contained more nitrate than those under organic management. Conventional fields had about three times more NO_3-N and were about 50-100 times more acidic than organic fields (Table 1.2). Copper availability was higher in organic than in conventional coffee (Table 1.2). Leaf litter was about 50% deeper in organic than conventional fields (Table 1.2). Calcium was lowest in conventional fields at both sites, highest in Monteverde organic, and had intermediate levels at San Vito organic fields (Table 1.2). Magnesium was highest in Monteverde organic fields, lowest in Monteverde conventional, and intermediate in San Vito fields of both types (Table 1.2). Copper

Slope, coffee plant density, age of coffee plants, electrical conductivity, organic material, and manganese were all measured, did not differ by field type (Table 1.3).

Community Composition

The NMS ordination plot (Fig. 1.3) showed that root fungal community composition overlapped in fields under conventional and minimal conventional management, but both showed separation from organic fields along Axis 2. Axis 1 accounted for 54.2% of the observed variation in community composition while Axis 2 accounted for 22.7%. These two axes accounted for 76.9% of the variation in root fungal communities. Along Axis 2, which was the primary axis indicating differences in fungal community composition based on field type, fungal community composition in organic and conventionally-managed fields was correlated with differences in NO_3-N, shade and pH, as well as levels of calcium, copper, potassium and magnesium (Fig. 3). Elevation, zinc, phosphorus, iron, sodium, and the N:P ratio were all correlated with Axis 1, which was not associated with differences related to field type.

Guild Composition

Guild structure differed with field management. There were fewer species of saprotrophs in conventionally-managed than in organic fields ($F_{3,18}$=11.18, P =0.0036). A significant interaction between field type and region showed Monteverde conventional fields had lower richness than either Monteverde organic fields or San Vito fields of both types ($F_{7,14}$ = 7.6946, P = 0.0125; Fig.1.4). Saprotroph abundance did not differ by field type ($F_{7,14}$ = 0.0018, P = 0.9666). Mycoparasite richness showed the same pattern as saprotrophs, with an interaction of field type and region such that richness was lowest in Monteverde conventional fields, highest in Monteverde organic, and intermediate in San Vito fields ($F_{7,14}$ = 4.73, P = 0.0108; Fig. 1.4). Mycoparasite abundance also did not differ by field type ($F_{7,14}$ = 1.83, P = 0.1585; Fig.1.4).

Plant pathogens were less abundant in conventional fields than in organic fields ($F_{7,14}$ = 6.6945, P = 0.0194; Fig. 1.4). There was an interaction of field type and region for plant pathogen richness ($F_{7,14}$ =21.42, P = 0.0004) in which Monteverde conventional fields had lowest richness, Monteverde organic had highest richness, and San Vito fields of both types had intermediate richness. Plant pathogen abundance showed the same pattern for an interaction of field type and region ($F_{7,14}$ =5.01, P = 0.0419, Fig. 1.4). Saprotroph-plant pathogens in organic fields had lower sequence abundance than in conventional fields ($F_{7,14}$ = 7.06, P = 0.0188). Saprotroph-plant pathogen abundance showed an interaction of field type and region, such that Monteverde conventional fields had higher abundance than in Monteverde conventional fields or San Vito fields of both types ($F_{7,14}$ = 13.26, P = 0.0027; Fig. 1.4). Saprotroph-plant pathogen richness did not differ between conventional and organic fields ($F_{7,14}$ = 1.44, P = 0.2666; Fig. 1.4).

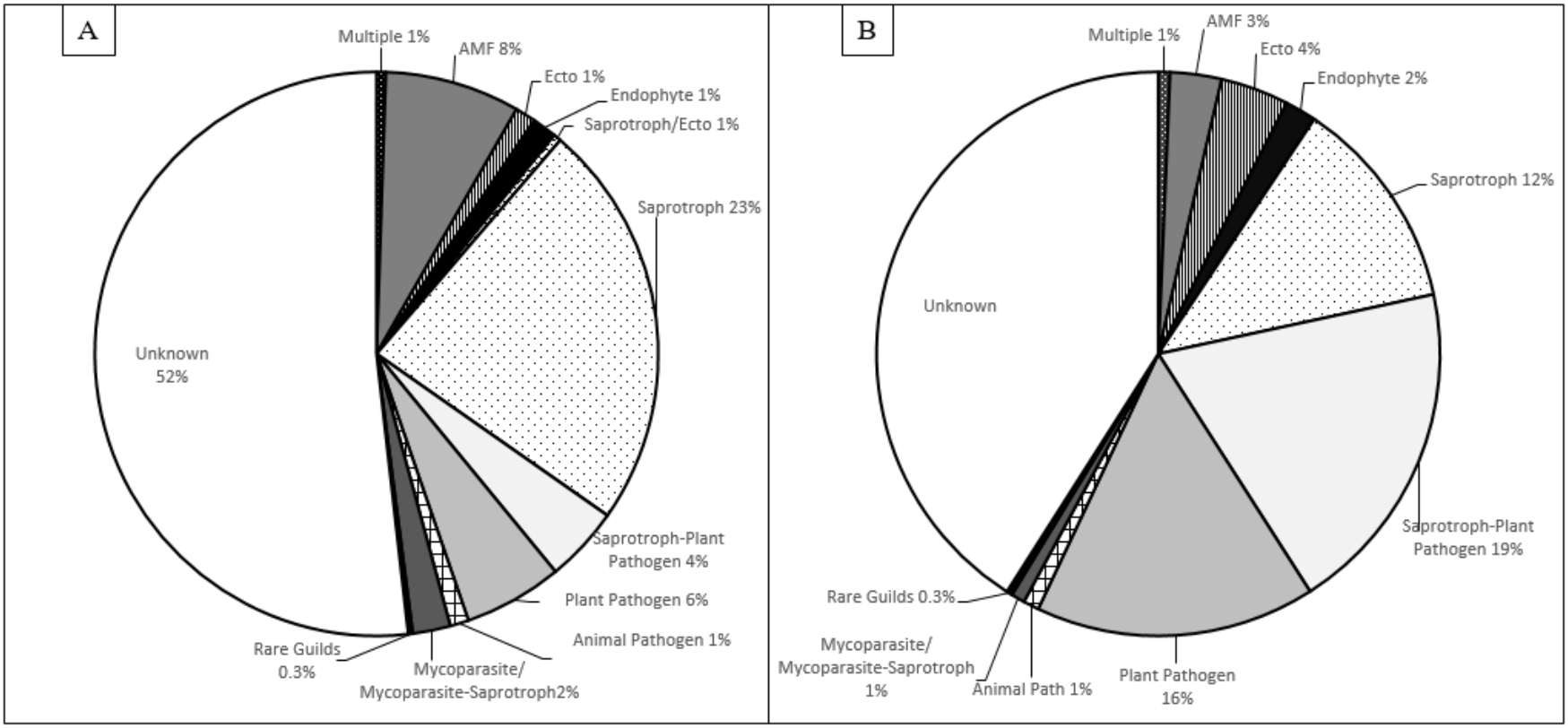

Figure 1.1. Fungal guild composition by a) OTU richness and b) sequence abundance for 25 coffee fields under organic and conventional management in Costa Rica. Samples were rarefied prior to analysis. Fungi that could be assigned to more than two of these simplified guilds were referred to as "multiple." Rare guilds include those with a richness of fewer than 10 OTUs and/or an abundance of less than 10,000 sequences.

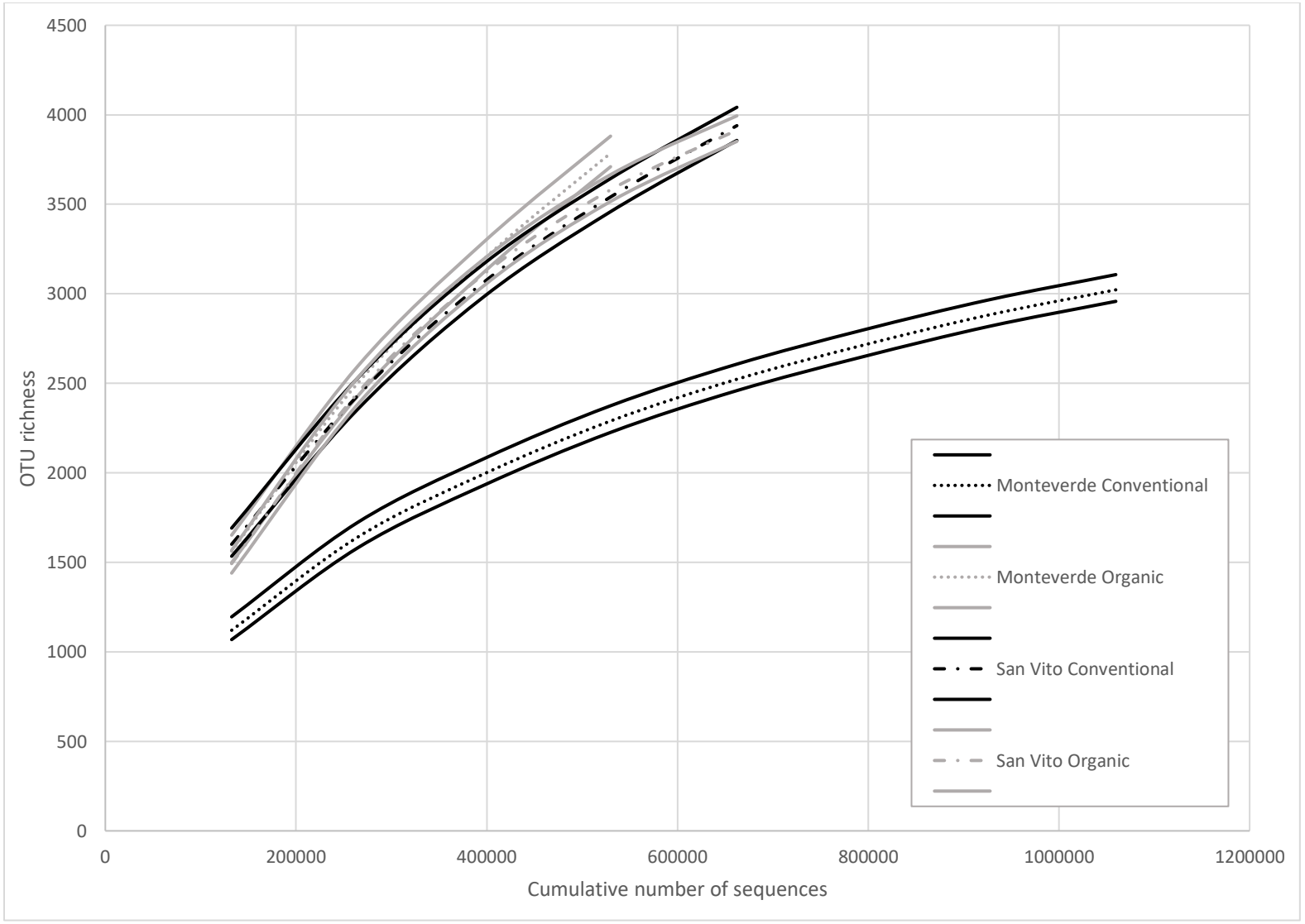

Figure 1.2. OTU richness with accumulation of sequences of root fungi in the roots of organic and conventionally-managed coffee in Monteverde and San Vito, Costa Rica. Solid lines indicate a 95% confidence interval based on the Chao I estimator.

Table 1.1. The most abundant OTUs and their closest taxonomic match in Warcup. Rank of OTU in each field type (conventional or organic) is presented. Function is guild or life strategy as best determined from literature. Warcup confidence shows level of certainty in the taxonomic identification from the Warcup database.

Rank Abundance/ Hellinger	Closest match (WARCUP)	Function	Phylum	Hellinger Rank (Conv/Org)	Warcup confidence
1/1	*Edenia gomezpompae*	Endophyte, possibly mycorrhizal (Gonzalez *et al.* 2007).	Ascomycota	3/1	0.86
2/2	Unknown		Basidiomycota	2/2	0.61
3/3	*Knufia sp.*	Plant pathogen (Hutchison, Untereiner, and Hiratsuka 1995)	Ascomycota	1/10	0.75
4/4	Xylariales	Multiple (Nannfeldt 1932)	Ascomycota	4/4	0.88
5/5	*Rhyzopycnis vagum*	Sugarcane and cantaloupe root pathogen (Farr, Miller, and Bruton 1998)	Ascomycota	6/3	1.0
6/6	*Fusarium oxysporum*	Saprotroph-plant pathogen (Rodriguez et al. 1996, Larkin et al. 1993)	Ascomycota	5/5	1.0
7/7	*Campylospora parvula*	Endophyte (Fiuza 2013)	Ascomycota	8/7	1.0
8/8	Unknown		Basidiomycota	7/14	0.53
11/9	*Mycena sp.*	Saprotroph-plant pathogen (Avelino et al. 2007; Selosse, Schneider-Maunoury, and Martos 2018)	Basidiomycota	9/12	0.82
13/10	*Fusarium oxysporum*	Saprotroph-plant pathogen (Rodriguez et al. 1996, Larkin et al. 1993)	Ascomycota	12/8	0.77
9/11	Pezizomycotina	Multiple (Ekanayaka et al. 2018)	Ascomycota	10/15	0.67
12/12	Pezizomycotina	Multiple (Ekanayaka et al. 2018)	Ascomycota	13/11	0.76
15/13	*Cylindrocarpon pauciseptatum*	Plant pathogen (Abreo et al. 2010)	Ascomycota	25/6	0.99
19/14	*Dokmaia monthadangii*	Saprotroph-endophyte (Promputtha et al., n.d.)	Ascomycota	20/9	1.0
17/15	Sordariomycetidae	Multiple (Maharachchikumbura et al., 2015)	Ascomycota	14/30	0.7
10/16	Helotiales	Multiple	Ascomycota	11/140	0.56
23/17	Agaricales	Multiple	Basidiomycota	16/25	0.78
20/18	Pezizomycotina	Multiple (Ekanayaka et al. 2018)	Ascomycota	15/43	0.88
14/19	Unknown		Basidiomycota	17/37	0.65
27/20	*Pochonia chlamydospora*	Nematode pathogen (X. Z. Liu and Chen 2003)	Ascomycota	21/26	0.58
21/21	*Phoma putaminum*	Plant pathogen (Evidente 1995)	Ascomycota	19/42	0.92
25/22	*Ceratobasidium sp. AG A*	Pathogen, orchid mycorrhizal (Bidartondo et al. 2004)	Basidiomycota	30/16	0.97
35/23	Sordariomycetidae	Multiple (Maharachchikumbura et al., 2015)	Ascomycota	18/92	0.59
40/24	*Cladosporium perangustum*	Plant pathogen (Oliviera *et al.* 2014)	Ascomycota	28/32	1.0
38/25	Agaricomycetes	Saprotroph (Morgenstern, Klopman, and Hibbett 2008)	Basidiomycota	35/24	0.67

Table 1.2. Environmental characteristics from 22 coffee fields under organic and conventional management in Monteverde and San Vito, two regions of Costa Rica. Sample size indicated in parentheses. Means that share a letter do not differ statistically by Tukey HSD. "Test" indicates whether difference arose from field type or from the interaction of field type and region (F x R). Only characteristics with statistically significant differences are shown.

	Monteverde		San Vito				
Variable	Conventional (8)	Organic (4)	Conventional (5)	Organic (5)	$F_{1,14}$	p	Test
Shade (%)	9 ± 3^{a}	64 ± 11^{b}	36 ± 10^{ab}	53 ± 8^{b}	6.40	0.024	F x R
Shade Tree Richness	0.5 ± 0.3^{a}	4.5 ± 0.6^{b}	4 ± 1.2^{b}	4.2 ± 1.6^{b}	12.50	0.004	F x R
NO_3^--N (kg/ha)	164 ± 31^{b}	54 ± 15^{a}	103 ± 5^{b}	33 ± 6^{a}	33.81	<0.0001	Field Type
Leaf Litter (cm)	1.43 ± 0.21^{b}	2.13 ± 0.31^{a}	1.44 ± 0.14^{b}	1.86 ± 0.18^{a}	6.42	0.0238	Field Type
pH	5.18 ± 0.15^{a}	6.09 ± 0.06^{b}	5.25 ± 0.07^{a}	5.72 ± 0.3^{b}	17.07	0.001	Field Type
Cu (ppm)	1.87 ± 0.21^{a}	2.52 ± 0.45^{b}	3.94 ± 0.32^{c}	6.25 ± 0.86^{d}	9.12	0.009	Field Type
Ca^{+2}(ppm)	1769 ± 203^{a}	3750 ± 580^{b}	1622 ± 323^{a}	2199 ± 669^{ab}	12.87	0.003	F x R
Mg^{+2}(ppm)	89 ± 7^{a}	294 ± 78^{c}	185 ± 35^{b}	185 ± 55^{b}	9.67	0.008	F x R

Table 1.3. Environmental variables in 25 coffee fields in Costa Rica that did not show statistical differences in a three-way ANOVA of year, region and field type. Data were transformed as necessary prior to analysis to meet assumptions of ANOVA.

Environmental variable	***F***	***P***
Coffee density (# plants/100 m2 plot)	1.140191	0.393382
EC (mmhos/cm)	0.993927	0.474235
K (ppm)	0.990206	0.476459
Coffee field age (years)	0.742804	0.642176
Coffee plant age (years)	0.560163	0.773562
Slope	0.332723	0.80298
Deviation from SE (neg = more NE/N)	0.272956	0.954522
Zn (ppm)	1.56	0.227185
Mn (ppm)	1.06	0.438805

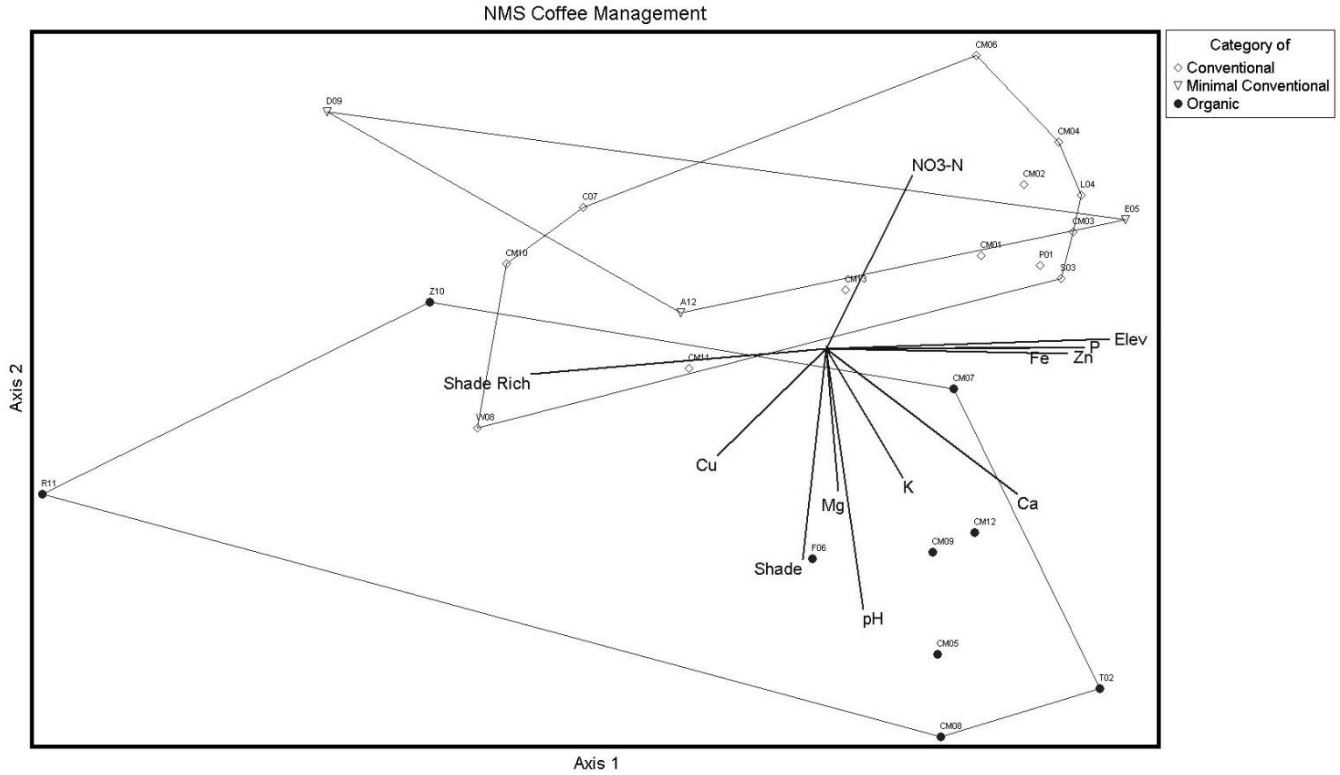

Figure 1.3. Non-metric multidimensional scaling ordination of root fungal communities in coffee fields under conventional, minimal conventional and organic management (final stress = 8.995). Ordination with 999 randomizations. The best solution was three dimensional, and two axes are shown here. Axis 1 represents 54.2% and Axis 2 accounts for 22.7% of the total variation in fungal community composition. Environmental characteristics were transformed when necessary and bi-plot overlay shows these data in relation to fungal communities. Fungal OTU abundances were rarefied and Hellinger-transformed prior to analysis. Each point represents the fungal community composition of one coffee field.

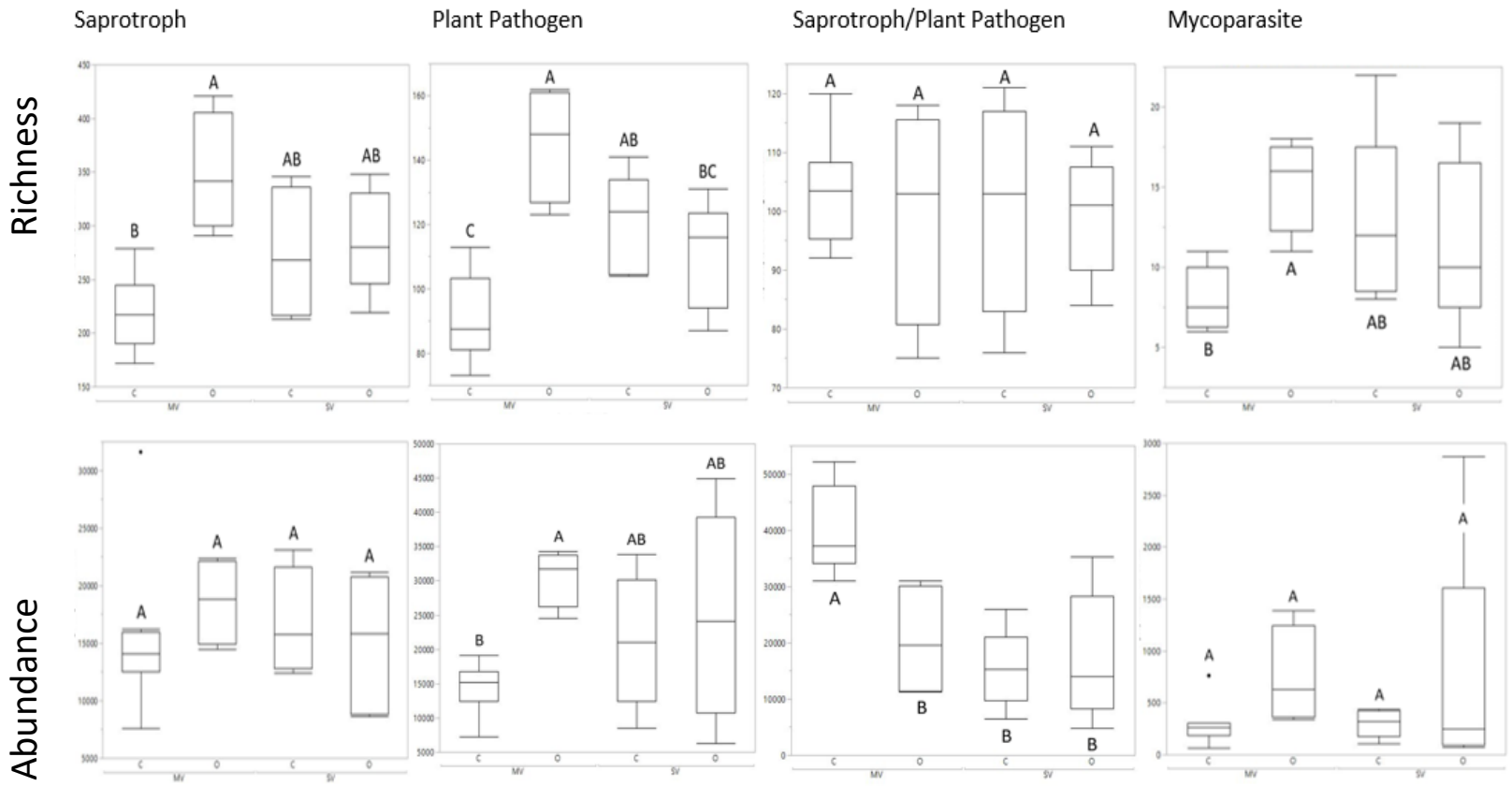

Figure 1.4. OTU richness and sequence abundance of fungal guilds in roots of coffee under conventional (C) or organic (O) management in Monteverde (MV) and San Vito (SV), Costa Rica. Centerline of each boxplot is the median of the data. Whiskers are drawn to the furthest data point within 1.5 x the interquartile range of the data, and all values outside that range are considered outliers and are represented as dots. Means that share a letter do not differ statistically based on a three-factor ANOVA followed by Tukey HSD to assess the effects of year, region and field type. All plots show untransformed data from a rarefied dataset. Only guilds that showed a statistically significant ($\alpha = 0.05$) interaction of field type and region for richness or abundance are shown. No interactions of field type and year were observed.

Discussion

Fungal Diversity Overall

In this study, I gained an understanding of the relationship of various soil characteristics with the diversity and abundance of coffee root fungi. I also explored relationships between guilds of fungi to elucidate how trophic structure of root fungal communities might change under the different soil conditions created by coffee field type.

The structure of fungal communities corresponded to differences in coffee management. Overall OTU richness was greater in organic than in conventional coffee. The most abundant OTUs were abundant regardless of management. Thus, it follows that the effect of coffee field types on fungal richness was due to changes in the diversity of rarer species, a pattern observed in prior work (Suding et al. 2005). The findings of this study show that organic coffee management is helpful for preserving fungal biodiversity.

Environmental Characteristics Affecting Fungal Community Composition

My dataset showed that NO_3^-N was higher in conventionally-managed coffee fields than in organic fields and that NO_3^--N was associated with differences in fungal communities between the two management types. In a meta-analysis of studies on the response of plant communities to nitrogen fertilization, Suding *et al*. (2005) found that communities with high N inputs were less diverse because species with high N requirements were able to outcompete other species. Furthermore, studies in grain and sugarcane agricultural systems found that fungal communities were modified by N input from fertilizers containing both ammonium and NO_3^-N such that fungal diversity showed a negative association with N fertilization (Paungfoo-Lonhienne et al., 2015; Zhou et al., 2016).

Nitrate also helps to explain observed differences in fungal guilds. Differences in nitrogen availability influence which types of fungi are supported within plant roots (Rai and Agarkar 2014). Previous studies in agricultural and forest systems have shown that high N enrichment shifts rhizosphere fungal communities so that plant pathogens become more abundant (Liu et al. 2017; Zhou et al. 2016; Paungfoo-Lonhienne et al. 2015; Edwards et al. 2011). My study, however, showed reduced richness and abundance of plant pathogens in conventional fields compared to organic fields in Monteverde, and no difference in San Vito. This is likely because foliar fungicides, which can enter the soil and restructure fungal communities (de Boer et al. 2012; Keiblinger et al. 2018; Wang, Zhou, and Cang 2014) were used to combat fungal pathogens in conventional fields. Saprotrophic fungi grow more slowly and perform less decomposition in soils enriched with N in the form of both ammonium and NO_3^-N relative to unenriched soils (Diepen et al. 2017). Additionally, mycoparasitic fungi tend to be less abundant in soils with high N inputs (Paungfoo-Lonhienne et al. 2015). Though we found no management effect on the abundance of mycoparasites or saprotrophs, these guilds did have the highest richness in Monteverde organic fields, where soil nitrate was low. The tendency for rare species to be eliminated in high N, combined with the inhibiting effect of N on growth and abundance of saprotrophs and mycoparasites could have led to the higher richness of these guilds in organic coffee fields. In other words, when fungal growth was suppressed, more species of fungi became rare enough to be eliminated from the community.

Some fungi can act as opportunists, shifting from one guild to another based on dynamic relationships between host, fungus and environment (Kuo et al. 2015; Schulz and Boyle 2005). While plant pathogens appeared to be less abundant in conventional fields than in organic fields in Monteverde and all fields in San Vito, saprotroph-plant pathogens were most abundant in

Monteverde conventional fields. It is possible that obligate plant pathogens are suppressed in the presence of fungicides but that lifestyle-switching fungi capable of surviving as saprotrophs proliferate and recolonize plant roots as pathogens once fungicide levels decline.

Organic fields were less acidic than conventionally-managed fields. This may be due to an interaction of shade trees and N application. Conventional fertilizers contain either nitrates or ammonium, and both of these can cause soil acidification (Barak et al. 1997). Aboveground biomass, in this study taking the form of shade trees, has been linked to a reduction in soil acidity and soil nitrification (Fu et al., 2015a; Matsushima and Chang, 2007; Wartenberg et al., 2017). Saprotrophs degrade litter less efficiently in acidic soils (Gao et al. 2016; Maaroufi et al. 2015; Gramss, Ziegenhagen, and Sorge 1999). Thus, the higher pH in organic fields may explain the greater richness of saprotrophic fungi found there relative to conventionally-managed fields.

Shade and magnesium (Mg^{+2}) were also related to differences in fungal communities between management type. Shade was lowest in Monteverde organic fields compared to all other fields. Shade leads to a cooler and more humid microclimate which allows soil fungi to proliferate (Melloni et al., 2018; Niether et al., 2018). Mg^{+2} was higher in Monteverde organic fields than in Monteverde conventional and San Vito fields of both types. The difference in Mg^{+2} may be due to fertilizer application in conventional management, as Mg^{+2} deficiency can be exacerbated by fertilizer application (Yu-Chuan et al., n.d.). Saprotroph richness showed the same pattern as Mg^{+2} with Monteverde organic fields showing the highest richness of all fields. Saprotrophic fungi help with plant uptake of Mg^{+2} by breaking down nutrient-rich leaf litter and releasing the mineral back into the soil, where it can be accessed by plants (Kimmig, Holmden, and Bélanger 2018). Thus, it is possible that the rich saprotroph communities in Monteverde organic fields were releasing more Mg^{+2} into these fields.

Guild Composition and Trophic Structure of the Root Fungal Community

Changes in communities at one trophic level can affect diversity and abundance at other levels. In marine communities, extinctions due to human activities tended occur among species at the highest trophic levels, while invasions tended to occur more frequently at the lowest trophic levels (Byrnes, Reynolds, and Stachowicz 2007; Reynolds and Bruno 2012). These changes led to a trophic cascade resulting in the enrichment of diversity at lower trophic levels and losses of diversity among predators (Reynolds and Bruno 2012). Few studies have applied this concept to fungi, but following the same line of reasoning, fungi at the highest trophic level might have lower richness and abundance than those at lower trophic levels, especially in human-dominated systems. Guild is related to a fungus' mode of acquiring nutrition (Nguyen et al. 2016) and thus can be used to indicate its trophic level. In my study, mycoparasites would form the top of the trophic pyramid, while plant pathogens and saprotrophs would act as primary consumers. Conventional coffee fields in Monteverde had less diverse fungal communities, and showed a shift toward saprotroph-plant pathogens, a guild at the second trophic level. Mycoparasites, a guild at the third level of the trophic pyramid, were richest in Monteverde organic fields, as were saprotrophs and plant pathogens, two guilds found in the second trophic level. This could mean either that conditions favoring diversity in one of these fungal guilds favors diversity in all three, or that greater opportunity for cross-guild interactions in a community lead to increased diversity(Kulmatiski et al., 2014).

Limitations

While the techniques used in this study are on the forefront of current research methods for studying fungal communities, there are some associated limitations of the findings. The unknown fungi in this study made up a large portion of the dataset. This proportion, however,

comparable to the amount of unknowns in other contemporary studies, where anywhere from 40-80% of OTUs cannot be assigned taxonomy (Anthony et al., 2017; Nguyen et al., 2016). Since the databases used to assign taxonomy and guild information are a conglomeration of current knowledge, the proportions of these identified fungi may reflect the depth of study on particular groups of fungi (Nguyen et al., 2016). Additionally, the sequence-based measure of abundance allows us to assess prevalence of fungal DNA in the root environment but does not give absolute fungal abundance (Fitzpatrick et al., 2018). Despite these limitations, the results of this study reveal important patterns in fungal diversity, fungal community composition and in guilds of known fungi as they relate to differences in coffee management.

Conclusions

This research serves as an important link between agricultural practices and their implications for belowground fungal communities. This study suggests that fungal communities in coffee roots are structured differently based on coffee field type. Organic and conventional coffee management resulted in different soil environments. Field type also corresponded to differences in fungal richness and abundance in saprotrophs, plant pathogens, mycoparasites, and species that can be both saprotrophic and plant pathogenic. These disparities in fungal guilds may influence trophic structure of the fungal community and thereby have profound impacts on soil ecosystem function. In the future, it will be important to test how fungal community composition and trophic structure change when individual aspects of management such as the amount of fertilizer and fungicide are experimentally manipulated in the field or laboratory. Such a test will help further disentangle how fungal communities respond to common management strategies.

CHAPTER TWO: LIGHT AVAILABILITY AND HOST RELATEDNESS CONTRIBUTE TO COMMUNITY COMPOSITION OF BELOWGROUND FUNGI ON COFFEE (*COFFEA ARABICA*) AND NATIVE RUBIACEAE IN COSTA RICA[2]

Introduction

Belowground fungi interact with plants in a multitude of important ways, and their diversity and ecological importance merits a degree of study not yet achieved (Dassen et al., 2017; Fuhrman, 2009; Jeffries et al., 2003; Tedersoo et al., 2014). Belowground fungi in soils and plant roots include plant symbionts such as fungal pathogens that attack plants at the root, mycorrhizal fungi that provide the plant with nutrition and resistance to drought and pathogens (Tedersoo et al., 2016, 2014), commensal endophytic fungi (Schulz and Boyle, 2005) and decomposers that aid in soil carbon cycling (Delgado-Baquerizo et al., 2015)**.**

Environmental filters can structure fungal communities by encouraging or inhibiting certain fungal species. Fungi may depend on certain edaphic or biotic characteristics of their environment, or they may become more competitive under certain conditions (Högberg et al., 2003; Prescott and Grayston, 2013; Suding et al., 2005; Voříšková and Baldrian, 2013; Xiao et al., 2018). Soil temperature and moisture are two environmental characteristics commonly considered important for determining degree of fungal growth (Castaño et al., 2018). Changes in soil temperature can cause shifts in the composition of belowground fungal communities (Zhang

[2]The material in this chapter was co-authored by Elizabeth Sternhagen and Laura Aldrich-Wolfe. Elizabeth Sternhagen had primary responsibility for collecting samples and taking measurements in the field, for carrying out lab work, and for executing the bioinformatic and statistical analyses. Elizabeth Sternhagen was the primary developer of the conclusions that are advanced here. Elizabeth Sternhagen also drafted and revised all versions of this chapter. Laura Aldrich-Wolfe served as proofreader and checked the math in the statistical analysis conducted by Elizabeth Sternhagen.

et al., 2005). Soil moisture changes belowground fungal communities by allowing microbes to increase their activity and population size (Li et al., 2018). Light availability can influence fungi as well, particularly because solar radiation of the soil causes changes to soil temperature and moisture, inducing microbes to grow and respire more in response to those factors (Tang et al., 2005; Zhang et al., 2018).

Phylogenetic relatedness of the host plant is another factor that could influence fungal communities. The symbiotic relationships between many fungi and plants mean that these organisms have evolved together for millions of years (Fitzpatrick et al., 2018). Some evidence for a phylogenetic signal in fungal communities has been found, as by Parker et al. (2015) and Gilbert et al. (2007) , who observed that more closely-related plants shared more fungal pathogens than plants that were distantly related. Phylogenetically-conserved plant attributes such as root length and diameter are important for determining which fungi can colonize the plant (Comas et al., 2014). Additionally, a plant's root exudates create a resource pool that fungi can exploit (Nakayama and Tateno, 2018; Rasmann and Turlings, 2016).

Prior studies have addressed how fungi respond to various environmental filters, and how plant hosts might foster different fungal communities. DeBeenhouwer *et al.* (2016) found that community composition of AM fungi on coffee was influenced by agricultural intensification and the associated changes in soil environment. A study by Saucedo-Garcia *et al.* (2014) showed that community composition of endophytic fungi in coffee leaves was influenced by the coffee host's microclimate. The relative importance of environment and host relatedness for fungal community structure, however, still has not been thoroughly addressed. In this study, we used metabarcoding and high throughput sequencing of the ITS2 region of fungal DNA to assess whether fungal community structure differed between the roots and rhizosphere of coffee and

native forest Rubiaceae, and how fungal community in the bulk soil compared with these communities. I hypothesized that, because host relatedness encompasses plant traits that are important for fungal community structure (Mazel et al., 2018), fungal communities on coffee roots would be more similar to those of closely related tree species than to those of more distantly related species in the plant family Rubiaceae. Therefore, host phylogenetic distance from coffee should be an important predictor of differences in fungal community composition. Alternatively, if environment is more important than host relatedness in shaping root and rhizosphere fungal communities, I expected to observe a closer correspondence between coffee root fungal communities and coffee soil fungal communities than between coffee root fungal communities and related forest trees in the Rubiaceae.

Methods

Description of Field Sites

This study was conducted in the Monteverde cloud forest region of Costa Rica. Samples were taken from three coffee fields that had forest adjacent to them (Cañitas:10.3249, -84.8416; Monteverde: 10.3008, -84.8152; San Luis: 10.2865, -84.8024; Fig. 2.1). Coffee farmers were interviewed to establish previous land use of the coffee field and adjoining forest, the age of the coffee plants, and the type and frequency of chemical treatments applied to the field.

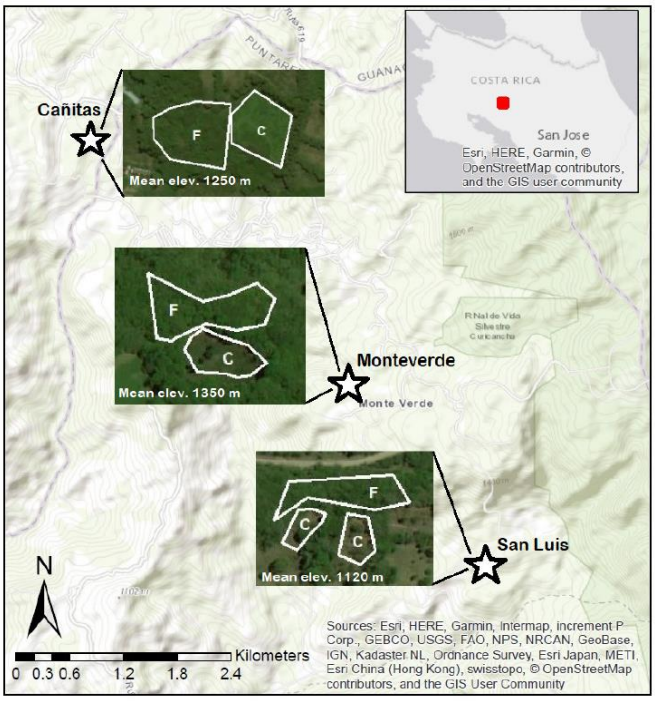

Figure 2.1. Map of three sites in the Monteverde region of Costa Rica. Stars indicate sites, and each associated inset is a magnification of the site. Polygons outline forest (F) and coffee (C) habitat at each site. Mean elevation of the site is indicated in each inset.

Root and Soil Sampling

Thirty coffee plants were selected from across the entire area of each coffee field for root and rhizosphere soil sampling. In forest habitat, 15 trees from each of 4-5 species of Rubiaceae were haphazardly selected along compass-line transects that started at the edge of the coffee field and entered the forest perpendicular to this edge. Approximately 1g of fine roots (~20 roots, each about four cm in length) was taken from each forest tree and coffee plant. By carefully extracting the finest roots so that the soil naturally clinging to the root's surface remained attached, rhizosphere soil could be separated from fine roots using a 2mm sieve. The sieve was soaked in 10% sodium hypochlorite for 10 min between each sample to minimize cross-contamination. Root samples were collected from 305 trees (*Coffea arabica*, N=90; *Psychotria panamensis*, N=49; *Psychotria subsessilis*, N=43; *Palicourea valerioana* N=31; *Hamelia patens*, N=30; *Randia matudae*, N=16; *Randia grandifolia*, N=16; *Psychtoria monteverdensis*, N=15;

Palicourea pubescens, N=15;), and rhizosphere soil was collected from 258 of those trees. On some trees (*Pa. valerioana,* N=19; *C. arabica* N= 11; *Ps. subsessilis,* N=5; *R. matudae* N=4; *Ps. panamensis* N=3; *R. grandifolia* N=2; and N=1 for *Pa. pubescens, Ps. monteverdensis,* and *H. patens*) only roots were collected because rhizosphere soil did not remain attached to the root and thus could not be collected. In order to characterize the soil fungal community of each coffee field and forest at each site, three soil cores, each 2 cm in diameter and 20 cm deep, were taken from 30 points haphazardly selected around each field or forest habitat. These cores were pooled for each habitat at each site and mixed thoroughly. The soil was placed in a cooler along with all root and rhizosphere samples. In the lab, the root samples were rinsed with tap water and dried with a paper towel, and then all roots, rhizosphere soil and bulk soil were stored in a manual-defrost freezer at approximately -15° C ≤ 24 hours after field collection. Frozen samples were transported from Costa Rica to the United States on dry ice. From ten coffee plants and five forest trees per species at each site, a subsample of fresh roots was placed in 1% KOH for subsequent analysis of fungal root colonization.

Environmental Data

In each habitat at each site, the abiotic environment was characterized. A single integrated measurement of photosynthetically-active radiation (PAR) was taken for each tree at a height of approximately 1 m above the ground, between 1100-1300 h on cloudless days using a LI-COR 191R line quantum sensor (Li-COR, Inc., Lincoln Nebraska). Soil temperature was measured continuously over the course of the sampling period at each site (Cañitas: March 3 - April 3, 2017; Monteverde: April 11 - April 22, 2017; San Luis: April 22 - May 23, 2017) using three HOBO MX2304 data loggers (Onset Corporation, Bourne Massachusetts) distributed across the area of each habitat. Soil moisture was measured for the same subset of trees that were

used for mycorrhizal colonization by drying to constant mass and calculating mass loss. From this same subset of trees, 300-500 grams of soil was sieved through a 2mm sieve and analyzed at North Dakota State University Soils Lab. to determine pH (measured 1:1 in water), levels of nitrate, ammonium, P (Olsen method), Zn, Fe, Mn, Cu, Ca^{+2}, Mg^{+2}, and K^{+}, and percent organic material ("% OM" measured by loss on ignition). Density of the host trees in each habitat was measured using the variable area transect method (Parker, 1979).

Root Staining and Scoring

Root samples in 1% KOH were transferred to 10% KOH (*w/v*) at approximately 95° C until roots cleared. Coffee roots cleared in 15 min while more lignified roots (e.g. *Ps. panamensis* and *Ps. subsessilis,* by visual assessment) took about 45 min to clear. Cleared roots were then rinsed under tap water for 10 min, acidified in 2% HCl (*w/v*) for 15 minutes at room temperature and stained with 0.05% Trypan blue (dH_2O, 85% lactic acid and glycerol (1:1:1) with 0.05% (*w/v*) Trypan blue) at room temperature for 5 min. Samples were rinsed in tap water for > 1h to remove excess stain. Roots from *H. patens* did not clear fully using this process, so roots from this species were placed in 2.5% KOH at 90° C for 20 min, rinsed under tap water for 10 min, placed in 3% H_2O_2 at ~140° C for 10 min, then acidified and stained as above.

About 20 root segments per tree were mounted on a microscope slide in polyvinyl-lacto-glycerol. A minimum of 100 intersections were scored on each slide for the presence of structures from arbuscular mycorrhizal (AM) fungi (arbuscules, vesicles, hyphae) and non-AM fungi at 200 × magnifications (method adapted from McGonigle *et al.* 1990) using a Zeiss Axio Scope.A1 with DIC (Carl Zeiss Microscopy, Göttingen Germany).

Molecular Analyses

All root and soil samples were frozen to keep the DNA stable until extraction. Root tissue subsamples of ~100mg and rhizosphere soil subsamples of ~250mg were kept frozen with liquid N and ground using a Tissuelyser II (QIAGEN Inc., Hilden Germany). Root DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN Inc., Hilden Germany) and rhizosphere soil DNA was extracted using the MoBio PowerSoil Kit (QIAGEN Inc., Hilden Germany), following the manufacturer's protocols except that purified DNA extracts were stored in TE buffer. All extracts were stored at -20˚ C. For each sample, 20 µL of extract was shipped on dry ice to the Genomics Center at the University of Minnesota, where the internal transcribed spacer (ITS2) region was amplified using the ITS4 forward primer (TCCTCCGCTTATTGATATGC; White *et al.* 1990) and the 5.8SR reverse primer (TCGATGAAGAACGCAGCG; https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/) and were sequenced using Illumina high-throughput sequencing with a unique index adapter for each sample.

Sequence Data Processing

Forward and reverse reads were paired using PEAR 0.9.8 (http://www.exelixis-lab.org/pear), then quality filtered with the FASTX-toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). The ITS2 region was extracted using ITSx 1.0.11 (Bengtsson-Palme et al., 2013), singletons and sequences less than 100bp were removed and sequences were then clustered into groups with a 97% similarity threshold and were assigned taxonomy based on the Warcup database (Deshpande et al., 2016). A representative sequence from each of these groups was compared with the UNITE curated database of known fungal ITS sequences and by the Basic Local Alignment and Search Tool (BLAST) with sequences in Genbank. Any non-fungal OTUs were removed from the data set. OTUs with high prevalence in

controls, defined as those OTUs present in > 10% of controls, or that had an abundance ≤ 1% of the abundance of the smallest sample, were removed from all samples. If the relative abundance of an OTU in a control was more than 1% in each sample, it was removed from the dataset. Samples were then rarefied by sample type: root samples were rarefied to 103 sequences, rhizosphere samples to 5692 sequences, and background samples to 35919 sequences.

Data Analysis

The effects of host species, habitat and site on environmental variables were evaluated using analysis of variance (ANOVA) in JMP Pro (JMP®, Version <*13.0*>. SAS Institute Inc., Cary, NC, 1989-2007, hereafter "JMP"). Environmental data were transformed as necessary to fit the assumptions of ANOVA and tests were conducted using response screening and the false discovery rate (FDR) *P*-value to control for Type I error (Benjamini and Hochberg, 1995).

OTUs that had an abundance of 100 or more sequences in root samples were pooled by genus. For trees that occurred at all three sites, a Venn diagram was created using the Bioinformatics and Evolutionary Genomics Venn Diagram Tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) to compare how many fungal genera were unique to individual tree species or shared between tree species. Shared fungal genera were also compared between coffee roots and bulk soil in coffee or forest. Since sequence abundance was intrinsically different in each sample type, a universal cutoff was not applicable, so the 50 most abundant OTUs from each sample type were pooled by genus and assembled into a Venn diagram using the same process as above. A relative abundance chart was made with genera that had an abundance of at least 200 sequences. The abundances of these genera across species were compared using a Pearson's Chi Squared test in JMP for each site.

Sequence abundance matrices were Hellinger-transformed in PC-ORD to weight OTUs with low or high abundance appropriately relative to one another. Non-metric multidimensional scaling (NMS) ordination using the Sorenson distance measure was carried out on the rarefied, Hellinger-transformed OTU abundance tables. Ordinations allowed for visualization of fungal community composition in root, rhizosphere and background soil (sample type) as a function of tree species, site, and sample type were produced using PC-ORD Version 7.02 (MjM Software, Gleneden Beach, Oregon, U.S.A.). A randomized Monte Carlo test was run to ensure that the ordination output was different from the result due to chance. When individual data points were missing in the environmental matrix, mean values by site and tree species were supplied for the given variable to ensure that main and secondary matrices were the same size. All environmental variables were transformed as necessary and overlaid onto the NMS as a biplot indicating correlations with fungal communities.

PerMANOVAs were used to test the effect of site, tree species, sample type and the interaction of these factors on fungal community composition. Fungal communities on tree species at all three sites, and those occurring at only two sites, were tested separately for each sample type (root, rhizosphere and bulk soil). Because PerMANOVA allows no more than two factors, separate tests were run for each two-factor combination of site, tree species, and sample type. To ensure sufficient degrees of freedom, site-by-species combinations with low sample size were excluded (e.g. *Pa. valerioana* had only two rhizosphere samples from San Luis). Because PerMANOVA requires a balanced design, samples were excluded at random for each test, so that every site-by-species combination had the same number of samples as the group with the smallest sample size.

Analysis of Phylogenetic Relatedness Among Plant Species

Because the current published phylogeny of the family Rubiaceae does not include all of the plant species sampled in this study, a phylogeny was constructed using NCBI sequences in the MEGA version 10.0.4 (Kumar, Stecher, Li, Knyaz, and Tamura 2018). Sequences were acquired from the NCBI database for each of the plant species used in the study (*Coffea arabica,* KP776623.1; *Randia grandifolia,* AF493455.1; *Psychotria monteverdensis,* KC480521; *Palicourea pubescens,* AF071997.1; *Psychotria panamensis,* FJ208642.1; *Palicourea valerioana* KC480546; *Psychotria quinqueradiata,* FJ208618.1; *Hamelia patens,* JX468353.1). The two Palicourea species were listed as members of the Psychotria, a designation based on the former understanding of phylogenetic relatedness, which has now been updated (http://www.tropicos.org/Name/40000063?projectid=34). One species (*Randia matudae*) did not have any accessions in NCBI and therefore could not be included in the tree. Included sequences were 605-711 bp in length and spanned the 18S, ITS1, 5.8S, ITS2 and 26S segments of DNA. All sequences had a complete 5.8S segment, and other portions were either complete, partial, or absent depending on the species. Pairwise phylogenetic distances were calculated in MEGA using the Tamura-Nei model. The arrangement of the resulting phylogenetic tree corresponded to plant relationships within the Rubiaceae (Dr. Charlotte Taylor, *personal communication with the author*; Bremer and Eriksson, 2009; Razafimandimbison et al., 2014). This matrix was simplified and only the phylogenetic distance of each tree from coffee was used in statistical analyses. The distance for *R. matudae* was assumed to be identical to that of *R. grandifolia.*

Results

Host Phylogeny

Nine tree species were included in this study, encompassing five genera and three subfamilies within the Rubiaceae (Fig. 2.3). Randia species were the most closely related to coffee, followed by *H. patens*. Two species in the Psychotria came next, *Ps. monteverdensis* followed by *Ps. panamensis.* The three most forest Rubiaceae most distantly-related to coffee were, in order, *Pa. pubescens, Ps. quinqueradiata, and Pa. valerioana.* Species in each subfamily were as follows: *C. arabica, R. grandifolia,*and *R. matudae,* in the Ixoroideae, *H. patens* in the Cinchonoideae, and *Ps. quinqueradiata, Ps. panamensis, Ps. monteverdensis, Pa. valerioana,* and *Pa. pubescens* in the Rubioideae.

Environmental Differences

A number of environmental characteristics differed between coffee field and forest habitats. Soils in coffee were more acidic than forest soils (Table 2.1; $F_{1,89}$ = 107.60, P <0.0001). PAR and soil phosphorous were higher in coffee than in forest (PAR $F_{1,89}$ = 146.35, P = <0.0001; P $F_{2,89}$ = 15.32, P = <0.0001). Phosphorus was about 10 times higher in coffee than in forest soils, except in San Luis where levels did not differ between habitats (Table 2.1). In Cañitas and in Monteverde, organic matter, manganese and magnesium availabilities were higher in forest than in coffee, but these characteristics did not differ by habitat in San Luis (Table 2.1). Availability of zinc, copper and calcium differed by habitat, only at a single site, showing no difference at the other two sites. Zinc and copper were higher in coffee than in forest soils in Monteverde while calcium availability was higher in forest than in coffee soil in Cañitas (Table 2.1). Mean, minimum and maximum daily temperature were all higher in coffee than in forest across all three sites (Table 2.1).

Differences in environment based on tree species were also observed. Most of these, however, could be attributed to a strong effect of site, or seemed to be driven by the strong differences between coffee and forest trees. Phosphorus and pH was higher in coffee soils than in forest soils, but did not differ consistently among soils of different tree species (Table 2.2). Light environment differed by tree species (Fig. 2.2; $F_{8,86}$=3.7630, P = 0.0008). Availability of PAR was highest in coffee, about four times lower for *H. patens,* and about 200 times lower for *Palicourea valerioana*. All other tree species occurred at intermediate light levels, 10-100 times lower than the light availability in coffee.

Fungal Community Composition

Fungal community composition differed between root and rhizosphere samples (pseudo$F_{2, 324}$= 4.3678, P = 0.002). Root and rhizosphere samples were differentiated along Axis 1 of the NMS plot (Fig. 2.4A). Community composition also differed by host tree species (pseudo$F_{5, 155}$ = 3.5746, P = 0.002), and these differences were associated with Axis 2 of the NMS (Fig. 2.4B). Several environmental characteristics were also associated with differences in community composition along Axis 2. Shade, phylogenetic distance from coffee, host tree density, phosphorus and soil temperature were each correlated with this axis.

When root, rhizosphere and bulk soils fungal communities were plotted using NMS ordination, fungal community composition did not differ between rhizosphere and bulk soils while root fungal communities were distinct from both rhizosphere and bulk soil communities (Fig. 2.5. Root fungal communities differed from fungal communities of rhizosphere and bulk soil along Axis 1, and to a lesser extent, along Axis 2. As in the previous ordination (Fig. 2.4), soil temperature and host density were correlated with differences in fungal community composition within each sample type. Environmental characteristics correlated with the

differences in fungal communities between soil and roots included Ca^{+2}, Mg^{+2}, Mn, Fe, Cu, Nitrate, shade, ammonium, P, K^{+}, soil humidity, pH and fungal root colonization. Phylogenetic distance to coffee could not be included in this biplot because the bulk soil samples were associated with habitats rather than with individual tree species.

Among the fungal genera with highest sequence abundance in Rubiaceae roots, the only fungus found in all nine tree species was an unnamed species from the order Pleosporales (Fig. 2.6). *Randia matudae* was the only tree with a single fungal genus, an unidentified Helotiales, dominating the composition of its most abundant genera. This Helotiales had significantly higher abundance than expected on *Randia matudae,* X^2 (95, N =3462) = 1601.05, P = <0.0001. One unidentified Ascomycota ("Ascomycota 1", Fig. 2.6) was found significantly more than expected on coffee at all three sites (Cañitas: X^2 (76, N =3337) = 107.808, P = <0.0001; Monteverde: X^2 (95, N =3462) = 280.931, P = <0.0001; San Luis: X^2 (102, N =3759) = 209.994, P = <0.0001).

For the four tree species that occurred at all three sites, approximately 61% of the abundant fungal genera (27 out of 44) were found on roots of the three forest trees as well as on coffee roots, 11% (5 out of 44) were found only on coffee roots, and approximately 11% (5 out of 44) were found only on forest tree roots (i.e. they were not found on coffee; Fig. 2.7A). In comparing abundant fungal genera in coffee roots to those in bulk soils, it was found that 12% of genera were held in common between coffee roots, forest soils and coffee field soils, whereas coffee roots and coffee field bulk soils shared almost 50% (Fig. 2.7B). Almost one-third (27%) of the abundant fungal genera occurred forest soils but not in coffee roots or in coffee field bulk soils (Fig. 2.7B).

Table 2.1. Site and habitat effects on environmental characteristics for coffee fields and adjacent forest fragments at three sites in the Monteverde region of Costa Rica. Values are means ± standard error. Means that share a letter do not differ statistically by Tukey HSD. Only variables that had at least one significant effect are shown. Soil temperature values are the mean of continuous daily measurements at each site for 2-4 weeks.

	Cañitas (elev: 1250masl; 10.3008, -84.8152)		Monteverde (elev: 1350masl; 10.2865, -84.8024)		San Luis (elev: 1120masl 10.3249, -84.8416)		Habitat		Site		Site×Habitat	
	Coffee	Forest	Coffee	Forest	Coffee	Forest	$F_{1, 89}$	p	$F_{2, 89}$	p	$F_{2, 89}$	p
pH	5.6 ± 0.11^{b}	6.3 ± 0.08^{a}	5.8 ± 0.05^{b}	6.1 ± 0.05^{a}	5.6 ± 0.05^{b}	6.3 ± 0.05^{a}	107.60	9.392 E-16	0.08	0.9248	4.91	0.01544
NO-3-N (kg/ha)	75 ± 15.0^{d}	120 ± 8.61^{bc}	133 ± 13.1^{bc}	153 ± 10.5^{b}	264 ± 36.1^{a}	102 ± 6.51^{cd}	1.12	0.3884	17.93	1.236 E-06	31.96	2.980 E-10
% Organic Matter	11.6 ± 0.46^{c}	16.8 ± 0.94^{b}	15.7 ± 0.62^{b}	21.8 ± 0.59^{a}	18.9 ± 0.88^{ab}	19.7 ± 0.56^{a}	38.26	9.797 E-08	22.62	6.553 E-08	7.01	0.002990
P (ppm)	29 ± 4.69^{a}	2.5 ± 0.24^{d}	20.6 ± 4.07^{ab}	2.8 ± 0.22^{d}	7.2 ± 0.53^{b}	3.8 ± 0.19^{c}	179.59	3.188 E-21	0.30	0.7728	15.32	6.274 E-06
K (ppm)	158.5 ± 25.73^{ab}	204.9 ± 25.16^{a}	78 ± 9.56^{c}	121.6 ± 19.08^{bc}	256.5 ± 41.82^{a}	264.4 ± 22.19^{a}	7.00	0.01544	42.99	1.015 E-12	0.42	0.5117
Zn (ppm)	3.2 ± 0.5^{b}	3.8 ± 0.35^{b}	12.5 ± 3.53^{a}	4 ± 0.59^{b}	3.3 ± 0.33^{b}	3.5 ± 0.22^{b}	4.44	0.05699	6.44	0.004555	9.16	0.0005732
Fe (ppm)	50.4 ± 4.39^{c}	63.9 ± 3.46^{bc}	62.5 ± 1.94^{c}	60.7 ± 2.11^{c}	83.9 ± 3.22^{a}	74 ± 2.01^{ab}	0.07	0.8171	28.95	1.581 E-09	7.05	0.002990
Mn (ppm)	4.4 ± 0.5^{c}	9.4 ± 0.82^{ab}	7.5 ± 0.53^{b}	11 ± 0.38^{a}	11.4 ± 0.57^{a}	10.2 ± 0.47^{a}	23.06	1.743 E-05	17.35	1.758 E-06	13.06	2.825 E-05
Cu (ppm)	2.6 ± 0.38^{b}	3.6 ± 0.25^{ab}	4.5 ± 0.3^{a}	3 ± 0.15^{b}	2.6 ± 0.25^{b}	3 ± 0.13^{b}	0.03	0.8720	9.78	0.0003577	15.20	6.583 E-06
Ca^{+2} (ppm)	1169 ± 153^{c}	2912 ± 232^{a}	1916 ± 157^{bc}	2538 ± 138^{ab}	2475 ± 161^{ab}	3091 ± 126^{a}	42.16	2.927 E-08	8.20	0.001194	5.41	0.01044
Mg^{+2}(ppm)	97.1 ± 13.99^{d}	216.4 ± 11.97^{bc}	162.2 ± 21.74^{cd}	257.2 ± 16.05^{ab}	252.4 ± 33.07^{abc}	304.2 ± 15.27^{a}	28.50	2.581 E-06	16.58	2.621 E-06	1.33	0.3657
NH4-N ppm	8.8 ± 0.94^{b}	8.4 ± 0.37^{b}	9.9 ± 0.87^{a}	11.6 ± 0.61^{a}	9.1 ± 0.41^{ab}	9.7 ± 0.47^{ab}	1.52	0.3117	6.44	0.004555	1.08	0.4335
% Soil Humidity	20 ± 1^{b}	20 ± 1^{b}	20 ± 1^{b}	30 ± 2^{a}	30 ± 1^{a}	30 ± 1^{a}	24.83	1.004 E-05	34.75	1.252 E-10	8.16	0.001270
PAR (umol m^{-2} s^{-1})	1170.7 ± 159.22^{a}	71.8 ± 36.76^{b}	1185.3 ± 259.79^{a}	73.1 ± 64.81^{b}	934 ± 232.21^{a}	64.7 ± 40.33^{b}	146.35	1.417 E-17	0.71	0.5826	0.69	0.5826
							$F_{1, 435}$	p	$F_{2, 435}$	p	$F_{2, 435}$	p
Mean Soil Temp. (°C)	19.9 ± 0.15^{c}	18.08 ± 0.06^{d}	20.96 ± 0.15^{b}	18.31 ± 0.07^{d}	22.22 ± 0.07^{a}	20.22 ± 0.04^{c}	553.78	<0.0001	326.02	<0.0001	5.76	0.0034
Min. Soil Temp. (°C)	18.11 ± 0.13^{c}	17.77 ± 0.05^{d}	19.84 ± 0.1^{b}	18.09 ± 0.06^{cd}	21.59 ± 0.06^{a}	19.63 ± 0.06^{b}	281.0	<0.0001	598.91	<0.0001	59.37	<0.0001
Max. Soil Temp. (°C)	23.82 ± 0.73^{b}	18.39 ± 0.06^{a}	22.51 ± 0.29^{d}	18.65 ± 0.09^{a}	22.85 ± 0.08^{e}	20.92 ± 0.06^{c}	562.56	<0.0001	340.97	<0.0001	6.96	0.0011

Table 2.2. Soil environmental characteristics for nine plant species in the family Rubiaceae at three sites in the Monteverde region of Costa Rica. Values are means ± SE. Means that share a letter do not differ statistically by Tukey HSD. F-ratios and false discovery rate P-values from a response screening of the data are presented. Only variables that had at least one significant effect are shown.

Site	Tree Species	pH	NO-3-N (kg/ha)	% Organic material	P (ppm)	Fe (ppm)	Mn (ppm)	% Humidity	Ca (ppm)
Cañitas	*Coffea arabica*	5.56 ± 0.11^{d}	75.1 ± 15.0^{c}	11.6 ± 0.46^{e}	29 ± 4.69^{a}	50.4 ± 4.39^{c}	4.4 ± 0.5^{d}	15.7 ± 0.9^{e}	1169 ± 153^{c}
	Hamelia patens ¥	6.33 ± 0.03	112 ± 7.46	19 ± 2.09	2.7 ± 0.67	61.8 ± 4.91	11.2 ± 3.44	20.4 ± 2.3	2640 ± 277
	Psychotria panamensis	6.77 ± 0.32^{a}	109 ± 29.6^{bc}	11.7 ± 2.15^{de}	3 ± 1.15^{c}	47.2 ± 14.33^{c}	7.3 ± 0.6^{bcd}	20.9 ± 2.4^{cde}	3667 ± 1158^{a}
	Psychotria quinqueradiata	6.30 ± 0.09^{bc}	99.5 ± 7.66^{bc}	15.7 ± 0.77^{bcde}	2 ± 0.32^{c}	68.4 ± 2.74^{abc}	7.3 ± 0.8^{cd}	21.3 ± 1.7^{de}	2310 ± 267^{abc}
	Palicourea valerioana	6.25 ± 0.08^{ab}	146 ± 15.0^{ab}	19.1 ± 1.21^{abc}	2.7 ± 0.21^{c}	69.6 ± 4.4^{abc}	11.4 ± 0.98^{ab}	23.5 ± 1.1^{bcde}	3172 ± 137^{a}
Monteverde	*Coffea arabica*	5.75 ± 0.05^{cd}	133 ± 13.1^{b}	15.7 ± 0.62^{cd}	20.6 ± 4.07^{a}	62.5 ± 1.94^{bc}	7.5 ± 0.53^{c}	20.3 ± 0.6^{e}	1916 ± 157^{bc}
	Randia matudae¥	6.05 ± 0.06	136 ± 3.81	22.3 ± 0.5	2.5 ± 0.1	67 ± 1.2	13 ± 8.7	23.7 ± 2.3	2347 ± 159
	Psychotria monteverdensis ¥	6.03 ± 0.02	138 ± 5.27	22.2 ± 0.1	2.3 ± 0.1	52.8 ± 0.8	8.7 ± 8.7	22.1 ± 2	2897 ± 35
	Psychotria panamensis	6.15 ± 0.14^{bc}	165 ± 14.2^{ab}	22.3 ± 1.76^{a}	3 ± 0.32^{bc}	60 ± 6.74^{bc}	11.3 ± 0.58^{ab}	31.6 ± 3.4^{abc}	2464 ± 327^{ab}
	Psychotria quinqueradiata	6.27 ± 0.08^{b}	174 ± 34.8^{ab}	21.7 ± 1.06^{a}	2.6 ± 0.43^{c}	64.6 ± 3.8^{bc}	11.9 ± 0.77^{a}	32.9 ± 2.3^{a}	2766 ± 218^{ab}
	Palicourea valerioana	6.08 ± 0.04^{bc}	149 ± 15.9^{ab}	21.2 ± 1.59^{a}	3.6 ± 0.87^{bc}	53.3 ± 4.06^{c}	9.3 ± 0.52^{abc}	33.6 ± 4.9^{a}	2376 ± 282^{abc}
San Luis	*Coffea arabica*	5.62 ± 0.05^{d}	264 ± 36.1^{a}	18.9 ± 0.88^{abc}	7.2 ± 0.53^{ab}	83.9 ± 3.22^{a}	11.4 ± 0.57^{a}	31.3 ± 0.6^{ab}	2475 ± 161^{ab}
	Randia grandifolia¥	6.36 ± 0.03	110 ± 3.92	21 ± 0.3	3.4 ± 0.1	71.9 ± 1.3	9.7 ± 8.7	27.7 ± 2.1	3344 ± 69
	Palicourea pubescens¥	6.28 ± 0.03	102 ± 5.38	18.9 ± 0.5	4.8 ± 0.2	79.9 ± 0.8	13 ± 8.7	31 ± 0.4	3218 ± 67
	Hamelia patens ¥	6.03 ± 0.07	128 ± 6.85	19.4 ± 0.55	4.3 ± 0.33	79.6 ± 2.4	10.6 ± 0.64	29.3 ± 2.9	3080 ± 277
	Psychotria panamensis	6.22 ± 0.08^{ab}	89.9 ± 9.52^{bc}	18 ± 0.46^{abcd}	3.4 ± 0.24^{bc}	76.7 ± 3.03^{ab}	9.5 ± 0.72^{abc}	30.9 ± 1.1^{abcd}	3102 ± 175^{a}
	Psychotria quinqueradiata	6.43 ± 0.07^{ab}	97.2 ± 8.45^{bc}	22 ± 0.87^{ab}	3.3 ± 0.33^{bc}	65.3 ± 4.68^{abc}	8.7 ± 0.52^{abc}	33.2 ± 1.5^{abc}	2347 ± 459^{abc}
	Palicourea valerioana	6.60 ± 0^{ab}	51.6 ± 0^{bc}	18.1 ± 0^{abcde}	4 ± 0^{abc}	57.2 ± 0^{abc}	7.8 ± 0^{abcd}	27.7 ± 0^{abcde}	3520 ± 0^{ab}
Site	$F_{2,58}$	3.29	8.43	28.73	1.01	4.48	10.76	27.72	1.99
	p	0.08737	0.002013	2.940 E-08	0.4262	0.03465	0.0004306	6.005 E-08	0.2179
Species	$F_{3,58}$	43.83	0.94	10.88	41.96	0.94	4.54	8.71	13.29
	p	2.188 E-13	0.4691	5.196 E-05	3.458 E-13	0.4691	0.01503	0.0003824	7.144 E-06
Site × Species	$F_{6,58}$	4.81	9.71	4.37	3.23	5.2	11.43	3.43	4
	p	0.001653	1.870 E-06	0.002912	0.01911	0.0009291	2.100 E-07	0.01501	0.005358

¥This species did not occur at all three sites.

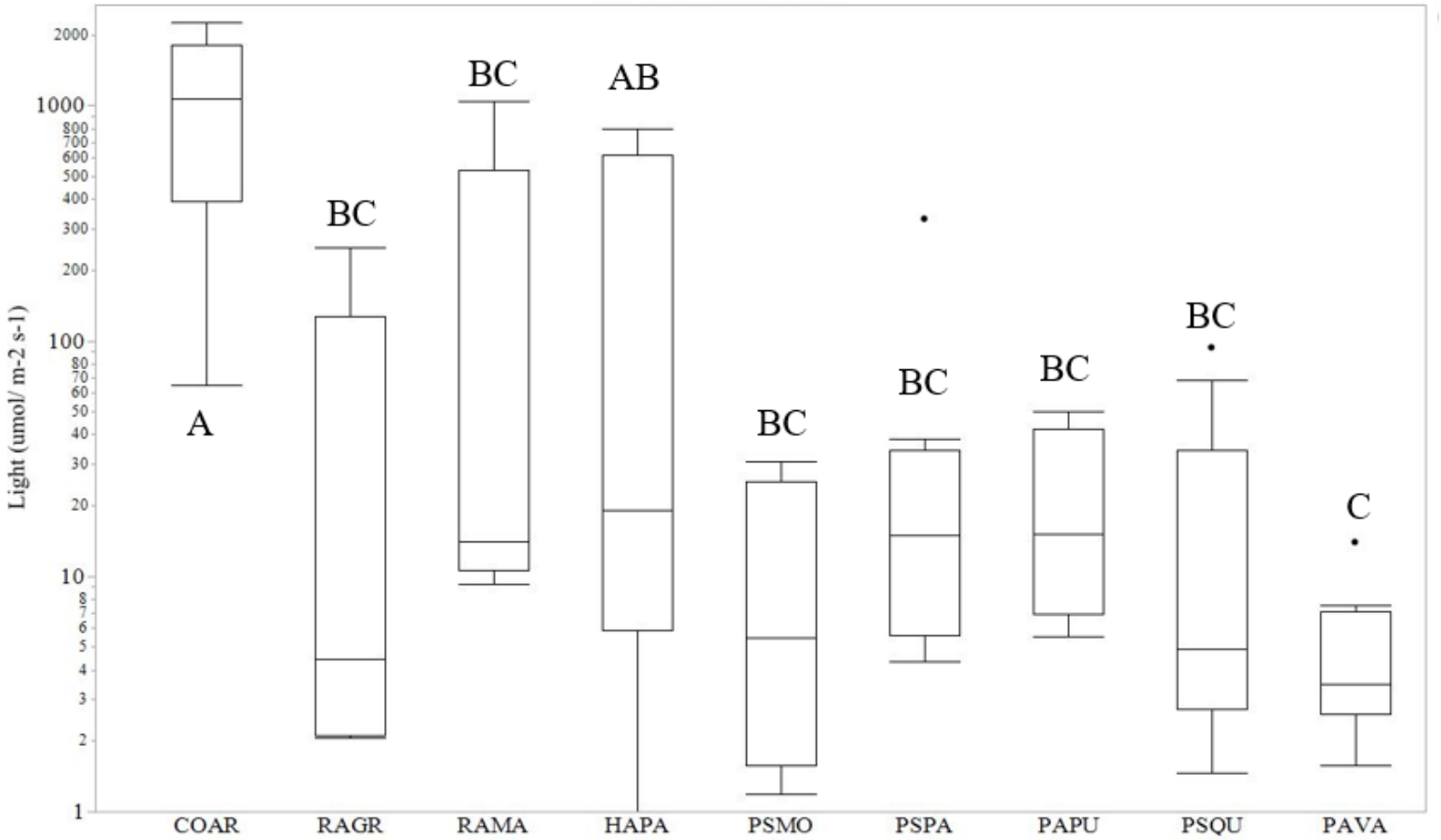

Figure 2.2. Light availability for nine plant species in the Rubiaceae, ranked in order of increasing phylogenetic distance to coffee and labeled using the first two letters of the genus and first two letters of the species. Values for light are plotted on a log scale. Light values with a shared letter did not differ statistically by Tukey HSD.Light availability differed by species ($F_{8,86}$=3.7630, P=0.0008).

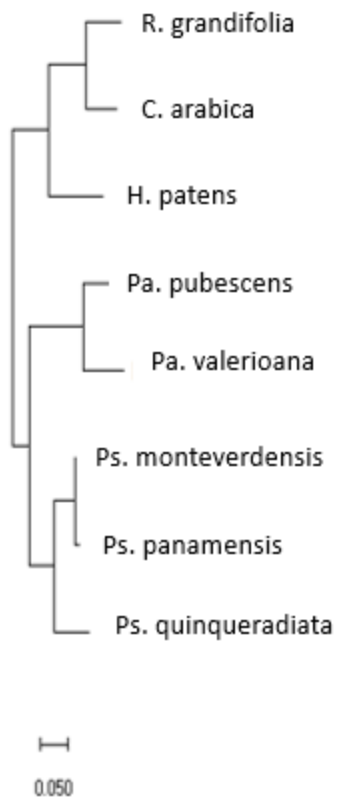

Figure 2.3. Phylogeny of eight Rubiaceae species in three sites near Monteverde, Costa Rica. Tree was constructed using NCBI sequences 605-711bp long, and which spanned the 18S, ITS1, 5.8S, ITS2 and 26S regions of plant DNA. Branch length is number of nucleotide substitutions. Sequences were aligned using MUSCLE and maximum likelihood tree was generated using the TN93 model.

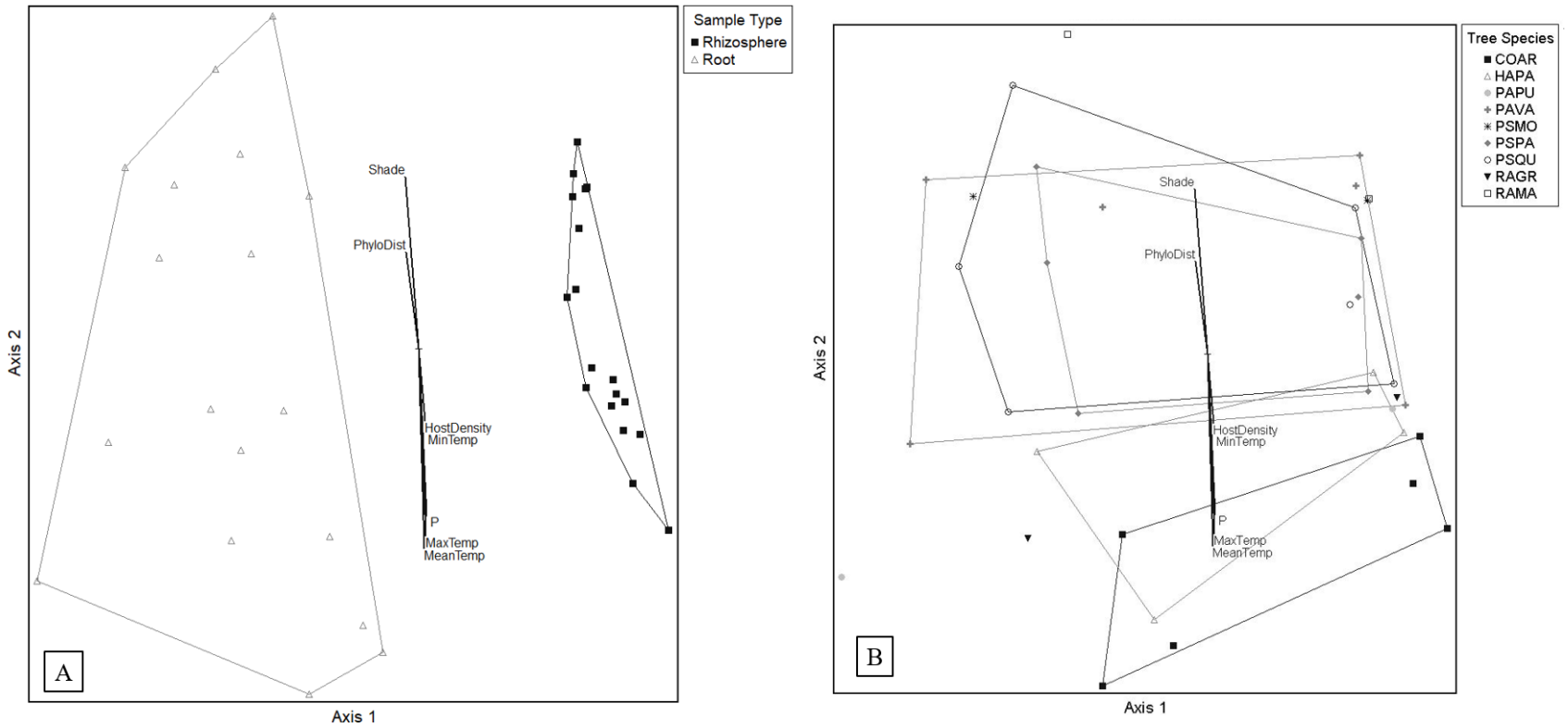

Figure 2.4. Non-metric multidimensional scaling ordination of fungal communities in forest and coffee fields in Costa Rica (final stress = 9.34). The best solution was three dimensional and two axes are shown here. Environmental characteristics and phylogenetic distance from coffee ("PhyloDist") were transformed as necessary and the bi-plot overlay shows these data in relation to fungal communities. OTU abundances were rarefied and Hellinger-transformed prior to analysis, and only OTUs that occurred in >= 10% of samples were included. Ordinations show root (N=18) and rhizosphere (N=18) fungal communities in coffee and forest trees grouped by A.) sample type and B.) host tree species, abbreviated with the first two letters of the plant's genus followed by the first two letters of its species. Axis 1 accounted for 46.6%, Axis 2 accounted for 28.2%, and Axis 3 (not shown) accounted for 11.5% of the variation in fungal communities among samples.

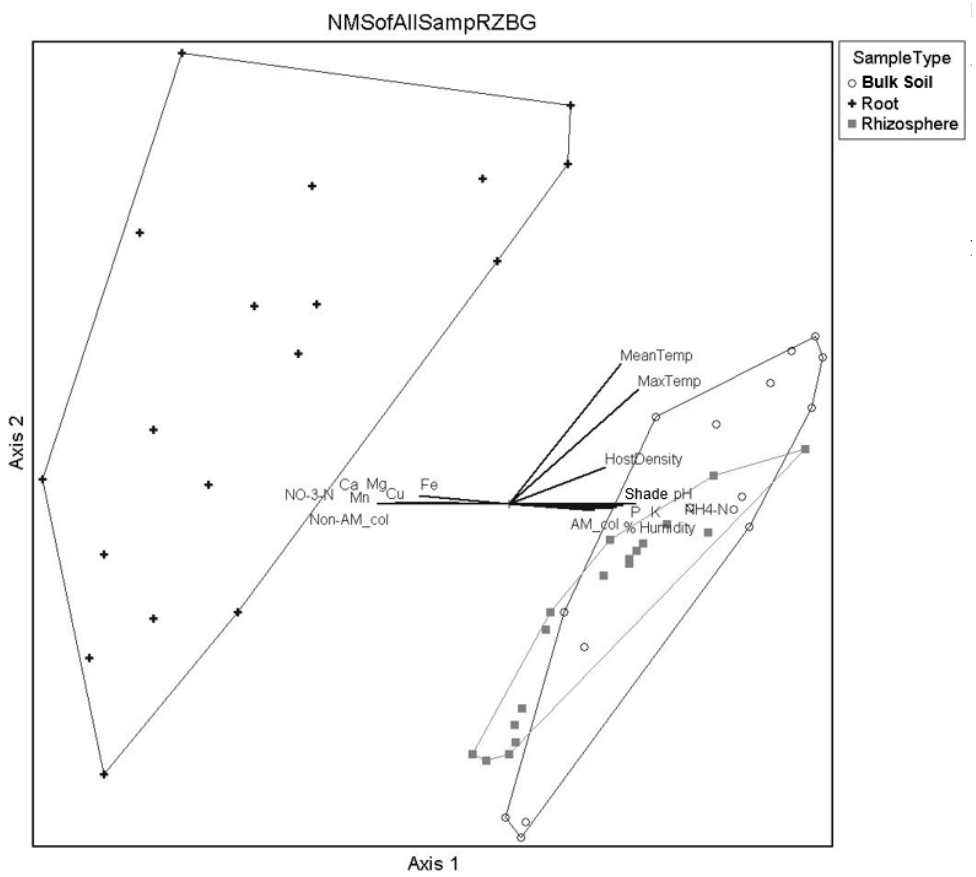

Figure 2.5. Non-metric multidimensional scaling ordination of fungal communities in forest and coffee fields in Costa Rica (final stress = 8.59705). The best solution was three-dimensional and two of three axes are shown. Environmental characteristics were transformed as necessary and the bi-plot overlay shows these data in relation to fungal communities. OTU abundances were rarefied and Hellinger-transformed prior to analysis, and only OTUs that occurred in >= 10% of samples were included. Ordinations show root (N=18), rhizosphere (N=18) and bulk soil (N=16) fungal communities in coffee and forest trees grouped by sample type. Axis 1 accounted for 43.6%, Axis 2 accounted for 31.0%, and Axis 3 (not shown) accounted for 11.7% of the variation in fungal communities among samples.

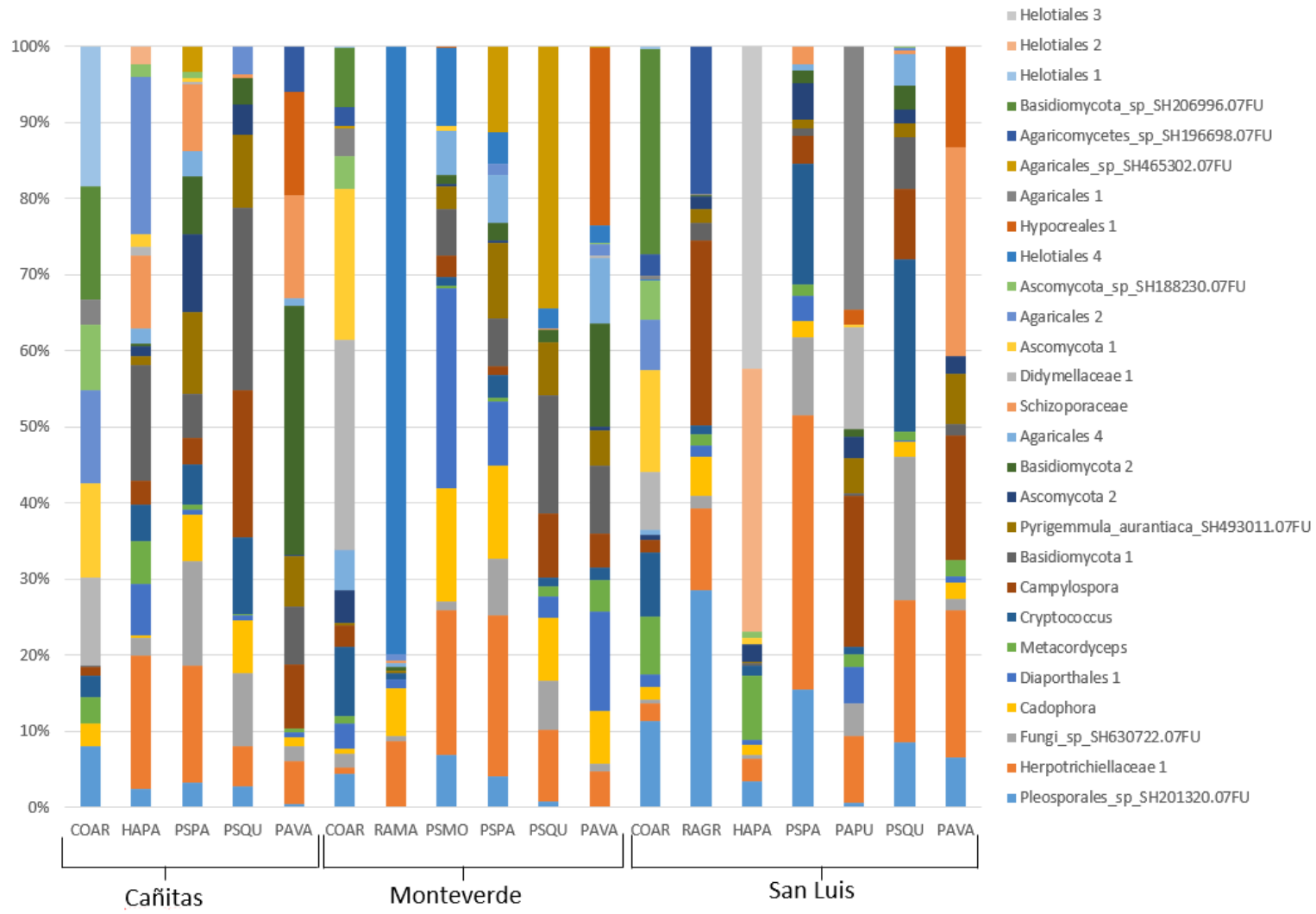

Figure 2.6. Relative abundance of fungal genera with a sequence abundance greater than 200 across all nine species of Rubiaceae at three sites in the Monteverde region of Costa Rica.

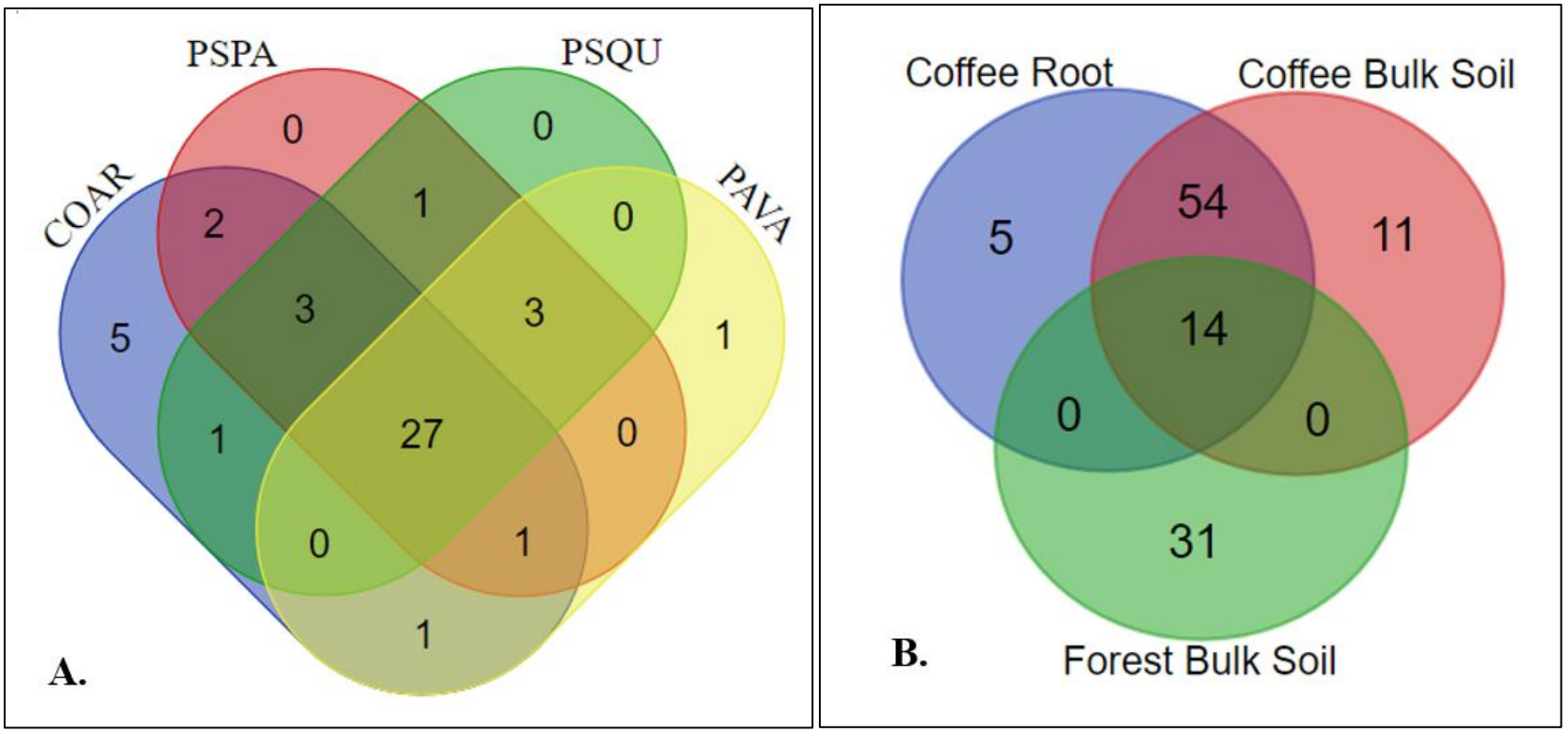

Figure 2.7. Venn diagrams showing number of shared and unique fungal genera in A.) roots of four plant species in the Rubiaceae found at three sites in Costa Rica, and B.) coffee roots, and bulk soil from coffee fields and bulk soil from forest habitats at the same three sites. Fungal OTUs with a sequence abundance ≥ were included in A. and the 50 most abundant OTUs from each sample type were included in B. Four-letter abbreviations are the first two letters of genus and species names for the Rubiaceae species at all sites.

Discussion

My results showed that 1.) fungal community composition differed between sites and tree species, 2.) soil environment differed by site and tree species, and 3.) light availability and phylogenetic relatedness of the plant were both important for describing differences in root fungal communities.

Coffee is cultivated extensively in the Americas, outside its native range, and thus grows in a different environment from that in which it evolved (De Beenhouwer et al., 2015). Since belowground fungi are an important part of the plant's biotic environment, it is important to discover what influences the community composition of these fungi. According to our study, host microclimate, and light availability in particular, seems to be important for differences in fungal community composition of roots across plant species in the Rubiaceae. Additionally, phylogenetically conserved plant traits of the host tree may be important for structuring fungal community structure, though fungal community differences that are correlated with host relatedness may instead be responses to differences in host microclimate.

Light Availability

This dataset showed that light availability was the only environmental characteristic that differed between tree species regardless of site. Small variations in light availability can have important consequences for tree niche (Chazdon and Fetcher, 1984). In forest habitats, canopy gaps and heavily shaded areas create nuanced light niches (Chazdon and Fetcher, 1984; Feng et al., 2018). Even sporadic sun flecks can drastically increase the carbon gain of a plant (Chazdon and Fetcher, 1984). In areas of full sun, small variations in light may not be as important since the plant is at full photosynthetic capacity much of the time (Chazdon and Fetcher, 1984). Tree species with lower light requirements have a lower photosynthetic rate, which they compensate

for with reduced respiration and less allocation of photosynthate to functions beyond basic growth and reproduction. Some of the tree species in this study had light environments that were easily perceptible in the field. Coffee was grown in full sun, and accordingly it had the highest light availability. *Hamelia patens* was most commonly found on the forest edge, where the tree was only partially shaded. Other species in the forest were less easily differentiated. *Palicourea valerioana* had the lowest light availability, and though this was not distinguishable to the eye, it was reflected in the general growth pattern of the tree, which was consistently a small shrubby tree that never grew above two meters while other species sometimes reached several meters in height (*personal observation*). This difference based on species could also explain why shade and phylogenetic distance correlate tightly when looking at differences in fungal communities

Differences in light availability can translate to effects on belowground microbial communities. In this study, root communities showed that coffee has the most unique species, and *Pa. valerioana* has the fewest shared OTUs with coffee. In roots as compared to bulk soils, the most shared OTUs were in coffee fields, while forest soils tended to have fewer of the fungi that were present in coffee roots or soils. This could mean that light creates niches that influence which fungi can survive there, whether because of the responses of the plant host to light availability, or because of changes in the soil environment due to light. Plants use photosynthesis to convert light energy to potential energy stored in carbon-to-carbon bonds. Some of these molecules leave the plant in the form of carbon-rich root exudates (Nakayama and Tateno, 2018). Quantity of root exudates produced by a plant is dependent on how much solar radiation the plant receives (Nakayama and Tateno, 2018). High soil temperature and high light exposure work together to generate more root exudates by altering the plant's rate of photosynthesis and respiration (Nakayama and Tateno, 2018; Pramanik, Tagai, Asao, Matsui, 2000; Vuković et al.,

2013). The plant secretes the exudates into the soil and microbes use these compounds as energy sources to initiate the process of decomposition (Nakayama and Tateno, 2018). In this way, light availability of a plant influences the resources allocated to belowground fungi. Additionally, in areas of high light availability, more solar radiation reaches the soil, increasing soil temperature and stimulating microbial activity and decomposition (Tang et al., 2005; Zhang et al., 2018). A link between high light and increased decomposition is supported by our data because in the coffee habitat, light availability was highest, as was soil temperature, and organic matter was lowest. Solar radiation and the accompanying increase in soil temperature may have stimulated saprobes and thereby led to more decomposition, releasing organic matter from the soil through cellular respiration and thereby decreasing amounts of organic matter in the soil. The difference in organic matter could also be affected by intentional human removal of organic matter from coffee fields in the form of routine weed removal or as coffee berries collected during harvest.

Phylogenetic Distance

Phylogenetically conserved plant traits can be important for shaping belowground fungal communities, and this could help explain why we found phylogenetic distance of the plant host to be related to differences in fungal communities in the roots and rhizosphere. Comas et al (2014) found that root length, diameter, and branching intensity differed between plant species and showed a phylogenetic signal, with each of these variables showing patterns that are more similar in closely related plants than in plants that were distantly related. Root fungi such as pathogens and those forming mycorrhizas selectively exploit roots based on their morphology (Comas et al., 2014). Similarly, aboveground fungal plant pathogens exhibit similar levels of pathogenicity on closely-related plants and are less likely to infect phylogenetically distant hosts (Parker et al., 2015). Differences in physiology mean that plant species release root exudates at

different rates, and in different quantities, and of differing compositions (Epron et al., 2011). Root exudates serve as resource pools for fungi, so a plant attracts fungi by exuding compounds that the fungus can use (Rasmann and Turlings, 2016). Plants not only attract mutualists and commensals with these compounds, but pathogens as well (Rasmann and Turlings, 2016). In both the case of root morphology and exudates, fungi respond to a phylogenetically conserved plant trait, so, transitively, the fungal community is influenced by plant phylogeny. Although we did not measure plant traits such as root exudate production and composition in this study, to the extent that such traits are conserved across closely-related plant species, phylogenetic distance can act as a proxy (Mazel et al., 2018).

Other Environmental Differences

Several environmental characteristics with important effects on fungal communities differed between forest and coffee habitats. Phosphorus availability was correlated with differences in community composition based on tree species. Phosphorus availability differed by tree species, and this difference was driven by the high P levels in coffee fields. Since high phosphorus naturally occurs in soils that are only slightly acidic (Roques et al., n.d.), it is likely that the high P levels in coffee were related to fertilization.. Additionally, mycorrhizal fungi are important for P uptake by plants, so it is possible that low soil P was due to forest fungal communities that were more effective at taking up and using P (Bulgarelli et al., 2017). Soils in coffee fields were more acidic than forest soils. This may be related to the effects of common agricultural practices on soils. Chemical fertilizers tend to contain nitrates or ammonium that cause soil acidification (Barak et al., 1997). Additionally, increased aboveground biomass has been linked to higher soil pH (Fu et al., 2015b; Wartenberg et al., 2017). Since diverse and multi-storied forests have much higher aboveground biomass than coffee fields, which have a single

predominant plant species, this could be another explanation for the higher pH in forest soils. Micronutrients can contribute to soil acidity (Roques et al., n.d.), and in this study, several micronutrients (e.g. Mn, Cu) differed by habitat, and were correlated with differences in fungal community composition between roots and soils across tree species. Since both forest and coffee soils were acidic, the ranges where these micronutrients were most influential applies to both habitats. Magnesium is one exception, since it tends to be high from between 6.5 and 8.5 pH (Roques et al., n.d.). The less-acidic forest soils align more closely with this nutrient, and indeed, we saw that Mg^{+2} was higher in these soils. Fertilization can spur a magnesium deficiency in the soil (Yu-Chuan et al., n.d.) so it is possible this difference is related to agricultural management in the coffee field. Also, saprotrophic fungi help plants take up Mg^{+2} by breaking down leaf litter and releasing the nutrients back into the soil where plants can access it (Kimmig et al., 2018) so it is possible that forest fungal communities had a saprotroph community that was effective at replenishing soil magnesium.

Conclusion

Belowground fungi form diverse communities that are essential to plant health and ecosystem function (Tedersoo et al., 2014), but our understanding of how these communities are structured is still narrow. This study shows that both the microclimate and the phylogenetic relatedness of the host plant are important in predicting belowground fungal community composition. We found that light availability is an important environmental predictor, but that because light differences were strongly linked to individual tree species, the effect of light on fungal community composition cannot be definitively separated from a more direct host effect.

LITERATURE CITED

Abreo, E., Martinez, S., Bettucci, L., Lupo, S., 2010. Morphological and molecular characterisation of Campylocarpon and Cylindrocarpon spp. associated with black foot disease of grapevines in Uruguay. Australas. Plant Pathol. 39, 446. https://doi.org/10.1071/AP10021

Agtmaal, M. van, Straathof, A., Termorshuizen, A., Teurlincx, S., Hundscheid, M., Ruyters, S., Busschaert, P., Lievens, B., Boer, W. de, 2017. Exploring the reservoir of potential fungal plant pathogens in agricultural soil. Appl. Soil Ecol. 121, 152–160. https://doi.org/10.1016/j.apsoil.2017.09.032

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Andrade, S. a. L., Mazzafera, P., Schiavinato, M.A., Silveira, A.P.D., 2009. Arbuscular mycorrhizal association in coffee. J. Agric. Sci. 147, 105–115. https://doi.org/10.1017/S0021859608008344

Anthony, M.A., Frey, S.D., Stinson, K.A., 2017. Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. Ecosphere 8, n/a-n/a. https://doi.org/10.1002/ecs2.1951

Avelino, J., Cabut, S., Barboza, B., Barquero, M., Alfaro, R., Esquivel, C., Durand, J.-F., Cilas, C., 2007. Topography and Crop Management Are Key Factors for the Development of American Leaf Spot Epidemics on Coffee in Costa Rica. Phytopathology 97, 1532–1542. https://doi.org/10.1094/PHYTO-97-12-1532

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C., Wall, D.H., 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biol. Biochem. 41, 606–610. https://doi.org/10.1016/j.soilbio.2008.12.022

Bagyaraj, D.J., Thilagar, G., Ravisha, C., Kushalappa, C.G., Krishnamurthy, K.N., Vaast, P., 2015. Below ground microbial diversity as influenced by coffee agroforestry systems in the Western Ghats, India. Agric. Ecosyst. Environ. 202, 198–202. https://doi.org/10.1016/j.agee.2015.01.015

Barak, P., Jobe, B.O., Krueger, A.R., Peterson, L.A., Laird, D.A., 1997. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. Plant Soil 197, 61–69.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4, 914–919. https://doi.org/10.1111/2041-210X.12073

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300.

Bidartondo, M.I., Burghardt, B., Gebauer, G., Bruns, T.D., Read, D.J., 2004. Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. Proc. R. Soc. B Biol. Sci. 271, 1799–1806. https://doi.org/10.1098/rspb.2004.2807

Bremer, B., Eriksson, T., 2009. Time Tree of Rubiaceae: Phylogeny and Dating the Family, Subfamilies, and Tribes. Int. J. Plant Sci. 170, 766–793. https://doi.org/10.1086/599077
Bulgarelli, R.G., Marcos, F.C.C., Ribeiro, R.V., de Andrade, S.A.L., 2017. Mycorrhizae enhance nitrogen fixation and photosynthesis in phosphorus-starved soybean (Glycine max L. Merrill). Environ. Exp. Bot. 140, 26–33. https://doi.org/10.1016/j.envexpbot.2017.05.015
Buttigieg, P.L., Ramette, A., 2014. A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. FEMS Microbiol. Ecol. 90, 543–550. https://doi.org/10.1111/1574-6941.12437
Byrnes, J.E., Reynolds, P.L., Stachowicz, J.J., 2007. Invasions and Extinctions Reshape Coastal Marine Food Webs. PLoS ONE 2, e295. https://doi.org/10.1371/journal.pone.0000295
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303
Castaño, C., Lindahl, B.D., Alday, J.G., Hagenbo, A., Martínez de Aragón, J., Parladé, J., Pera, J., Bonet, J.A., 2018. Soil microclimate changes affect soil fungal communities in a Mediterranean pine forest. New Phytol. 220, 1211–1221. https://doi.org/10.1111/nph.15205
Chazdon, R.L., Fetcher, N., 1984. Photosynthetic Light Environments in a Lowland Tropical Rain Forest in Costa Rica. J. Ecol. 72, 553. https://doi.org/10.2307/2260066
Comas, L.H., Callahan, H.S., Midford, P.E., 2014. Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground strategies. Ecol. Evol. 4, 2979–2990. https://doi.org/10.1002/ece3.1147
Dassen, S., Cortois, R., Martens, H., de Hollander, M., Kowalchuk, G.A., van der Putten, W.H., De Deyn, G.B., 2017. Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. Mol. Ecol. 26, 4085–4098. https://doi.org/10.1111/mec.14175
De Beenhouwer, M., Muleta, D., Peeters, B., Van Geel, M., Lievens, B., Honnay, O., 2015a. DNA pyrosequencing evidence for large diversity differences between natural and managed coffee mycorrhizal fungal communities. Agron. Sustain. Dev. 35, 241–249.
De Beenhouwer, M., Van Geel, M., Ceulemans, T., Muleta, D., Lievens, B., Honnay, O., 2015b. Changing soil characteristics alter the arbuscular mycorrhizal fungi communities of Arabica coffee (Coffea arabica) in Ethiopia across a management intensity gradient. Soil Biol. Biochem. 91, 133–139. https://doi.org/10.1016/j.soilbio.2015.08.037
de Boer, T.E., Taş, N., Braster, M., Temminghoff, E.J.M., Röling, W.F.M., Roelofs, D., 2012. The Influence of Long-Term Copper Contaminated Agricultural Soil at Different pH Levels on Microbial Communities and Springtail Transcriptional Regulation. Environ. Sci. Technol. 46, 60–68. https://doi.org/10.1021/es2013598
Delgado-Baquerizo, M., García-Palacios, P., Milla, R., Gallardo, A., Maestre, F.T., 2015. Soil characteristics determine soil carbon and nitrogen availability during leaf litter decomposition regardless of litter quality. Soil Biol. Biochem. 81, 134–142. https://doi.org/10.1016/j.soilbio.2014.11.009
Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives

multifunctionality in terrestrial ecosystems. Nat. Commun. 7, 10541. https://doi.org/10.1038/ncomms10541
Deshpande, V., Wang, Q., Greenfield, P., Charleston, M., Porras-Alfaro, A., Kuske, C.R., Cole, J.R., Midgley, D.J., Tran-Dinh, N., 2016. Fungal identification using a Bayesian classifier and the Warcup training set of internal transcribed spacer sequences. Mycologia 108, 1–5. https://doi.org/10.3852/14-293
Diepen, L.T.A. van, Frey, S.D., Landis, E.A., Morrison, E.W., Pringle, A., 2017. Fungi exposed to chronic nitrogen enrichment are less able to decay leaf litter. Ecology 98, 5–11. https://doi.org/10.1002/ecy.1635
Dixon, E.R., Cardenas, L., Alfaro, M., Salazar, F., Hatch, D.J., 2011. High rates of nitrogen cycling in volcanic soils from Chilean grasslands: High rates of nitrogen cycling in volcanic soils. Rapid Commun. Mass Spectrom. 25, 1521–1526. https://doi.org/10.1002/rcm.4969
Edwards, I.P., Zak, D.R., Kellner, H., Eisenlord, S.D., Pregitzer, K.S., 2011. Simulated Atmospheric N Deposition Alters Fungal Community Composition and Suppresses Ligninolytic Gene Expression in a Northern Hardwood Forest. PLoS ONE 6, e20421. https://doi.org/10.1371/journal.pone.0020421
Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., Eisen, J.A., Sundaresan, V., 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl. Acad. Sci. 112, E911–E920. https://doi.org/10.1073/pnas.1414592112
Ekanayaka, A.H., Hyde, K.D., Jones, E.B.G., Zhao, Q., 2018. Taxonomy and phylogeny of operculate discomycetes: Pezizomycetes. Fungal Divers. 90, 161–243. https://doi.org/10.1007/s13225-018-0402-z
Epron, D., Ngao, J., Dannoura, M., Bakker, M.R., Zeller, B., Bazot, S., Bosc, A., Plain, C., Lata, J.C., Priault, P., Barthes, L., Loustau, D., 2011. Seasonal variations of belowground carbon transfer assessed by in situ; pulse labelling of trees. Biogeosciences 8, 1153–1168. https://doi.org/10.5194/bg-8-1153-2011
Esmaeili Taheri, A., Hamel, C., Gan, Y., 2015. Pyrosequencing reveals the impact of foliar fungicide application to chickpea on root fungal communities of durum wheat in subsequent year. Fungal Ecol. 15, 73–81. https://doi.org/10.1016/j.funeco.2015.03.005
Evidente, A., 1995. Putaminoxin, a phytotoxic nonenolide from Phoma putaminum. Phytochemistry 40, 1637–1641. https://doi.org/10.1016/0031-9422(95)00505-2
Farr, D.F., Miller, M.E., Bruton, B.D., 1998. Rhizopycnis vagum Gen. et sp. nov., a New Coelomycetous Fungus from Roots of Melons and Sugarcane. Mycologia 90, 290–296. https://doi.org/10.2307/3761305
Feng, J., Zhao, K., He, D., Fang, S., Lee, T., Chu, C., He, F., 2018. Comparing shade tolerance measures of woody forest species. PeerJ 6, e5736. https://doi.org/10.7717/peerj.5736
Fitzgerald, D.B., Winemiller, K.O., Sabaj Pérez, M.H., Sousa, L.M., 2017. Using trophic structure to reveal patterns of trait-based community assembly across niche dimensions. Funct. Ecol. 31, 1135–1144. https://doi.org/10.1111/1365-2435.12838
Fitzpatrick, C.R., Copeland, J., Wang, P.W., Guttman, D.S., Kotanen, P.M., Johnson, M.T.J., 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. Proc. Natl. Acad. Sci. 115, E1157–E1165. https://doi.org/10.1073/pnas.1717617115

Fiuza, P., 2013. Ingoldian fungi from semiarid Caatinga biome of Brazil. The genus Campylospora. Mycosphere 4, 559–565. https://doi.org/10.5943/mycosphere/4/3/9
Fu, X., Yang, F., Wang, J., Di, Y., Dai, X., Zhang, X., Wang, H., 2015a. Understory vegetation leads to changes in soil acidity and in microbial communities 27years after reforestation. Sci. Total Environ. 502, 280–286. https://doi.org/10.1016/j.scitotenv.2014.09.018
Fu, X., Yang, F., Wang, J., Di, Y., Dai, X., Zhang, X., Wang, H., 2015b. Understory vegetation leads to changes in soil acidity and in microbial communities 27years after reforestation. Sci. Total Environ. 502, 280–286. https://doi.org/10.1016/j.scitotenv.2014.09.018
Fuhrman, J.A., 2009. Microbial community structure and its functional implications. Nature 459, 193–199. https://doi.org/10.1038/nature08058
Gao, W., Kou, L., Yang, H., Zhang, J., Müller, C., Li, S., 2016. Are nitrate production and retention processes in subtropical acidic forest soils responsive to ammonium deposition? Soil Biol. Biochem. 100, 102–109. https://doi.org/10.1016/j.soilbio.2016.06.002
Gramss, G., Ziegenhagen, D., Sorge, S., 1999. Degradation of Soil Humic Extract by Wood- and Soil-Associated Fungi, Bacteria, and Commercial Enzymes. Microb. Ecol. 37, 140–151. https://doi.org/10.1007/s002489900138
Grinhut, T., Hadar, Y., Chen, Y., 2007. Degradation and transformation of humic substances by saprotrophic fungi: processes and mechanisms. Fungal Biol. Rev. 21, 179–189. https://doi.org/10.1016/j.fbr.2007.09.003
Gweon, H.S., Oliver, A., Taylor, J., Booth, T., Gibbs, M., Read, D.S., Griffiths, R.I., Schonrogge, K., 2015. PIPITS: an automated pipeline for analyses of fungal internal transcribed spacer sequences from the Illumina sequencing platform. Methods Ecol. Evol. 6, 973–980. https://doi.org/10.1111/2041-210X.12399
Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K., Högberg, P., 2003. Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - a hypothesis based on field observations in boreal forest. New Phytol. 160, 225–238. https://doi.org/10.1046/j.1469-8137.2003.00867.x
Hutchison, L.J., Untereiner, W.A., Hiratsuka, Y., 1995. Knufia cryptophialidica gen. et sp. nov., a Dematiaceous Hyphomycete Isolated from Black Galls of Trembling Aspen (Populus tremuloides). Mycologia 87, 902–908. https://doi.org/10.2307/3760868
Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., Barea, J.-M., 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol. Fertil. Soils 37, 1–16. https://doi.org/10.1007/s00374-002-0546-5
Jezeer, R.E., Santos, M.J., Boot, R.G.A., Junginger, M., Verweij, P.A., 2018. Effects of shade and input management on economic performance of small-scale Peruvian coffee systems. Agric. Syst. 162, 179–190. https://doi.org/10.1016/j.agsy.2018.01.014
Kamiyama, A., Maria, I.C. de, Souza, D.C.C. de, Silveira, A.P.D. da, 2011. Percepção ambiental dos produtores e qualidade do solo em propriedades orgânicas e convencionais. Bragantia 70, 176–184. https://doi.org/10.1590/S0006-87052011000100024
Keiblinger, K.M., Schneider, M., Gorfer, M., Paumann, M., Deltedesco, E., Berger, H., Jöchlinger, L., Mentler, A., Zechmeister-Boltenstern, S., Soja, G., Zehetner, F., 2018. Assessment of Cu applications in two contrasting soils—effects on soil microbial activity and the fungal community structure. Ecotoxicology 27, 217–233. https://doi.org/10.1007/s10646-017-1888-y

Kimmig, S.R., Holmden, C., Bélanger, N., 2018. Biogeochemical cycling of Mg and its isotopes in a sugar maple forest in Québec. Geochim. Cosmochim. Acta 230, 60–82. https://doi.org/10.1016/j.gca.2018.03.020
Kivlin, S.N., Hawkes, C.V., 2016. Tree species, spatial heterogeneity, and seasonality drive soil fungal abundance, richness, and composition in Neotropical rainforests. Environ. Microbiol. 18, 4662–4673. https://doi.org/10.1111/1462-2920.13342
Kulmatiski, A., Anderson-Smith, A., Beard, K.H., Doucette-Riise, S., Mazzacavallo, M., Nolan, N.E., Ramirez, R.A., Stevens, J.R., 2014. Most soil trophic guilds increase plant growth: a meta-analytical review. Oikos 123, 1409–1419. https://doi.org/10.1111/oik.01767
Kumar S, Stecher G, Li M, Knyaz C, and Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
Kuo, H.-C., Hui, S., Choi, J., Asiegbu, F.O., Valkonen, J.P.T., Lee, Y.-H., 2015. Secret lifestyles of Neurospora crassa. Sci. Rep. 4. https://doi.org/10.1038/srep05135
Lammel, D.R., Azevedo, L.C.B., Paula, A.M., Armas, R.D., Baretta, D., Cardoso, E.J.B.N., 2015. Microbiological and faunal soil attributes of coffee cultivation under different management systems in Brazil. Braz. J. Biol. 75, 894–905. https://doi.org/10.1590/1519-6984.02414
Li, G., Kim, S., Han, S.H., Chang, H., Du, D., Son, Y., 2018. Precipitation affects soil microbial and extracellular enzymatic responses to warming. Soil Biol. Biochem. 120, 212–221. https://doi.org/10.1016/j.soilbio.2018.02.014
Lichtfouse, E. (Ed.), 2011. Genetics, Biofuels and Local Farming Systems, Sustainable Agriculture Reviews. Springer Netherlands, Dordrecht. https://doi.org/10.1007/978-94-007-1521-9
Liu, X., Lyu, S., Sun, D., Bradshaw, C.J.A., Zhou, S., 2017. Species decline under nitrogen fertilization increases community-level competence of fungal diseases. Proc. R. Soc. B Biol. Sci. 284, 20162621. https://doi.org/10.1098/rspb.2016.2621
Liu, X.Z., Chen, S.Y., 2003. Nutritional requirements of Pochonia chlamydospora and ARF18, fungal parasites of nematode eggs. J. Invertebr. Pathol. 83, 10–15. https://doi.org/10.1016/S0022-2011(03)00037-5
López-Rodríguez, G., Sotomayor-Ramírez, D., Amador, J.A., Schroder, E.C., 2015. Contribution of nitrogen from litter and soil mineralization to shade and sun coffee (Coffea arabica L.) agroecosystems 13.
Lyngbæk, A.E., Muschler, R.G., Sinclair, F.L., 2001. Productivity and profitability of multistrata organic versus conventional coffee farms in Costa Rica. Agrofor. Syst. 53, 205–213. https://doi.org/10.1023/A:1013332722014
Maaroufi, N.I., Nordin, A., Hasselquist, N.J., Bach, L.H., Palmqvist, K., Gundale, M.J., 2015. Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils. Glob. Change Biol. 21, 3169–3180. https://doi.org/10.1111/gcb.12904
Maharachchikumbura, S.S.N., Hyde, K.D., Jones, E.B.G., McKenzie, E.H.C., Huang, S.-K., Abdel-Wahab, M.A., Daranagama, D.A., Dayarathne, M., D'souza, M.J., Goonasekara, I.D., Hongsanan, S., Jayawardena, R.S., Kirk, P.M., Konta, S., Liu, J.-K., Liu, Z.-Y., Norphanphoun, C., Pang, K.-L., Perera, R.H., Senanayake, I.C., Shang, Q., Shenoy, B.D., Xiao, Y., Bahkali, A.H., Kang, J., Somrothipol, S., Suetrong, S., Wen, T., Xu, J., 2015. Towards a natural classification and backbone tree for Sordariomycetes. Fungal Divers. 72, 199–301. https://doi.org/10.1007/s13225-015-0331-z

Matsushima, M., Chang, S., 2007. Effects of understory removal, N fertilization, and litter layer removal on soil N cycling in a 13-year-old white spruce plantation infested with Canada bluejoint grass. https://doi.org/10.1007/s11104-007-9220-x

Mazel, F., Davis, K.M., Loudon, A., Kwong, W.K., Groussin, M., Parfrey, L.W., 2018. Is Host Filtering the Main Driver of Phylosymbiosis across the Tree of Life? mSystems 3. https://doi.org/10.1128/mSystems.00097-18

Melloni, R., Costa, N.R., Melloni, E.G.P., Lemes, M.C.S., Alvarenga, M.I.N., Nunes Neto, J., 2018. Sistemas agroflorestais cafeeiro-araucária e seu efeito na microbiota do solo e seus processos. Ciênc. Florest. 28, 784. https://doi.org/10.5902/1980509832392

Morgenstern, I., Klopman, S., Hibbett, D.S., 2008. Molecular Evolution and Diversity of Lignin Degrading Heme Peroxidases in the Agaricomycetes. J. Mol. Evol. 66, 243–257. https://doi.org/10.1007/s00239-008-9079-3

Nakayama, M., Tateno, R., 2018. Solar radiation strongly influences the quantity of forest tree root exudates. Trees 32, 871–879. https://doi.org/10.1007/s00468-018-1685-0

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Niether, W., Armengot, L., Andres, C., Schneider, M., Gerold, G., 2018. Shade trees and tree pruning alter throughfall and microclimate in cocoa (Theobroma cacao L.) production systems. Ann. For. Sci. 75. https://doi.org/10.1007/s13595-018-0723-9

Nilsson, R.H., Tedersoo, L., Ryberg, M., Kristiansson, E., Hartmann, M., Unterseher, M., Porter, T.M., Bengtsson-Palme, J., Walker, D.M., de Sousa, F., Gamper, H.A., Larsson, E., Larsson, K.-H., Kõljalg, U., Edgar, R.C., Abarenkov, K., 2015. A Comprehensive, Automatically Updated Fungal ITS Sequence Dataset for Reference-Based Chimera Control in Environmental Sequencing Efforts. Microbes Environ. 30, 145–150. https://doi.org/10.1264/jsme2.ME14121

Padovan, M.P., Cortez, V.J., Navarrete, L.F., Navarrete, E.D., Deffner, A.C., Centeno, L.G., Munguía, R., Barrios, M., Vílchez-Mendoza, J.S., Vega-Jarquín, C., Costa, A.N., Brook, R.M., Rapidel, B., 2015. Root distribution and water use in coffee shaded with Tabebuia rosea Bertol. and Simarouba glauca DC. compared to full sun coffee in sub-optimal environmental conditions. Agrofor. Syst. 89, 857–868. https://doi.org/10.1007/s10457-015-9820-z

Parker, I.M., Saunders, M., Bontrager, M., Weitz, A.P., Hendricks, R., Magarey, R., Suiter, K., Gilbert, G.S., 2015. Phylogenetic structure and host abundance drive disease pressure in communities. Nature 520, 542–544. https://doi.org/10.1038/nature14372

Parker, K.R., 1979. Density Estimation by Variable Area Transect. J. Wildl. Manag. 43, 484–492. https://doi.org/10.2307/3800359

Paungfoo-Lonhienne, C., Yeoh, Y.K., Kasinadhuni, N.R.P., Lonhienne, T.G.A., Robinson, N., Hugenholtz, P., Ragan, M.A., Schmidt, S., 2015. Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. Sci. Rep. 5. https://doi.org/10.1038/srep08678

Pramanik, Tagai, Asao, Matsui, 2000. Effects of Temperature and Photoperiod on Phytotoxic Root Exudates of Cucumber (Cucumis sativus) in Hydroponic Culture. J. Chem. Ecol. 15.

Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. For. Ecol. Manag. 309, 19–27. https://doi.org/10.1016/j.foreco.2013.02.034
Promputtha, I., Hyde, K.D., Lumyong, P., McKenzie, E.H.C., Lumyong, S., n.d. Dokmaia monthadangii gen. et sp. nov., a synnematous anamorphic fungus on Manglietia garrettii 5.
Promputtha, I., Lumyong, S., Dhanasekaran, V., McKenzie, E.H.C., Hyde, K.D., Jeewon, R., 2007. A Phylogenetic Evaluation of Whether Endophytes Become Saprotrophs at Host Senescence. Microb. Ecol. 53, 579–590. https://doi.org/10.1007/s00248-006-9117-x
Rai, M., Agarkar, G., 2014. Plant–fungal interactions: What triggers the fungi to switch among lifestyles? Crit. Rev. Microbiol. 1–11. https://doi.org/10.3109/1040841X.2014.958052
Rasmann, S., Turlings, T.C., 2016. Root signals that mediate mutualistic interactions in the rhizosphere. Curr. Opin. Plant Biol. 32, 62–68. https://doi.org/10.1016/j.pbi.2016.06.017
Razafimandimbison, S.G., Taylor, C.M., Wikstrom, N., Pailler, T., Khodabandeh, A., Bremer, B., 2014. Phylogeny and generic limits in the sister tribes Psychotrieae and Palicoureeae (Rubiaceae): Evolution of schizocarps in Psychotria and origins of bacterial leaf nodules of the Malagasy species. Am. J. Bot. 101, 1102–1126. https://doi.org/10.3732/ajb.1400076
Reynolds, P.L., Bruno, J.F., 2012. Effects of Trophic Skewing of Species Richness on Ecosystem Functioning in a Diverse Marine Community. PLoS ONE 7, e36196. https://doi.org/10.1371/journal.pone.0036196
Rodriguez, A., Perestelo, F., Carnicero, A., Regalado, V., Perez, R., Fuente, G., Falcon, M.A., 1996. Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti. FEMS Microbiol. Ecol. 21, 213–219. https://doi.org/10.1111/j.1574-6941.1996.tb00348.x
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584
Root, R.B., 1967. The Niche Exploitation Pattern of the Blue-Gray Gnatcatcher. Ecol. Monogr. 37, 317–350. https://doi.org/10.2307/1942327
Roques, S., Kendall, S., Smith, K., Price, P.N., Berry, P., n.d. A review of the non-NPKS nutrient requirements of UK cereals and oilseed rape 109.
Schuff-Werner, P., Gohlke, H., Bartmann, U., Baggio, G., Corti, M.C., Dinsenbacher, A., Eisenhauer, T., Grützmacher, P., Keller, C., Kettner, U., others, 1994. The HELP-LDL-apheresis multicentre study, an angiographically assessed trial on the role of LDL-apheresis in the secondary prevention of coronary heart disease. II. Final evaluation of the effect of regular treatment on LDL-cholesterol plasma concentrations and the course of coronary heart disease. Eur. J. Clin. Invest. 24, 724–732.
Schulz, B., Boyle, C., 2005. The endophytic continuum. Mycol. Res. 109, 661–686. https://doi.org/10.1017/S095375620500273X
Selosse, M.-A., Schneider-Maunoury, L., Martos, F., 2018. Time to re-think fungal ecology? Fungal ecological niches are often prejudged. New Phytol. 217, 968–972. https://doi.org/10.1111/nph.14983
Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., Milchunas, D.G., Pennings, S., 2005. Functional-and abundance-based mechanisms explain diversity loss due to N fertilization. Proc. Natl. Acad. Sci. U. S. A. 102, 4387–4392.

Tang, J., Qi, Y., Xu, M., Misson, L., Goldstein, A.H., 2005. Forest thinning and soil respiration in a ponderosa pine plantation in the Sierra Nevada. Tree Physiol. 25, 57–66. https://doi.org/10.1093/treephys/25.1.57

Tedersoo, L., Bahram, M., Cajthaml, T., Põlme, S., Hiiesalu, I., Anslan, S., Harend, H., Buegger, F., Pritsch, K., Koricheva, J., Abarenkov, K., 2016. Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. ISME J. 10, 346–362. https://doi.org/10.1038/ismej.2015.116

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., Kesel, A.D., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346, 1256688. https://doi.org/10.1126/science.1256688

Voříšková, J., Baldrian, P., 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME J. 7, 477–486.

Vuković, R., Bauer, N., Ćurković-Perica, M., 2013. Genetic elicitation by inducible expression of β-cryptogein stimulates secretion of phenolics from Coleus blumei hairy roots. Plant Sci. 199–200, 18–28. https://doi.org/10.1016/j.plantsci.2012.10.009

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, Q.-Y., Zhou, D.-M., Cang, L., 2014. Bioavailability of Soil Copper from Different Sources: Integrating Chemical Approaches with Biological Indicators. Pedosphere 24, 145–152. https://doi.org/10.1016/S1002-0160(13)60089-2

Wardle, D.A., 2004. Ecological Linkages Between Aboveground and Belowground Biota. Science 304, 1629–1633. https://doi.org/10.1126/science.1094875

Wartenberg, A.C., Blaser, W.J., Gattinger, A., Roshetko, J.M., Van Noordwijk, M., Six, J., 2017. Does shade tree diversity increase soil fertility in cocoa plantations? Agric. Ecosyst. Environ. 248, 190–199. https://doi.org/10.1016/j.agee.2017.07.033

White, T.M., Bruns, T., Lee, S., Taylor, J., 1990. White, T. M., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–327. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), in: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.M. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA, pp. 315–327.

Xiao, D., Huang, Y., Feng, S., Ge, Y., Zhang, W., He, X., Wang, K., 2018. Soil organic carbon mineralization with fresh organic substrate and inorganic carbon additions in a red soil is controlled by fungal diversity along a pH gradient. Geoderma 321, 79–89. https://doi.org/10.1016/j.geoderma.2018.02.003

Yu-Chuan, D., Chun-Rong, C., Wen, L., Yan-Shou, W., Xiao-Li, R., Ping, W., Guo-Hua, X., n.d. High Potassium Aggravates the Oxidative Stress Inducedy by Magnesium Deficiency in Rice Leaves∗ 12.

Zhang, W., Parker, K.M., Luo, Y., Wan, S., Wallace, L.L., Hu, S., 2005. Soil microbial responses to experimental warming and clipping in a tallgrass prairie. Glob. Change Biol. 11, 266–277. https://doi.org/10.1111/j.1365-2486.2005.00902.x

Zhang, X., Guan, D., Li, W., Sun, D., Jin, C., Yuan, F., Wang, A., Wu, J., 2018. The effects of forest thinning on soil carbon stocks and dynamics: A meta-analysis. For. Ecol. Manag. 429, 36–43. https://doi.org/10.1016/j.foreco.2018.06.027

Zhou, J., Jiang, X., Zhou, B., Zhao, B., Ma, M., Guan, D., Li, J., Chen, S., Cao, F., Shen, D., Qin, J., 2016. Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. Soil Biol. Biochem. 95, 135–143. https://doi.org/10.1016/j.soilbio.2015.12.012

APPENDIX

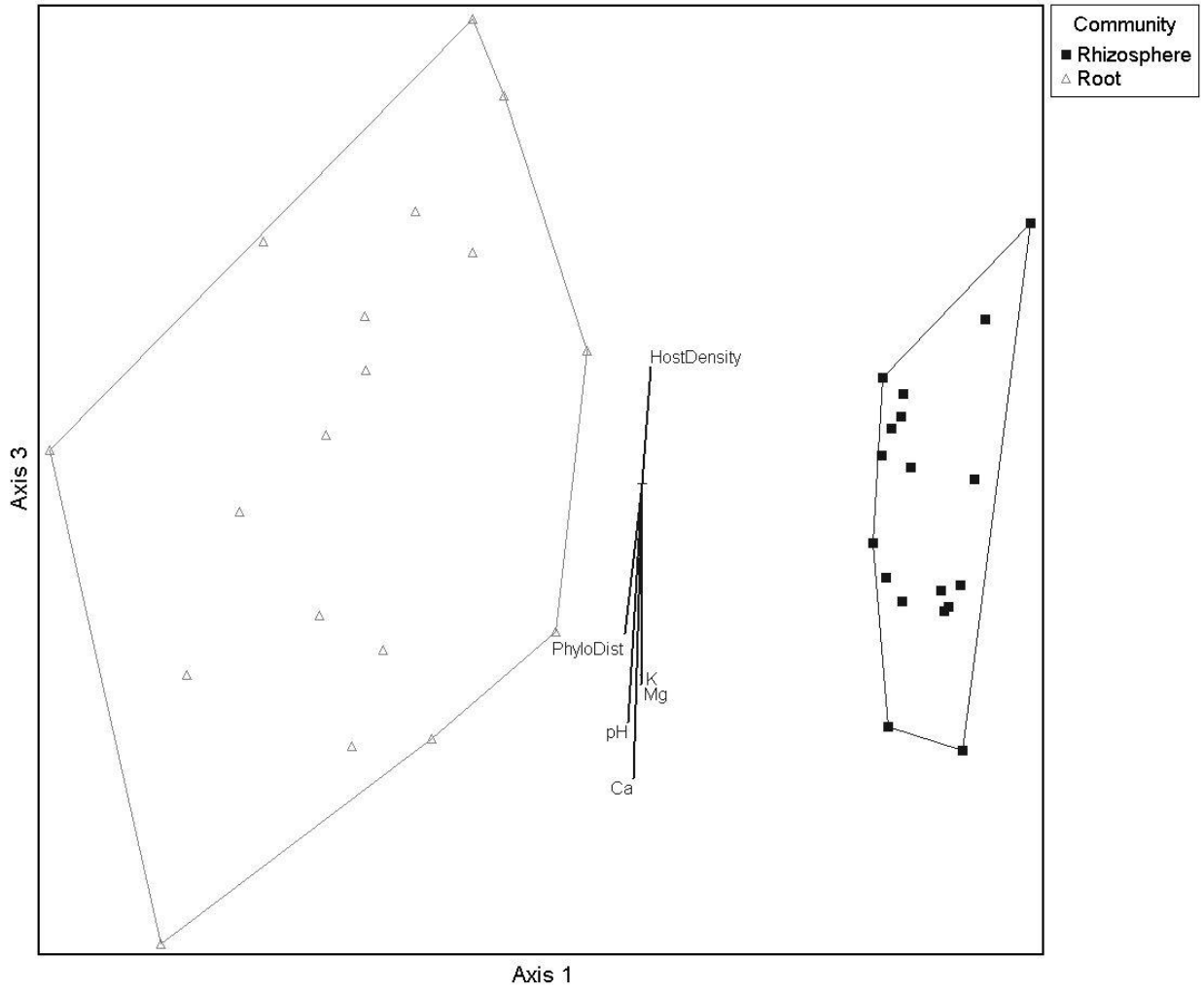

Figure A1. Non-metric multidimensional scaling ordination of fungal communities in forest and coffee fields in Costa Rica (final stress = 9.34). The best solution was three dimensional and two axes are shown here. Environmental characteristics and phylogenetic distance from coffee ("PhyloDist") were transformed as necessary and the bi-plot overlay shows these data in relation to fungal communities. OTU abundances were rarefied and Hellinger-transformed prior to analysis, and only OTUs that occurred in >= 10% of samples were included. Ordinations show root (N=18) and rhizosphere (N=18) fungal communities in coffee and forest trees grouped by sample type. Axis 1 accounted for 46.6%, Axis 2 (not shown) accounted for 28.2%, and Axis 3 accounted for 11.5% of the variation in fungal communities among samples.

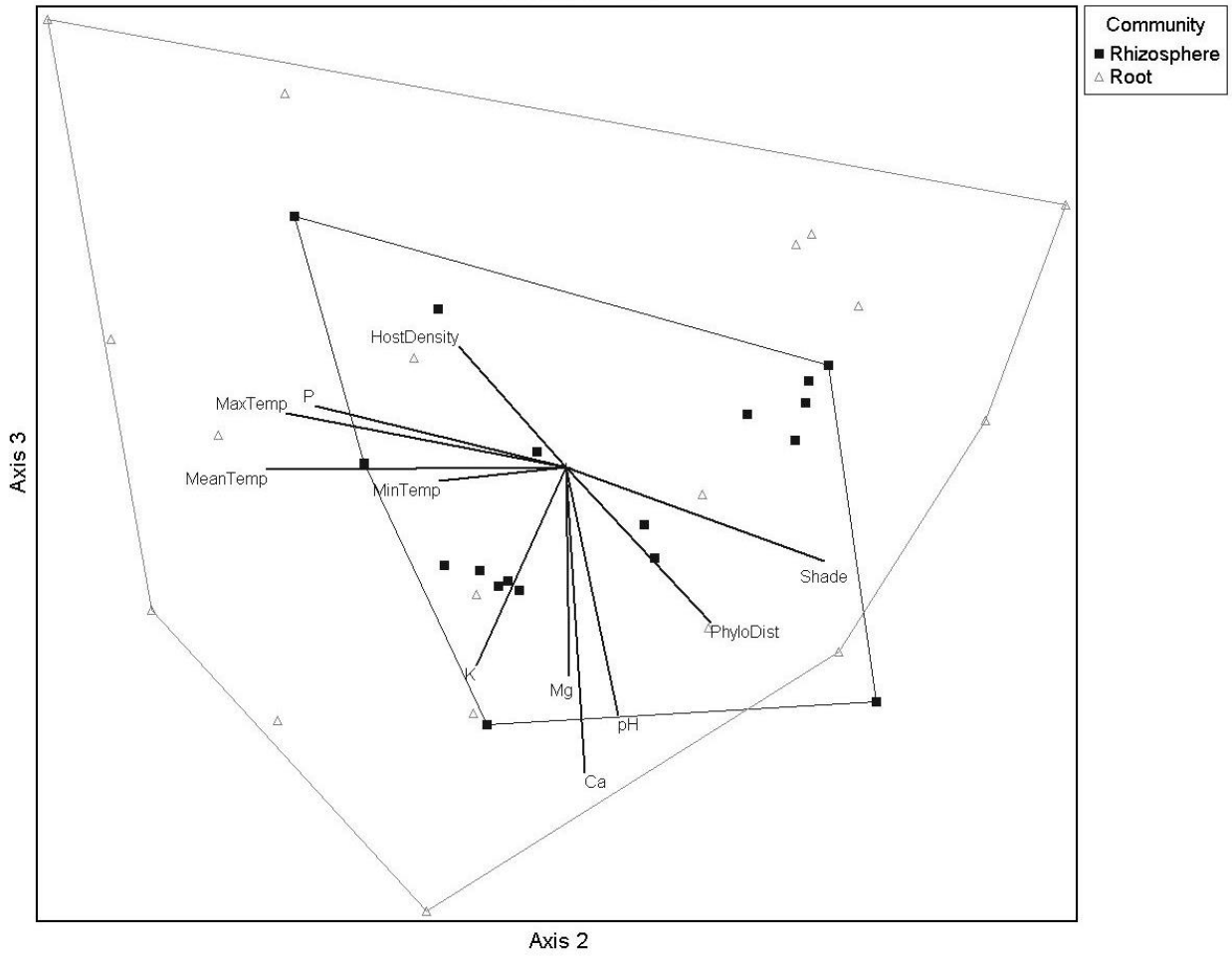

Figure A2. Non-metric multidimensional scaling ordination of fungal communities in forest and coffee fields in Costa Rica (final stress = 9.34). The best solution was three dimensional and two axes are shown here. Environmental characteristics and phylogenetic distance from coffee ("PhyloDist") were transformed as necessary and the bi-plot overlay shows these data in relation to fungal communities. OTU abundances were rarefied and Hellinger-transformed prior to analysis, and only OTUs that occurred in >= 10% of samples were included. Ordinations show root (N=18) and rhizosphere (N=18) fungal communities in coffee and forest trees grouped by sample type. Axis 1 (not shown) accounted for 46.6%, Axis 2 accounted for 28.2%, and Axis 3 accounted for 11.5% of the variation in fungal communities among samples.

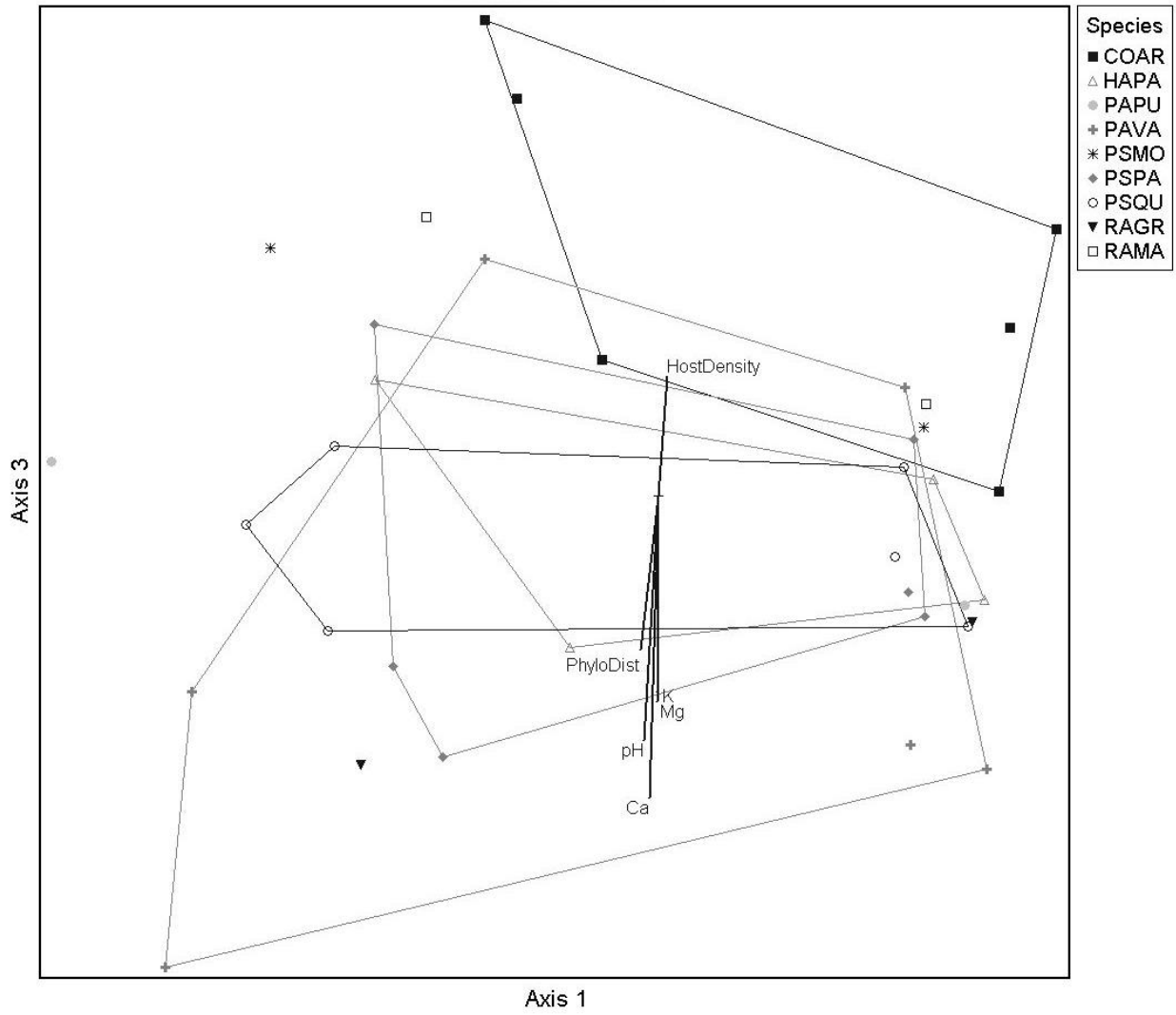

Figure A3. Non-metric multidimensional scaling ordination of fungal communities in forest and coffee fields in Costa Rica (final stress = 9.34). The best solution was three dimensional and two axes are shown here. Environmental characteristics and phylogenetic distance from coffee ("PhyloDist") were transformed as necessary and the bi-plot overlay shows these data in relation to fungal communities. OTU abundances were rarefied and Hellinger-transformed prior to analysis, and only OTUs that occurred in >= 10% of samples were included. Ordinations show root (N=18) and rhizosphere (N=18) fungal communities in coffee and forest trees grouped by host tree species, abbreviated with the first two letters of the plant's genus followed by the first two letters of its species. Axis 1 accounted for 46.6%, Axis 2 (not shown) accounted for 28.2%, and Axis 3 accounted for 11.5% of the variation in fungal communities among samples.

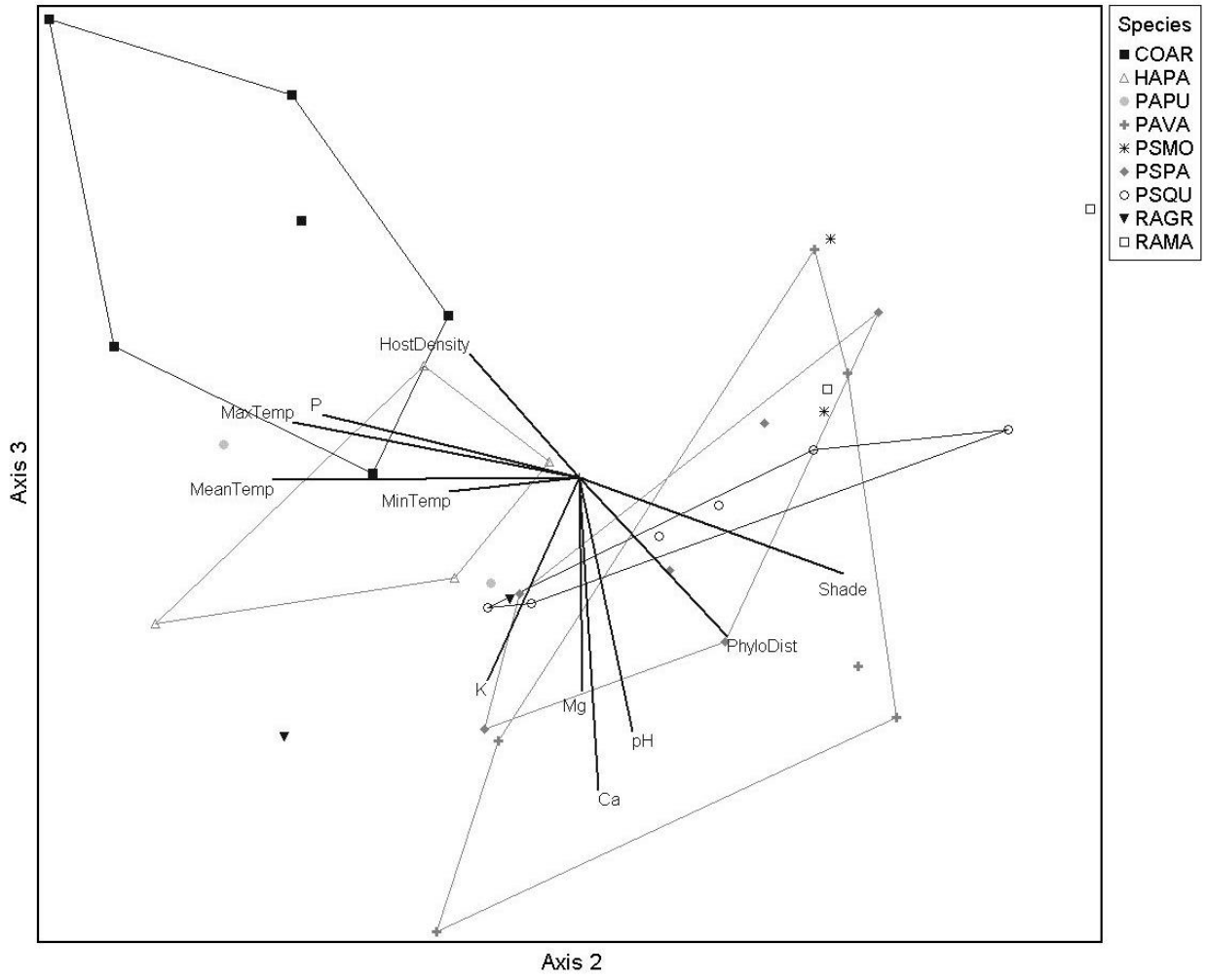

Figure A4. Non-metric multidimensional scaling ordination of fungal communities in forest and coffee fields in Costa Rica (final stress = 9.34). The best solution was three dimensional and two axes are shown here. Environmental characteristics and phylogenetic distance from coffee ("PhyloDist") were transformed as necessary and the bi-plot overlay shows these data in relation to fungal communities. OTU abundances were rarefied and Hellinger-transformed prior to analysis, and only OTUs that occurred in >= 10% of samples were included. Ordinations show root (N=18) and rhizosphere (N=18) fungal communities in coffee and forest trees grouped by host tree species, abbreviated with the first two letters of the plant's genus followed by the first two letters of its species. Axis 1 (not shown) accounted for 46.6%, Axis 2 accounted for 28.2%, and Axis 3 accounted for 11.5% of the variation in fungal communities among samples.

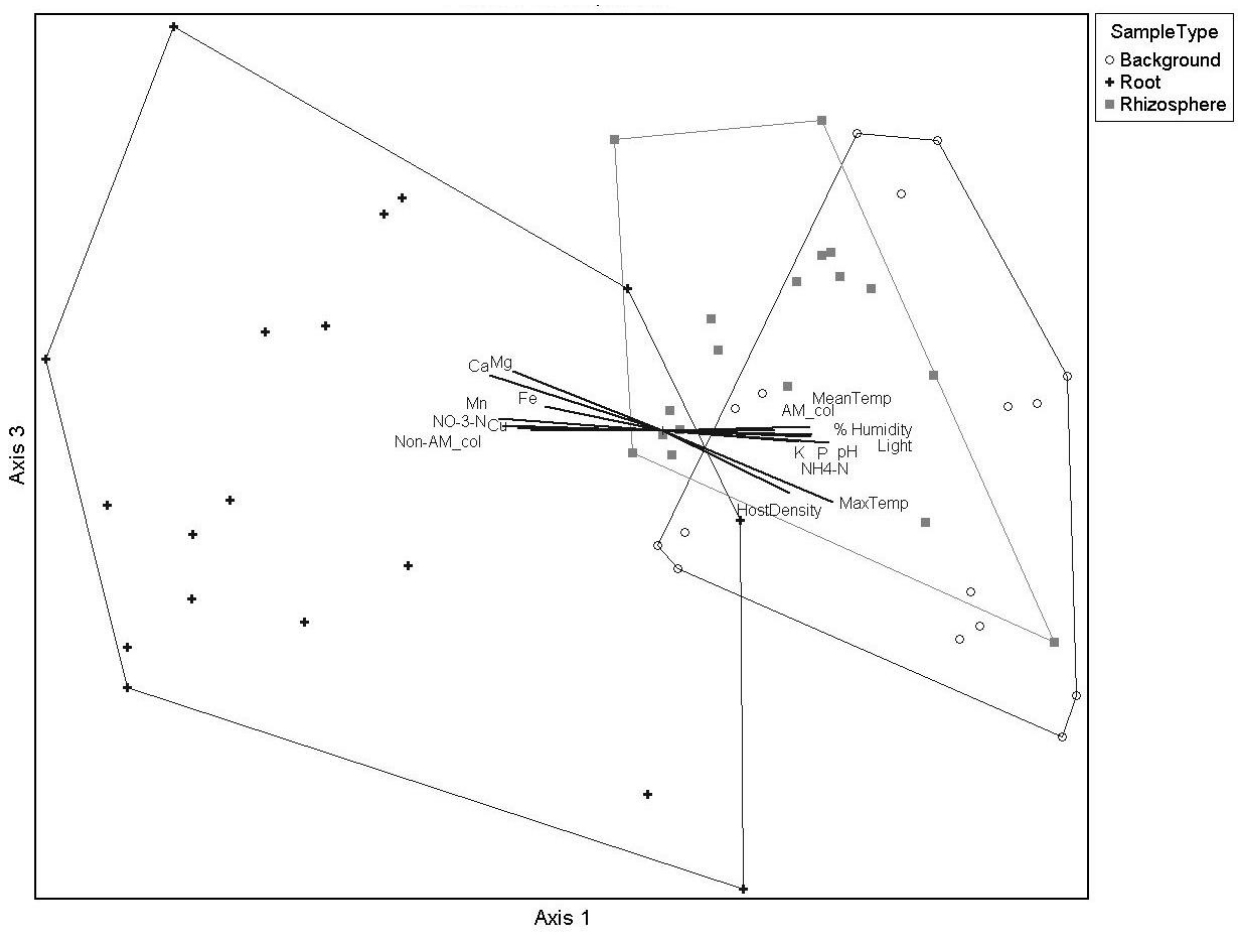

Figure A5. Non-metric multidimensional scaling ordination of fungal communities in forest and coffee fields in Costa Rica (final stress = 8.59705). The best solution was three-dimensional and two of three axes are shown. Environmental characteristics were transformed as necessary and the bi-plot overlay shows these data in relation to fungal communities. OTU abundances were rarefied and Hellinger-transformed prior to analysis, and only OTUs that occurred in >= 10% of samples were included. Ordinations show root (N=18), rhizosphere (N=18) and bulk soil (N=16) fungal communities in coffee and forest trees grouped by sample type. Axis 1 accounted for 43.6%, Axis 2 (not shown) accounted for 31.0%, and Axis 3 accounted for 11.7% of the variation in fungal communities among samples.

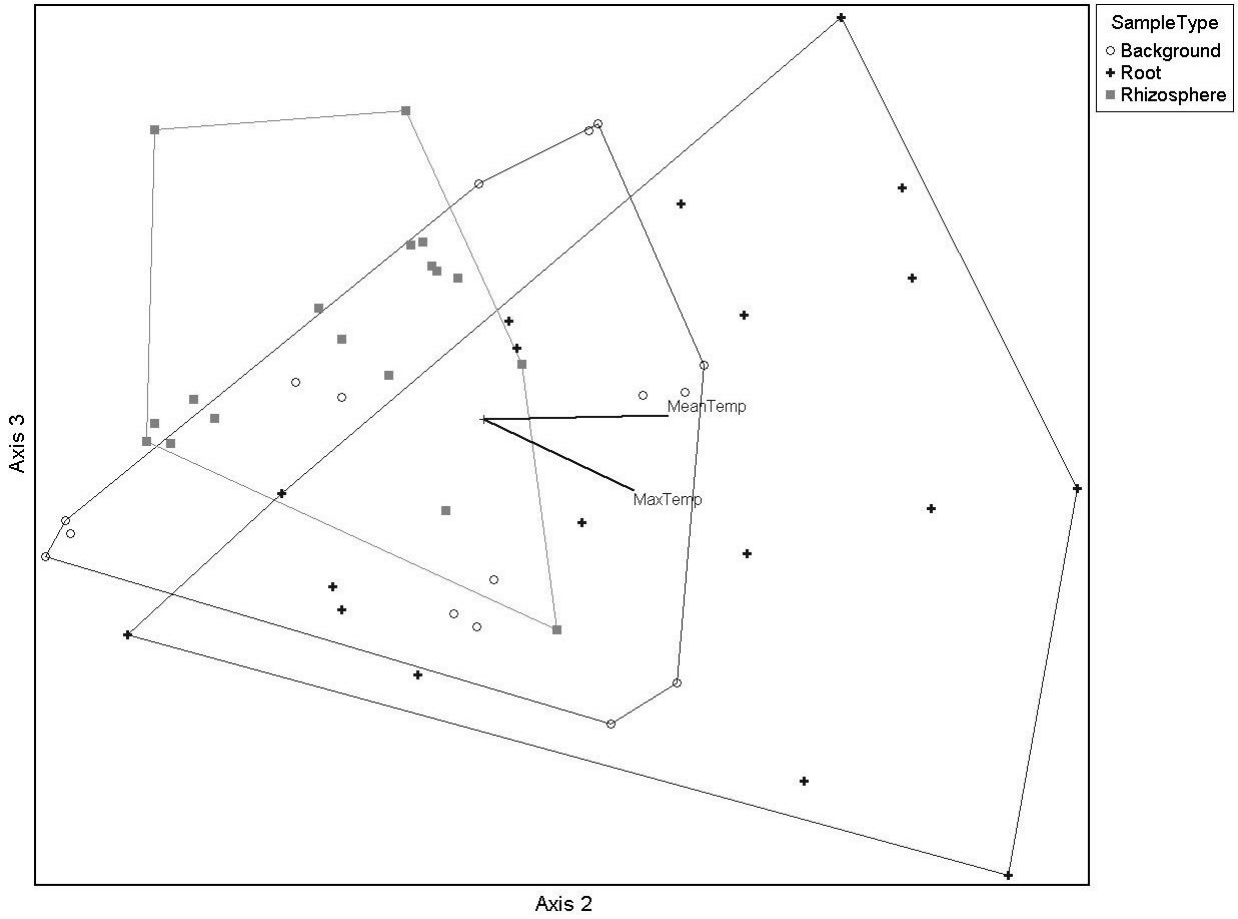

Figure A6. Non-metric multidimensional scaling ordination of fungal communities in forest and coffee fields in Costa Rica (final stress = 8.59705). The best solution was three-dimensional and two of three axes are shown. Environmental characteristics were transformed as necessary and the bi-plot overlay shows these data in relation to fungal communities. OTU abundances were rarefied and Hellinger-transformed prior to analysis, and only OTUs that occurred in >= 10% of samples were included. Ordinations show root (N=18), rhizosphere (N=18) and bulk soil (N=16) fungal communities in coffee and forest trees grouped by sample type. Axis 1 (not shown) accounted for 43.6%, Axis 2 accounted for 31.0%, and Axis 3 accounted for 11.7% of the variation in fungal communities among samples.