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Published in:
Applied and environmental microbiology

DOI:
10.1128/AEM.00052-20

Publication date:
2020

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

MICROBIAL ECOLOGY

Contrasting Patterns of Functional Diversity in Coffee Root Fungal Communities Associated with Organic and Conventionally Managed Fields

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ABSTRACT The structure and function of fungal communities in the coffee rhizosphere are influenced by crop environment. Because coffee can be grown along a management continuum from conventional application of pesticides and fertilizers in full sun to organic management in a shaded understory, we used coffee fields to hold host constant while comparing rhizosphere fungal communities under markedly different environmental conditions with regard to shade and inputs. We characterized the shade and soil environment in 25 fields under conventional, organic, or transitional management in two regions of Costa Rica. We amplified the internal transcribed spacer 2 (ITS2) region of fungal DNA from coffee roots in these fields and characterized the rhizosphere fungal community via high-throughput sequencing. Sequences were assigned to guilds to determine differences in functional diversity and trophic structure among coffee field environments. Organic fields had more shade, a greater richness of shade tree species, and more leaf litter and were less acidic, with lower soil nitrate availability and higher soil copper, calcium, and magnesium availability than conventionally managed fields, although differences between organic and conventionally managed fields in shade and calcium and magnesium availability depended on region. Differences in richness and community composition of rhizosphere fungi between organic and conventionally managed fields were also correlated with shade, soil acidity, and nitrate and copper availability. Trophic structure differed with coffee field management. Saprotrophs, plant pathogens, and mycoparasites were more diverse, and plant pathogens were more abundant, in organic than in conventionally managed fields, while saprotroph-plant pathogens were more abundant in conventionally managed fields. These differences reflected environmental differences and depended on region.

IMPORTANCE Rhizosphere fungi play key roles in ecosystems as nutrient cyclers, pathogens, and mutualists, yet little is currently known about which environmental factors and how agricultural management may influence rhizosphere fungal communities and their functional diversity. This field study of the coffee agroecosystem suggests that organic management not only fosters a greater overall diversity of fungi, but it also maintains a greater richness of saprotrophic, plant-pathogenic, and mycoparasitic fungi that has implications for the efficiency of nutrient cycling and regulation of plant pathogen populations in agricultural systems. As well as influencing community composition and richness of rhizosphere fungi, shade management and use of fungicides and synthetic fertilizers altered the trophic structure of the coffee agroecosystem.

KEYWORDS coffee, field management, functional diversity, fungi, guild, metabarcoding, nitrogen, organic agriculture, shade, soil ecology

Citation Sternhagen EC, Black KL, Hartmann EDL, Shivega WG, Johnson PG, McGlynn RD, Schmaltz LC, Asheim Keller RJ, Vink SN, Aldrich-Wolfe L. 2020. Contrasting patterns of functional diversity in coffee root fungal communities associated with organic and conventionally managed fields. Appl Environ Microbiol 86:e00052-20. https://doi.org/10.1128/AEM.00052-20.

Editor Harold L. Drake, University of Bayreuth

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Received 6 January 2020
Accepted 16 March 2020
Accepted manuscript posted online 27 March 2020
Published 19 May 2020

Land plants have diverse interactions with belowground fungal communities, and these relationships are understudied relative to their ecological importance (1, 2). Belowground plant-fungus relationships span the range of symbioses and include fungal pathogens that attack plant roots, mycorrhizal fungi that improve plant nutrition and pathogen resistance, commensal endophytes that live inside plant tissues usually asymptomatically, and fungi that benefit their host indirectly via attack on root parasites (3–6). Advancing an understanding of the nature and prevalence of these relationships entails a firm grasp of which taxa are present in the rhizosphere and how they respond to changes in their environment.

Guild assessments of fungal communities provide valuable information on possible plant-fungus functional relationships and therefore complement more traditional taxonomic studies (7). A guild is a group of species employing similar strategies to exploit the same type of environmental resource (8). Guild composition provides insights into the functionality of a system in terms of interspecific competition for similar resources, interactions between trophic levels of a community, and nutrient cycling (7, 9). Additionally, a fungal species' guild may change depending on environmental conditions, such as plant pathogens that become saprotrophic in the absence of a host or saprotrophs that occur in plants as asymptomatic endophytes until the plant begins to senesce (3, 10–12).

The environment acts as an important filter, with environmental changes altering fungal communities by benefiting or harming particular species or guilds. Edaphic and biotic properties can support or suppress guilds of belowground fungi depending on nutritional needs and the competitive ability of the fungi in different environments (13–15). For example, increased soil nitrogen availability may be associated with decreased decomposition rates and changes in the species composition of saprotrophic communities (16). Veach et al. (17) found that several fungal guilds showed changes in richness and abundance that were related to differences in environmental factors, such as the availability of N and C in the soil, soil pH, and site elevation. Changes in the soil environment can also lead to unfavorable conditions for certain species. For example, soil acidification filters out fungi unable to tolerate highly acidic conditions (18).

The influence of anthropogenic environmental filters should be clearly detectable in agricultural systems. Management strategy can affect soil properties and create environmental filters that change fungal communities (19). In some agricultural systems, N fertilization has been observed to acidify soils and decrease fungal diversity (20–22). Similarly, herbicides and fungicides may alter the guild compositions of root and soil fungal communities by favoring pathogens and suppressing mutualists (23, 24). In agroforests, leaf litter, tree roots, and their associated exudates create a variety of fungal niches and nutrient sources which also influence fungal community composition (21, 25, 26).

Coffee (*Coffea arabica* L.) is a particularly tractable system for studying environmental influences on fungal community composition, given the range of conditions under which it is grown. An important crop throughout the tropics (27), coffee can be managed as a monocrop in full sun or as an understory species in seminatural agroforests, under a wide range of management strategies aimed at maximizing the crop's health and/or productivity (28–30). In conventional management, productivity is typically emphasized through extensive application of synthetic fertilizers and fungicides (30). Organic agriculture foregoes these synthetic products and instead seeks to support the crop with methods emphasizing the natural ecology of the system (30, 31). Common management of organic coffee includes the use of shade trees for reducing soil erosion and nitrogen-fixing trees to increase nitrogen availability, as well as inputs such as compost and biocontrol fungi to manage fertility and pathogens, respectively (19, 30, 32, 33). While both conventional and organic management types include the use of shade trees and biocontrol fungi, these practices are more prevalent in organic fields (34–37).

Through this study of root fungal community composition, we sought to improve

TABLE 1 Environmental differences between organic and conventionally managed fields in Monteverde and San Vito, Costa Rica, by field type or by F × R[a]

Variable	Mean ± SE (*n*) by field type for location:						$F_{1,14}$	*P*	Effect
	Monteverde			San Vito					
	Conventional (8)	Minimal (1)	Organic (4)	Conventional (5)	Minimal (2)	Organic (5)			
Shade (%)	9 ± 3 A	<1	64 ± 11 B	36 ± 10 AB	17	53 ± 8 B	6.40	0.0241	F × R
Shade tree richness	0.5 ± 0.3 A	0	4.5 ± 0.6 B	4.0 ± 1.2 B	5	4.8 ± 1.9 B	12.50	0.0039	F × R
Leaf litter depth (cm)	2.3 ± 0.7 B	1.3	4.8 ± 1.2 A	2.2 ± 0.4 B	4.7	3.6 ± 0.6 A	6.42	0.0248	Field
pH in water	5.18 ± 0.15 A	5.30	6.09 ± 0.06 B	5.25 ± 0.07 A	5.20	5.72 ± 0.3 B	17.07	0.0010	Field
NO_3^--N (kg/ha)	164 ± 31 B	74	54 ± 15 A	103 ± 5 B	31	33 ± 6 A	33.81	<0.0001	Field
Cu concn (ppm)	1.87 ± 0.21 A	1.76	2.52 ± 0.45 B	3.94 ± 0.32 C	3.73	6.25 ± 0.86 D	9.12	0.0092	Field
Ca^{2+} concn (ppm)	1,769 ± 203 A	1012	3,570 ± 580 B	1,622 ± 323 A	706	2,199 ± 667 AB	12.87	0.0030	F × R
Mg^{2+} concn (ppm)	89 ± 7 A	81	294 ± 78 C	185 ± 35 B	142	185 ± 55 B	9.67	0.0077	F × R

[a]F × R, field type and field type by region. Means that share a letter did not differ statistically by Tukey's HSD test. Values are also shown for fields with minimal conventional management for comparison, but these were too few to include in the statistical analysis.

current understanding of effects of agricultural management on the structure of belowground fungal communities. Previous research has assessed fungal responses to different environmental factors, provided support for the use of guilds in assessing fungal communities, and demonstrated environmental differences between organic and conventional agricultural systems (35, 38, 39). However, the relationship between agricultural management and guild composition of root fungi remains to be clarified. In this study, we characterized the environment of coffee fields managed conventionally, organically, or in transition from conventional to organic management. We used high-throughput sequencing of the internal transcribed spacer 2 (ITS2) region of fungal DNA to elucidate how coffee rhizosphere fungal communities in conventionally managed fields differed from those under organic management.

Our goal was to determine whether environmental differences between conventionally managed and organic coffee were associated with differences in the guild composition of coffee rhizosphere fungal communities. We expected that coffee management would be correlated with differences in fungal community structure. We anticipated that conventional management might generate a simpler or more homogeneous environment, reducing niche diversity for fungi and thereby resulting in lower diversity overall relative to rhizosphere fungal communities in organic fields. We hypothesized that differences in nutrient and substrate availability would lead to differences in the richness and abundance of fungal species within guilds. Specifically, we predicted that the high nitrogen availability and acidity in conventionally managed fields would be associated with a loss of fungal diversity relative to organic fields, and that the more diverse nutrient sources in organic coffee would lead to a more complex fungal trophic structure and greater fungal diversity overall in organic fields.

RESULTS

Environmental differences between coffee field management types. Management resulted in environmental differences between organic and conventionally managed fields, but the extent of these differences varied between Monteverde and San Vito (Table 1). In Monteverde, shade and shade tree richness were greater in organic than in conventionally managed fields, while in San Vito, shade and shade tree richness did not differ between organic and conventionally managed fields and were similar to richness measures of organic fields in Monteverde. Leaf litter depth was greater in organic than in conventionally managed fields in both Monteverde and San Vito. Soils of coffee fields under conventional management had higher levels of nitrate (NO_3^--N) availability and were more acidic than those under organic management in both regions. Copper availability was higher in organic than in conventionally managed fields. Plant-available calcium and magnesium were higher in soils of organic fields than in those of conventionally managed fields in Monteverde, but in San Vito, they did not differ between field management types (here called "field types"). Soil organic matter and available phosphorus, potassium, zinc, and manganese did not differ by field type

(see Table S2 in the supplemental material). Aspects of management, such as field and plant age, location of fields with regard to slope, aspect, and elevation, and coffee plant density also did not differ between conventionally managed and organic fields.

Overall patterns of functional diversity. After rarefaction, 6,668 fungal operational taxonomic units (OTUs) were detected in coffee roots from the three field types. Using FUNGuild, 48% of OTUs (59% of sequences; Fig. 1) could be assigned to possible and probable guilds (here called "guilds"). Of the OTUs that could be assigned to guilds, saprotrophs and arbuscular mycorrhizal (AM) fungi were the most diverse (Fig. 1A), while saprotroph-plant pathogens, plant pathogens, and saprotrophs were the most frequently detected (Fig. 1B). Almost 20% of the detected sequences belonged to saprotroph-plant pathogens, yet this guild represented only 4% of the observed OTU richness. As expected from classical ecological theory (40), guilds at higher trophic levels (e.g., animal pathogens and mycoparasites) were less diverse and abundant than those of primary consumers (plant pathogens, saprotrophs, and mycorrhizal fungi).

The sequence counts per OTU after rarefaction ranged from 1 to 238,381. Forty-six OTUs had sequence counts of >10,000 (Table 2 and Fig. S1). Of these most abundant OTUs, only one (OTU 6468, most closely matched to *Knufia* sp.) differed in abundance between organic and conventionally managed fields ($F_{1,14} = 43.14$, $P < 0.0001$). This OTU was almost eight times more frequent in conventionally managed than in organic fields (data not shown). However, field type interacted with region ($F_{1,14} = 13.64$, $P = 0.0024$), such that this OTU was over five times more abundant in conventionally managed than organic fields in Monteverde but did not differ in abundance between organic fields in Monteverde and either field type in San Vito. More than half of these OTUs (28) belonged to the subphylum Pezizomycotina, 12 OTUs belonged to the subphylum Agaricomycotina, and the remainder were unknown Basidiomycota and a single OTU of unknown phylum (Table 2). Of these 46 most abundant OTUs, only five OTUs could be assigned to a species with 97% or greater confidence. Eighteen of these 46 OTUs (39%) could not be placed in guilds, 14 OTUs (30%) were possible plant pathogens or saprotrophs, 4 OTUs were considered possible endophytes, 4 OTUs were possibly saprotrophs or plant pathogens in clades known to form ectomycorrhizae, 3 OTUs were likely plant pathogens, 2 OTUs were likely saprotrophs, and 1 OTU was most closely related to pathogens that specialize on root-parasitic nematodes.

Effects of field management on diversity and community composition. The richness of OTUs differed by field type ($F_{1,14} = 6.97$, $P = 0.0194$), but, as for key environmental variables, this effect depended on region ($F_{1,14} = 5.01$, $P = 0.0419$). The estimated richness of OTUs was greater in organic than in conventionally managed fields in Monteverde (Fig. S2a). In San Vito, the OTU richness was similar to the OTU richness observed in organic fields in Monteverde and did not differ between conventionally managed and organic fields (Fig. S2b). OTU richness was positively correlated with shade ($r = 0.56$, $P = 0.0039$), shade tree richness ($r = 0.48$, $P = 0.0172$), pH ($r = 0.42$, $P = 0.0380$), and soil copper availability ($r = 0.49$, $P = 0.0132$) and negatively correlated with soil nitrate availability ($r = -0.44$, $P = 0.0264$) and phosphorus availability ($r = -0.57$, $P = 0.0029$; data not shown).

Conventionally managed and organic fields differed in fungal community composition ($F_{1,14} = 1.92$, $P = 0.0066$; Fig. 2), while fields under minimal conventional management did not appear to differ in community composition from conventionally managed fields. Differences in community composition between conventionally managed and organic fields were primarily along axis 2 of the nonmetric multidimensional scaling (NMS) plot and correlated with differences in soil nitrate availability and acidity, shade, and cation and copper availability. Elevation, shade tree richness, and soil availability of phosphorus, zinc, and iron were correlated with differences in fungal community composition along axis 1, which was less strongly associated with differences related to field type than was axis 2.

Effects of field management on functional diversity. The functional diversity of the identified OTUs differed between conventionally managed and organic fields, but

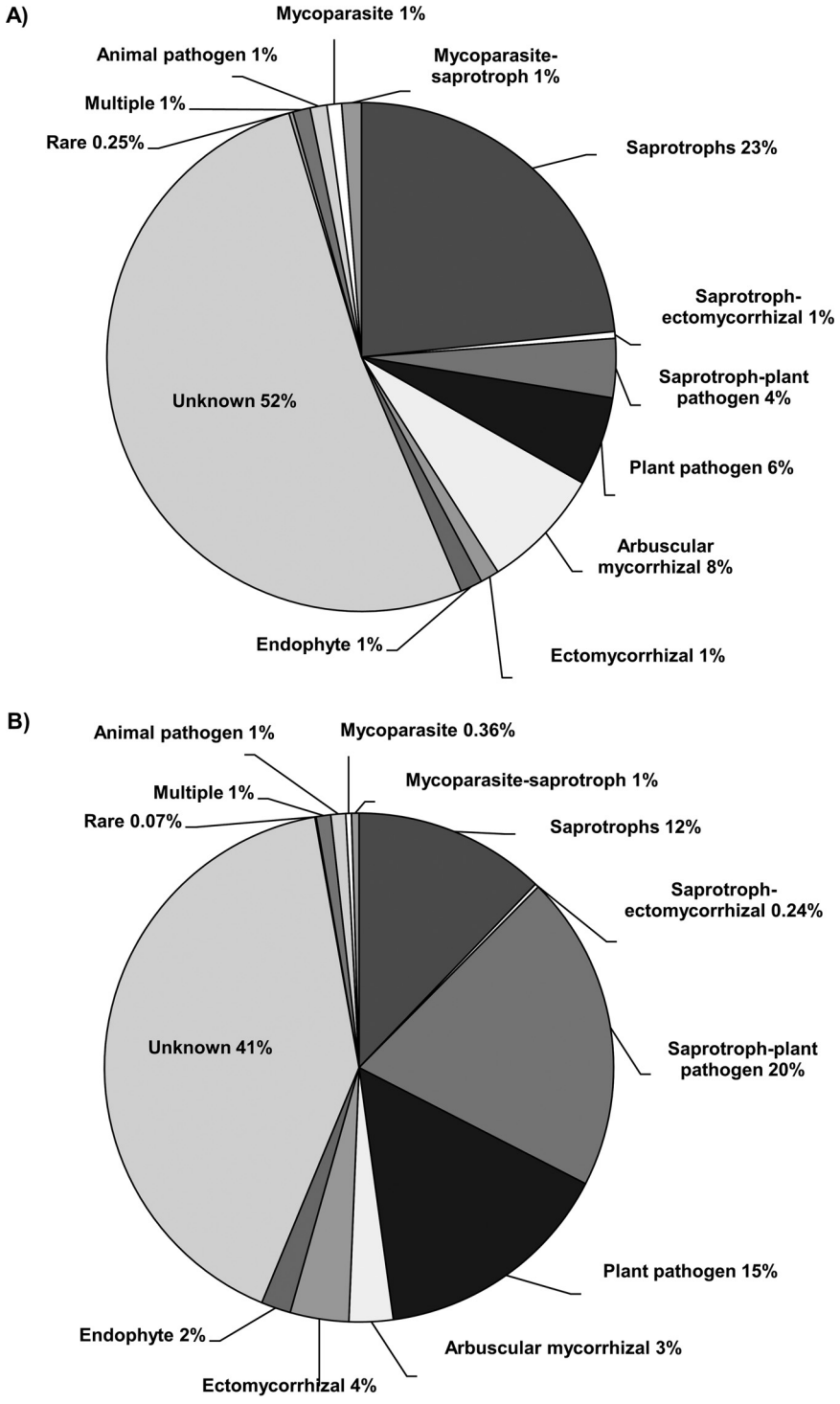

FIG 1 (A to B) Fungal guild composition by OTU richness (A) and sequence abundance (B) for 25 coffee fields under organic or conventional management in Costa Rica. Samples were rarefied prior to analysis. Fungi that could be assigned to more than two guilds were referred to as "multiple." Rare guilds include those with a richness of fewer than 10 OTUs and/or an abundance of <1,500 sequences.

there was an interaction of field type with region (Fig. 3). There were fewer saprotroph species ($F_{1,14} = 8.83$, $P = 0.0101$), fewer plant pathogen species ($F_{1,14} = 8.08$, $P = 0.0130$), and fewer mycoparasite species ($F_{1,14} = 4.60$, $P = 0.0499$) in conventionally managed than in organic fields. For each of these guilds, richness was lowest in conventionally managed fields in Monteverde, highest in organic fields in Monteverde,

TABLE 2 OTUs with the highest sequence counts after rarefaction, ranked from most to least abundant, with the identity and confidence of the closest match to each ITS2 sequence according to the Warcup data set (93)[a]

Phylum	Subphylum	Class	Order	Family	Species	Confidence	Possible guild (reference)	OTU	No. of sequences
Basidiomycota	Unknown				Unassigned	0.61	Unknown	1880	238,381
Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pleosporaceae	*Edenia gomezpompae*	0.86	Endophyte (101)	9344	230,312
Ascomycota	Pezizomycotina	*Incertae sedis*	*Incertae sedis*	*Incertae sedis*	*Knufia* sp.	0.75	Plant pathogen-saprotroph (102)	6468	174,704
Ascomycota	Pezizomycotina	Sordariomycetes	Xylariales	Unknown	Unassigned	0.88	Unknown	5805	117,117
Ascomycota	Pezizomycotina	Dothideomycetes	*Incertae sedis*	*Incertae sedis*	*Rhizopycnis vagum*	1	Endophyte-plant pathogen (103)	8402	104,774
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium oxysporum*	1	Plant pathogen-saprotroph (104)	9601	100,613
Ascomycota	Pezizomycotina	*Incertae sedis*	*Incertae sedis*	*Incertae sedis*	*Campylospora parvula*	1	Saprotroph (105)	5752	78,721
Ascomycota	Pezizomycotina	Unknown			Unassigned	0.67	Unknown	1891	68,607
Basidiomycota	Unknown				Unassigned	0.53	Unknown	2630	59,736
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	*Mycena* sp.	0.82	Plant pathogen-saprotroph (41)	1767	58,566
Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Unknown	Unassigned	0.56	Unknown	10037	48,594
Ascomycota	Pezizomycotina	Unknown			Unassigned	0.76	Unknown	1255	47,714
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	*F. oxysporum*	0.77	Plant pathogen-saprotroph	9265	42,491
Basidiomycota	Unknown				Unassigned	0.65	Unknown	1806	32,492
Ascomycota	Pezizomycotina	Sordariomycetes	Unknown		Unassigned	0.70	Unknown	9359	27,181
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	*Cylindrocarpon pauciseptatum*	0.99	Plant pathogen (106)	7563	26,079
Basidiomycota	Agaricomycotina	Agaricomycetes	Boletales	Sclerodermataceae	*Scleroderma*	0.82	Ectomycorrhiza-saprotroph (107)	607	25,638
Ascomycota	Pezizomycotina	*Incertae sedis*	*Incertae sedis*	*Incertae sedis*	*Dokmaia monthadangii*	1	Endophyte-saprotroph (108)	9893	23,968
Ascomycota	Pezizomycotina	*Incertae sedis*	*Incertae sedis*	*Incertae sedis*	*Knufia* sp.	0.82	Plant pathogen-saprotroph	6670	23,646
Ascomycota	Pezizomycotina	*Incertae sedis*	*Incertae sedis*	*Incertae sedis*	*Knufia* sp.	0.88	Plant pathogen-saprotroph	7039	23,211
Unknown					Unassigned	1	Unknown	599	21,659
Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Unknown	Unassigned	0.74	Unknown	10151	20,063
Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	*Incertae sedis*	*Phoma putaminum*	0.92	Plant pathogen-saprotroph (109)	8246	19,052
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Unknown	Unassigned	0.78	Unknown	368	16,762
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	*Mycena* sp.	0.64	Plant pathogen-saprotroph	2148	16,495
Ascomycota	Pezizomycotina	Sordariomycetes	*Incertae sedis*	Plectosphaerellaceae	*Plectosphaerella cucumerina*	0.94	Plant pathogen (110)	6360	16,470
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae	*Pochonia chlamydosporia*	0.58	Animal pathogen (111)	3444	15,452
Ascomycota	Pezizomycotina	*Incertae sedis*	*Incertae sedis*	*Incertae sedis*	*Knufia* sp.	0.73	Plant pathogen-saprotroph	7477	15,327
Ascomycota	Pezizomycotina	*Incertae sedis*	*Incertae sedis*	*Incertae sedis*	*Knufia* sp.	0.69	Plant pathogen-saprotroph	6851	14,536
Basidiomycota	Agaricomycotina	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Ceratobasidium* sp.	0.97	Ectomycorrhiza-plant pathogen (1, 42)	1265	14,129
Basidiomycota	Agaricomycotina	Agaricomycetes	Unknown		Unassigned	0.72	Unknown	1614	13,874
Basidiomycota	Unknown				Unassigned	0.60	Unknown	2317	12,474
Basidiomycota	Unknown				Unassigned	0.59	Unknown	3165	12,142
Basidiomycota	Agaricomycotina	Agaricomycetes	Atheliales	Atheliaceae	*Cristinia helvetica*	0.97	Saprotroph (112)	1183	11,952
Ascomycota	Pezizomycotina	Sordariomycetes	Unknown		Unassigned	0.59	Unknown	6182	11,762
Basidiomycota	Agaricomycotina	Agaricomycetes	Unknown		Unassigned	0.67	Unknown	3641	11,521
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	*Mycena* sp.	0.84	Plant pathogen-saprotroph	718	11,479
Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Sarcoscyphaceae	Unassigned	0.79	Unknown	5955	11,425
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Porotheleaceae	*Phloeomana speirea*	0.71	Plant pathogen-saprotroph (113)	3755	11,222
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	*Incertae sedis*	*Nectria mauritiicola*	0.57	Plant pathogen-saprotroph (114)	6887	11,204
Basidiomycota	Agaricomycotina	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Ceratobasidium* sp.	0.72	Ectomycorrhiza-plant pathogen	990	10,687
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	*C. pauciseptatum*	0.99	Plant pathogen	7576	10,575
Basidiomycota	Agaricomycotina	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Ceratobasidium* sp.	0.89	Ectomycorrhiza-plant pathogen	1068	10,130
Ascomycota	Pezizomycotina	*Incertae sedis*	*Incertae sedis*	*Incertae sedis*	*Knufia* sp.	0.82	Plant pathogen-saprotroph	6859	10,124
Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Unknown	Unassigned	0.84	Unknown	8625	10,058
Ascomycota	Pezizomycotina	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Cladosporium perangustum*	1	Endophyte-plant pathogen (115, 116)	9431	10,049

[a]Possible guild was determined from published literature. Only the 46 OTUs with sequence counts of >10,000 are shown; of these, only OTU 6468 differed in abundance between coffee roots of conventionally managed and organic fields.

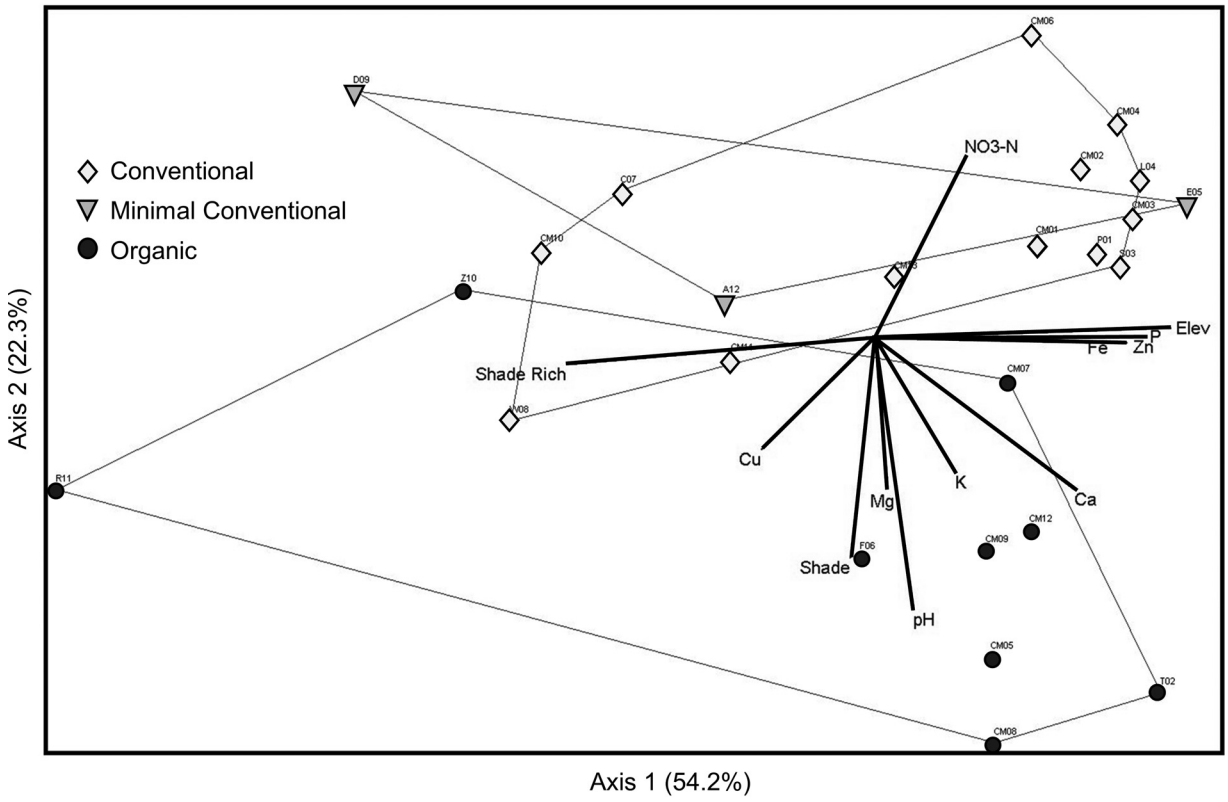

FIG 2 Biplot of nonmetric multidimensional scaling ordination of root fungal communities in coffee fields under conventional, minimal conventional, and organic management (final stress of three-dimensional solution, 8.994; 80 iterations, Monte Carlo $P = 0.0196$) with environmental variables. The two axes associated with differences in community composition by field management are shown here. The amount of variation in community composition represented by each axis is shown in parentheses. Fungal OTU abundances were rarefied and Hellinger-transformed prior to analysis. Each point represents the community composition of one coffee field based on root samples from 8 to 10 coffee plants. Elev, elevation.

and intermediate in both types of fields in San Vito ($F_{1,14} = 6.18$, $P = 0.0262$; $F_{1,14} = 23.08$, $P = 0.0003$; and $F_{1,14} = 8.63$, $P = 0.0108$, respectively). The richness of saprotroph-plant pathogens and root endophytes did not differ by field type ($F_{1,14} = 4.03$, $P = 0.0643$; ($F_{1,14} = 4.19$, $P = 0.0599$; data not shown for endophytes). Richness of fungal OTUs that FUNGuild identified as potentially belonging to multiple (three or more) guilds also differed by field type ($F_{1,14} = 19.13$, $P = 0.0006$; data not shown), with an interaction between field type and region ($F_{1,14} = 14.57$, $P = 0.0019$). On average, there were 12 OTUs in this group in conventionally managed and 26 OTUs in organic fields in Monteverde, while there were 18 and 19 OTUs in conventionally managed and organic fields, respectively, in San Vito.

The relative abundances of saprotrophs ($F_{1,14} = 0.002$, $P = 0.9666$) and mycoparasites ($F_{1,14} = 2.94$, $P = 0.1084$) did not differ by field type (Fig. 3B). Plant pathogens were less abundant in conventionally managed than in organic fields ($F_{1,14} = 6.96$, $P = 0.0194$). Plant pathogens were most frequently detected in organic fields in Monteverde, were detected at the lowest frequency in conventionally managed fields in Monteverde, and showed intermediate and highly varied frequencies in both types of coffee fields in San Vito ($F_{1,14} = 5.01$, $P = 0.0419$). In contrast, saprotroph-plant pathogens were more common in conventionally managed than in organic fields ($F_{1,14} = 8.18$, $P = 0.0126$). Saprotroph-plant pathogens were most frequent in conventionally managed fields in Monteverde and did not differ in relative abundance among Monteverde organic fields and both types of fields in San Vito ($F_{1,14} = 14.17$, $P = 0.0021$). On average, root endophytes were twice as abundant in organic than in conventionally managed fields ($F_{1,14} = 8.47$, $P = 0.0114$; data not shown). There were

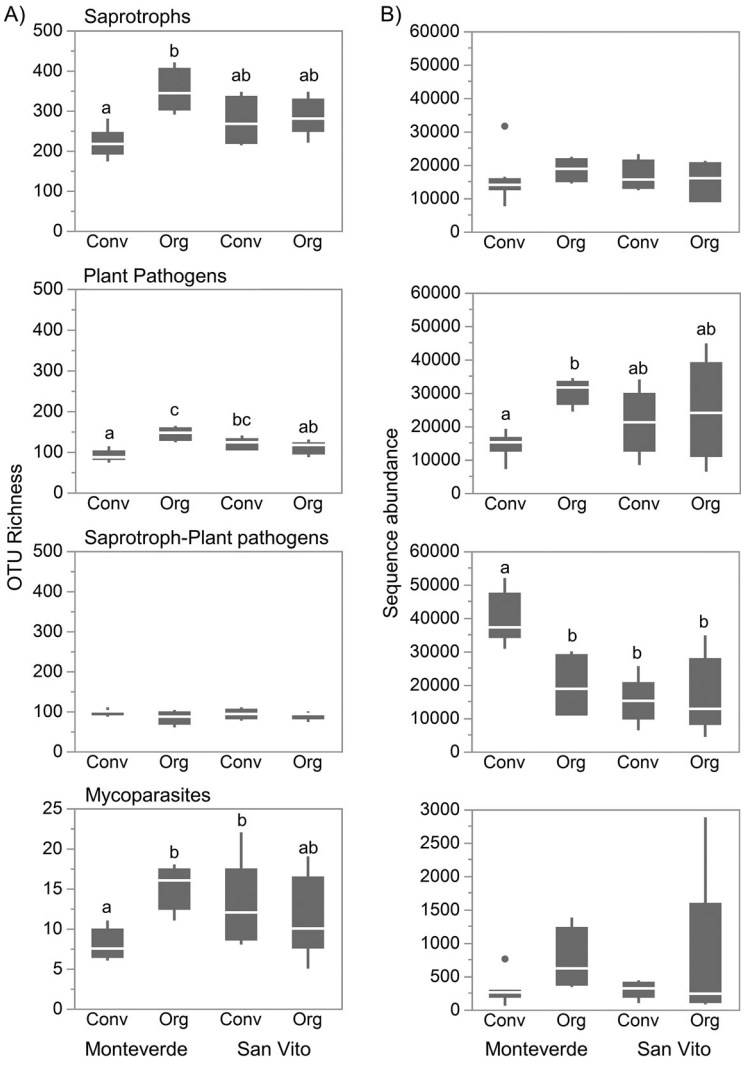

FIG 3 (A and B) Box plots of OTU richness (A) and sequence abundance (B) of four fungal guilds in roots of coffee under conventional (Conv) and organic (Org) management in Monteverde ($n_{conv} = 8$; $n_{org} = 4$) and San Vito ($n = 5$), Costa Rica. Box plots indicate the median and 25th and 75th quartiles, and the whiskers represent 1.5× interquartile distance. Means within a panel that share a letter did not differ statistically at an α of 0.05 by three-way ANOVA followed by Tukey's honestly significant difference (HSD) test to assess the effects of field type, region, and year. Year did not interact with field type.

also more sequences of OTUs that could be assigned to multiple guilds in organic than in conventionally managed fields ($F_{1,14} = 7.18$, $P = 0.0179$; data not shown).

Of the 538 OTUs found in three or more coffee fields and with sequence counts of >500, 34 OTUs differed in abundance by field type (Table 3). The majority of these OTUs belonged to the Pezizomycotina. Roughly one-quarter could not be assigned to a possible guild, and a number could not be assigned to a class or order. *Knufia* spp., saprotroph-plant pathogens, were the closest known relatives for six OTUs, which were all more abundant in roots of conventionally managed than in organic coffee. Of the other nine OTUs that were overrepresented in conventionally managed coffee fields, only one was closely related to known facultative pathogens of coffee, *Mycena* spp. (41). OTUs with greater abundance in conventionally managed fields were primarily from saprotroph-plant pathogen guilds (7 OTUs) but also included AM fungi (3 OTUs), saprotrophs, and three unknowns in the Sordariomycetes.

Nineteen OTUs were more abundant in organic than in conventionally managed fields (Table 3). OTUs overrepresented in organic fields appear to belong to diverse guilds, including saprotrophs (7 OTUs), an endophyte, a pathogen and a saprotroph-

TABLE 3 Fungal OTUs that differed in abundance between coffee roots from conventionally managed and organic fields by three-way ANOVA, with year, region, and field type as main effects and all interactions at an α of 0.05[a]

Phylum	Subphylum	Class	Order	Family	Species	Conf	Possible guild	OTU	No. of occurrences by field type (mean ± SE)		$F_{1,14}$	FDR *P*
									Conventional	Organic		
Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Unknown	Unassigned	0.52	Unknown	9400	9 ± 4	155 ± 63	26.66	0.0102
Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala* sp.	1	S	3736	3 ± 3	286 ± 142	47.92	0.0016
Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala* sp.	1	S	4272	9 ± 6	176 ± 74	17.00	0.0333
Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala pisciphila*	0.97	S	4246	473 ± 77	96 ± 48	27.36	0.0096
Ascomycota	Pezizomycotina	*Incertae sedis*			*Campylospora parvula*	1	S	7040	3 ± 2	510 ± 334	17.62	0.0301
Ascomycota	Pezizomycotina	*Incertae sedis*			*Dokmaia monthadangii*	1	Endophyte	9531	23 ± 9	187 ± 64	15.93	0.0389
Ascomycota	Pezizomycotina	*Incertae sedis*			*Knufia* sp.	0.75	S-PP	6468	10,866 ± 2,241	1,028 ± 255	67.57	0.0004
Ascomycota	Pezizomycotina	*Incertae sedis*			*Knufia* sp.	0.82	S-PP	7025	37 ± 8	2 ± 1	80.45	0.0003
Ascomycota	Pezizomycotina	*Incertae sedis*			*Knufia* sp.	0.63	S-PP	7027	36 ± 8	3 ± 1	78.26	0.0003
Ascomycota	Pezizomycotina	*Incertae sedis*			*Knufia* sp.	0.72	S-PP	7053	66 ± 15	8 ± 3	43.92	0.0019
Ascomycota	Pezizomycotina	*Incertae sedis*			*Knufia* sp.	0.71	S-PP	7059	85 ± 19	9 ± 3	79.82	0.0003
Ascomycota	Pezizomycotina	*Incertae sedis*			*Knufia* sp.	0.71	S-PP	7070	38 ± 8	3 ± 1	45.97	0.0017
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	*Incertae sedis*	*Acremonium furcatum*	0.99	Multiple	6073	7 ± 4	78 ± 33	24.18	0.0129
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	*Incertae sedis*	*Acremonium stromaticum*	0.67	Multiple	6237	4 ± 2	43 ± 19	31.06	0.0072
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	*Incertae sedis*	*Myrothecium cinctum*	0.98	S	8768	5 ± 2	58 ± 25	20.80	0.0192
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	*Incertae sedis*	*Myrothecium roridum*	1	S	4618	5 ± 3	170 ± 82	23.27	0.0146
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	*Cylindrocarpon pauciseptatum*	0.99	PP	7563	459 ± 176	2,190 ± 646	15.30	0.0416
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium equiseti*	0.92	S-PP	9134	108 ± 37	481 ± 233	15.49	0.0413
Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Nectriaceae	Unknown	0.6	S	5297	43 ± 22	0 ± 0	14.24	0.0493
Ascomycota	Pezizomycotina	Sordariomycetes	*Incertae sedis*		*Myrmecridium schulzeri*	0.98	S	9604	20 ± 5	108 ± 36	19.20	0.0234
Ascomycota	Pezizomycotina	Sordariomycetes	Sordariales	Chaetomiaceae	Unassigned	0.58	Unknown	8505	44 ± 24	0 ± 0	19.19	0.0234
Ascomycota	Pezizomycotina	Sordariomycetes	Unknown		Unassigned	0.84	Unknown	5069	7 ± 7	62 ± 30	21.69	0.0177
Ascomycota	Pezizomycotina	Sordariomycetes	Unknown		Unassigned	0.59	Unknown	6182	724 ± 149	147 ± 71	15.72	0.0402
Ascomycota	Pezizomycotina	Sordariomycetes	Unknown		Unassigned	0.52	Unknown	7523	50 ± 22	1 ± 0	17.12	0.0330
Ascomycota	Pezizomycotina	Unknown			Unassigned	1	Unknown	3916	1 ± 1	86 ± 30	71.98	0.0003
Ascomycota	Pezizomycotina	Unknown			Unassigned	0.97	Unknown	4271	2 ± 1	99 ± 57	94.53	0.0003
Ascomycota	Pezizomycotina	Unknown			Unassigned	0.6	Unknown	7282	2 ± 2	62 ± 32	24.44	0.0129
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Agaricaceae	Unknown	0.71	S	3456	0 ± 0	68 ± 55	19.77	0.0220
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Mycenaceae	*Mycena* sp.	0.87	S-PP	2014	170 ± 110	0 ± 0	73.55	0.0003
Basidiomycota	Agaricomycotina	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Ceratobasidium*	0.96	PP-Ecto	972	3 ± 3	75 ± 50	24.02	0.0129
Glomeromycota	Glomeromycotina	Glomeromycetes	Diversisporales	Gigasporaceae	*Gigaspora* sp.	0.87	AM	5119	82 ± 30	0 ± 0	25.32	0.0119
Glomeromycota	Glomeromycotina	Glomeromycetes	Diversisporales	Gigasporaceae	*Gigaspora* sp.	0.71	AM	5518	115 ± 45	0 ± 0	17.21	0.0328
Glomeromycota	Glomeromycotina	Glomeromycetes	Diversisporales	Gigasporaceae	*Gigaspora margarita*	0.95	AM	5234	205 ± 86	0 ± 0	15.97	0.0389
Unknown					Unassigned	1	Unknown	1774	9 ± 5	367 ± 185	22.27	0.0161

[a]Only OTUs with sequence counts of >500 and found in >3 coffee fields were included in the analysis. Possible guild was as assigned by FUNGuild, with the identity and confidence (Conf) of the closest match to each ITS2 sequence according to the Warcup data set (93). S, saprotroph; S-PP, saprotroph-plant pathogen; AM, arbuscular mycorrhizal; Ecto, ectomycorrhizal; FDR *P*, false-discovery rate *P* value.

plant pathogen, two OTUs related to *Acremonium* (a genus with multiple functional roles), a plant-pathogenic or ectomycorrhizal fungus, and six OTUs with unknown function. Four of these OTUs appear to be closely related to species previously identified as pathogens on coffee, as follows: *Fusarium stilboides*, which causes a coffee bark disease in Africa; *Myrothecium roridum*, which causes a coffee leaf spot disease; and *Ceratobasidium* spp., the causal agents of white thread blight (41, 42).

DISCUSSION

Our comparisons of environmental characteristics and root fungal communities in conventionally managed and organic coffee fields suggest that the diversity and community compositions of root fungal communities in coffee are influenced by a series of factors, some of which are directly related to the type of field management. Root fungal diversity was lowest in conventionally managed fields in Monteverde, which were also the fields exposed to fungicides and with the lowest shade and shade tree richness, lowest leaf litter, highest soil nitrate availability, and lowest magnesium availability. The increased light and nitrate availability, increased soil acidity, and decreased calcium, copper, magnesium, and potassium availability associated with conventional management were correlated with differences in root fungal community composition. Shade tree diversity, elevation, and availability of phosphorus, iron, and zinc were also correlated with differences in fungal community composition but did not differ consistently between field types.

Saprotrophs and AM fungi were the most diverse guilds in these coffee agroecosystems, while saprotroph-plant pathogens and plant pathogens were the most abundant. Type of field management also influenced the trophic structure of root fungal communities. Saprotroph, plant-pathogenic, and mycoparasitic fungi were least diverse, and saprotroph-plant-pathogenic fungi were most abundant in conventionally managed fields with the lowest shade and shade tree richness, highest soil nitrate availability, and lowest magnesium availability.

Species loss. While organic coffee fields differed in the compositions of their root fungal communities relative to those of conventionally managed fields, the most abundant OTUs were abundant regardless of management. However, on average OTU richness was 20% higher in organic than in conventionally managed fields, suggesting that conventional management results in the loss of rarer fungal taxa. Organic agriculture has generally been found to be more favorable to the maintenance of biodiversity than has conventional management (43). Shade coffee, which includes an overstory component as well as minimal inputs of synthetic fertilizers and fungicides (44), has been to shown to maintain species richness of animals across a range of taxonomic groups (mammals, birds, and invertebrates [33, 45, 46]). Most studies comparing soil fungi across agricultural management regimes have focused on AM fungi. Organic farming of apples, maize, and potatoes fostered a greater diversity of AM fungi than did conventional farming (47, 48). The richness and diversity of AM fungi declined with increasing management intensity in coffee in Ethiopia and Brazil (32, 49). In this study, we documented greater total fungal diversity in organic coffee than in conventionally managed coffee.

Environmental filtering. Several environmental characteristics appear to have acted as filters on root fungi in the coffee fields in this study, including shade, nitrate availability, and soil acidity. The conventionally managed fields in Monteverde, which had the lowest OTU richness of root fungi, also had the highest light availability. Shade produces a cooler and more humid microclimate that is more conducive to the growth of most fungi than are high-sunlight environments in the tropics (50, 51). The three fields we sampled which had previously been managed conventionally but were currently not receiving inputs of synthetic fertilizers, insecticides, and fungicides exhibited root fungal communities that were more similar to those of conventionally managed fields than of organic fields. This similarity suggests that the high light availability in these three fields may be an important determinant of fungal species composition belowground.

Nitrogen fertilization may negatively affect the diversity of root fungi. We found that soil NO_3^--N availability was higher in conventionally managed fields than in organic fields and that conventionally managed fields in Monteverde had the highest soil nitrate availability and the lowest fungal OTU richness, although conventionally managed fields in San Vito also had higher soil nitrate availability than did organic fields and yet no corresponding difference in fungal richness. Nitrogen fertilization in sugarcane did not reduce soil fungal diversity but did alter fungal community composition (52). In a meta-analysis of studies on the response of plant communities to nitrogen fertilization, Suding et al. (53) found that communities with high N inputs were less diverse because plant species with high N requirements were able to outcompete other species. Because shade was positively correlated with soil pH and soil availability of calcium, copper, magnesium, and potassium, while being negatively correlated with soil nitrate availability, the low species richness of root-associated fungi in conventionally managed fields in Monteverde may be the result of nitrogen fertilization but could also reflect the importance of these other differences in the soil environment for fungal species diversity.

Fungal guilds and trophic structure. The shifts in composition of coffee root fungal communities were associated with measures of soil fertility and aspects of shade. Fields with high light and nitrate availability, correspondingly high soil acidity, and lower availability of cations (calcium, copper, magnesium, and potassium) tended to be those that were conventionally managed and were associated with distinct root fungal communities that exhibited an altered guild structure relative to most organic fields. Plant pathogens were less abundant in conventionally managed fields than in organic fields in Monteverde, as expected given the use of synthetic fungicides in the former and their strict absence (other than use of biocontrol agents such as *Trichoderma* spp.) in the latter.

Differences in nitrogen availability and form influence which types of fungi are supported within plant roots (16, 54). Previous studies in agricultural and forest systems have shown that nitrogen enrichment shifts rhizosphere fungal communities so that plant pathogens become more abundant (15, 16, 52, 55). However, we found reduced richness and abundance of plant pathogens in conventionally managed fields compared to those of organic fields in Monteverde and no difference in San Vito. This suggests that the systemic fungicides applied to conventionally managed fields, which can enter the soil and restructure soil fungal communities (56, 57), may have played a stronger role in structuring coffee root fungal communities than did soil nitrate availability.

In conventionally managed fields, plant pathogens appeared to have been replaced by saprotroph-plant pathogens. Some fungi can act as opportunists, shifting from one guild to another based on dynamic relationships between host, fungus, and environment (3, 11). It is possible that obligate plant pathogens are more effectively suppressed by the use of fungicides (58), while pathogenic fungi also capable of surviving as saprotrophs proliferate and recolonize plant roots as pathogens once systemic fungicide levels decline.

While there was no effect of management on the abundances of saprotrophs or mycoparasites, these guilds were the most diverse in Monteverde organic fields, in which soil nitrate availability was low. Saprotrophic fungi grow more slowly and perform less decomposition in soils enriched with combined ammonium and NO_3^--N than in unfertilized soils (59). Nitrate fertilization has been shown to reduce the activity of ligninolytic enzymes and the flow of carbon through soil food webs (60). Thus, the lower soil nitrate availability in organic fields may explain the greater richness of saprotrophic fungi in organic relative to conventionally managed fields. Paungfoo-Lonhienne et al. (52) found that mycoparasitic fungi were less abundant in soils with high nitrogen inputs. In our study, mycoparasite sequences were quite rare in conventionally managed fields but were varied enough in abundance in organic fields that we were unable to detect a statistical difference between organic and conventionally

managed fields with our sample size. However, the OTU richness of mycoparasites was 2 to 3 times higher in organic than conventionally managed fields in Monteverde. The tendency for rare species to be eliminated by high N inputs, combined with the inhibiting effects of nitrogen on growth and abundance of saprotrophs and mycoparasites, may have led to the lower richness of these guilds in conventionally managed fields (52, 53, 59).

Organic fields were less acidic than were conventionally managed fields. This may be due to both the greater density and diversity of shade trees and the absence of inputs of chemical fertilizers. Nitrogen fertilizers can cause soil acidification (20). The presence of shade trees is associated with lower rates of nitrification and soil acidity relative to coffee grown in full sun (61). Saprotrophs degrade litter less efficiently in acidic soils (62, 63). Thus, the higher pH in organic fields may explain the greater richness of saprotrophic fungi in those fields relative to conventionally managed fields. However, because leaf litter was deeper in organic fields, saprotrophs may also have been more abundant in organic fields in Monteverde due to an increase in substrate availability.

Calcium and magnesium were more available in organic than in conventionally managed field soils in Monteverde. Because fertilizer application in conventional management can acidify the soil, cations may be lost more rapidly (64). Alternatively, the species-rich saprotroph communities in Monteverde organic fields may have resulted in more rapid release from organic matter, and consequently, greater soil availability of cations in these fields (65).

Changes in communities at one trophic level can affect diversity and abundance at other levels (66). In marine communities, extinctions are more frequent at the highest trophic levels, while invasions tend to occur more frequently at lower trophic levels (40), resulting in the enrichment of diversity at lower trophic levels and a disproportionate loss of diversity at the highest trophic level (67). Few studies have examined how management of natural or agricultural systems affects the trophic structure of belowground fungal communities (68–71). In this study, we did not observe an increase in the richness of any guild in conventionally managed fields relative to that in organic fields. Instead, saprotroph, plant pathogen, and mycoparasite richness were all lower in conventionally managed fields in Monteverde. If richness at lower trophic levels sustains richness at higher trophic levels, perhaps due to a role for interspecific competition in reducing the strength of trophic cascades (69, 72), the reduction in saprotroph and plant pathogen richness may have contributed to the loss of mycoparasitic species in conventionally managed fields. Mycoparasites were much less diverse in conventionally managed than in organic coffee in Monteverde, consistent with an increased risk of extinction at the highest trophic level in response to more intensive management (40, 69).

The role of mycoparasites belowground in either agricultural or natural systems is only beginning to be elucidated. Several recent studies have examined the potential for mycoparasites to combat fungal plant pathogens on crops (73, 74), and mycoparasites have been used successfully for biological control of the soilborne fungal pathogen *Sclerotinia sclerotiorum* in some field crops (75). Certainly, the loss of diversity in mycoparasites, whether or not it is accompanied by a reduction in abundance, could influence the extent to which plant pathogens can be regulated without resorting to fungicides. *Beauveria bassiana*, a mycoparasite and the primary fungus used for control of the coffee berry borer, has been shown to be more successful in attacking the coffee berry borer in organically grown shade coffee than in conventionally managed coffee in high-light environments (37). With increased recognition of the importance of natural enemies for successful control and reduction in the frequency of outbreaks of pests and diseases, we urgently need a better understanding of how mycoparasites can be reintroduced into agricultural systems once they have been eliminated.

Limitations. Next-generation sequencing has revolutionized our ability to detect microorganisms but also has its limitations (7, 76, 77). Approximately 40% of the

sequences in our data set (more than half the OTUs) could not be identified below the kingdom level. These percentages are similar to values obtained previously in both tropical and temperate studies of root and soil fungal diversity (7, 78) and are unsurprising given the cryptic nature of root fungal communities, the continued paucity of molecular work in the tropics, and the difficulty in culturing many root fungi (79, 80). Since the databases used to assign taxonomy and guild reflect current knowledge, the proportions of these identified fungi may be biased by the various depths of study for different groups of fungi (7). Additionally, species at the highest trophic levels are likely to be the least abundant and therefore the hardest to detect. Here, we use sequence abundance as a proxy for the relative abundances of different OTUs or guilds, yet these measures may differ from the actual abundances of different fungi in the root environment (81). However, there is some evidence from sequencing of mock communities of known abundances that sequence abundances can be strongly correlated with actual abundances (82).

This study used existing variation in coffee field management to examine how environmental factors influence root fungal diversity and community structure. Because fertilizer (and fungicide) applications were negatively correlated with shade, we were unable to assess the effect of one independent of the other. Additionally, the decision to farm organically or using conventional management may have been influenced by underlying environmental conditions at these sites. Future work should involve manipulation of shade, synthetic fungicide, and fertilizer use within individual coffee farms to disentangle the effects of each factor and determine their relative importance in driving the observed loss in diversity and changes in community composition. Despite these limitations, the results of this study reveal important patterns in fungal diversity, fungal community composition, and guilds of known fungi as they relate to differences in coffee management.

Conclusions. We found that fungal communities in coffee roots were structured differently depending on the type of field management. While soil nitrate availability was higher in conventionally managed fields than in organic fields, other indicators of soil fertility were lower in conventionally managed fields. The species richness of root fungi was lowest in fields with the lowest shade and highest nitrate availability. In addition to having the lowest abundance of plant pathogens, coffee roots in these fields also had the lowest richness of saprotrophic and mycoparasitic fungi and the highest abundance of saprotroph-plant pathogens. These results suggest a potential shift in the trophic structure of conventionally managed fields such that obligate plant pathogens are replaced by facultative plant pathogens, while the diversity of the mycoparasites important for the regulation of plant pathogens is reduced. 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. Such a test will help further disentangle how fungal communities respond to common agricultural management strategies and which factors are critical for the maintenance of belowground fungal diversity.

MATERIALS AND METHODS

Site description and study design. Two coffee-growing regions of Costa Rica with a premontane wet forest climate (83) were selected for this study, Monteverde (10°19′27.8″N, 084°50′30.1″W) and San Vito (08°52′41.1″N, 082°57′03.1″W). Soils in both Monteverde and San Vito are Andisols, a volcanic soil type with high organic matter, high leaching capacity, and pH of 5.6 to 5.8 (84). Monteverde experiences slightly lower rainfall, on average (300 cm year^{-1} versus 400 cm year^{-1} in San Vito [85]).

Twenty-five coffee fields were included in this study. Thirteen fields were sampled in Monteverde, with six fields sampled between 25 and 28 May 2011 and seven fields sampled between 1 and 4 June 2012. In San Vito, six fields each were sampled between 31 May to 3 June 2011 and 7 to 11 June 2012. At each site, the farmer or farm manager was interviewed to determine types of herbicides, pesticides, fungicides, and fertilizers used on the field, as well as the cultivars present, age of the field and coffee plants, prior land use, and pruning regimen. Fields were designated "conventionally managed" if farmers reported using synthetic fertilizers and pesticides, as "organic" if farmers reported that fields were certified organic or reported no use of synthetic fertilizers and pesticides in the previous 5 years, and as "minimal conventional" if farmers reported that they were in the process of transitioning from conven-

tional to organic management or had not used synthetic fertilizers or pesticides in the preceding 1 to 3 years.

Field sampling. For each field, the species richness of shade trees, type of windbreak, and phenological status of coffee plants (vegetative, flowering, green or mature fruit) were recorded. All fields except one, in which plants were vegetative, were producing green (immature) or green and red (mature) fruits at the time of sampling. In each field, a 20-m by 20-m plot was established >5 m from the edge and representative of the shade tree density of the field. Approximate elevation was recorded with an eTrex Venture HC global positioning system (GPS) receiver (Garmin Corp., Schaffhausen, Switzerland). The percent canopy cover at the center of the plot was calculated using a spherical densiometer with convex mirror (Forestry Suppliers, Jackson, MS, USA), according to the manufacturer's instructions. Plot aspect was measured by compass; plot slope was measured qualitatively in 2011 and using a clinometer in 2012. Coffee plant density was estimated by averaging the distance between rows for five rows and the distance between plants within a row for five pairs of plants.

Within each plot, one coffee plant was sampled every 5 m along every other row, for a total of 20 plants per plot. At each plant, leaf litter depth was measured at the dripline, and a soil sample was taken using a 2-cm-diameter corer to a depth of approximately 20 cm. From every other sampled plant, root samples were taken at 1 to 15 cm of depth from 3 to 5 sections of fine roots and combined, for a total of 10 plants per plot. Soil samples within a field were pooled, air-dried in paper bags, and stored at room temperature.

In the lab, each root sample was rinsed with tap water and divided in two. One subsample from each plant was stored in 1% (wt/vol) KOH for analysis of root colonization by AM fungi (L. Aldrich-Wolfe, K. L. Black, E. D. L. Hartmann, W. G. Shivega , L. C. Schmaltz, R. D. McGlynn, P. G. Johnson, R. J. Asheim Keller, and S. N. Vink, submitted for publication), while the second subsample was dried in the presence of Drierite (W. A. Hammond Company, Xenia, OH, USA) for DNA extraction. Drying roots results in no reduction in DNA yield relative to isolation from fresh or frozen samples, although it may reduce the yield of fungal DNA (86), and it eliminates the risk of DNA degradation when frozen samples thaw in transit (87). At the end of each year's sampling period, soils and dried root samples for DNA extraction were transported to the United States and stored at room temperature. Two to three soil subsamples from each field were analyzed for soil nutrient availability, pH in water, and organic matter by loss on ignition (LOI) at the Soils Testing Laboratory, North Dakota State University, Fargo, ND, USA. The mean measures per field were subsequently used for all statistical analyses.

Molecular detection of root fungi. Dried root samples were pulverized using six 2.33-mm-diameter chrome-steel beads (Biospec Products, Bartlesville, OK, USA) in a vortex adapter (Mo Bio Laboratories, Carlsbad, CA, USA) on a Vortex-Genie 2 mixer for 1 h (Scientific Industries, Inc., Bohemia, NY, USA). DNA was isolated from 20 mg of each sample for 8 to 10 root samples per field using the DNeasy plant minikit (Qiagen, Germantown, MD, USA), following the manufacturer's protocol (with two elution volumes of 50 μl each), and stored at −20°C.

The internal transcribed spacer region 2 (ITS2) was amplified by PCR for each DNA extract using 12.5 μl of 2× HiFi HotStart ready mix (Kapa Biosystems, Wilmington, MA, USA), 10 μl nuclease-free water, 0.8 μl each of 10 mM fungus-specific high-performance liquid chromatography (HPLC)-purified primers 5.8SR and ITS4 (88), 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 of 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× Tris-borate-EDTA (TBE), followed by staining with ethidium bromide. Extracts which failed to produce PCR products were diluted 10-fold and amplified using the above-described 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, IN, USA), following the manufacturer's protocol, with two washes with ethanol and elution in 10 mM Tris. The concentration of double-stranded DNA (dsDNA) in each sample was measured using a Qubit 2.0 fluorimeter (Invitrogen, Carlsbad, CA, USA). Eight (2011) or 10 (2012) samples per field were pooled at equal DNA concentrations in 10 mM Tris, and 3 to 5 ng of DNA per field was shipped frozen on dry ice for sequencing at the University of Minnesota Genomics Center (UMGC, St. Paul, MN, USA).

PCR products from each field were amplified using Nextera indexing primers (Illumina, San Diego, CA, 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. The 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 platform using reagent kit v3 with separate index reads. Preliminary quality control (QC) and demultiplexing were conducted by the UMGC.

Sequence data processing. Sequences were processed with the PIPITS 1.4.0 pipeline (89), 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 fungus-specific ITS2 region using ITSx 1.0.11 (90). Dereplication, removal of singleton sequences and those that were <100 bp, clustering to 97% sequence identity, and chimera detection, using the UNITE uchime 7.1 data set (91) as a reference, were conducted using VSEARCH 2.3.0 (92). Representative sequences were taxonomically assigned using the Warcup_retrained V2 ITS training set (93) with RDP Classifier 2.11 (94) to a taxonomic confidence level of 50% to retain a greater level of taxonomic resolution in the

downstream analyses. An abundance table was generated that clustered sequences in OTUs at 97% similarity. Samples were rarefied to 132,460 sequences (the number of fungal sequences observed in the smallest sample) in QIIME 1.9.1 (95) to remove the effect of differences in sequencing depth among samples on fungal OTU diversity. The rarefied OTU table was used in all statistical analyses.

Statistical analyses. OTU abundances were Hellinger transformed in R version 3.4.1 (https://cran.r-project.org/) using the vegan package (96) to downweight low-abundance OTUs (97). We used response screening in JMP Pro v13.0 (SAS Institute, Cary, NC, USA) to determine which of the 46 most abundant OTUs (OTUs with sequence counts of >10,000 after rarefaction and Hellinger transformation) and which of the OTUs that were common enough to compare across field types (we excluded OTUs with sequence counts of <500 and found in <4 fields) differed by year, region, or field type. Our model also included all possible interactions of these three factors. We adjusted *P* values using the false-discovery rate method to control the type I error rate (98). We excluded minimal conventional fields from this and all subsequent statistical analyses due to small sample size ($n = 3$). Species accumulation curves were calculated in PC-ORD v7.07 (99) by sampling with replacement ($n = 500$).

OTUs were assigned to functional guilds using FUNGuild, currently the largest database of fungal guilds (7), and then pooled into simplified guilds that reflected trophic level and type of interaction (e.g., "wood saprotroph" and "leaf saprotroph" were both considered saprotrophs; Table S1). We used a three-factor, full factorial analysis of variance (ANOVA) in JMP Pro v13 to assess the effects of field type, region, and year on environmental characteristics, total fungal OTU richness and abundance, and richness and abundance of fungal guilds. Because there was no effect of year on saprotroph richness, year was excluded from the final model for this guild. To meet model assumptions, canopy cover was arcsine square root transformed; leaf litter depth, elevation, pH, zinc, copper, and calcium were square root transformed; shade tree species richness, number of fungicides, age of coffee field, soil electrical conductivity, nitrate, phosphorus, potassium, manganese, and magnesium availability, and mycoparasite richness were log transformed, and the reciprocal was taken for percent organic matter prior to analysis. We used pairwise correlations in JMP Pro v13 to test for associations between fungal richness and environmental variables.

To visualize differences in fungal community composition, a nonmetric multidimensional scaling (NMS) ordination was carried out on the rarefied, Hellinger-transformed OTU data using the Sorenson distance measure, a random starting configuration, and 50 runs each with randomized and real data in PC-ORD v7.02. We used two-way permutational multivariate analysis of variance (PERMANOVA) (100) to compare fungal community composition in organic and conventionally managed fields as a function of each possible two-factor combination of year, region, and field type. For each PERMANOVA, six fields were excluded at random to obtain a balanced design. To visualize correlations of root fungal community composition with environmental variables, we constructed a biplot with the ordination (transforming environmental variables as indicated above to satisfy assumptions of normality and homogeneity of variance).

Data availability. Environmental and site history data for each field and the OTU table used for all data analyses are available at Dryad (https://datadryad.org/stash/dataset/doi:10.5061/dryad.q2bvq83g1). The ITS2 DNA sequences generated and analyzed during this study are publicly available under BioProject number PRJNA531329, BioSample numbers SAMN11371063 to SAMN11371087, and Sequence Read Archive numbers SRR8868669 to SRR8868693 at the National Center for Biotechnology Information, USA.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

ACKNOWLEDGMENTS

We thank the many farmers in Costa Rica who made this work possible; Guillermo Vargas Leitón, Ana Ovares Ugalde, Finca LifeMonteverde, Las Cruces Biological Station, the Organization for Tropical Studies, and Javier Guevara Sequiera at SINAC for logistical support; and the Minnesota Supercomputing Institute (MSI) at the University of Minnesota for providing resources that contributed to the research results reported in this paper (https://www.msi.umn.edu/). Comments from two anonymous reviewers strengthened the manuscript.

Funding for this research was provided by the Office of Academic Affairs, Biology Department Fuglestad-Torstveit Fund, NSF award DUE-0969568 to the Division of Science and Mathematics at Concordia College, and NSF EPSCoR-Track I cooperative agreement OIA-1355466 to North Dakota State University.

Any opinions, findings, conclusions, or recommendations expressed here are those of the authors and do not necessarily reflect the views of the National Science Foundation.

R.D.M., L.C.S., E.D.L.H., P.G.J., E.C.S., and L.A.-W. contributed to the study conception and design. Data were collected by L.C.S., R.D.M., E.D.L.H., P.G.J., W.G.S., K.L.B., and

R.J.A.K. L.A.-W., K.L.B., E.C.S., and S.N.V. conducted data processing and analysis. The first draft of the manuscript was written by E.C.S. All authors read and commented on previous drafts and read and approved the final manuscript.

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