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Occurrence of arbuscular mycorrhizal fungi in leaf litter and roots of shaded coffee plantations under organic and conventional management

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ABSTRACT: Evidence of arbuscular mycorrhizal fungal colonization of mat litter in various ecosystems plus previous reports of external mycelium of those fungi and mycorrhizal roots in litter from coffee plants and shade trees on coffee plantations suggest that they have a relationship with closed direct nutrient cycling between organic matter and living roots. This relationship was first proposed more than 50 years ago. Mycorrhizal symbiosis in tropical crops is affected by agricultural management practices. This study aimed to assess the occurrence of arbuscular mycorrhizal fungi in leaf litter from three shaded Colombian coffee agroecosystems under organic and conventional management. One is managed chemically, one organically, and one with a combined use of organic and chemical inputs. Leaf litter and roots were collected from the three coffee plots at three decomposition stages. Each plot represented a distinct fertilization and tree dominance pattern different from the other two plots. Arbuscular mycorrhizal fungi were found in decomposing leaves. The chemically managed plot showed statistical differences ($p<0.05$) with respect to the other plots, it had the greatest amounts of arbuscular mycorrhizal fungal root colonization (48.76–70.51 %), litter colonization (36.2–69.91 %), external mycelium length (28.66–48.33 m g^{-1}), and spore number (451.27–681.2 spores in 20 g of dry soil). In contrast, conditions on the combined management coffee plot results in smaller means of the variables evaluated. Arbuscular mycorrhizal fungal root colonization and nitrogen content of leaf litter varied among the decomposition stages ($p<0.05$). Litter quality of different tree species may have influenced colonization of plant matter within each plot. We found evidence of typical structures of arbuscular mycorrhizal fungi within and among decomposing leaf litter and roots growing into the mat litter in tropical agroecosystems. This supports the thought that these fungi have a role in carbon and nutrient recycling, which are influenced by agricultural management practices and plant population composition.

Keywords: nutrient cycling, *Coffea arabica*, organic agriculture, agroforestry.

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INTRODUCTION

Arbuscular mycorrhizal fungi colonize leaf litter across a wide range of natural ecosystems and seem to have a key role in the decomposition of organic matter and subsequent transference of nutrients to plants (Bunn et al., 2019). These microorganisms are presumed to have no saprophytic capacity (Herman et al., 2012), but can induce a faster and more intense leaf litter decomposition (Gui et al., 2017). It has been proposed that direct nutrient cycling is the main energy-saving mechanism in tropical ecosystems where arbuscular mycorrhizal plants are dominant (Diederichs and Moawad, 1993; Cuenca, 2015). Several reports about arbuscular mycorrhizal fungi in seeds, arthropod skeletons, and decomposing leaves have been published (Pirozynski and Dalpé, 1989; Rivera and Guerrero, 1998; Aristizábal et al., 2004; Posada et al., 2012). These fungi can minimize losses of mineralized nutrients via lixiviation, leaching, or chemical fixation to soil components since these nutrients are directly transported from the colonized leaf litter to the host plant's roots (Rivera and Guerrero et al., 1998). Aristizábal et al. (2004) conclude that arbuscular mycorrhizal fungi are more important for recycling mineral nutrients in tropical areas than previously recognized. Since there have only been a few studies documenting arbuscular mycorrhizal fungal (AMF) colonization of leaf litter, and it has been proposed to evaluate it in different types of ecosystems (Bunn et al., 2019), new research on those systems with significant amounts of mulch and high rates of nutrient losses could improve our understanding of nutrient cycling in key terrestrial ecosystems.

These ecosystems include coffee farms and plantations where the mycorrhizal association is critical for growth, nutrition, and stress tolerance of plants (Cogo et al., 2017). Sustainable shaded coffee ecosystems are common on both conventional and organic farms in Latin America. Shade is commonly provided by plants from *Alchornea*, *Cecropia*, *Cedrela*, *Citrus*, *Cordia*, *Croton*, *Erythrina*, *Eucalyptus*, *Ficus*, *Fraxinus*, *Hyeronima*, *Inga*, *Musa*, *Nectandra*, *Ocotea*, *Persea*, *Prunus*, *Solanum*, *Vernonia,* and *Viburnum*. Nutrient and water cycling vary according to vegetation structure and composition as well as to shade conditions (Beer et al., 1997; Toledo and Moguel, 2012; Haggar et al., 2015). In most Colombian coffee-growing zones, coffee is planted close to shade trees which provide ecological benefits as well as wood and other types of fruit. Producers locate coffee plants depending on the availability of sunlight, water, and nutrients on the particular plot (DaMatta and Rodríguez, 2007).

Sixty-seven percent of Colombian coffee is grown under shade or partial shade (Cardona-Calle and Sadeghian, 2005). Some shade trees provide natural mulch in the form of abundant leaf litter, which is a significant source of organic matter (Romero-Alvarado et al., 2002; Zhang et al., 2017) and also provides a habitat for mycorrhizal fungi to colonize (Aristizábal et al., 2004; Posada et al., 2012; Bunn et al., 2019). Litter quality varies among plant species and is a key factor in the decomposition of organic matter in tropical forest ecosystems (Krishna and Mohan, 2017). The C:N ratio has been used as a predictor of decomposition (Mafongoya et al., 1997), for example in tropical agroecosystems containing coffee, the decomposition rates of *Erythrina* and *Musa* litter depend on the specific mixture of leaves (Tully and Lawrence, 2012). As leaf litter decomposes, it becomes a potential source of mineral nutrients for all plants within the system (Hodge et al., 2001). However, in the coffee-growing soils of Colombia's Andean region, nutrients released from organic matter are quickly fixed in the soil and become unavailable to plants. Consequently, biological mechanisms that facilitate nutrient cycling and reduce in-soil losses need to be introduced into these ecosystems (Cavagnaro et al., 2015). Leaf litter colonization by arbuscular mycorrhizal fungi, which take up recently mineralized nutrients and transfer them to plant roots, is one such system with potential for future use, taking into account the influence that shade tree composition and decomposition rates may have on the mycorrhizal colonization.

Recently, coffee producers have increased their use of organic production systems by employing compost and vermicompost instead of the chemical inputs used by conventional growing coffee systems (Ibanez and Blackman, 2016). Given the role of mycorrhizae in coffee plantations, a better understanding of the effects of low-impact management on beneficial microorganisms could allow them to become an even more widespread alternative to high-impact techniques employed in Colombian mountain slope agriculture.

This study aimed to evaluate the occurrence of arbuscular mycorrhizal fungi in leaf litter at three decomposition stages in shaded coffee under organic and conventional management systems, in plots with different shade tree composition. This could support the possibility that AMF natural leaf litter colonization in managed ecosystems is a global phenomenon.

MATERIALS AND METHODS

Study sites and sampling

This study was conducted on the Altamira coffee farm in San Francisco, Cundinamarca, Colombia at 04° 58' 38" N, 74° 17' 32" W, and 1,800 m above sea level. Soils are mainly Andisols (Parra Ardila et al., 1974). The mean annual temperature is approximately 20 °C and the average annual rainfall is 1,830 mm. There are two rainy seasons (April to June and September to November). Sampling was conducted in May, during the rainy season. Samples of leaf litter from shade trees were collected from plots under organic management (OR), conventional management with chemical inputs (CH), and under combined organic and chemical management (ORCH). The OR plot had 86 shade trees while the CH plot and the ORCH plot had 23 shade trees. Three months prior to sampling the OR plot was fertilized with 20 m^3 ha^{-1} of biodigester effluent derived from pig and cow manure and one month before sample collection, 2 kg per plant of vermicompost was applied around each plant. The CH plot was fertilized one month before sampling with 2 kg of 25-4-24 NPK fertilizer per coffee plant at 0.10 m from the stem. The ORCH plot was fertilized with the biodigester effluent as previously described, 0.8 kg of chicken manure, and 80 g of 25-4-24 NPK fertilizer per plant three months before sampling. In addition, 2 kg of vermicompost per plant was applied one month before sampling.

Three quadrants of 400 cm^2 were established within each plot. As described in table 1, three stages of decomposing leaf litter were sampled for a total of 9 samples per plot, as proposed by Zheng et al. (2018). Leaf litter and roots present in the litter were collected. The amount of sample collected in each stage ranged from 23-137, 73-352, and 174-934 g for stages 1, 2, and 3, respectively, occupying the same volume. The areas of the leaf fragments were measured and the average was estimated (Table 1). Plant species near each sampling site were recorded. After collecting the leaf litter, soil samples were taken in each sampling site at a depth of 0 to 0.15-0.20 m. A composite sample from each plot was prepared to perform physical-chemical soil analyses. In addition, two coffee root samples were collected from each plot and stored in plastic bags at 4 °C to assess their fungal colonization.

Soil characterization and leaf litter carbon and nitrogen content

To determine the total carbon and nitrogen contents of plant material, nine composite samples of leaf litter fragments were created from samples at each of the three decomposition stages from each plot. Soil and litter were sent to the Soil Laboratory at the Instituto Geográfico Agustín Codazzi (IGAC) in Bogotá, Colombia. The laboratory measured soil texture, pH, aluminum content, aluminum saturation percentage, organic carbon content, extractable phosphorus content, base saturation, exchangeable complex, and carbon and nitrogen contents (Aristizábal et al., 2004; IGAC, 2006).

Table 1. Traits of three different stages of leaf litter decomposition within sampled plots

Stage	Description	Leaf area			Decomposition layers reported in literature
		OR	CH	ORCH	
		cm^2			
1	Upper litter layer composed mainly of not-yet decomposed plant matter and already fragmented leaves	1.0	2.2	2.3	L: composed of recognizable plant remains
2	Fragmented leaves with the greatest proportion of roots and soil. Leaves between topmost layer and soil layer	0.7	0.8	1.0	LF: mixture of organic matter in different stages of 3 decomposition
3	Highly fragmented leaves in contact with roots and incorporated into the upper layer of soil	0.3	0.4	0.3	LF: mixture of organic matter in different stages of 3 decomposition

H (humus) layer was not considered in the present study. OR: organic; CH: conventional; ORCH: combined; L: fresh litter layer; LF: fresh litter and fermentation layer.

Clearing and staining of plant material and fungal colonization assessment

Conventional techniques were adapted to our research conditions for analysis of AMF and non-arbuscular mycorrhizal fungal (NAMF) colonization of both leaf litter and roots. Arbuscular mycorrhizal fungi were identified by their coenocytic hyphae with unilateral angular projections (Friese and Allen, 1991) while non-arbuscular mycorrhizal fungi were identified by their dematiaceous and septate hyphae with thin cell walls. Arbuscular mycorrhizal fungi and non-arbuscular mycorrhizal fungi were cleared, stained, and analyzed separately. A total of 23 clearing and staining assays were performed on leaf litter samples. The concentrations of KOH and H_2O_2 proposed by Phillips and Hayman (1970) were modified and measured against exposure times and treatment temperatures. Cleared samples were acidified and stained (Phillips and Hayman, 1970).

Colonization was assessed through observation of typical structures of arbuscular mycorrhizal fungi including hyphae, vesicles, and spores. We analyzed two to four slides per sample. The colonization percentage was estimated to be the number of colonized intersections divided by the total number of observed intersections. Similarly, root samples were mounted on microscope slides for assessment according to McGonigle et al. (1990). We estimated amounts of AMF leaf litter colonization, AMF colonization of roots within leaf litter, NAMF leaf litter colonization, and NAMF colonization of roots within leaf litter.

Spore number

Arbuscular mycorrhizal fungal spores were isolated from a 20-g subsample of leaf litter or soil by wet sieving and decanting followed by centrifugal flotation (Gerdemann and Nicholson, 1963; Jenkins, 1964). Spore number was reported as the number of spores per 20 g of dry soil sample.

External mycelium

Isolation and quantification of external mycelium lengths were performed as described by Herrera et al. (1986) from 5 g of soil or leaf litter. Results were expressed in m g^{-1} of sample.

Statistical analyses

All statistical analyses were performed with R (StatR, RWizard platform version 4.2) (Guisande et al., 2014). Data from the three decomposition stages of the leaf litter for all three plots, OR, CH, and ORCH were analyzed with nested ANOVA. To reach data

normality and homogeneity of variance, logarithm transformation was used for spore number and external mycelium length while arcsine transformation was used for AMF litter colonization, NAMF litter colonization, and NAMF root colonization. Differences of means among plots were revealed with a Tukey's range test when the ANOVA p-value was significant.

One-way ANOVA was used to analyze differences among data obtained from leaf litter carbon and nitrogen contents as well as C:N ratios of all three plots. Then one-way ANOVA was used to analyze differences among data among decomposition stages. Spearman correlations among carbon and nitrogen contents, C:N ratios, and fungal variables were established using means for each variable while correlations between fungal variables were estimated through analysis across the whole data set. Relative frequencies of coffee shade trees in OR, ORCH, and CH were estimated by calculating the number of plants of each species in each plot divided by the total number of plants other than coffee plants in that plot. Coffee plants were not taken into account in calculations of relative frequencies.

RESULTS

Most of the plants associated with coffee crops that contributed to leaf litter were AMF hosts. Organic treatment contained a greater number of different tree species than CH and ORCH. In OR, *Musa paradisiaca* and *Citrus* sp. were predominant with relative frequencies of 0.337 and 0.139. Meanwhile, other plants within the plot included various Fabaceae trees belonging to genera *Inga, Erythrina,* and *Leucaena*. In ORCH, *Musa paradisiaca* presented the highest relative frequency with a value of 0.740. It was followed by *Nectandra* sp. A smaller proportion of *Citrus* sp. trees were present in ORCH than in OR. *Fraxinus* sp. and *Ficus* sp. were predominant in CH. Their relative frequencies were 0.156 and 0.125. They were followed by *Citrus* sp., *Vernonia* sp., and *Inga* sp., which had higher relative frequencies than in OR (Table 2).

Nitrogen contents decreased from 2.13 % in stage 1 to 1.56 % in stage 3 as decomposition progressed ($p<0.05$) (Table 3). Carbon content values were 44.1, 37.6, and 29.34 % for stages 1, 2, and 3, respectively. At 41.67 %, carbon content was highest in OR where *Musa paradisiaca* and *Citrus* sp. predominated.

Arbuscular mycorrhizal fungal colonization of material from all plots included external mycelium and colonization of roots and decomposing leaf litter in veins and blades. Figure 1 shows typical structures of arbuscular mycorrhizal fungi observed in stage 3 in both leaf litter and roots. There were significant differences in spore number, external mycelium length, AMF leaf litter colonization, and AMF root colonization among plots, which represented the primary factor. There were also differences in AMF root colonization between and among decomposition stages, which represented the nested secondary factor (Table 4). Conventional counts of AMF variables were significantly different from ORCH counts. In CH, we found spore densities ($p<0.01$) higher than 451 spores per 20 g dry soil, external mycelium ($p<0.01$) values greater than 20 m g^{-1}, AMF litter colonization ($p<0.05$) means between 36.2–69.9 %, and AMF root colonization ($p<0.01$) values between 48.76–70.51 %. Similarly, spore number and AMF root colonization measurements were higher ($p<0.01$) in CH than in OR which had means ranging between 27.35-51.61 spores per 20 g of dry soil and 18.83-35.68 % for AMF root colonization. Average external mycelium lengths peaked in stage 2 samples from OR and ORCH with values of 27.54 and 9.69 m g^{-1}, respectively. Only in CH the average length continued to increase into stage 3. They grew to as long as 48.33 m g^{-1}. Coefficients of variation (CV) for all measurements were generally above 30 % (Table 4). The lowest CV of 9.88 % was obtained in colonized roots from CH leaf litter from stage 1. This indicates lower spatial variation than in the other sampling areas.

We observed arbuscular and non-arbuscular mycorrhizal fungi coexisting in coffee roots. The means for mycorrhizal root colonization were 43 % in CH, 44.5 % in ORCH,

Table 2. Relative frequency of coffee shade trees and associated plants in sampled plots

Tree	Family	Plot		
		OR	CH	ORCH
Vernonia sp.	Asteraceae	0.023	0.094	0.000
Protium sp.	Burseraceae	0.012	0.000	0.000
Carica pubescens	Caricaceae	0.012	0.000	0.000
Clethra sp.	Clethraceae	0.000	0.031	0.000
Cupressus lusitanica	Cupressaceae	0.035	0.000	0.000
Macleania rupestris	Ericaceae	0.000	0.031	0.000
Escallonia sp.	Escalloniaceae	0.000	0.031	0.000
Leucaena leucocephala	Fabaceae	0.046	0.000	0.000
Erythrina edulis	Fabaceae	0.035	0.000	0.043
Erythrina rubrinervia	Fabaceae	0.012	0.000	0.000
Inga sp.	Fabaceae	0.035	0.094	0.000
Persea americana	Lauraceae	0.070	0.062	0.000
Ocotea sp.	Lauraceae	0.012	0.000	0.000
Nectandra sp.	Lauraceae	0.012	0.031	0.087
Tibouchina sp.	Melastomataceae	0.012	0.062	0.000
Ficus sp.	Moraceae	0.012	0.125	0.000
Clarisia biflora	Moraceae	0.000	0.031	0.000
Morus insignis	Moraceae	0.000	0.031	0.000
Musa paradisiaca	Musaceae	0.337	0.000	0.740
Eucalyptus globulus	Myrtaceae	0.105	0.031	0.000
Fraxinus sp.	Oleaceae	0.012	0.156	0.000
Retrophyllum rospigliosii	Podocarpaceae	0.035	0.000	0.043
Roupala sp.	Proteaceae	0.012	0.031	0.000
Prunus integrifolia	Rosaceae	0.023	0.031	0.000
Citrus sp.	Rutaceae	0.139	0.094	0.087
Cyphomandra sp.	Solanaceae	0.023	0.000	0.000
Solanum inopinum	Solanaceae	0.000	0.031	0.000

OR: organic; CH: conventional; ORCH: combined.

Table 3. Leaf litter carbon, nitrogen, and carbon to nitrogen ratio within plots and composite averages for three decomposition stages

Plot	C	N	C:N ratio
	%		
OR	41.67 a	1.9 a	22.29 a
CH	36.36 a	2.04 a	17.72 a
ORCH	31.3 a	1.64 a	19.04 a
Stage			
1	44.1 a	2.13 a	21.02 a
2	37.6 a	1.77 ab	21.57 a
3	29.34 a	1.56 b	18.65 a

OR: organic; CH: conventional; ORCH: combined. Means of C, N or C:N ratio followed by the same letter are not significantly different at $p<0.05$ by Tukey's range test. C and N content were determined by dry combustion using a Leco CN 2000 analyzer.

and 37 % in OR while NAMF colonization's mean was 3 % in CH, 2.6 % in ORCH, and 0.7 % in OR. Correlations of AMF leaf litter colonization ($\rho = 0.733$; $p = 0.031$) and AMF root colonization ($\rho = 0.817$; $p = 0.011$) with external mycelium were positive (Table 5).

Further, we found positive correlations of leaf litter carbon with NAMF root colonization ($\rho = 0.833$; $p<0.01$) (Table 6).

Finally, the physicochemical analyses revealed various soil textures and low-nutrient conditions. Despite the large content of organic carbon (5.00-8.33 %) and extractable

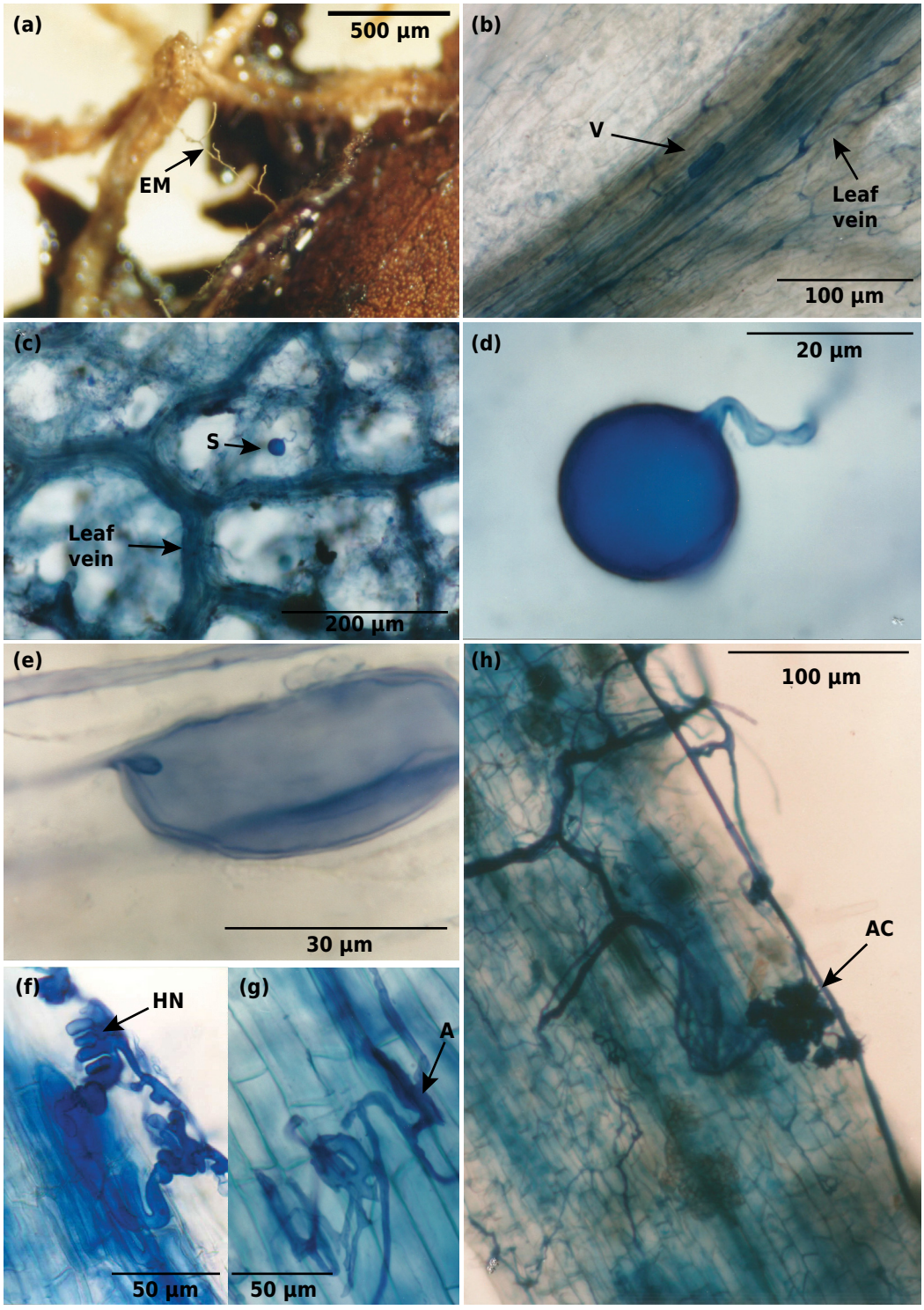

Figure 1. Colonization of leaf litter and roots by AMF. External mycelium (a). Hyphae and a vesicle colonizing a leaf vein in a sample from CH (b). Spore found in leaf tissue from OR (c). Magnified AMF spore found in leaf litter from plot OR (d). Arbuscular mycorrhizal fungal vesicle found in *Fraxinus* sp. leaf litter from plot CH (e). Arbuscular mycorrhizal fungal hyphal infection networks (f). Hyphae and appressorium formation (g). External mycelium and auxiliary cells typical of some Gigasporaceae fungi in roots from CH (h). EM: external mycelium; V: vesicle; S: spore; HN: hyphal infection networks; A: appressorium; AC: auxiliary cells.

Table 4. Fungal parameters from the plots sampled at three different stages of leaf litter decomposition

Plot	Stage	Arbuscular mycorrhizal fungi												Non-arbuscular mycorrhizal fungi					
		Spore number			External mycelium length			Leaf litter colonization			Root colonization			Leaf litter colonization			Root colonization		
		spores per 20 g of dry soil		%	— m g^{-1} —		%	%											
		Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
OR	1	51.61	39.21	93.04	23.18	2.94	15.52	22.55	22.55	141.42	18.83	9.07	59.01	12.29	8.29	95.37	7.15	9.10	155.86
	2	39.24	9.68	30.22	27.54	7.23	32.17	54.44	13.34	34.64	35.68	9.01	30.92	11.73	8.28	99.81	4.51	1.78	48.35
	3	27.35	11.92	53.36	19.25	8.26	52.55	56.12	43.88	110.58	31.84	6.59	25.36	8.33	1.88	31.85	5.63	1.18	25.60
CH	1	681.20	493.53	88.73	28.66	20.52	87.69	36.20	17.56	59.41	70.51	5.69	9.88	12.06	9.30	94.46	7.22	4.72	80.09
	2	483.41	276.03	69.93	36.24	27.37	92.50	42.32	24.29	70.30	67.38	9.53	17.33	5.21	3.52	82.70	3.44	1.48	52.66
	3	451.27	260.39	70.67	48.33	26.17	66.33	69.91	29.96	52.50	48.76	21.18	53.21	14.23	10.12	87.05	0.87	0.99	139.70
ORCH	1	45.74	29.94	80.18	7.31	4.28	71.73	6.86	9.70	173.21	29.55	11.81	48.93	6.60	9.33	173.21	2.42	2.17	109.71
	2	40.76	10.20	30.66	9.69	9.28	117.24	9.55	2.95	43.69	18.85	4.83	31.36	11.38	11.38	141.42	3.31	4.68	173.21
	3	41.78	18.91	55.44	5.48	2.81	62.91	14.80	0.00	0.00	7.56	6.21	116.18	ND	ND	ND	1.04	1.04	141.42
ANOVA p-value																			
Plot		1.23e^{-05}			0.00543			0.0352			2.29e^{-06}			0.437			0.199		
Stage		0.935			0.66184			0.2172			0.0457			0.977			0.328		

OR: organic; CH: conventional; ORCH: combined; ND: not determined.

Table 5. Correlations among fungal variables. Spearman coefficient (p-value)

	Spore number	External mycelium length	AMF in litter	AMF in roots	Non-AMF in litter
External mycelium length	0.567 (0.121)				
AMF litter colonization	0.000 (>0.999)	0.733 (0.031)			
AMF root colonization	0.517 (0.162)	0.817 (0.011)	0.567 (0.121)		
NAMF litter colonization	0.250 (0.521)	0.583 (0.108)	0.417 (0.270)	0.283 (0.463)	
NAMF root colonization	0.083 (0.843)	0.183 (0.644)	0.100 (0.810)	0.267 (0.493)	0.267 (0.493)

Table 6. Correlations among carbon, nitrogen, carbon to nitrogen ratio, and fungal variables. Spearman coefficient (p-value)

	C	N	C:N ratio
Spore number	0.117 (0.776)	0.633 (0.076)	_0.517 (0.162)
External mycelium length	0.117 (0.776)	0.417 (0.270)	0.000 (>0.999)
AMF litter colonization	-0.100 (0,810)	-0.050 (0.912)	0.100 (0.810)
AMF root colonization	0.233 (0.552)	0.467 (0.213)	0.100 (0.810)
NAMF litter colonization	0.450 (0.230)	0.400 (0.291)	0.383 (0.312)
NAMF root colonization	0.833 (0.008)	0.600 (0.097)	-0.517 (0.162)

phosphorus (118.2-174.5 mg dm^{-3}), the pH (4.6-5.7) and the exchangeable aluminum in ORCH (6.1 cmol$_c$ kg^{-1}) indicated that phosphorus availability was limited due to fixation (Table 7).

DISCUSSION

Results showing different fungal responses among the sampled plots can be partly explained by the variation of traits associated with different vegetation dominance

Table 7. Physicochemical soil properties in sampled plots

	OR	CH	ORCH
Sand (g kg^{-1})	660	340	500
Loam (g kg^{-1})	220	260	180
Clay (g kg^{-1})	120	400	320
pH(H_2O)	5.1	5.7	4.6
Al ($cmol_c$ kg^{-1})	0.3	ND	6.1
AlS (g kg^{-1})	0.09	ND	0.43
OC (g kg^{-1})	0.83	0.50	0.50
EP (ppm)	174.5	118.2	155.2
BS (%)	62.3	55.2	25.2
Exchangeable complex ($cmol_c$ kg^{-1})			
CEC	50.6	35.4	32.7
Ca^{2+}	24.01	17.47	7.22
Mg^{2+}	6.11	1.73	0.64
K^+	1.16	0.31	0.34
Na^+	0.27	0.03	0.05
TB	31.55	19.54	8.25
Cation ratios			
Ca/Mg	3.93	10.29	12.03
Mg/K	5.08	5.60	2.00
Ca/K	20.00	58.30	24.06
(Ca + Mg)/K	25.08	64.00	26.06

OR: organic; CH: conventional; ORCH: combined; Al: aluminum content; AlS: aluminum saturation percentage; OM: organic matter; EP: extractable phosphorus; BS: base saturation; CEC: cation exchange capacity; TB: total base; ND: not determined. Sand, loam, and clay contents were determined with Bouyoucos method. pH(H_2O) was measured in saturated paste extract with a soil:solution ratio of 1:1. EP were extracted with Bray II solution. Al^{3+} were extracted by KCl 1 mol L^{-1}. OM (organic matter) = 1.72 × OC (Organic Carbon) determined by Walkley and Black method. Ca^{2+}, Mg^{2+}, K^+, and Na^+ were extracted by ammonium acetate solution. CEC pH 7 is the sum of the contents of Ca^{2+}, Mg^{2+}, K^+, and Na^+.

patterns. Likewise, our CV values for the variables in decomposing plant material may in part be explained by the plant species composition in the plots. In general, CV ranged from moderate, defined as 15 to 35 %, to high, defined as over 35 % (Wilding and Drees, 1983). Shrubs patterns measured 1 to 5 m, while tree patterns measured from 5 to 10 m. The plots had tree-crop combinations with shrubs and trees and this composition could influence patterns. Involved factors include canopies, litter quality, and aboveground and belowground litter input rates (Binkley and Giardina, 1998).

As leaf litter is fragmented by soil fauna, it is colonized by fungi and bacteria. Fungi are more active in deeper layers and are able to grow as the physical properties of leaf litter change (Dix and Webster, 1995). In our case, we found no correlations between AMF litter colonization and NAMF litter colonization.

Counts of mycorrhizal variables were generally higher in CH. The tree species in CH with the highest relative frequencies were *Fraxinus* sp. and *Ficus* sp. The CH also had the highest relative frequency of *Inga* sp. Microbial community structures that degrade lignocellulosic compounds in the first few centimeters may be determined by litter quality (Zheng et al., 2018). According to Stevenson and Cole (1999), nitrogen content over approximately 2.5 % promotes mineralization. Leaves with high nitrogen content and low lignin and polyphenol contents decompose quickly (Mafongoya et al., 1997). Fabaceae leaves are known to have higher nitrogen levels than leaves of non-legumes (McKey, 1994). The CH plot's nitrogen content and the low C:N ratio of its tree leaves

indicate higher levels of mineralization than those of the other plots. This could promote colonization in the most decomposed levels where roots, soil, and plant material are in contact with each other. On the other hand, previous reports that leguminous plants are more mycorrhizal-dependent than other plants presumably explain the abundance of spores in CH (Bainard et al., 2011). While tree and crop combinations can affect the diversity of arbuscular mycorrhizal fungi in agroforestry systems composed of coffee and plants from Fabaceae and Musaceae families (Dobo et al., 2018), fertilization may also influence AMF spore abundance (Cuenca, 2015).

Greater root AMF colonization was found by Sánchez (2017) in coffee plants fertilized with conventional products, which is similar to our finding that there was greater AMF root colonization in CH than in ORCH and OR. These findings contrast with reports in the literature that the use of readily soluble fertilizers negatively impacts this variable (Gosling et al., 2006). In addition, it has been previously suggested that the application of chemical compounds may affect certain fungal species that adapt to these fertilizer conditions (Sieverding et al., 1991).

The lowest means of external mycelium and root colonization were found in ORCH, which differed more from CH than OR did. The combination of organic inputs and NPK may dramatically depress AMF colonization as we observed in this study. Moreover, overuse of organic amendments, including chicken manure, may be harmful to arbuscular mycorrhizal fungi (Gosling et al., 2006).

According to the number of coffee plants and shade trees per square meter in the plots, coffee plant densities in OR, ORCH, and CH were similar, but shade tree density was greatest in ORCH. This particular characteristic of ORCH may have been related to slower decomposition rates and lower colonization values. In coffee ecosystems in tropical forest areas, coffee leaf litter decomposes first, with $k = 10$ while shade tree leaves decompose more slowly, with $k = 4$ (Cuenca et al., 1983). Soil management can also affect mycorrhizal responses. High phosphorus levels, such as those in ORCH, combined with low levels of soluble carbohydrates in roots are related to mechanisms by which plants regulate colonization (Azcón-Aguilar and Bago, 1994). On the other hand, organic matter enhances hyphal lengths (St. John et al., 1983). High phosphorus contents may have inhibited AMF colonization in OR, but high organic carbon content may have offset this effect leaving colonization unchanged.

In accordance with previous studies of tropical and temperate forests, we observed mycorrhizal fungal structures colonizing leaf litter from the beginning of leaf decomposition to the final stages of highly fragmented leaf litter (Bunn et al., 2019). Fungal colonization of leaf veins and blades was similar to that reported by Aristizábal et al. (2004), who described thick hyphae resembling runner hyphae and thin branch hyphae in decomposing leaves. Auxiliary cell formation on the extraradical mycelium is a distinguishing characteristic of certain Gigasporaceae genera such as those found in CH (Figure 1h) (Dodd et al., 2000). This taxon has been frequently encountered in coffee and forest soil studies that include morphological descriptions and molecular analysis (Cogo et al., 2017). Arbuscular mycorrhizal fungal diversity has implications for plant communities and plant growth promotion that include the possibility that functional traits of different taxa are related to mineral nutrient acquisition and that their sensitivity to environmental factors may vary (Girlanda et al., 2007). Both of these are relevant for ecosystem functioning and nutrient cycling. Several species of coffee shade trees are native to tropical forests. According to Cuenca et al. (1983), decomposition of organic matter and nutrient cycling in coffee agroecosystems are similar to those in tropical forest systems. A research in Brazil indicates that AMF diversity is greater under agroecological coffee management than under conventional management and that fungal community composition of forest fragments is more similar to fungal community composition in plots managed organically than that in conventional plots (Prates Júnior et al., 2019).

The average external mycelium length was slightly shorter in the fresh litter layer than in the layer of fresh litter and organic fermentation products. Those layers were described previously by Zheng et al. (2018). Larger amounts of extraradical hyphae in the bottom layer, where the leaf litter is more highly decomposed than in the top layer, has been previously reported (Aristizábal et al., 2004). Increasing mycorrhizal fungal growth as decomposition advances from one stage to another indicates that arbuscular mycorrhizal fungi take up recently mineralized nutrients. Arbuscular mycorrhiza formation is commonly highest in the first centimeters of soil around coffee roots, and roots of *Coffea arabica* are abundant in the distinctive litter layer of shade systems (Cuenca, 2015). Recently mineralized nutrients are transferred to the plant via arbuscular mycorrhizal fungi thereby avoiding fixation and lixiviation-related losses (Cuenca et al., 1983; Cavagnaro et al., 2015). In this process, the root colonization is especially important. In addition to the colonization of the coffee roots themselves, colonization occurred in the fresh litter layer and the fermentation layer.

We found a statistically significant correlation between external mycelium length and AMF litter colonization. This is comparable to results by Aristizábal et al. (2004), who detected a positive correlation between external AMF hyphae in litter and AMF vesicles in leaves collected below the canopy of *Morella parvifolia*.

CONCLUSIONS

Our results provide insight into how strategies of fertilization and tree-crop combinations can influence fungal litter and root colonization in agroforestry systems. The large amounts of spores, root colonization, and decomposing leaves colonization found in this study suggest that the leaf litter layers in agroecosystems harbor arbuscular mycorrhizal fungi. Therefore, maintaining the layer of leaf litter colonized by arbuscular mycorrhizal fungi rather than removing it is a promising sustainable agricultural practice for the cultivation of coffee and other crops. This study demonstrates the occurrence of arbuscular mycorrhizal fungi in decomposing plant material in managed ecosystems and supports the idea that these fungi may be involved in transferring nutrients released by decomposing leaf litter to host plants following the hypothesis of direct nutrient cycling in tropical Andean agroecosystems. Further research into the role of mycorrhizae in nutrient cycling and the importance of arbuscular mycorrhizal fungal community composition in nutrient dynamics within managed soils is needed to improve our understanding of how to minimize nutrient losses.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://www.rbcsjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-45-e0200110/1806-9657-rbcs-45-e0200110-suppl01.pdf

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