# Taxonomic shifts in arbuscular mycorrhizal fungal communities with shade and soil nitrogen across conventionally managed and organic coffee agroecosystems

Aldrich-Wolfe, Laura; Black, Katie L.; Hartmann, Eliza D. L.; Shivega, W. Gaya; Schmaltz, Logan C.; McGlynn, Riley D.; Johnson, Peter G.; Asheim Keller, Rebecca J.; Vink, Stefanie N.

*Published in:*
Mycorrhiza

*DOI:*
10.1007/s00572-020-00967-7



*Publication date:*
2020

Mycorrhiza (2020) 30:513–527
https://doi.org/10.1007/s00572-020-00967-7

ORIGINAL ARTICLE

# Taxonomic shifts in arbuscular mycorrhizal fungal communities with shade and soil nitrogen across conventionally managed and organic coffee agroecosystems

Laura Aldrich-Wolfe[1] · Katie L. Black[2] · Eliza D. L. Hartmann[2] · W. Gaya Shivega[2] · Logan C. Schmaltz[2] · Riley D. McGlynn[2] · Peter G. Johnson[2] · Rebecca J. Asheim Keller[2] · Stefanie N. Vink[3]

Received: 28 February 2020 / Accepted: 20 May 2020 / Published online: 4 June 2020


## Abstract

The composition of arbuscular mycorrhizal fungal (AMF) communities should reflect not only responses to host and soil environments, but also differences in functional roles and costs vs. benefits among arbuscular mycorrhizal fungi. The coffee agroecosystem allows exploration of the effects of both light and soil fertility on AMF communities, because of the variation in shade and soil nutrients farmers generate through field management. We used high-throughput ITS2 sequencing to characterize the AMF communities of coffee roots in 25 fields in Costa Rica that ranged from organic management with high shade and no chemical fertilizers to conventionally managed fields with minimal shade and high N fertilization, and examined relationships between AMF communities and soil and shade parameters with partial correlations, NMDS, PERMANOVA, and partial least squares analysis. Gigasporaceae and Acaulosporaceae dominated coffee AMF communities in terms of relative abundance and richness, respectively. Gigasporaceae richness was greatest in conventionally managed fields, while Glomeraceae richness was greatest in organic fields. While total AMF richness and root colonization did not differ between organic and conventionally managed fields, AMF community composition did; these differences were correlated with soil nitrate and shade. OTUs differing in relative abundance between conventionally managed and organic fields segregated into four groups: Gigasporaceae associated with high light and nitrate availability, Acaulosporaceae with high light and low nitrate availability, Acaulosporaceae and a single relative of *Rhizophagus fasciculatus* with shade and low nitrate availability, and *Claroideoglomus*/*Glomus* with conventionally managed fields but uncorrelated with shade and soil variables. The association of closely related taxa with similar shade and light availabilities is consistent with phylogenetic trait conservatism in AM fungi.

**Keywords** Arbuscular mycorrhizal fungi · Nitrate · Light availability · Acaulosporaceae · Gigasporaceae · Glomeraceae

## Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with many terrestrial plants, in which the AM fungal partners provide an array of benefits—improved plant uptake of poorly mobile mineral nutrients and protection from pathogens, heavy metals, salinity, or drought—in exchange for C from the host plant (Augé 2001; Borowicz 2001; Smith and Read 2008; Ferrol et al. 2016). This partnership is estimated to cost the host as much as 20% of C fixed by photosynthesis (Jakobsen and Rosendahl 1990) and is asymmetric. While the resources provided by fungal partners can also be acquired by the plant host on its own, the fungi are entirely dependent upon their hosts for the energy they require for growth and maintenance (Smith and Read 2008). The nature of this symbiosis thus implies that factors influencing plant mineral

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00572-020-00967-7) contains supplementary material, which is available to authorized users.

✉ Laura Aldrich-Wolfe
laura.aldrichwolfe@ndsu.edu

[1] Department of Biological Sciences, North Dakota State University, Fargo, ND, USA

[2] Biology Department, Concordia College, Moorhead, MN, USA

[3] Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, the Netherlands

nutrition, exposure to pathogens and other stressors, and photosynthetic rate should exert strong influences on the extent and types of partnerships formed between plants and AM fungi.

If different AM fungi provide different benefits and vary in their C cost to their hosts, then AM fungal (AMF) communities should be shaped by factors influencing the relative importance of different benefits of the fungi to their hosts and also by host photosynthetic rate. Several studies have demonstrated both differences in plant C allocation to different AM fungal partners and differences among AM fungal partners in plant benefit (e.g., Bever et al. 2009; Kiers et al. 2011; Argüello et al. 2016). Research to date suggests the existence of trade-offs among AM fungi in the benefits they provide and their C demand (Chagnon et al. 2013).

The general architecture of hyphal growth in AM fungi has been shown to be conserved at the family level (Hart and Reader 2002; Maherali and Klironomos 2007). Fungi in the family Gigasporaceae colonize plant root systems to a lesser extent than fungi in the family Glomeraceae and produce primarily extraradical mycelia that extend a greater distance from the root than those of Glomeraceae, while members of the Glomeraceae produce primarily intraradical hyphae (Hart and Reader 2002; Powell et al. 2009). Consequently, taxa in the Gigasporaceae may primarily improve nutrient uptake, while those in the Glomeraceae may play the larger role in protection of roots from pathogens (Newsham et al. 1995; Maherali and Klironomos 2007). Sikes et al. (2009) provide experimental support for this divergence of function with divergence in morphology.

The light environment of the host also should play a strong role in determining AMF communities. While the currency with which mycorrhizal fungi pay their host may depend on environment and host requirements, the host currency is invariably C fixed via photosynthesis (Smith and Read 2008; Konvalinková et al. 2015). Different AMF species differ in their C cost and consequently their relative benefit to their host (Pearson and Jakobsen 1993; Lendenmann et al. 2011). Plants grown under low light conditions are less likely to be colonized and are more weakly colonized by AM fungi than plants grown under high light (Whitbeck 2001; Gehring and Connell 2006; Menezes et al. 2016; Koorem et al. 2017). AM fungi may be less important for plant nutrient uptake but more important for pathogen protection when photosynthesis is light-limited, because plants in low light environments are more likely than their full sun counterparts to succumb to disease (Ballaré et al. 2012). Despite differences in root colonization and mycorrhizal growth response under varying light conditions (e.g., (Konvalinková et al. 2015; Konvalinková and Jansa 2016), manipulations of light environment have not resulted in changes in AMF community composition in the few studies to examine this parameter (Heinemeyer et al. 2004; Menezes et al. 2016; Koorem et al. 2017). However, those studies involved the exposure of plant species to light environments that they would not typically experience.

The facultative nature of the symbiosis on the part of plant hosts has been well-documented, particularly in agricultural systems. Multiple studies have observed lower root colonization by AM fungi in crops that have been fertilized with P (Menge et al. 1978; Jensen and Jakobsen 1980; Siqueira et al. 1998; Kahiluoto et al. 2000; Gosling et al. 2013), suggesting a reduced dependence of plants on arbuscular mycorrhizas in the absence of P limitation. Plants with high P status have been shown to suppress the mycorrhizal pathway for acquisition of P (Nagy et al. 2009) and reduce their allocation of C to mycorrhizal roots (Ji and Bever 2016).

Hosts may exercise control over the association through differential allocation of resources to their partner fungi (Werner and Kiers 2015; Wipf et al. 2019). Therefore, while conditions that reduce the importance of AM fungi to their hosts' survival and reproduction (i.e., fitness) should result in an overall decline in abundance of AM fungi, differences in environmental conditions that change the relative importance of different AM fungi for host fitness should result in shifts in the AMF community toward those taxa that maximize host benefit. If closely related AM fungi tend to play similar roles in the plant community, then AMF communities will not differ in idiosyncratic ways between different environments. Instead, closely related taxa would be expected to respond similarly to environmental differences.

Coffee (*Coffea arabica* L.) is in many ways an ideal crop with which to study the roles of soil fertility and light environment in shaping AMF communities. In contrast to most other crops, coffee is deliberately grown by farmers under a range of shade and soil fertility conditions depending on whether the crop is being managed for yield or quality, organic certification, or longevity (Muschler 2001; Lyngbæk et al. 2001; Valencia et al. 2015; Allinne et al. 2016). Under conventional management, coffee is typically grown under very little shade and regularly fertilized with N, P, and K, as well as other macro- and micronutrients; and synthetic fungicides are applied throughout the year. In contrast, coffee produced organically is usually grown under moderate shade and with or without applications of organic fertilizers (Haggar et al. 2011; Schnabel et al. 2018).

In this study, we used high-throughput sequencing of the ribosomal RNA gene's internal transcribed spacer 2 (ITS2) to characterize the AMF communities of coffee fields representing a diverse array of conditions with respect to shade, soil fertility, and other aspects of field management. We hypothesized that AMF communities on coffee grown organically would differ from those on coffee under conventional management. We expected that coffee on organic farms would support a more diverse AMF community than coffee on conventionally managed farms, because of lower soil macronutrient availability on farms that do not experience regular additions of synthetic fertilizer and increased disease as a result of higher shade and the

absence of applications of synthetic fungicides. In shaded coffee fields, we expected to observe a distinct suite of AM fungi, assuming that those fungi that confer high C costs on their hosts would exhibit diminished abundances and that shade would emphasize pathogen protection over nutrient uptake. Because different AM fungi are expected to provide different benefits for their plant hosts, and function is expected to be conserved phylogenetically, we predicted that closely related taxa would differ in abundance in similar ways under different environmental conditions in coffee fields.

## Materials and methods

### Field sites

Twenty-five coffee fields were included in this study from two regions of Costa Rica, Santa Elena de Monteverde (hereafter "Monteverde") and San Vito de Coto Brus (hereafter "San Vito"). Both regions have a tropical premontane climate and Andisol soils (Janzen 1983). Mean annual rainfall is higher in San Vito with a more pronounced dry season in Monteverde. The owner or manager of each coffee field was interviewed to determine prior use of the coffee field; length of time in coffee production; coffee cultivars and plant ages; frequency of pruning; and use of fertilizers, herbicides, pesticides, and fungicides. Fields were designated as "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 conventional to organic management or had used no synthetic fertilizers or pesticides within the preceding 1–3 years. Thirteen fields were sampled in Monteverde (N 10° 16′ to 10° 22′, W 084° 45′ to 084° 54′): three conventionally managed, one minimal conventional, and two organic fields, 25–28 May 2011; and five conventionally managed and two organic fields, 1–4 June 2012. In San Vito (N 08° 52′ to 08° 57′, W 082° 47′ to 082° 60′), twelve fields were sampled: two conventionally managed, two minimal conventional, and two organic fields, 31 May–3 June 2011; and three organic and three conventionally managed fields, 7–11 June 2012. For each field, species richness of shade trees, type of windbreak, and phenological status of coffee plants were recorded. All fields except one, in which plants were vegetative, were producing green (immature) or green and red (mature) fruit at the time of sampling.

### Sample collection

In each field, a 20-m × 20-m plot was established > 5 m from the edge and representative of the shade tree density of the field. Elevation, percent canopy cover by shade trees (hereafter "shade (%)"), aspect, slope, coffee plant density, leaf litter depth, and soil characteristics were measured for each plot (see Sternhagen et al. (2020) for details). Coffee plants were 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. Root samples were taken at a depth of 1–15 cm from 3 to 5 distinct points along the root system (i.e., from laterals arising from different secondary roots) of the nearest coffee plant to every other soil sampling point, for a total of 10 plants per plot. Approximately 100 cm of fine roots were collected from each plant. Root samples from each plant were rinsed individually in tap water and divided into two subsamples. One subsample from each plant was stored in 1% KOH (*w/v*) for analysis of root colonization by AM fungi, while the second was dried in the presence of Drierite (W.A. Hammond Company, Xenia, OH, USA) for DNA extraction. Soil samples within a field were air-dried in paper bags, pooled, and stored at room temperature.

At the end of each year's sampling period, samples were transported to the USA. Soils and dried root samples for DNA extraction were stored at room temperature for < 2 weeks prior to further processing. Root samples in 1% KOH were refrigerated at 4 °C. Two (2011) or three (2012) soil analytical subsamples from each field were assessed for soil nutrient availability (nitrate, Olsen P, K, Ca, Mg, Cu, Fe, Mn, Zn), pH in water, and organic matter (by loss on ignition) at the Soils Testing Laboratory, North Dakota State University, Fargo, ND, USA. Means per field were subsequently used for all statistical analyses.

### Root colonization by AM fungi

Root samples in 1% KOH were transferred to 10% KOH (*w/v*) for clearing at approximately 95 °C for 10 min, rinsed in tap water for 10 min, acidified in 2% HCl (*v/v*) solution for 15 min at room temperature, and stained with trypan blue ($dH_2O$, 85% lactic acid and glycerol (1:1:1) with 0.05% (*w/v*) trypan blue) at 70 °C for 5 min. Samples were then rinsed in tap water > 1 h to remove excess stain. Twenty ~ 2.5-cm-long root segments per plant were mounted on a microscope slide in polyvinyl-lactoglycerol and scored at a minimum of 100 intersections for the presence of AMF structures (arbuscules, vesicles, intraradical hyphae) at × 200 magnification (technique modified from McGonigle et al. (1990)) using a Nikon Eclipse 80*i* microscope with DIC (Nikon Instruments, Inc., Melville, NY, USA). Root samples from eight plants per field were scored in 2011 and from 10 plants per field in 2012.

### Molecular detection of root fungi

DNA was extracted from 20 mg of dried root sample for each of 8–10 plants per field using the Qiagen DNeasy Plant Mini

Kit (Qiagen, Germantown, MD, USA), and the fungal ITS2 region was amplified in triplicate using the 5.8SR and ITS4 primers (White et al. 1990) and stored at − 20 °C, as described in Sternhagen et al. (2020). After amplification and purification, eight (2011) or ten (2012) samples per field were pooled at equal DNA concentration in 10 mM Tris to ensure that each sample was equally represented in the library for each coffee field. Frozen PCR products (3–5 ng of DNA per field) were submitted for sequencing at the University of Minnesota Genomics Center (UMGC, St. Paul, MN, USA). Preliminary quality control and demultiplexing were conducted by the UMGC.

### Sequence data processing

Sequences were processed using the PIPITS 1.4.0 pipeline (Gweon et al. 2015) with the standard settings. Briefly, forward and reverse reads were merged by PEAR 0.9.8 (http://www.exelixis-lab.org/pear). The FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit) was used for quality filtering, followed by the extraction of the fungal-specific ITS2 region using ITSx 1.0.11 (Bengtsson-Palme et al. 2013). VSEARCH 2.3.0 (Rognes et al. 2016) was used for dereplication, removal of singletons and of sequences < 100 bp, clustering to 97% sequence identity, and subsequent chimera detection with the UNITE Uchime 7.1 dataset (Nilsson et al. 2015) as the reference. Representative sequences were taxonomically assigned with RDP Classifier 2.11 (Wang et al. 2007) using the Warcup_retrained V2 ITS training dataset (Deshpande et al. 2016). To retain a greater level of taxonomic resolution in downstream analyses, a taxonomic confidence level of 50% was used. At the final step, an abundance table was generated clustering sequences in OTUs at 97% identity, which generally reflects phylogenetic relationships at the species level in fungi (Lindahl et al. 2013). Samples were rarefied to 132,460 sequences per sample (the minimum number of fungal sequences observed in a single sample) in QIIME 1.9.1 (Caporaso et al. 2010), to take any difference in sequencing depth across samples into account. The rarefied OTU table was used in the statistical analyses. Only OTUs that were assigned to the class Glomeromycetes were included in the final dataset.

### Statistical analysis

Differences in environmental variables, root colonization by AM fungi, and sequence relative abundance and richness of AM fungi between organic and conventionally managed coffee fields were compared by three-way analysis of variance with field type, region, and sampling year as fixed effects and including all possible interactions. To control the family-wise type I error rate, $p$ values were adjusted using the false discovery rate (Benjamini and Hochberg 1995). Differences among means for interactions were determined by Tukey HSD.

Relationships among environmental variables and AMF abundance and richness were examined using partial correlations. Associations with environmental variables of those AMF OTUs that differed in abundance between conventionally managed and organic fields were tested by partial least squares analysis. Dependent variables were transformed as needed to meet model assumptions. The above analyses were conducted in JMP Pro® version 13 (SAS Institute, Inc. 2016).

Similarities among AMF communities were visualized using nonmetric multidimensional scaling (NMDS) on Sørensen distances in PC-ORD version 7 (McCune and Mefford 2016). Two-way PERMANOVA was used to test for a statistical difference in community composition between organic and conventionally managed fields, with either field type and region or field type and year as fixed effects and including the respective interaction. To maintain a balanced design, only the sixteen fields with equal representation across field types, regions, and years were included in each PERMANOVA. Abundances of OTUs were Hellinger-transformed (Legendre and Gallagher 2001) prior to analysis. There were too few minimal conventional fields to include in ANOVA and PERMANOVA, but they were included in correlation and partial least squares analyses.

## Results

### Diversity and abundance of AMF families in coffee fields in Costa Rica

A total of 517 AMF taxa (defined as OTUs at 97% sequence identity for ITS2) were detected in the roots of coffee in the 25 fields sampled (Online Resource Table S1). Of these, 496 belonged to seven described families (Fig. 1). Based on sequence abundance, the most common family was Gigasporaceae, followed by Acaulosporaceae and Glomeraceae (Fig. 1a). In terms of OTU richness, the most diverse family was Acaulosporaceae, followed by Gigasporaceae and Glomeraceae (Fig. 1b). Only 4% of the AMF sequences (21 OTUs) could not be assigned to a described AMF family.

### Environmental differences between coffee fields by field type

Leaf litter depth, soil pH, and soil Cu availability were higher in organic than in conventionally managed fields, while soil nitrate availability was, on average, three times higher in conventionally managed than organic fields (Table 1). Shade, shade tree richness, and soil Ca and Mg availability were higher in organic fields than in conventionally managed fields

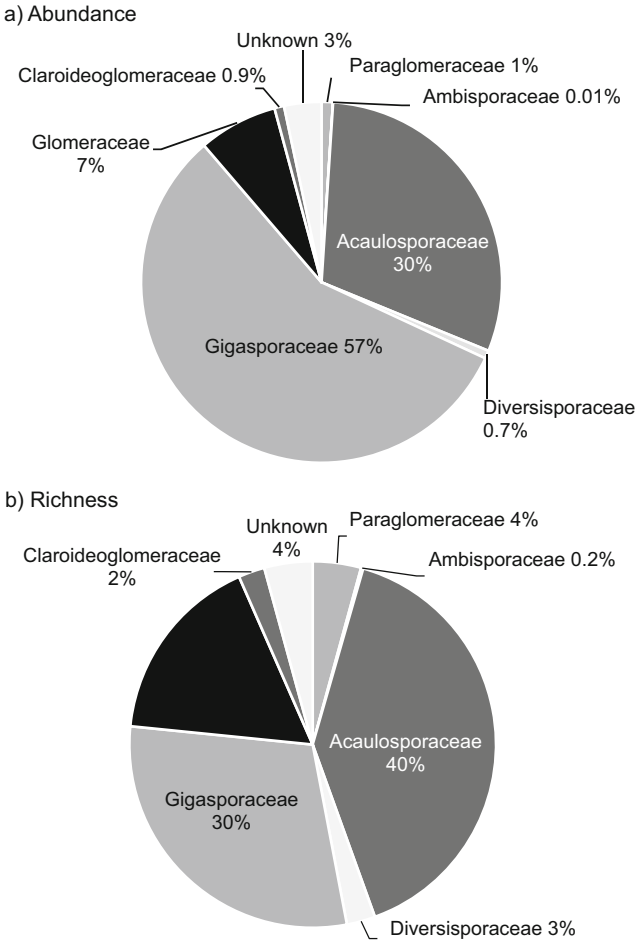


**Fig. 1** Relative abundance (**a**) and OTU richness (**b**) by AMF family for ITS2 sequences amplified from 8 to 10 coffee root samples collected in each of 25 coffee fields in two regions of Costa Rica in late May and early June 2011–2012

in Monteverde, but were intermediate in value, and did not differ statistically between conventionally managed and organic fields, in San Vito (Sternhagen et al. 2020). Conventionally managed and organic fields did not differ in elevation, slope, or slope aspect; density or age of coffee plants; or age of coffee fields (Online Resource TableS2). Organic matter and soil availability of P, K, Fe, Mn, Na, and Zn also did not differ between conventionally managed and organic fields.

## Abundance and richness of AM fungi in relation to field type and environmental variables

Coffee roots in these fields were extensively colonized by AM fungi; mean root length colonization was 45 ± 2% ($n$ = 8–10 per field). On average, 3699 ± 884 ITS2 sequences of AM fungi were detected per field. Neither root colonization by AM fungi nor AMF sequence abundance differed between organic and conventionally managed coffee fields (data not shown; $F_{1,\ 14} = 3.53$, FDR $p = 0.293$; $F_{1,\ 14} = 0.06$, FDR $p = 0.923$, respectively).

On average, 83 ± 9 AMF OTUs were detected per coffee field ($n$ = 8–10). Overall richness of AM fungi did not differ between organic and conventionally managed fields (Online Resource Fig. S1; $F_{1,\ 14} = 0.26$, FDR $p = 0.798$). However, two of the three most frequently detected AMF families did differ in richness between field types. Mean richness (± SE) of Gigasporaceae was higher in conventionally managed (39 ± 4 OTUs) than in organic fields (22.5 ± 5 OTUs; $F_{1,\ 14} = 6.57$, $p = 0.0225$), while mean richness of Glomeraceae was lower in conventionally managed (6 ± 2 OTUs) than in organic fields (13.5 ± 2 OTUs; $F_{1,\ 14} = 6.63$, $p = 0.0221$). There was no difference in richness of Acaulosporaceae between field types (33 ± 5 OTUs; $F_{1,\ 14} = 1.34$, $p = 0.2663$).

Root colonization by AM fungi was strongly positively correlated with soil Cu availability (Fig. 2a; Table 2), while sequence counts of AM fungi were positively correlated with soil P availability (Table 2). Overall richness of AMF was not strongly correlated with any of the environmental variables (data not shown). However, richness of OTUs in the Acaulosporaceae was positively correlated with shade tree richness and soil Ca and Mn availability and negatively correlated with shade, soil pH and soil P (Fig. 2b), and Fe availability (Table 2). Richness of OTUs in the Gigasporaceae was positively correlated with soil Mg availability and negatively correlated with soil K availability. Richness of OTUs in the Glomeraceae was positively correlated

**Table 1** Environmental variables that differed between conventionally managed (conventional) and organic coffee fields with no interaction with region or year, by three-way ANOVA with field type, region, and year as main effects and including all possible interactions, for fields sampled either in 2011 or 2012 in Monteverde and San Vito, Costa Rica

| | Conventional (13) | Minimal (3) | Organic (9) | $F_{1,\ 14}$ | $P$ |
|---|---|---|---|---|---|
| Leaf litter (cm) | 2.27 ± 0.43 | 3.56 ± 1.31 | 4.15 ± 0.6 | 6.42 | 0.0248 |
| pH in $H_2O$ | 5.21 ± 0.09 | 5.23 ± 0.12 | 5.89 ± 0.17 | 17.07 | 0.0010 |
| $NO_3^-$-N | 141 ± 20 | 46 ± 15 | 42 ± 8 | 33.81 | < 0.0001 |
| Cu (ppm) | 2.66 ± 0.34 | 3.07 ± 0.73 | 4.59 ± 0.82 | 9.12 | 0.0092 |

Values are means ± SE (sample sizes in parentheses). Minimal conventional (minimal) fields are included here for comparison but were too few to include in the statistical analyses

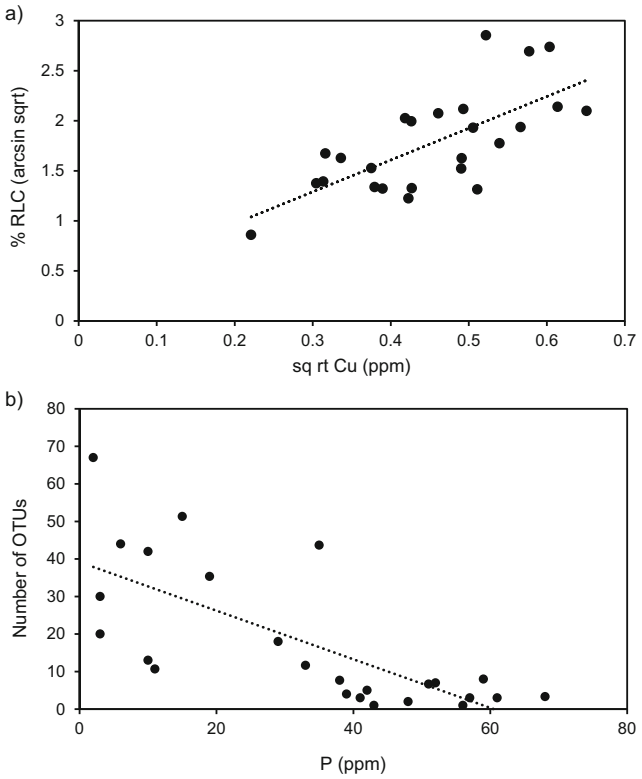


**Fig. 2** Correlation between (**a**) root length colonized by arbuscular mycorrhizal fungi (% RLC) and soil copper availability, and (**b**) richness of Acaulosporaceae OTUs and soil phosphorus availability for 25 coffee fields in Costa Rica sampled in 2011–2012. Values of % RLC are means for samples from 8 to 10 coffee plants per field

with shade, soil pH, and soil Zn availability and negatively correlated with shade tree richness.

## Differences in community composition of AM fungi in relation to field type and environment

Communities of AM fungi differed between organic and conventionally managed fields (PERMANOVA $F_{1,\ 12} = 1.72$, $p = 0.0204$; Fig. 3). However, the three fields recently converted to organic management or lacking recent synthetic inputs did not appear to differ in composition from conventionally managed fields. There was a weak interaction between field type and region ($F_{1,\ 12} = 1.48$, $p = 0.0574$) and no interaction of field type with sampling year ($F_{1,\ 12} = 1.04$, $p = 0.3598$; data not shown). Axis 1 of the NMDS plot was correlated with richness of shade trees per coffee field and soil P availability, while axis 2 was correlated with soil pH and cation ($Ca^{2+}$, $K^{+}$, $Mg^{2+}$) availability. Differentiation of AMF communities between organic and conventionally managed fields was correlated with shade and soil nitrate availability (Fig. 3).

Of the 517 AMF OTUs detected in this study, many were too rare to be able to distinguish differences in relative abundance by field type. However, thirty-three AMF OTUs did differ in sequence counts between conventionally managed and organic fields (Fig. 4; Online Resource Table S1), and nine of these were among the 20 most abundant OTUs detected in this study (Online Resource Table S1). In Monteverde, 12 OTUs, all in the Gigasporaceae, differed in relative abundance between organic and conventionally managed fields, including three OTUs whose closest known relative is *Gigaspora margarita* and seven OTUs that likely belong to the genus *Gigaspora* but do not match any species currently in published sequence databases (Fig. 4a). All of these OTUs had greater mean abundance in conventionally managed than

**Table 2** Partial correlations of measures of richness and relative abundance of arbuscular mycorrhizal (AM) fungi with environmental variables for 25 coffee fields in two regions of Costa Rica (RLC = root length colonized by AM fungi)

| | Mean %RLC (arcsin sq rt) | Sequence count (sq rt) | Acaulosporaceae OTU richness | Gigasporaceae OTU richness | Glomeraceae OTU richness |
|---|---|---|---|---|---|
| Elev m (sq) | 0.35 | 0.05 | 0.04 | 0.25 | 0.08 |
| Shade % (arcsin sq rt) | 0.15 | 0.32 | *− 0.66* | 0.31 | *0.73* |
| Leaf litter cm (sq rt) | 0.32 | − 0.21 | 0.00 | − 0.15 | 0.24 |
| No. of shade tree spp. (log) | 0.32 | 0.09 | *0.60* | − 0.18 | *− 0.58* |
| pH (sq) | − 0.22 | − 0.08 | *− 0.64* | 0.26 | *0.51* |
| $NO_3$-N ppm (log) | 0.17 | − 0.20 | − 0.22 | 0.47 | 0.27 |
| P ppm (log) | 0.14 | *0.65* | *− 0.52* | 0.22 | 0.19 |
| K ppm (log) | − 0.11 | − 0.26 | 0.42 | *− 0.56* | − 0.30 |
| Ca ppm (sq rt) | − 0.02 | − 0.03 | *0.62* | − 0.37 | − 0.30 |
| Mg ppm (log) | − 0.15 | − 0.44 | − 0.43 | *0.58* | 0.31 |
| Cu ppm (sq rt) | *0.77* | 0.12 | 0.37 | − 0.14 | − 0.09 |
| Fe ppm | 0.05 | 0.26 | *− 0.55* | 0.35 | 0.20 |
| Mn ppm (log) | − 0.10 | − 0.40 | *0.57* | − 0.16 | − 0.36 |
| Zn ppm (sq rt) | − 0.30 | − 0.37 | − 0.39 | 0.22 | *0.50* |

Positive and negative correlations > 0.5 are shown in italics. Data transformations prior to analysis are shown in parentheses

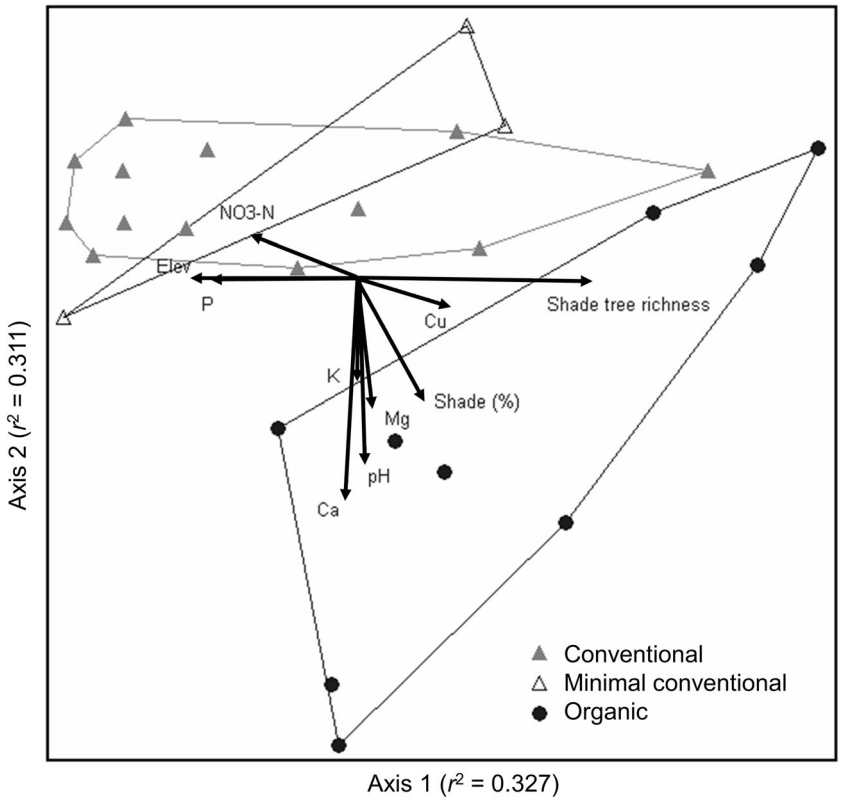


**Fig. 3** Nonmetric multidimensional scaling (NMDS) plot of AM fungal communities in coffee roots for 25 coffee fields under three types of management (organic = no synthetic inputs; conventional = applications of fungicides and synthetic fertilizers; minimal conventional = transitioning to organic or lacking synthetic inputs for 2–3 years) indicated by polygons, with overlay of environmental variables correlated with the first two axes of the NMDS. Length of vectors indicates strength of correlation. Community composition was estimated using high-throughput sequencing of the ITS2 region of the fungal rDNA repeat. Final stress of 3D solution = 11.75; axis 3 (not shown) $R^2 = 0.158$

organic fields. No OTUs in Monteverde were observed to have greater relative abundance in organic fields than conventionally managed ones.

In San Vito, 18 OTUs were more abundant in conventionally managed than in organic fields (Fig. 4b), including the same 12 OTUs with greater relative abundances in

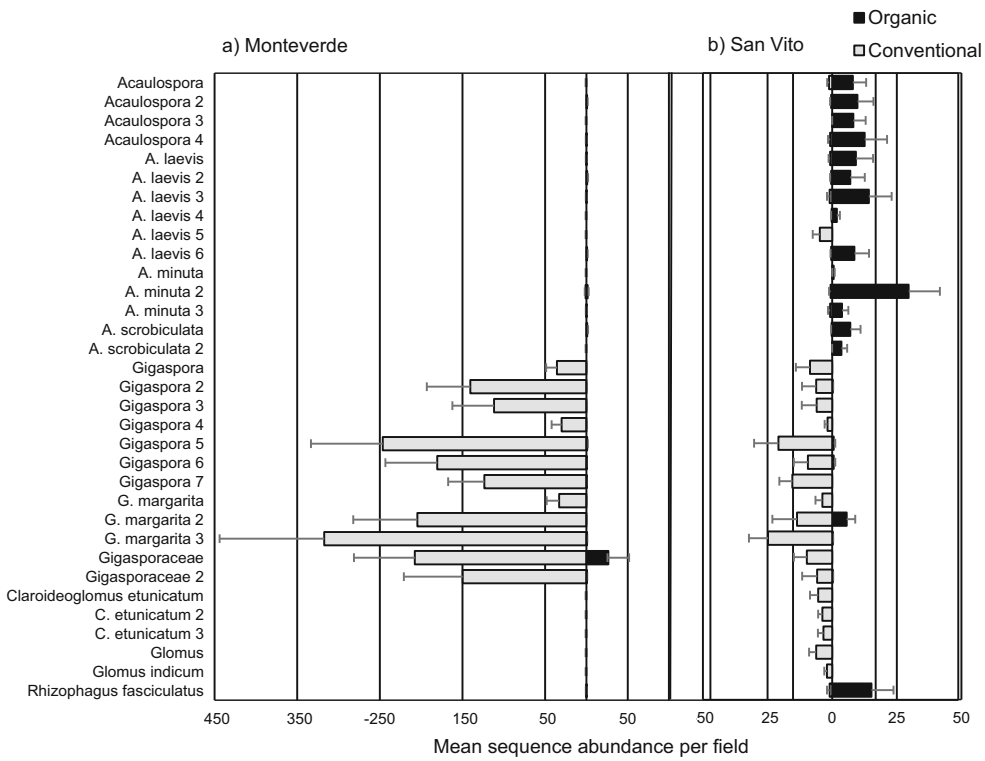


**Fig. 4** Relative abundance of ITS2 sequences ± SE of 33 AMF OTUs (at 97% sequence identity) that differed in abundance at $\alpha = 0.05$ in coffee roots collected from organic and conventionally managed coffee fields in (**a**) Monteverde ($N_c = 8$, $N_o = 4$) and (**b**) San Vito ($N_c = 5$, $N_o = 5$), Costa Rica. See Online Resource Table S1 for test statistics and $p$ values

conventionally managed coffee fields in Monteverde. Additionally, there was one OTU whose closest known relative is *Acaulospora laevis*, three closely related to *Claroideoglomus etunicatum*, and two OTUs related to the genus *Glomus*, which also were more abundant in conventionally managed coffee fields than organic fields. In contrast to Monteverde, there were also 16 OTUs that were more abundant, on average, in organic fields than in conventionally managed ones: 15 OTUs in the Acaulosporaceae and one OTU whose closest known relative is *Rhizophagus fasciculatus*.

Partial least squares analysis revealed that the 33 AMF OTUs that differed in relative abundance between organic and conventionally managed fields fell into three discernible groups according to differences in environmental variables (Fig. 5). Five *Acaulospora* OTUs, including two OTUs whose closest relative is *A. scrobiculata* and one whose closest relative is *A. minuta*, in addition to one OTU whose closest relative is *R. fasciculatus*, were all more abundant in coffee fields with high shade and less acidic soils with low nitrate availability. Ten *Acaulospora* OTUs, including six OTUs related to *A. laevis* and two related to *A. minuta*, were all more abundant in coffee fields with low shade and low soil nutrient availability. Twelve *Gigaspora* OTUs were more abundant in coffee fields with low shade and high soil nitrate availability, although for *Gigaspora* 7, the relationship between abundance and nitrate availability was weak, and for one OTU in the Gigasporaceae, abundance was most strongly correlated with availability of P, Fe, and Zn. For a fourth group composed of three OTUs whose closest relatives are *C. etunicatum* and two OTUs in the genus *Glomus*, higher relative abundance in conventionally managed fields in San Vito (Fig. 4b) was not correlated with any environmental variables measured in this study.

## Discussion

In our characterization of AMF communities and soil and shade environments across coffee field types, we found that measures of overall AMF abundance and richness did not differ between conventionally managed and organic fields. However, differences in richness emerged at the family level, with proportionately more OTUs in the Gigasporaceae in conventionally managed and Glomeraceae in organic fields. Environmental correlates of AMF abundance and richness differed, and richness of each of the three predominant AMF families was correlated with a different suite of environmental variables. Four groups of AMF OTUs differed in abundance between conventionally managed and organic fields: a group composed primarily of *Acaulospora* OTUs associated with high shade and low nitrate; a second group of *Acaulospora* associated with low shade and low nitrate; *Gigaspora* OTUs associated with low shade and high nitrate availability; and finally a group composed of *Glomus* and *Claroideoglomus*

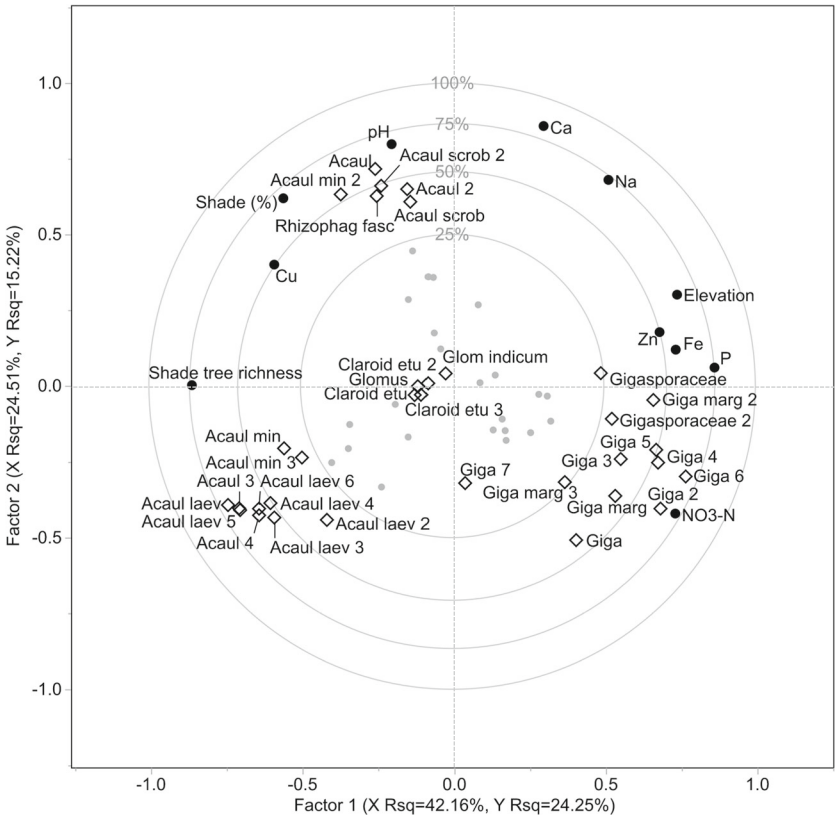


**Fig. 5** Correlation loading plot of partial least squares (PLS) analysis examining relationships between environmental variables (large black circles) and abundance for 33 OTUs (open diamonds) differing in abundance between field types. Eleven environmental variables with variable importance in projection (VIP) scores > 0.8 were included in the final model, which reduced to two factors explaining 67% of the variation in environmental parameters (*X*) and 39.5% of the variation in OTU abundance (*Y*) for 23 coffee fields (small gray circles) in which these OTUs were detected. OTU labels indicate the closest match for that OTU in the Warcup training dataset (Deshpande et al. 2016)

OTUs associated with unknown environmental characteristics of conventionally managed fields.

## Diversity and abundance of AMF families

In contrast to most published studies of AMF community composition, in which members of the family Glomeraceae tend to dominate both in terms of abundance and richness (e.g., Camenzind et al. 2014; Alguacil et al. 2016; Rodríguez-Echeverría et al. 2017), the most frequently encountered family in these Costa Rican coffee fields was Gigasporaceae and the most diverse family was Acaulosporaceae. Spores of Gigasporaceae and Acaulosporaceae predominate in forest communities in Costa Rica (Picone 2000; Lovelock et al. 2003). In one of the few studies to examine AMF communities from tropic to polar regions, Stürmer et al. (2018) observed a higher abundance of Acaulosporaceae and a greater richness of Gigasporaceae among spores of the Neotropics than in the Arctic. In the Cerrado of Brazil, Gigasporaceae was the most frequently encountered family and Acaulosporaceae was the most diverse family across multiple land uses, and a species of *Gigaspora* (*G. decipiens*) was the most common species in coffee plantations (Fernandes et al. 2016).

These patterns stand in contrast to the predominance of the Glomerales in many temperate and some tropical sites (Camenzind et al. 2014; Rodríguez-Echeverría et al. 2017; Van Geel et al. 2017), including in coffee agroecosystems in East Africa (Muleta et al. 2008; De Beenhouwer et al. 2015a). Perhaps the overrepresentation of Gigasporaceae in the tropical Americas documented here and its underrepresentation in Africa (Öpik et al. 2010) reflect a center of origin in the Americas. Because colonization and sporulation exhibit seasonality (Merryweather and Fitter 1998), the prevalence of Gigasporaceae and Acaulosporaceae also may reflect our sampling during the transition from the dry to wet season.

Because fungicides are used consistently throughout the growing season in conventionally managed coffee fields and these fields typically are heavily fertilized (Hernández-Martínez et al. 2009; Cerda et al. 2017), we expected to observe lower root colonization by AM fungi in conventionally managed than in organic fields. Instead, there was no difference in root colonization between the two field types. Fungicides used in coffee, which typically are applied to the foliage using backpack-sprayers (de Souza et al. 2011), may have little lasting systemic effect in the root system (Rivera-Becerril et al. 2017) or may have had no effect on root colonization but still reduced hyphal growth in the soil (which was not measured in this study) and/or contributed to the differences in community composition of AM fungi observed between organic and conventionally managed fields (Ipsilantis et al. 2012; Jin et al. 2013; Rivera-Becerril et al. 2017).

## Soil fertility correlates of AMF abundance and richness

The importance of AM fungi for plant uptake of poorly mobile soil nutrients such as Cu and P is well-established (Li et al. 1991; Smith and Smith 2011; Lehmann and Rillig 2015). In this study, root colonization by AM fungi was positively correlated with soil Cu availability, while relative abundance of AMF sequences was positively correlated with soil P availability. Many studies have observed a negative correlation between AMF abundance and soil P availability (Treseder 2004; Kahiluoto et al. 2001; Chen et al. 2014; Liu et al. 2014; Williams et al. 2017), consistent with declining plant investment in mycorrhizas as soil mineral availability increases (Graham et al. 1991; Johnson 2010; Williams et al. 2017). However, other studies in low P soils also have found positive correlations of AMF abundance with soil P availability (Lekberg et al. 2008; Liu et al. 2013; Teste et al. 2016). If the increased capacity for photosynthesis due to mineral nutrient acquisition via mycorrhizas results in a greater investment by the host in its mycorrhizal associates, then, as long as those mineral nutrients remain limiting to plant performance, we would expect to observe a positive correlation between AMF abundance and nutrient availability (Smith and Gianinazzi-Pearson 1990).

Richness of OTUs was correlated with a different combination of environmental parameters for each of the families Acaulosporaceae, Gigasporaceae, and Glomeraceae. Richness of Acaulosporaceae was positively correlated with Mn availability and negatively correlated with soil pH and P availability, consistent with its importance for P uptake in acidic soils (Kawahara et al. 2016). AMF richness and soil P availability are typically negatively correlated (Treseder 2004; Verbruggen et al. 2012; Camenzind et al. 2014; Liu et al. b; Van Geel et al. 2017), including along a traditional to conventional coffee agroecosystem gradient in Ethiopia (De Beenhouwer et al. 2015b). Plants are able to differentially allocate C to their AMF associates (Kiers et al. 2011; Ji and Bever 2016) and reduce C allocation to their mycorrhizas in proportion to increased plant P concentration (Menge et al. 1978), which suggests that AMF species may be progressively excluded from the root system as plant P concentration increases (Van Geel et al. 2017). Conversely, AMF richness may be high when the minerals they provide are scarce, if the presence of competing associates reduces the cost-to-benefit ratio for the host (Werner and Kiers 2015).

Soil nitrate availability was not strongly correlated with either measures of overall AMF richness or abundance. Many studies of N fertilization have observed declines in AMF richness with increased levels of N fertilization (e.g., Liu et al. 2012, 2014; Camenzind et al. 2014; Leff et al. 2015; Allen et al. 2016; Williams et al. 2017; Sheldrake et al. 2018). Nitrogen fertilization may be directly harmful to

many fungi or indirectly harmful through soil acidification (Lilleskov et al. 2019). However, Egerton-Warburton et al. (2007) found that N fertilization only reduced AM richness in P-rich soils, in which N fertilization may have alleviated N limitation thereby reducing plant reliance on AM fungi for nutrient uptake. In low P soils, AMF productivity and richness increased in response to N fertilization, presumably by increasing plant P limitation and consequently plant C allocation to mycorrhizas. In the coffee fields in our study, the loss of diversity attributable to soil acidification may be offset by increased plant demand for soil P. It is also possible that fields low in nitrate may have had appreciable levels of ammonium, which we did not measure.

### Light environment

Root colonization by AM fungi typically declines with shading (Konvalinková and Jansa 2016; Menezes et al. 2016), but the majority of studies have been conducted with plant species adapted to high light environments (reviewed in Konvalinková and Jansa 2016) or seedlings of tropical forest canopy trees (Whitbeck 2001; Gehring 2003; Gehring and Connell 2006). In our study, there was little correlation between shade and AMF root colonization or sequence counts in coffee fields, suggesting that AM fungi play important roles for coffee in both shade and sun environments. AMF abundance, either by measures of root colonization or spore number, has previously been shown to be higher in coffee fields with shade trees than in those without (Muleta et al. 2008) and to increase with richness of shade trees (Arias et al. 2012; Bagyaraj et al. 2015), although Muleta et al. (2007) found no correlation between shade and spore abundance.

There also was little correlation between shade and AMF richness across the coffee fields in our study. Richness of AM fungi has been shown to decline in experimentally shaded alpine meadows (Shi et al. 2014; Liu et al. 2015b). However, in a study of forest plant species, richness of AM fungi was lower for shade-avoidant herbs in shade than in a clearcut, but did not differ between shade and the clearcut for shade-tolerant plant species (Koorem et al. 2017). Taken together, these findings suggest that the number of AMF species that a plant species supports depends on the light environment to which the plant species is adapted rather than exclusively on light availability.

Because AM fungi differ in their C requirements (Pearson and Jakobsen 1993; Lendenmann et al. 2011; Ji and Bever 2016), some species would be expected to be restricted to hosts with high photosynthetic rates in high light environments, while others might be better able to form mycorrhizal associations with plants in shade (Chagnon et al. 2013). While there was no apparent relationship between total AMF richness and light availability across these coffee fields, richness of Acaulosporaceae was negatively correlated with shade and positively correlated with shade tree richness and vice versa for richness of Glomeraceae. We would expect to observe these contrasting patterns in richness in response to shade, if Acaulosporaceae taxa tend to place a higher C demand on their hosts than members of the Glomeraceae and/or if taxa in the Acaulosporaceae are important for nutrient uptake while taxa in the Glomeraceae are important for protection from root pathogens. Diversifying shade trees may reduce disease incidence (Parker et al. 2015), thereby reducing the importance of protection from pathogens. Coffee plants in fields with more shade tree species also may be colonized by additional AMF species if host diversity reduces competitive exclusion or increases the number of AMF species that can be supported (Hiiesalu et al. 2014).

### Differences in community composition of AM fungi between field types

Studies of variation in community composition of AM fungi at the landscape scale often concern natural plant communities or seminatural grasslands, in which shifts in AMF community composition usually are accompanied by a marked change in the plant community (Dumbrell et al. 2010; Lekberg et al. 2011; Antoninka et al. 2015; Kohout et al. 2015; Bainard et al. 2017; Chaudhary et al. 2018). Because plant hosts also influence the AMF community (Vandenkoornhuyse et al. 2003; Aldrich-Wolfe 2007; Gosling et al. 2013), this makes it difficult to separate responses of AM fungi to environmental factors from effects of plant community composition (Leff et al. 2015). Here we held the host constant and observed shifts in fungal community composition associated with aspects of nutrient availability (soil P and nitrate; soil pH and cations) and shade (both canopy cover and shade tree richness). Using pyrosequencing, De Beenhouwer et al. (2015b) found that AMF community composition varied with coffee management intensity in Ethiopia and was influenced by soil pH and N and P availability. Prates Júnior et al. (2019) did not observe a difference in AMF communities with differing management intensity in Brazil by DGGE-PCR, but did observe differences between coffee and forest AMF communities that were associated with differences in pH, P, and organic matter.

Fertilization (of primarily natural plant communities) with N and P shifts AMF community composition (Jumpponen et al. 2005; Liu et al. 2015a), but just as we observed in coffee, the community responds differently to fertilization with N than P, highlighting that the AM fungi influenced by changes in N availability are not the same taxa influenced by changes in P availability. Chen et al. (2014) observed a decline in abundance but little change in AMF community composition in response to P fertilization, but a strong shift in composition in response to N fertilization. Camenzind et al. (2014) observed reductions in richness of the Diversisporales in response to N fertilization, consistent with our findings of

decreased abundance of some *Acaulospora* OTUs under conditions of elevated N availability but inconsistent with the increased abundance of *Gigaspora* OTUs that we observed in fields with high nitrate availability. To the extent that the use of synthetic fertilizers is correlated with the use of other agrochemicals, differences in AMF communities that correlated with soil nitrate availability may also reflect differences in fungicide use.

AM fungi that differed in abundance between conventionally managed and organic coffee fields fell into four groups. These groups reflected both apparent phylogenetic relationships and environmental differences, which is consistent with phylogenetic niche conservatism in AM fungi (Hart and Reader 2002; Maherali and Klironomos 2007; Powell et al. 2009). Treseder et al. (2018) predicted that *Gigaspora* would be associated with low and *Glomus* with high N availability, under the expectation that hosts would discriminate against those fungi with high C costs as soil fertility increased. In this study, OTUs in the family Gigasporaceae either failed to exhibit a difference in abundance between conventionally managed and organic coffee or were associated with coffee fields with high soil nitrate availability and high sunlight. Given the low P availability in many of these coffee field soils, it is likely that coffee plants in sunny environments were able to invest in *Gigaspora* to maximize P uptake. In low P tallgrass prairie, spore counts of *Gigaspora gigantea* increased with N fertilization (Eom et al. 1999), while Gigasporaceae was replaced by Glomeraceae with increasing soil nitrate availability in high P soils in California grasslands (Egerton-Warburton and Allen 2000). Our results are consistent with the model of Johnson (2010), in which AMF species with extensive extraradical mycelia that impose a high C cost on the host are favored under conditions of high N coupled with low P availability.

OTUs in the Acaulosporaceae either failed to exhibit a difference in abundance between conventionally managed and organic coffee or were associated with low soil nitrate availability. One subset of *Acaulospora* OTUs, along with a single OTU in the genus *Rhizophagus* (family Glomeraceae), was associated with low soil nitrate and light availability and less acidic soils, while another subset of *Acaulospora* appeared to be associated with a high light environment and low nutrient availability in general. The functional roles of members of the Acaulosporaceae are currently not well-characterized. Jakobsen et al. (1992) found that *A. laevis* was involved in P transport over greater distances than a species of *Glomus*. Maherali and Klironomos (2007) suggested that, given their low biomass both inside and outside the root, *Acaulospora* species were unlikely to play an important role in either P transport or protection from root pathogens. The association of some OTUs in this group with soils of low nutrient availability observed in our study suggests that they may be important for nutrient uptake, while the association of some OTUs with high and others with low light environments implies there may be important differences within the family in C cost to the host.

A group of OTUs in the families Claroideoglomeraceae and Glomeraceae were most likely to be detected in conventionally managed coffee fields, but their abundances were not strongly correlated with any of the environmental variables measured in this study. These taxa were presumably responding to one or more unknown factors characteristic of conventionally managed fields. *Claroideoglomus* and related taxa in the Glomeraceae have been considered ruderal species, in the sense that they are associated with the highly disturbed and relatively fertile environment of annual cropping systems (Oehl et al. 2010; Chagnon et al. 2013; Carballar-Hernández et al. 2017). Their association with coffee roots in conventionally managed fields also might reflect tolerance of fungicides or perhaps a protective role against the facultative root pathogens that increase in abundance in conventionally managed relative to organic fields (Sternhagen et al. 2020).

## Conclusions

AMF communities differed in composition between conventionally managed and organic coffee fields, and differences in community composition were associated with differences in shade and soil nitrate availability. In contrast to studies in temperate systems, the dominant families in these coffee fields were Gigasporaceae and Acaulosporaceae rather than Glomeraceae and Diversisporaceae. OTUs that differed in abundance between conventionally managed and organic fields fell into four groups: *Gigaspora* species associated with high nitrate availability and low shade; *Acaulospora* species associated with low nitrate and low shade; *Acaulospora* species and a single OTU related to *Rhizophagus* associated with low nitrate and high shade; and *Claroideoglomus* and *Glomus* species associated with conditions of conventionally managed fields not measured in this study. Measures of soil fertility that were positively correlated with AMF abundance tended to be negatively correlated with AMF richness and vice versa, consistent with the productivity–diversity relationship observed across ecosystems. Richness of different AMF families was correlated with different environmental parameters, suggestive of differences in function at the family level. The importance of both shade and soil nutrients for structuring AMF communities in the coffee agroecosystem underscores the need for more studies that examine the costs and benefits of mycorrhizas from the fungal and plant perspectives simultaneously.

**Acknowledgments** The authors acknowledge the many farmers who welcomed us onto their land and patiently answered our questions; Sydney Redmond for assistance in scoring root colonization; Ylva

Lekberg for her invaluable logistical assistance; David Janos and two anonymous reviewers, whose suggestions greatly improved the manuscript; and the Minnesota Supercomputing Institute (MSI) at the University of Minnesota for providing resources that contributed to the research results reported in this paper (URL: http://www.msi.umn.edu).

**Authors' contributions** LAW, RM, LS, EH, and PJ designed and executed the field study; RM, LS, EH, PJ, KB, WGS, and RA conducted the lab work; EH, PJ, RM, LS, KB, WGS, and RA contributed to data analysis; KB and SV conducted the bioinformatics; all authors contributed to the drafts of the manuscript; LAW wrote the final draft.

**Funding information** This research was funded by the Office of Undergraduate Research, the Biology Department Fuglestad-Torstveit Fund, and NSF award DUE-0969568 to the Division of Science and Mathematics at Concordia College, and EPSCoR Track 1 Award 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.

**Data availability** The ITS2 DNA sequences generated and analyzed during this study are publicly available as BioProject PRJNA531329, BioSamples SAMN11371063-87 and Sequence Read Archives SRR8868669-93 at the National Center for Biotechnology Information, USA. The environmental dataset for the 25 coffee fields is available through Dryad (https://doi.org/10.5061/dryad.q2bvq83g1).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

Aldrich-Wolfe L (2007) Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community. Ecology 88:559–566

Alguacil MDM, Torres MP, Montesinos-Navarro A, Roldán A (2016) Soil characteristics driving arbuscular mycorrhizal fungal communities in semiarid Mediterranean soils. Appl Environ Microbiol 82: 3348–3356

Allen EB, Egerton-Warburton LM, Hilbig BE, Valliere JM (2016) Interactions of arbuscular mycorrhizal fungi, critical loads of nitrogen deposition, and shifts from native to invasive species in a southern California shrubland. Botany 94:425–433

Allinne C, Savary S, Avelino J (2016) Delicate balance between pest and disease injuries, yield performance, and other ecosystem services in the complex coffee-based systems of Costa Rica. Agric Ecosyst Environ 222:1–12

Antoninka AJ, Ritchie ME, Johnson NC (2015) The hidden Serengeti—Mycorrhizal fungi respond to environmental gradients. Pedobiologia 58:165–176

Argüello A, O'Brien MJ, van der Heijden MGA, Wiemken A, Schmid B, Niklaus PA, Maherali H (2016) Options of partners improve carbon for phosphorus trade in the arbuscular mycorrhizal mutualism. Ecol Lett 19(6):648–656

Arias RM, Heredia-Abarca G, Sosa VJ, Fuentes-Ramírez LE (2012) Diversity and abundance of arbuscular mycorrhizal fungi spores under different coffee production systems and in a tropical montane cloud forest patch in Veracruz, Mexico. Agrofor Syst 85:179–193

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Bagyaraj DJ, Thilagar G, Ravisha C, Kushalappa CG, Krishnamurthy KN, Vaast P (2015) Below ground microbial diversity as influenced by coffee agroforestry systems in the Western Ghats, India. Agric Ecosyst Environ 202:198–202

Bainard LD, Chagnon P-L, Cade-Menun BJ, Lamb EG, LaForge K, Schellenberg M, Hamel C (2017) Plant communities and soil properties mediate agricultural land use impacts on arbuscular mycorrhizal fungi in the Mixed Prairie ecoregion of the North American Great Plains. Agric Ecosyst Environ 249:187–195

Ballaré CL, Mazza CA, Austin AT, Pierik R (2012) Canopy light and plant health. Plant Physiol 160:145–155

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

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 57:289–300

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M (2009) Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecol Lett 12(1):13–21

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068

Camenzind T, Hempel S, Homeier J, Horn S, Velescu A, Wilcke W, Rillig MC (2014) Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. Glob Chang Biol 20:3646–3659

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Carballar-Hernández S, Hernández-Cuevas LV, Montaño NM, Larsen J, Ferrera-Cerrato R, Taboada-Gaytán OR, Montiel-González AM, Alarcón A (2017) Native communities of arbuscular mycorrhizal fungi associated with *Capsicum annuum* L. respond to soil properties and agronomic management under field conditions. Agric Ecosyst Environ 245:43–51

Cerda R, Allinne C, Gary C, Tixier P, Harvey CA, Krolczyk L, Mathiot C, Clément E, Aubertot J-N, Avelino J (2017) Effects of shade, altitude and management on multiple ecosystem services in coffee agroecosystems. Eur J Agron 82:308–319

Chagnon P-L, Bradley RL, Maherali H, Klironomos JN (2013) A trait-based framework to understand life history of mycorrhizal fungi. Trends Plant Sci 18:484–491

Chaudhary VB, Cuenca G, Johnson NC (2018) Tropical-temperate comparison of landscape-scale arbuscular mycorrhizal fungal species distributions. Divers Distrib 24:116–128

Chen Y-L, Zhang X, Ye J-S, Han H-Y, Wan S-Q, Chen B-D (2014) Six-year fertilization modifies the biodiversity of arbuscular mycorrhizal fungi in a temperate steppe in Inner Mongolia. Soil Biol Biochem 69:371–381

De Beenhouwer M, Muleta D, Peeters B, Van Geel M, Lievens B, Honnay O (2015a) DNA pyrosequencing evidence for large diversity differences between natural and managed coffee mycorrhizal fungal communities. Agron Sustain Dev 35:241–249

De Beenhouwer M, Van Geel M, Ceulemans T, Muleta D, Lievens B, Honnay O (2015b) Changing soil characteristics alter the arbuscular mycorrhizal fungi communities of Arabica coffee (*Coffea arabica*)

in Ethiopia across a management intensity gradient. Soil Biol Biochem 91:133–139

de Souza AF, Zambolim L, de Jesus Júnior VC, Cecon PR (2011) Chemical approaches to manage coffee leaf rust in drip irrigated trees. Australas Plant Pathol 40:293–300

Deshpande V, Wang Q, Greenfield P, Charleston M, Porras-Alfaro A, Kuske CR, Cole JR, Midgley DJ, Tran-Dinh N (2016) Fungal identification using a Bayesian classifier and the Warcup training set of internal transcribed spacer sequences. Mycologia 108:1–5

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. The ISME Journal 4:337–345

Egerton-Warburton LM, Allen EB (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecol Appl 10:484–496

Egerton-Warburton LM, Johnson NC, Allen EB (2007) Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grassland. Ecol Monogr 774):527–544

Eom A-H, Hartnett DC, Wilson GWT, Figge DAH (1999) The effect of fire, mowing and fertilizer amendment on arbuscular mycorrhizas in tallgrass prairie. Am Midl Nat 142:55–70

Fernandes RA, Ferreira DA, Saggin-Junior OJ, Stürmer SL, Paulino HB, Siqueira JO, Carneiro MAC (2016) Occurrence and species richness of mycorrhizal fungi in soil under different land use. Can J Soil Sci 96:271–280

Ferrol N, Tamayo E, Vargas P (2016) The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. J Exp Bot 67:6253–6265

Gehring CA (2003) Growth responses to arbuscular mycorrhizae by rain forest seedlings vary with light intensity and tree species. Plant Ecol 167:127–139

Gehring CA, Connell JH (2006) Arbuscular mycorrhizal fungi in the tree seedlings of two Australian rain forests: occurrence, colonization, and relationships with plant performance. Mycorrhiza 16:89–98

Gosling P, Mead A, Proctor M, Hammond JP, Bending GD (2013) Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. New Phytol 198:546–556

Graham JH, Eissenstat DM, Drouillard DL (1991) On the relationship between a plant's mycorrhizal dependency and rate of vesicular-arbuscular mycorrhizal colonization. Funct Ecol 5:773–779

Gweon HS, Oliver A, Taylor J, Booth T, Gibbs M, Read DS, Griffiths RI, Schonrogge K (2015) PIPITS: an automated pipeline for analyses of fungal internal transcribed spacer sequences from the Illumina sequencing platform. Methods Ecol Evol 6:973–980

Haggar, J., M. Barrios, M. Bolaños, M. Merlo, P. Moraga, R. Munguia, A. Ponce, S. Romero, G. Soto, C. Staver, and E. de M. F. Virginio. 2011. Coffee agroecosystem performance under full sun, shade, conventional and organic management regimes in Central America. Agrofor Syst 82:285–301

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 153: 335–344

Heinemeyer A, Ridgway KP, Edwards EJ, Benham DG, Young JPW, Fitter AH (2004) Impact of soil warming and shading on colonization and community structure of arbuscular mycorrhizal fungi in roots of a native grassland community. Glob Chang Biol 10:52–64

Hernández-Martínez G, Manson RH, Hernández AC (2009) Quantitative classification of coffee agroecosystems spanning a range of production intensities in central Veracruz, Mexico. Agric Ecosyst Environ 134:89–98

Hiiesalu I, Pärtel M, Davison J, Gerhold P, Metsis M, Moora M, Öpik M, Vasar M, Zobel M, Wilson SD (2014) Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. New Phytol 203:233–244

Ipsilantis I, Samourelis C, Karpouzas DG (2012) The impact of biological pesticides on arbuscular mycorrhizal fungi. Soil Biol Biochem 45: 147–155

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115: 77–83

Jakobsen I, Abbott LK, Robson AD (1992) External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum,* L. New Phytol 120:509–516

Janzen DH (1983) Costa Rican natural history. University of Chicago Press, Chicago, Illinois

Jensen A, Jakobsen I (1980) The occurrence of vesicular-arbuscular mycorrhiza in barley and wheat grown in some Danish soils with different fertilizer treatments. Plant Soil 55:403–414

Ji B, Bever JD (2016) Plant preferential allocation and fungal reward decline with soil phosphorus: implications for mycorrhizal mutualism. Ecosphere 7:e01256

Jin H, Germida JJ, Walley FL (2013) Suppressive effects of seed-applied fungicides on arbuscular mycorrhizal fungi (AMF) differ with fungicide mode of action and AMF species. Appl Soil Ecol 72:22–30

Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales: Tansley review. New Phytol 185:631–647

Jumpponen A, Trowbridge J, Mandyam K, Johnson L (2005) Nitrogen enrichment causes minimal changes in arbuscular mycorrhizal colonization but shifts community composition—evidence from rDNA data. Biol Fertil Soils 41:217–224

Kahiluoto H, Ketoja E, Vestberg M (2000) Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization 1. Bioassays in a growth chamber. Plant Soil 227:191–206

Kahiluoto H, Ketoja E, Vestberg M, Saarela I (2001) Promotion of AM utilization through reduced P fertilization 2. Field studies. Plant & Soil 231:65–79

Kawahara A, An G-H, Miyakawa S, Sonoda J, Ezawa T (2016) Nestedness in arbuscular mycorrhizal fungal communities along soil pH gradients in early primary succession: acid-tolerant fungi are pH generalists. PLoS One 11:e0165035

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882

Kohout P, Doubková P, Bahram M, Suda J, Tedersoo L, Voříšková J, Sudová R (2015) Niche partitioning in arbuscular mycorrhizal communities in temperate grasslands: a lesson from adjacent serpentine and nonserpentine habitats. Mol Ecol 24:1831–1843

Konvalinková T, Jansa J (2016) Lights off for arbuscular mycorrhiza: on its symbiotic functioning under light deprivation. Front Plant Sci 7: 782

Konvalinková T, Püschel D, Janoušková M, Gryndler M, Jansa J (2015) Duration and intensity of shade differentially affects mycorrhizal growth- and phosphorus uptake responses of Medicago truncatula. Front Plant Sci 6:65

Koorem K, Tulva I, Davison J, Jairus T, Öpik M, Vasar M, Zobel M, Moora M (2017) Arbuscular mycorrhizal fungal communities in forest plant roots are simultaneously shaped by host characteristics and canopy-mediated light availability. Plant Soil 410:259–271

Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M, Steenbock C, Stevens CJ, Fierer N (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proc Natl Acad Sci 112:10967–10972

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280

Lehmann A, Rillig MC (2015) Arbuscular mycorrhizal contribution to copper, manganese and iron nutrient concentrations in crops – a meta-analysis. Soil Biol Biochem 81:147–158

Lekberg Y, Koide RT, Twomlow SJ (2008) Effect of agricultural management practices on arbuscular mycorrhizal fungal abundance in low-input cropping systems of southern Africa: a case study from Zimbabwe. Biol Fertil Soils 44:917–923

Lekberg Y, Meadow J, Rohr JR, Redecker D, Zabinski CA (2011) Importance of dispersal and thermal environment for mycorrhizal communities: lessons from Yellowstone National Park. Ecology 92: 1292–1302

Lendenmann M, Thonar C, Barnard RL, Salmon Y, Werner RA, Frossard E, Jansa J (2011) Symbiont identity matters: carbon and phosphorus fluxes between *Medicago truncatula* and different arbuscular mycorrhizal fungi. Mycorrhiza 21:689–702

Li X-L, Marschner H, George E (1991) Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover. Plant Soil 136:49–57

Lilleskov EA, Kuyper TW, Bidartondo MI, Hobbie EA (2019) Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. Environ Pollut 246:148–162

Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjøller R, Kõljalg U, Pennanen T, Rosendahl S, Stenlid J, Kauserud H (2013) Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol 199:288–299

Liu Y, Shi G, Mao L, Cheng G, Jiang S, Ma X, An L, Du G, Johnson NC, Feng H (2012) Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New Phytol 194:523–535

Liu L, Zhang T, Gilliam FS, Gundersen P, Zhang W, Chen H, Mo J (2013) Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest. PLoS One 8:e61188

Liu W, Jiang S, Zhang Y, Yue S, Christie P, Murray PJ, Li X, Zhang J (2014) Spatiotemporal changes in arbuscular mycorrhizal fungal communities under different nitrogen inputs over a 5-year period in intensive agricultural ecosystems on the North China Plain. FEMS Microbiol Ecol 90:436–453

Liu Y, Johnson NC, Mao L, Shi G, Jiang S, Ma X, Du G, An L, Feng H (2015a) Phylogenetic structure of arbuscular mycorrhizal community shifts in response to increasing soil fertility. Soil Biol Biochem 89:196–205

Liu Y, Mao L, Li J, Shi G, Jiang S, Ma X, An L, Du G, Feng H (2015b) Resource availability differentially drives community assemblages of plants and their root-associated arbuscular mycorrhizal fungi. Plant Soil 386:341–355

Lovelock CE, Andersen K, Morton JB (2003) Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. Oecologia 135:268–279

Lyngbæk AE, Muschler RG, Sinclair FL (2001) Productivity and profitability of multistrata organic versus conventional coffee farms in Costa Rica. Agrofor Syst 53:205–213

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316: 1746–1748

McCune B, Mefford MJ (2016) PC-ORD. Multivariate analysis of ecological data. MjM Software Design, Gleneden Beach, Oregon

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. The New Phytologist 115:495–501

Menezes KMS, Silva DKA, Queiroz MAA, Félix WP, Yano-Melo AM (2016) Arbuscular mycorrhizal fungal communities in buffelgrass pasture under intercropping and shading systems in Brazilian semi-arid conditions. Agric Ecosyst Environ 230:55–67

Menge JA, Steirle D, Bagyaraj DJ, Johnson ELV, Leonard RT (1978) Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytol 80:575–578

Merryweather J, Fitter A (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta* II. Seasonal and spatial patterns of fungal populations. New Phytol 138:131–142

Muleta D, Assefa F, Nemomissa S, Granhall U (2007) Composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest, southwestern Ethiopia. For Ecol Manag 241:145–154

Muleta D, Assefa F, Nemomissa S, Granhall U (2008) Distribution of arbuscular mycorrhizal fungi spores in soils of smallholder agroforestry and monocultural coffee systems in southwestern Ethiopia. Biol Fertil Soils 44:653–659

Muschler RG (2001) Shade improves coffee quality in a sub-optimal coffee-zone of Costa Rica. Agrofor Syst 51:131–139

Nagy R, Drissner D, Amrhein N, Jakobsen I, Bucher M (2009) Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. New Phytol 181:950–959

Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. Trends Ecol Evol 10:407–411

Nilsson RH, Tedersoo L, Ryberg M, Kristiansson E, Hartmann M, Unterseher M, Porter TM, Bengtsson-Palme J, Walker DM, de Sousa F, Gamper HA, Larsson E, Larsson K-H, Kõljalg U, Edgar RC, Abarenkov K (2015) A comprehensive, automatically updated fungal ITS sequence dataset for reference-based chimera control in environmental sequencing efforts. Microbes Environ 30:145–150

Oehl F, Laczko E, Bogenrieder A, Stahr K, Bösch R, van der Heijden M, Sieverding E (2010) Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biol Biochem 42:724–738

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241

Parker IM, Saunders M, Bontrager M, Weitz AP, Hendricks R, Magarey R, Suiter K, Gilbert GS (2015) Phylogenetic structure and host abundance drive disease pressure in communities. Nature 520: 542–544

Pearson JN, Jakobsen I (1993) Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. New Phytol 124:481–488

Picone C (2000) Diversity and abundance of arbuscular–mycorrhizal fungus spores in tropical forest and pasture. Biotropica 32:734–750

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proc R Soc Lond B Biol Sci 276:4237–4245

Prates Júnior, P., B. C. Moreira, M. de C. S. da Silva, T. G. R. Veloso, S. L. Stürmer, R. B. A. Fernandes, E. de S. Mendonça, and M. C. M. Kasuya. 2019. Agroecological coffee management increases arbuscular mycorrhizal fungi diversity. PLoS One 14:e0209093

Rivera-Becerril F, van Tuinen D, Chatagnier O, Rouard N, Béguet J, Kuszala C, Soulas G, Gianinazzi-Pearson V, Martin-Laurent F (2017) Impact of a pesticide cocktail (fenhexamid, folpel, deltamethrin) on the abundance of Glomeromycota in two agricultural soils. Sci Total Environ 577:84–93

Rodríguez-Echeverría S, Teixeira H, Correia M, Timóteo S, Heleno R, Öpik M, Moora M (2017) Arbuscular mycorrhizal fungi communities from tropical Africa reveal strong ecological structure. New Phytol 213:380–390

Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584

SAS Institute, Inc (2016) JMP. Cary, North Carolina

Schnabel, F., E. de Melo Virginio Filho, S. Xu, I. D. Fisk, O. Roupsard, and J. Haggar. 2018. Shade trees: a determinant to the relative success of organic versus conventional coffee production. Agrofor Syst 92:1535–1549

Sheldrake M, Rosenstock NP, Mangan S, Revillini D, Sayer EJ, Olsson PA, Verbruggen E, Tanner EVJ, Turner BL, Wright SJ (2018) Responses of arbuscular mycorrhizal fungi to long-term inorganic and organic nutrient addition in a lowland tropical forest. The ISME Journal 12:2433–2445

Shi G, Liu Y, Johnson NC, Olsson PA, Mao L, Cheng G, Jiang S, An L, Du G, Feng H (2014) Interactive influence of light intensity and soil fertility on root-associated arbuscular mycorrhizal fungi. Plant Soil 378:173–188

Sikes BA, Cottenie K, Klironomos JN (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. J Ecol 97:1274–1280

Siqueira JO, Saggin-Júnior OJ, Flores-Aylas WW, Guimarães PTG (1998) Arbuscular mycorrhizal inoculation and superphosphate application influence plant development and yield of coffee in Brazil. Mycorrhiza 7:293–300

Smith SE, Gianinazzi-Pearson V (1990) Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L.: effects of photon irradiance and phosphate nutrition. Aust J Plant Physiol 17:177–188

Smith S, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier Ltd.

Smith FA, Smith SE (2011) What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? Plant Soil 348:63–79

Sternhagen, E. C., K. L. Black, E. D. L. Hartmann, W. G. Shivega, P. G. Johnson, R. D. McGlynn, L. C. Schmaltz, R. J. A. Keller, S. N. Vink, and L. Aldrich-Wolfe. 2020. Contrasting patterns of functional diversity in coffee root fungal communities associated with organic and conventionally-managed fields. Applied and Environmental Microbiology 86. (DOI):https://doi.org/10.1128/AEM.00052-20

Stürmer SL, Oliveira LZ, Morton JB (2018) Gigasporaceae versus Glomeraceae (phylum Glomeromycota): a biogeographic tale of dominance in maritime sand dunes. Fungal Ecol 32:49–56

Teste FP, Laliberté E, Lambers H, Auer Y, Kramer S, Kandeler E (2016) Mycorrhizal fungal biomass and scavenging declines in phosphorus-impoverished soils during ecosystem retrogression. Soil Biol Biochem 92:119–132

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytol 164:347–355

Treseder KK, Allen EB, Egerton-Warburton LM, Hart MM, Klironomos JN, Maherali H, Tedersoo L (2018) Arbuscular mycorrhizal fungi as mediators of ecosystem responses to nitrogen deposition: a trait-based predictive framework. J Ecol 106:480–489

Valencia, V., P. West, E. J. Sterling, L. García-Barrios, and S. Naeem. 2015. The use of farmers' knowledge in coffee agroforestry management: implications for the conservation of tree biodiversity. Ecosphere 6:art122

Van Geel M, Verbruggen E, De Beenhouwer M, van Rennes G, Lievens B, Honnay O (2017) High soil phosphorus levels overrule the potential benefits of organic farming on arbuscular mycorrhizal diversity in northern vineyards. Agric Ecosyst Environ 248:144–152

Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. Mol Ecol 12:3085–3095

Verbruggen E, van der Heijden MGA, Weedon JT, Kowalchuk GA, Röling WFM (2012) Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. Mol Ecol 21:2341–2353

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73(16):5261–5267

Werner GDA, Kiers ET (2015) Partner selection in the mycorrhizal mutualism. New Phytol 205:1437–1442

Whitbeck JL (2001) Effects of light environment on vesicular-arbuscular mycorrhiza development in *Inga leiocalycina*, a tropical wet forest tree. Biotropica 33:303–311

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–327. PCR protocols: a guide to methods and applications. Academic, San Diego, CA

Williams A, Manoharan L, Rosenstock NP, Olsson PA, Hedlund K (2017) Long-term agricultural fertilization alters arbuscular mycorrhizal fungal community composition and barley (*Hordeum vulgare*) mycorrhizal carbon and phosphorus exchange. New Phytol 213:874–885

Wipf D, Krajinski F, van Tuinen D, Recorbet G, Courty P-E (2019) Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. New Phytol 223:1127–1142