***New Phytologist* Supporting Information**

Article title: Crop diversity enriches AMF communities in an intensive agricultural landscape
Authors: Aidee Guzman, Marisol Montes, Leslie Hutchins, Gisel DeLaCerda, Paula Yang, Anne Kakouridis, Ruth Dahlquist-Willard, Mary Firestone, Timothy Bowles and Claire Kremen

Article acceptance date: 15 February 2021

The following Supporting Information is available for this article:

Methods S1 Molecular analysis of AMF communities: primer selection, PCR conditions, and amplicon library preparation

Fig. S1 PCA of soil properties used to calculate the soil properties index. The ordination plot is color-coded to illustrate differences in soil properties between farm management (monoculture versus polyculture) and transect type (within-row versus across-row).

Fig. S2 The frequency of AMF taxa found in as few as 1 to as many as 100 of all 372 communities sampled. Of the 244 AMF OTUs, 167 occurred in fewer than 10 samples.

Fig. S3 The AMF taxa accumulation curve reaching a plateau of 214.778 ± 9.546 of 244 taxa after 167 samples.

Fig. S4 Boxplot for observed richness for (a) between farm management (monoculture versus polyculture) and transect type (within-row versus across-row) plus (b) focal crop (eggplant versus squash). The boxplot is bounded by the first and third quartile ranges with the line in the box representing the median. The whiskers extend from the first and third quartile to values that are not within the 1.5 interquartile range from both directions. Data beyond the whiskers are presented as individual circles.

Fig. S5 The boxplot for AMF (a) observed richness, (b) chao1 richness, and (c) diversity across the number of years in polyculture management (0, <10, and >10 years).

Fig. S6 The boxplot for the dispersion of the edaphic property dissimilarities from the centroid between farm management (monoculture versus polyculture).

Table S1. Site-by-site properties, including farm management (monoculture versus polyculture), focal crop (eggplant versus squash), plus the first year of polyculture management, number of years in polyculture, monoculture, or fallow.

Table S2. Results of the indicator species analysis for AMF taxa (listed by OTU plus their genus) of monoculture and polyculture fields.

Table S3. Model parameter estimates, with standard error in parentheses, of all soil properties.

Methods S1 *Molecular analysis of AMF communities: primer selection, PCR conditions, and amplicon library preparation*

AMF communities in root-zone soil were characterized using molecular methods. Soil samples for molecular measurements were immediately stored at -80°C upon return to the lab until DNA extractions could proceed. DNA was extracted from 0.25g of soil using the DNeasy PowerSoil Kit (Qiagen, location). DNA concentration was measured with the Quant-iT 1X dsDNA HS Assay kit (Life Technologies Inc., Gaithersburg, MD, USA) and concentrations were adjusted to 5ng/µl with ddH2O prior to PCR amplification. The ITS2 rRNA region (5.8Fun/ITS4Fun) was amplified to characterize the communities of fungi. Apart from providing more accurate measures of fungal diversity and abundance (Taylor *et al.*, 2016), since our root-zone samples could contain roots, despite best efforts to remove them, ITS2 primers were also used because they can better discriminate against plant DNA than other ITS primers (Taylor *et al.*, 2016). ITS2 primers have also matched well with all lineages in Glomeromycotina, the subphylum AMF belong to, (Spatafora *et al.*, 2016). Further, in the same study region, they have successfully been used to study fine-scale patterns of AMF community succession (Gao *et al.*, 2019). In addition, we also considered the differences in characterizing AMF communities using primers in the more variable ITS2 region versus AMF specific primers (i.e. NS31/AML2 primers; Simon *et al.*, 1992; Lee *et al.*, 2008) in the more well-conserved small subunit (SSU) rRNA region. First, ITS2 primers have been shown to detect similar environmental patterns to AMF-specific SSU primers (Berruti et al. 2017; Lekberg et al. 2018). However, while AMF specific primers are considered to resolve more "species-level" assignments of AMF taxa, the well-conserved SSU region may not be able to discriminate between taxon groups within some AMF families, namely in the Diversisporaceae and Glomeraceae, and, thus, potentially may lump several distinct AMF taxa from these families into single taxonomic units (Stockinger *et al.* 2012; Öpik *et al.*, 2013). In contrast, the more variable ITS2 region primers provide better separation between inter- and intra-species variation among fungi than the AMF-specific SSU primers (Schoch *et al.*, 2012). Therefore, while the ITS2 primers can potentially result in a larger number of unassigned AMF taxa at lower hierarchical levels, these primers could reduce lumping distinct AMF taxa into the same taxon. Since the main objective of our study was to examine how farm management impacted AMF community richness and diversity, we used the more variable ITS2 primers to characterize the AMF community in our soil samples to avoid potentially obscuring AMF community inter-species variation. The (5.8S) forward and (ITS4) reverse primers contained a 29 (forward) or 25 (reverse) base linker, a 12 base barcode, a 29 (forward) or 34 (reverse) base pad, a 0–8 base heterogeneity spacer (Fadrosh *et al.*, 2014). Sequencing of amplicon libraries was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) with 300bp paired-end reads at the Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley, CA. Detailed information about molecular analysis, specifically PCR conditions and amplicon library preparation, can be found in the supplementary methods.

PCR amplification for each sample was carried out in a 25 µl reaction mixture containing: 10µl 5PRIME HotMaster Mix (Eppendorf-5Prime, Gaithersburg, MD, USA), 2.5µl forward primer, 2.5µl reverse primer, 2µl template DNA, 3µl BSA, and 5µl nuclease-free water. PCR amplification was performed using the one-step PCR method in the Gene Amplification PCR System (BioRad Laboratories Inc.) with the following conditions: initial denaturation at 96°C for 2 min, followed by 35 cycles of 94°C for 30s, 58°C for 40s and 72°C for 2 min, and a final extension at 72°C for 10 min. In addition to soil samples, a synthetic mock community (Nguyen *et al.*, 2015), DNA extraction blanks and PCR blanks were also amplified during library preparation. The amplicon libraries were produced from a pool of three separate PCRs per sample. The quality of PCR products was evaluated by agarose gel electrophoresis. The PCR product yield was quantified using the Quant-iT 1X dsDNA HS Assay kit (Life Technologies Inc., Gaithersburg, MD, USA) and samples were pooled at equimolar concentrations (50ng of each of the 378 samples). Libraries were quality checked for correct amplicon size and purity using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) at the Functional Genomics Laboratory at the University of California, Berkeley, CA. Sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) with 300bp paired-end reads at the Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley, CA.

References

Berruti A, Desirò A, Visentin S, Zecca O, Bonfante P 2017. ITS fungal barcoding primers versus 18S AMF-specific primers reveal similar AMF-based diversity patterns in roots and soils of three mountain vineyards. *Environmental Microbiology Reports* **9**: 658–667.

Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, Ravel J, 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* **2**: 6.

Gao C, Montoya L, Xu L, Madera M, Hollingsworth J, Purdom E, Hutmacher RB, Dahlberg JA, Coleman-Derr D, Lemaux PG, Taylor JW, 2019. Strong succession in arbuscular mycorrhizal fungal communities. *The ISME Journal* **13**: 214–226.

Lee J, Lee S, Young JP 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiol Ecol* **65**: 339–349.

Lekberg Y, Vasar M, Bullington LS, Sepp SK, Antunes PM, Bunn R, Larkin BG, Opik M 2018. More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers. *New Phytologist* **220:** 971-976.

Simon L, Lalonde M, Bruns TD 1992. Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied and environmental microbiology* **58:** 291-295.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal BC, Fungal BCAL 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc Natl Acad Sci U S A* **109**: 6241–6246.

Spatafora JW, Chang Y, Benny GL, Lazarus KL, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A, et al. 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* **108**: 1028–1046.

Stockinger H, Krüger M, Schüssler A 2010. DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist* **187**: 461–474.

Taylor DL, Walters WA, Lennon NJ, Bochicchio J, Krohn A, Caporaso JG, Pennanen T, 2016. Accurate Estimation of Fungal Diversity and Abundance through Improved Lineage-Specific Primers Optimized for Illumina Amplicon Sequencing. *Applied and Environmental Microbiology* **82**: 7217–7226.

Figure S1. PCA of soil properties used to calculate the soil properties index. The ordination plot is color-coded to illustrate differences in soil properties between farm management (monoculture versus polyculture) and transect type (within-rows versus across-rows).

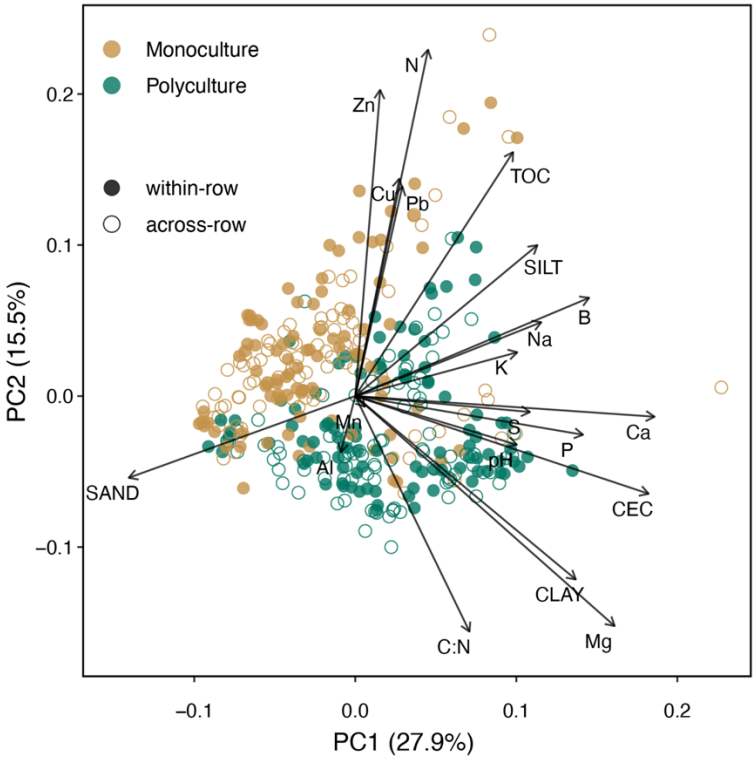

Figure S2. The frequency of AMF taxa found in as few as 1 to as many as 100 of all 372 communities sampled. Of the 243 AMF OTUs, 167 occurred in fewer than 10 samples.

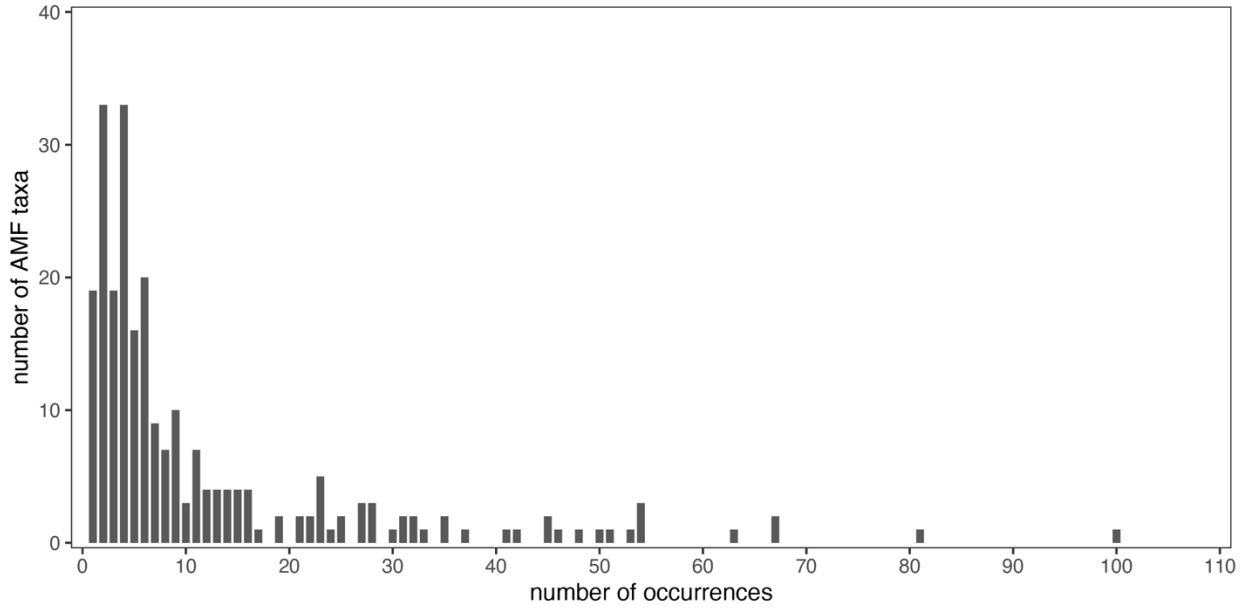

Figure S3. The AMF taxa accumulation curve reaching a plateau of 214.778 ± 9.546 of 243 taxa after 167 samples.

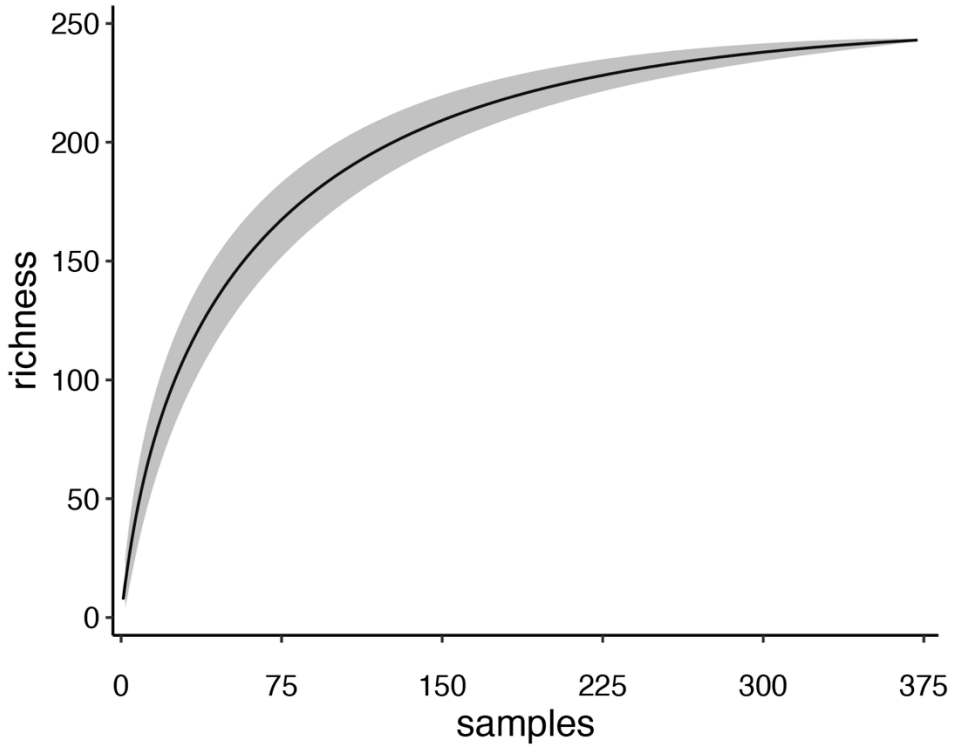

Figure S4. Boxplot for observed richness for (a) between farm management (monoculture versus polyculture) and transect type (within-row versus across-row) plus (b) focal crop (eggplant versus squash). The boxplot is bounded by the first and third quartile ranges with the line in the box representing the median. The whiskers extend from the first and third quartile to values that are not within the 1.5 interquartile range from both directions. Data beyond the whiskers are presented as individual circles.

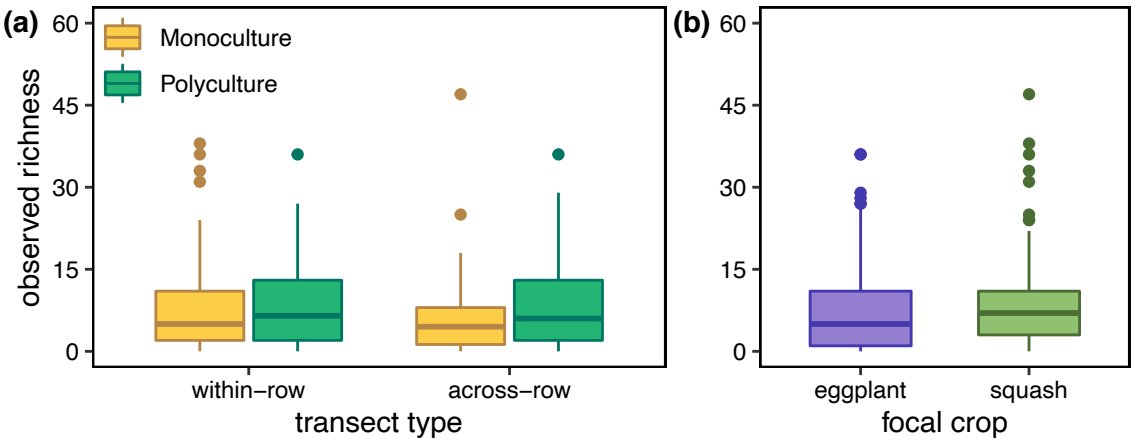

Figure S5. The boxplot for AMF (a) observed richness, (b) chao1 richness, and (c) diversity across the number of years in polyculture management (0, <10, and >10 years).

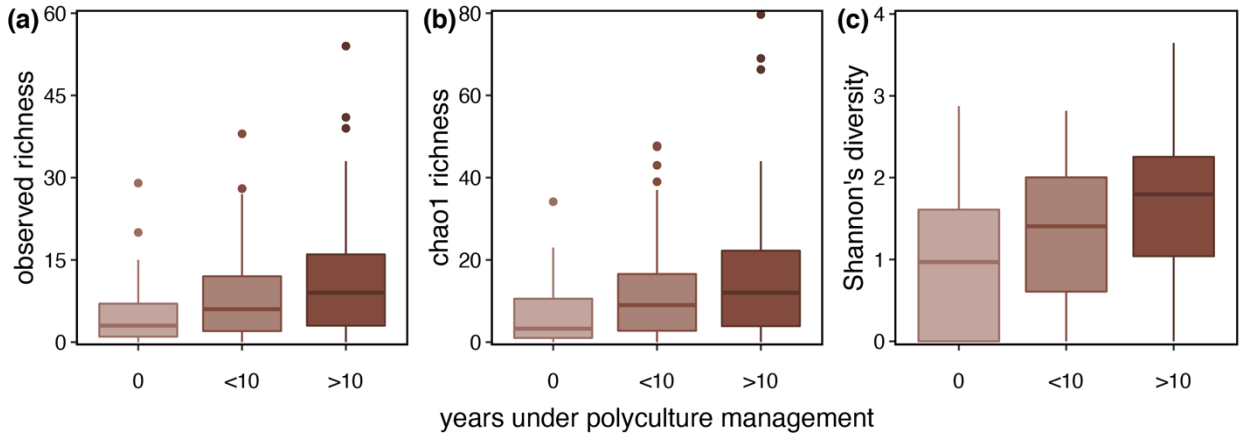

Figure S6. The boxplot for the dispersion of the edaphic property dissimilarities from the centroid between farm management (monoculture versus polyculture).

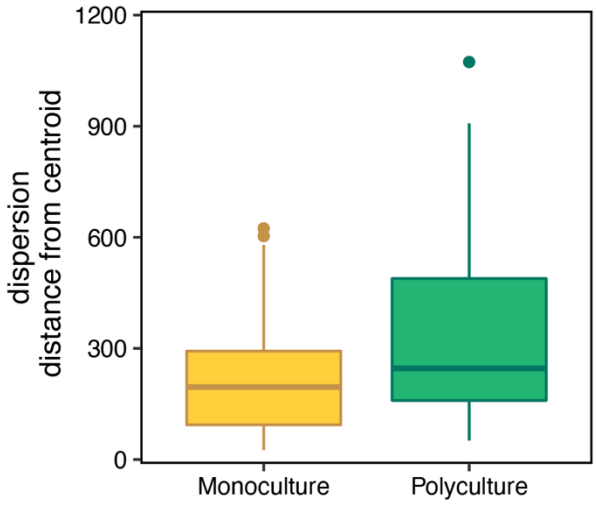

Table S1. Site properties, including farm management (monoculture versus polyculture), focal crop (eggplant versus squash), plus field area (in acres), number of crops, and number of years in polyculture, monoculture, or fallow.

Sampling unit	Farm site	Year sampled	Farm management	Focal crop	Area (ac)	Number of crops	Total years of polyculture management	Total years of monoculture management	Total years of fallow
1	A	2017	Monoculture	Eggplant	35.1	1	0	15	0
2	B	2017	Monoculture	Eggplant	35.2	1	10	5	0
3	C	2017	Monoculture	Eggplant	4.54	1	0	14	1
4	D	2017	Monoculture	Eggplant	6.13	1	0	2	13
5	E	2017	Monoculture	Eggplant	14.2	1	0	15	0
6	F	2017	Polyculture	Eggplant	3.901	31	13	2	0
7	G	2017	Polyculture	Eggplant	4.73	25	7	1	7
8	H	2017	Polyculture	Eggplant	11.4	46	7	8	0
9	I	2017	Polyculture	Eggplant	22.6	NA	15	0	0
10	J	2017	Polyculture	Eggplant	28.7	29	15	0	0
11	C	2018	Monoculture	Eggplant	4.54	1	0	14	1
12	D	2018	Monoculture	Eggplant	6.13	1	0	3	12
13	K	2018	Monoculture	Eggplant	7.32	1	0	15	0
14	L	2018	Monoculture	Eggplant	20.9	1	0	15	0
15	M	2018	Monoculture	Eggplant	8.42	1	0	15	0
16	G	2018	Polyculture	Eggplant	4.73	25	8	0	7
17	H	2018	Polyculture	Eggplant	11.4	37	8	7	0
18	I	2018	Polyculture	Eggplant	22.6	66	15	0	0
19	J	2018	Polyculture	Eggplant	28.7	30	15	0	0
20	N	2018	Polyculture	Eggplant	8.86	30	11	1	3
21	O	2018	Polyculture	Eggplant	4.56	27	9	6	0
22	P	2018	Monoculture	Squash	15.75	1	0	10	5
23	Q	2018	Monoculture	Squash	36.9	1	0	15	0
24	R	2018	Monoculture	Squash	7.74	1	0	15	0
25	S	2018	Monoculture	Squash	24.9	1	0	5	10
26	T	2018	Monoculture	Squash	10.5	1	0	15	0
27	U	2018	Polyculture	Squash	4.34	NA	8	0	7
28	V	2018	Polyculture	Squash	2.51	NA	7	8	0
29	W	2018	Polyculture	Squash	13.6	NA	7	8	0
30	X	2018	Polyculture	Squash	35.9	NA	15	0	0
31	Y	2018	Polyculture	Squash	38.25	55	11	4	0

Note: Data not available marked as "NA".

**Years under polyculture, monoculture, or fallow management in the 15 years prior to sampling*

Table S2. Results of the indicator species analysis for AMF taxa (listed by OTU plus their genus) of monoculture and polyculture fields.

Farm management	OTU_Genus	indval	*P* value
Monoculture	OTU574_Rhizophagus	0.429	0.001
	OTU222_Acaulospora	0.343	0.001
	OTU299_Acaulospora	0.320	0.001
	OTU2212_Diversispora	0.254	0.004
	OTU817_Claroideoglomus	0.245	0.001
	OTU2938_unassigned	0.236	0.002
	OTU2107_unassigned	0.197	0.004
	OTU1937_Claroideoglomus	0.167	0.029
	OTU2417_unassigned	0.167	0.023
	OTU2209_Claroideoglomus	0.167	0.032
Polyculture	OTU294_Glomus	0.552	0.001
	OTU1132_unassigned	0.411	0.001
	OTU759_unassigned	0.405	0.001
	OTU857_Rhizophagus	0.377	0.001
	OTU284_unassigned	0.375	0.001
	OTU786_unassigned	0.367	0.001
	OTU477_unassigned	0.348	0.001
	OTU606_unassigned	0.348	0.001
	OTU461_unassigned	0.347	0.001
	OTU679_unassigned	0.346	0.001
	OTU946_unassigned	0.325	0.001
	OTU422_Rhizophagus	0.319	0.003
	OTU751_unassigned	0.315	0.001
	OTU605_unassigned	0.308	0.008
	OTU1405_Paraglomus	0.289	0.001
	OTU1041_unassigned	0.283	0.001
	OTU793_Claroideoglomus	0.253	0.003
	OTU419_unassigned	0.249	0.003
	OTU1192_unassigned	0.244	0.003
	OTU2112_unassigned	0.244	0.005
	OTU617_unassigned	0.239	0.003
	OTU1549_Diversispora	0.239	0.003
	OTU2152_unassigned	0.239	0.002
	OTU1269_unassigned	0.219	0.011
	OTU789_Glomus	0.217	0.007
	OTU1419_unassigned	0.217	0.007
	OTU1297_Rhizophagus	0.217	0.004
	OTU1487_Rhizophagus	0.217	0.007
	OTU934_Glomus	0.204	0.008
	OTU1464_Acaulospora	0.204	0.008
	OTU1728_unassigned	0.204	0.007
	OTU898_Glomus	0.201	0.029
	OTU943_Glomus	0.198	0.05
	OTU2717_unassigned	0.197	0.041
	OTU1548_unassigned	0.196	0.039
	OTU820_Dominikia	0.194	0.027
	OTU1359_Claroideoglomus	0.191	0.015
	OTU1049_unassigned	0.191	0.016
	OTU1131_unassigned	0.191	0.013
	OTU2158_Diversispora	0.191	0.017
	OTU1954_Glomus	0.187	0.04
	OTU907_Glomus	0.177	0.029
	OTU1241_unassigned	0.177	0.038
	OTU1455_Glomus	0.177	0.042
	OTU2460_unassigned	0.177	0.043
	OTU2913_unassigned	0.177	0.035
	OTU1908_Claroideoglomus	0.161	0.048

Table S3. Model parameter estimates, with standard error in parentheses, of all soil properties.

Edaphic variable	Farm type	Transect type	Focal crop	Farm management x Transect Type
Al	- 0.253(0.458)	0.016(0.065)	0.638(0.49)	- 0.040(0.065)
B	- 0.016(0.026)	0.001(0.006)	0.012(0.028)	- 0.005(0.006)
C:N	- 1.240(0.534) *	- 0.469(0.105) ***	0.487(0.571)	0.232(0.105) *
Ca	- 97.562(57.601)	- 14.441(9.538)	13.787(61.578)	- 12.093(9.538)
CEC	- 1.092(0.383) **	- 0.047(0.057)	0.469(0.409)	- 0.096(0.057)
% clay	- 1.866(0.427) ***	- 0.102(0.088)	0.496(0.456)	0.040(0.088)
Cu	0.058(0.07)	0.008(0.014)	0.093(0.075)	- 0.012(0.014)
K	- 20.121(8.644) *	5.270(2.388) *	10.767(9.241)	- 5.074(2.388) *
Mg	- 77.962(17.616) ***	- 1.734(1.246)	24.493(18.832)	- 0.974(1.246)
Mn	- 0.88(0.772)	0.399(0.206)	1.512(0.826)	- 0.479(0.206) *
N	0.003(0.003)	0.002(0.001) **	0.001(0.003)	- 0.001(0.001)
Na	- 4.178(3.694)	1.309(0.838)	- 3.097(3.949)	- 1.748(0.838) *
P	- 6.477(3.214)	0.996(0.635)	3.329(3.436)	- 2.238(0.635) ***
Pb	0.069(0.028) *	0.014(0.009)	0.019(0.03)	0.004(0.009)
pH	- 0.155(0.146)	- 0.015(0.018)	- 0.009(0.156)	0.009(0.018)
S	- 5.681(1.84) **	0.815(0.801)	0.677(1.967)	- 2.113(0.801) **
% sand	0.944(2.017)	0.515(0.251) *	0.629(2.157)	0.232(0.251)
% silt	0.922(1.737)	- 0.413(0.258)	- 1.125(1.857)	- 0.271(0.258)
TOC	- 0.006(0.027)	0.005(0.005)	0.014(0.029)	- 0.006(0.005)
Zn	1.170(0.366) **	0.028(0.056)	0.849(0.392) *	- 0.071(0.056)

* P <0.01, ** P <0.05, *** P <0.001; Al, aluminum; B, boron; C:N, carbon-nitrogen ratio; Ca, calcium; CEC, cation exchange capacity; Cu, copper; K, potassium; Mg, magnesium; Mn, manganese; N, nitrogen; Na, sodiun P, phosphorus; Pb, lead; S, sulfur; TOC, total organic carbon; Zn, zinc.