APPENDIX

Supplementary material - Paper I: Püschel *et al*. (2017)

Püschel, D., Janoušková, M., Voříšková, A., Gryndlerová, H., Vosátka, M., Jansa, J., 2017. **Arbuscular mycorrhiza stimulates biological nitrogen fixation in two *Medicago* spp. through improved phosphorus acquisition.** Frontiers in Plant Science 8, 390.

Supplementary material - Paper 2: Voříšková *et al*. (2017)

Voříšková, A., Jansa, J., Püschel, D., Krüger, M., Cajthaml, T., Vosátka, M., Janoušková, M., 2017. **Real-time PCR quantification of arbuscular mycorrhizal fungi: does the use of nuclear or mitochondrial markers make a difference?** Mycorrhiza 27, 577–585.

Supplementary material - Paper 3: Voříšková *et al*. (2019)

Voříšková, A., Jansa, J., Püschel, D., Vosátka, M., Šmilauer, P., Janoušková, M., 2019. **Abiotic contexts consistently influence mycorrhiza functioning independently of the composition of synthetic arbuscular mycorrhizal fungal communities.** Mycorrhiza 29, 127–139

Supplementary material - Paper 4: Voříšková *et al*. (manuscript)

Voříšková, A., Jansa, J., Püschel, D., Vosátka, M., Janoušková, M. **Are mycorrhizal benefits influenced by the quantitative composition of arbuscular mycorrhizal fungal communities?**

Supplementary material - Paper I: Püschel *et al.* (2017)

TABLE S1 | Effects of experimental factors (plant: *Medicago truncatula* or *M. sativa*; soil: sand–zeolite substrate with 10% of LT or Tän soil; mycorrhizal inoculation: presence or absence of *Rhizophagus irregularis* 'PH5'; phosphorus (P) addition: 0, 10 or 40 mg of P per pot) and their interactions on various plant parameters according to a general linear model (GLM) analysis using the P addition level as the continuous predictor. BNF – biological nitrogen fixation; N – nitrogen; MGR – mycorrhizal growth response, MPR – mycorrhizal P-uptake response, MNR – mycorrhizal N-uptake response. Presented are *F* and *p* values for each of the separate analyses. Significant factors or their interactions ($p < 0.05$) are highlighted in red.

	Total dry weight		N derived from BNF		Total P content		Total N content	
	F	p	F	p	F	p	F	p
Plant (1)	8.59	0.0042	6.37	0.0131	6.71	0.0110	0.01	0.9224
Soil (2)	19.91	0.0000	4.97	0.0280	0.01	0.9118	21.43	0.0000
Inoculation (3)	21.13	0.0000	75.74	0.0000	239.76	0.0000	43.17	0.0000
Phosphorus (4)	3.63	0.0597	14.88	0.0002	26.56	0.0000	10.61	0.0015
1 × 2	5.59	0.0200	9.12	0.0032	0.22	0.6375	2.15	0.1455
1 × 3	21.78	0.0000	25.09	0.0000	0.04	0.8358	17.00	0.0001
2 × 3	0.52	0.4733	0.92	0.3387	0.12	0.7275	2.61	0.1092
1 × 4	11.44	0.0010	4.72	0.0321	10.85	0.0014	0.49	0.4860
2 × 4	12.05	0.0008	7.82	0.0062	14.28	0.0003	9.55	0.0026
3 × 4	13.29	0.0004	7.16	0.0087	27.73	0.0000	15.92	0.0001
1 × 2 × 3	6.55	0.0120	2.12	0.1486	3.00	0.0863	2.98	0.0873
1 × 2 × 4	2.93	0.0899	4.47	0.0370	2.49	0.1177	4.47	0.0370
1 × 3 × 4	2.13	0.1472	1.26	0.2641	1.53	0.2188	1.60	0.2095
2 × 3 × 4	7.98	0.0057	13.27	0.0004	8.63	0.0041	7.69	0.0066
1 × 2 × 3 × 4	5.55	0.0204	3.96	0.0492	2.13	0.1471	4.02	0.0476

	Shoot P concentration		Root P concentration		Shoot N concentration		Root N concentration	
	F	p	F	p	F	p	F	p
Plant (1)	5.94	0.0166	11.00	0.0013	18.86	0.0000	1.94	0.1670
Soil (2)	21.98	0.0000	40.90	0.0000	0.04	0.8420	6.38	0.0131
Inoculation (3)	178.76	0.0000	457.39	0.0000	2.90	0.0918	17.88	0.0001
Phosphorus (4)	45.42	0.0000	12.39	0.0006	8.43	0.0045	4.18	0.0434
1 × 2	10.91	0.0013	0.00	1.0000	2.36	0.1274	0.06	0.8137
1 × 3	14.95	0.0002	7.29	0.0081	0.81	0.3691	0.13	0.7201
2 × 3	0.04	0.8482	0.97	0.3271	0.82	0.3659	0.16	0.6915
1 × 4	1.56	0.2152	11.52	0.0010	18.91	0.0000	5.11	0.0259
2 × 4	0.91	0.3419	4.16	0.0441	0.53	0.4667	0.12	0.7282
3 × 4	5.28	0.0236	11.77	0.0009	0.05	0.8235	4.86	0.0297
1 × 2 × 3	2.42	0.1228	0.21	0.6463	2.02	0.1587	0.04	0.8333
1 × 2 × 4	0.00	0.9884	0.24	0.6235	0.11	0.7371	0.73	0.3960
1 × 3 × 4	4.89	0.0293	5.30	0.0233	0.03	0.8587	0.53	0.4677
2 × 3 × 4	0.01	0.9285	1.56	0.2152	0.00	0.9693	0.00	0.9960
1 × 2 × 3 × 4	0.06	0.8082	0.83	0.3639	0.65	0.4227	0.24	0.6243

	Mycorrhizal colonization		MGR		MPR		MNR	
	F	p	F	p	F	p	F	p
Plant (1)	12.15	0.0010	123.10	0.0000	6.86	0.0116	62.85	0.0000
Soil (2)	0.00	0.9845	16.14	0.0002	0.21	0.6515	19.68	0.0000
Phosphorus (3)	49.04	0.0000	53.27	0.0000	77.66	0.0000	38.53	0.0000
1 × 2	0.09	0.7626	48.26	0.0000	11.95	0.0011	21.17	0.0000
1 × 3	1.09	0.3008	19.91	0.0000	0.02	0.8904	10.14	0.0025
2 × 3	3.25	0.0773	32.34	0.0000	7.42	0.0088	21.69	0.0000
1 × 2 × 3	0.83	0.3664	29.64	0.0000	9.34	0.0036	15.38	0.0003

Supplementary material - Paper II: Voříšková *et al*. (2017)

Supplementary methods

Text S1

Real-time PCR quantification based on nuclear ribosomal DNA

Nuclear ribosomal DNA of each of the four species was quantified using *Taq*Man-based qPCR with hydrolysis probes with fluorescent reporter dye 6-carboxyfluorescein (6-FAM) at the 5′ end and quencher Black Hole Quencher (BHQ1) at the 3' end. qPCR was performed in 10 µl reaction volume with the following reagents and qPCR conditions: 1 x LightCycler 480 Probes Master (Roche, Penzberg, Germany), taxon-specific primers (for primers and concentrations see Table S1), 50 nM of TaqMan taxon-specific probes and 2.5 µl of 1:9 diluted DNA extract or the corresponding dilution of a standard. The thermal cycling program was the following: pre-incubation at 95 °C for 10 min, 40 cycles of 95 °C for 10 s, annealing for 30 s with primer–specific temperature (see Table S1) and elongation at 72 °C for 1 s. The 5-fold dilution series of the standards were amplified in technical triplicates, samples in technical duplicates. Standards were PCR products obtained from spores of the corresponding AMF isolate, serially diluted in 10mM Tris (seven five-fold dilutions from 1 pg DNA μl^{-1} to 0.000064 pg DNA μl^{-1}). Target DNA for the preparation of standards was extracted from ca. 100 spores per isolate using the DNA Plant Mini kit (Qiagen, Hilden, Germany), with the exception of *Claroideoglomus claroideum* BEG96, where DNA was extracted from a single spore according to Redecker et al. (1997). PCR products for the standards were prepared by PCR, using the primers LR1 (van Tuinen et al. 1998) and LSUmBr (Krüger et al. 2009). PCR was performed in 20 µl reaction volume with the following reagents and PCR conditions: 1x *Pfu* buffer (without $MgSO_4$) (ThermoFisher, Scientific, Waltham, MA, USA), 2 mM $MgSO_4$, 0.2 mM of each dNTP, 0.5 µM of each primer, 0.5 U *Pfu* DNA Polymerase (ThermoFisher) and 2 – 20 ng DNA template. The thermal cycling program was: initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 1 min, 58 °C for 45 sec, 72 °C for 2 min and a final elongation at 72 °C for 10 min. Each DNA extract was amplified in triplicates. Pooled amplicons were gel–purified using the Zymoclean Gel DNA Recovery kit (Zymo Research, Irvine, USA) and their final DNA concentration was measured spectrophotometrically (BioPhotometer, Eppendorf).

DNA concentrations of the amplified target region, according in samples, were calculated based on calibration curves derived from the serially diluted PCR products in the LightCycler 480 software version 1.5 (Roche). The DNA concentrations were calculated to copy numbers of the target region according to Krak et al. (2012): CN ng^{-1} DNA = (6.022 x 10^{23})/(amplicon length [bp] x 10^9 x 660).

Real-time PCR quantification based on mitochondrial ribosomal DNA

Mitochondrial ribosomal DNA of each of the four species was quantified by means of SYBR Green-based qPCR assays. qPCR was performed in 10 µl reaction volume with the following reagents and qPCR conditions: 1 x LightCycler 480 SYBR Green I Master (Roche), 0.5 µM of specific forward/reverse primers (see Table S1) and 2.5 µl of 1:10 diluted DNA extract or the corresponding dilution of a standard; pre-incubation at 95 °C for 5 min, 40 cycles of 95 °C for 10 s, annealing for

10 s with primer–specific temperature (see Table S1), elongation at 72 °C for 15 s and termination with a standard melting curve.

The employed standards were serially diluted plasmids prepared as described in Krak et al. (2012) with the following differences: The plasmids carrying *F. mosseae*, *G. margarita* and *C. claroideum* fragments were linearized using the restriction enzyme EcoRV (ThermoFisher, Scientific) and those carrying the *R. irregularis* fragment, using the enzyme EcoRI (ThermoFisher, Scientific) and purified by ZymoClean Gel DNA Recovery kit (ZymoReseach). Plasmids were quantified spectrophotometrically (BioPhotometer, Eppendorf) and serially diluted to seven or eight five-fold dilutions (25 - 0.00032 pg DNA μl^{-1}).

DNA concentrations of the amplified target region in samples were calculated based on calibration curves derived from the serially diluted plasmids in the LightCycler 480 software version 1.5 (Roche). The DNA concentrations were calculated to copy numbers of the target region in the same way as mentioned above.

Design of new qPCR assays

In addition to available qPCR assays, one new *Taq*Man-based assay targeted to the large subunit of nuclear ribosomal DNA (nrLSU) of *Gigaspora margarita* BEG34 and three SYBR Green-based assays targeting the large subunit of mitochondrial ribosomal DNA (mtLSU, *rnl* gene) of *Gigaspora margarita* BEG34, *Funneliformis mosseae* BEG95 and *Claroideoglomus claroideum* BEG96 was developed.

Purified amplicons of partial nrLSU of *G. margarita* BEG34, prepared in the same way as described above for the PCR product standards, were cloned, using TOPO TA Cloning Kit (ThermoFisher) according to the manufacturer's protocol and sequenced using M13 primers at Macrogen Inc. Company (Amsterdam, the Netherlands). New primers targeting *G. margarita* sequences and excluding other AMF species were designed in Allele ID version 6 software (Premier Biosoft International, Palo Alto, CA, USA).

For the design of primers targeting the mtLSU, first all available sequences of the mtLSU within the phylum Glomeromycota were downloaded from public databases (DDBJ/EMBL/GenBank) and aligned with MAFFT (Katoh et al. 2005). Based on this alignment, the general primer mtLSUallF01 (5' CAG CGT ACC TTT TGY ATA ATG G 3') and mtLSUallR01 (5' CCA GTG CCG TAC CRK CTA GTA AC 3') were designed to amplify for all three AMF species were designed. These newly designed primers were tested by PCR on DNA extracts from spores obtained as described above.

The PCR was performed in 20 µl reaction volume with the following reagents and PCR conditions: 1x *Pfu* buffer with 2 mM $MgCl_2$ (ThermoFisher, Scientific, Waltham, MA, USA), 0.25 mM of each dNTP, 0.5 µM of each primer, 0.025 U μl^{-1} Pfu DNA polymerase (ThermoFisher) and 2 – 20 ng DNA template; initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 45 sec, elongation at 72 °C for 1 min and a final elongation at 72 °C for 10 min. All three isolates were successfully amplified and sequenced at GATC (Konstanz, Germany). The newly derived sequences were checked for their identity using BLAST against public databases (DDBJ/EMBL/GenBank) and used to design new species-specific qPCR primer (SYBR Green) with the Primer3 (Untergasser et al. 2012) and Allele ID v. 6 software packages.

The PCR conditions for the new assays were optimized with serial dilutions of PCR products and plasmids (see standard curves in Fig. S1). Using DNA extracts from root samples colonized by the respective AMF isolate, it was confirmed that melting temperature (Tm) of products obtained from experimental samples was within the range of Tm obtained with plasmid standards (as given in Fig. S1). Cross-amplification tests were carried out with the new and previously available primers using DNA extracts from the root samples. The tests verified the specificity of the qPCR assays within the set of the four studied isolates (Table S2).

Supplementary references

Katoh K, Kuma KI, Toh H, Miyata T (2005) MAFFT version 5: Improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 33:511–518. doi: 10.1093/nar/gki198

Krak K, Janoušková M, Caklová P, Vosátka M, Štorchová H (2012) Intraradical dynamics of two coexisting isolates of the arbuscular mycorrhizal fungus *Glomus intraradices* sensu lato as estimated by real-time PCR of mitochondrial DNA. Appl Environ Microbiol 78:3630–3637. doi: 10.1128/AEM.00035-12

Krüger M, Stockinger H, Krüger C, Schüßler A (2009) DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol 183:212–223. doi: 10.1111/j.1469-8137.2009.02835.x

Redecker D, Thierfelder H, Walker C, Werner D, Redecker D, Thierfelder H, Walker C, Universita FB Der (1997) Restriction analysis of PCR-amplified internal transcribed spacers of ribosomal DNA as a tool for species identification in different genera of the order Glomales. Appl Environ Microbiol 63:1756–1761.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3-new capabilities and interfaces. Nucleic Acids Res 40:1–12. doi: 10.1093/nar/gks596

Trouvelot A (1986) Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une significantion fonctionnelle. Mycorrhizae Physiol Genet 217–221. doi: 10.1177/004057368303900411

van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Mol Ecol 7:879–887. doi: 10.1046/j.1365-294x.1998.00410.x

Supplementary tables

Table S1: Real-time PCR assays and reaction conditions used for the quantification of four arbuscular mycorrhizal fungal isolates, based on primers and hydrolysis probes targeted to the large subunit of nuclear ribosomal DNA (nrLSU) or the large subunit of mitochondrial ribosomal DNA (mtLSU, *rnl* gene).

target region	target isolate	type	sequence 5'-3'	References	Primers conc.	Annealing temperature (°C)	Size of amplicon (bp)
nrLSU	*Rhizophagus. irregularis* PH5	primerF	TTCGGGTAATCAGCCTTTCG	Thonar et al., 2012	400 nM	58	249
		primerR	TCAGAGATCAGACAGGTAGCC				
		hydrolysis probe	FAM-TTAACCAACCACACGGGCAAGTACA-BHQ1				
nrLSU	*Funneliformis mosseae* BEG95	primerF	GGAAACGATTGAAGTCAGTCATACCAA	Thonar et al., 2012	400 nM	58	121
		primerR	CGAAAAAGTACACCAAGAGATCCCAAT				
		hydrolysis probe	FAM-AGAGTTTCAAAGCCTTCGGATTCGC-BHQ1				
nrLSU	*Gigaspora margarita* BEG34	primerF	GAGGAAAAGAAACTAACAAGG	newly designed	500 nM	60	75
		primerR	AAACCAGGTAGATTTTAAATTTG				
		hydrolysis probe	FAM-CCGCTTCACTCGCCGTTACT-BHQ1				
nrLSU	*Claroideoglomus claroideum* BEG96	primerF	GCGAGTGAAGAGGGAAGAG	Thonar et al., 2012	300 nM	52	178
		primerR	TTGAAAGCGTATCGTAGATGAAC				
		hydrolysis probe	FAM-AACAGGACATCATAGAGGGTGACAATCCC-BHQ1				
mtLSU	*Rhizophagus. irregularis* PH5	primerF	GAGGGAGTGGCAGTTTCTT	Krak et al., 2012	500 nM	60	133
		primerR	GCATTCTTAGCCCAGCTATG				
mtLSU	*Funneliformis. mosseae* BEG95	primerF	GATTAGCTGGTCTTCCGCGA	newly designed	500 nM	60	98
		primerR	AAGGCCAAAGGTAAGGTCGG				
mtLSU	*Gigaspora margarita* BEG34	primerF	AGACACGAAGGAGCAGGGTA	newly designed	500 nM	60	100
		primerR	TCCAGTGCCGTACCAGCTAG				
mtLSU	*Claroideoglomus claroideum* BEG96	primerF	CCTGGCTTCGGGTCTGATG	newly designed	500 nM	62	100
		primerR	TTCAGGCTCACTGTCGTGAC				

Table S2: Cross-specificity tests of the real-time PCR assays used for the quantification of four arbuscular mycorrhizal fungal isolates (AMF). The assays targeted either the large subunit of nuclear ribosomal DNA (nrLSU) or the large subunit of mitochondrial ribosomal DNA (mtLSU, *rnl* gene), primer sequences and PCR conditions are summarized in Table S1. Values in bold are crossing point (Cp) values of specific tests, n.d. (not detected), i.e. no signal within 40 PCR amplification cycles.

AMF	Target region	Cp values			
		CC	FM	GM	RI
CC	nrLSU	**25.72**	35	35	n.d.
FM	nrLSU	n.d.	**26.09**	n.d.	n.d.
GM	nrLSU	35	35	**24.49**	33.77
RI	nrLSU	n.d.	35	35	**23.26**
CC	mtLSU	**24.92**	35	n.d.	n.d.
FM	mtLSU	n.d.	**23.94**	35	35
GM	mtLSU	n.d.	35	**22.23**	n.d.
RI	mtLSU	n.d.	35	n.d.	**20.81**

Targeted AMF*: Claroideoglomus claroideum* BEG96 (CC), *Funnelifomis mosseae* BEG95 (FM), *Gigaspora margarita* BEG34 (GM), *Rhizophagus irregularis* PH5 (RI)

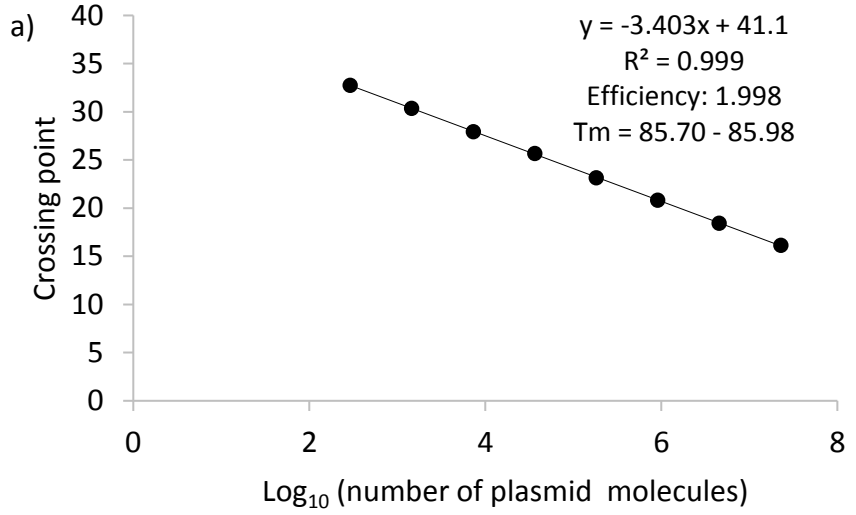
Table S3: Linear regression models of arbuscule abundance in mycorrhizal parts of root system (a%), vesicle abundance in whole root system (V%) and relative content of AMF-specific phospholipid fatty acids (PLFA), as predicted by arbuscular mycorrhizal fungal isolate (AMF) and root colonization intensity (M%). n.d. the interaction term was not included into the model. Significant effects are highlighted in bold. M%, a% and V% were estimated as described in Trouvelot (1986).

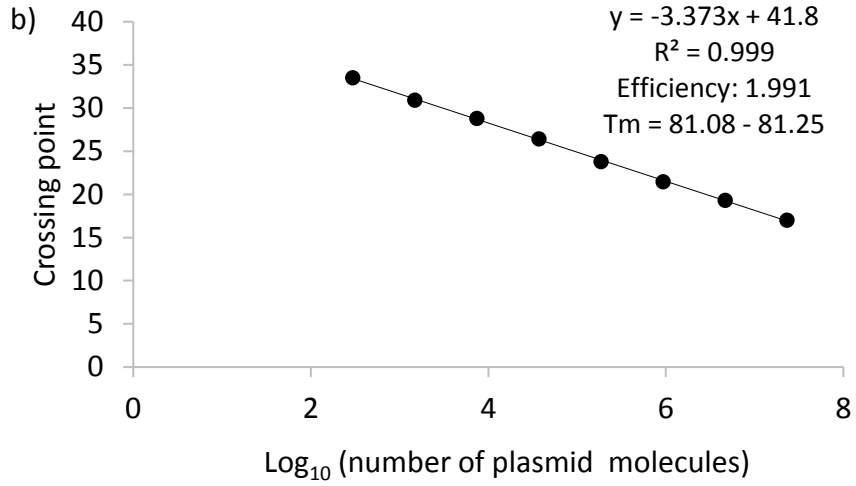
		a%		V%		PLFA	
	df	F value	P value	F value	P value	F value	P value
AMF	3	**53.52**	**< 0.001**	**82.70**	**< 0.001**	**13.55**	**< 0.001**
M%	1	2.10	0.162	**19.31**	**< 0.001**	**21.99**	**< 0.001**
AMF * M%	3	n.d.	n.d.	n.d.	n.d.	**3.64**	**0.032**

Table S4: Pair wise relationship between quantification parameters in each arbuscular mycorrhizal fungal isolate: *Claroideoglomus claroideum* BEG96 (CC), *Funnelifomis mosseae* BEG95 (FM), *Gigaspora margarita* BEG34 (GI), *Rhizophagus irregularis* PH5 (RI). M% – intensity of root colonization; a% - arbuscule abundance in mycorrhizal parts of root system, V% - vesicle abundance in whole root system, PLFA – relative content of AMF-specific phospholipid fatty acids; nrDNA CN – copy numbers of nuclear ribosomal DNA, mtDNA CN – copy numbers of mitochondrial ribosomal DNA, mt/nr – ratio of mtDNA CN to nrDNA CN. n.d. - not determined because most V% values = 0. Significant effects are highlighted in bold.

	CC		FM		GM		RI	
	adj. R^2	P value	adj. R^2	P value	adj. R^2	P value	adj. R^2	P value
M% - a%	**0.503**	**0.045**	-0.250	0.978	-0.178	0.772	-0.106	0.544
M% - V%	**0.594**	**0.026**	0.179	0.222	n.d.	n.d.	**0.510**	**0.042**
M% - PLFA	**0.880**	**0.001**	**0.748**	**0.016**	0.388	0.080	-0.153	0.673
M% - nrDNA CN	**0.506**	**0.044**	**0.656**	**0.031**	-0.163	0.708	0.220	0.162
M% - mtDNA CN	**0.496**	**0.047**	0.261	0.171	0.046	0.307	-0.151	0.066
M% - mt/nr	0.051	0.362	0.065	0.310	0.308	0.114	**0.887**	**0.001**
PLFA - nrDNA CN	0.326	0.105	**0.718**	**0.027**	-0.088	0.505	-0.105	0.541
PLFA - mtDNA CN	0.339	0.100	0.344	0.129	**0.521**	**0.041**	0.177	0.191
PLFA - mt/nr	-0.139	0.668	-0.081	0.474	**0.906**	**0.001**	-0.080	0.489
a% - nrDNA CN	0.022	0.335	-0.136	0.561	0.159	0.204	-0.171	0.741
a% - mtDNA CN	0.002	0.362	0.084	0.294	-0.093	0.516	-0.188	0.833
a% - mt/nr	-0.008	0.430	0.091	0.288	-0.182	0.795	-0.190	0.851
V% - nrDNA CN	0.204	0.172	0.318	0.142	n.d.	n.d.	-0.164	0.708
V% - mtDNA CN	0.190	0.181	0.031	0.342	n.d.	n.d.	-0.192	0.854
V% - mt/nr	-0.087	0.554	0.019	0.354	n.d.	n.d.	0.351	0.095

Supplementary figures





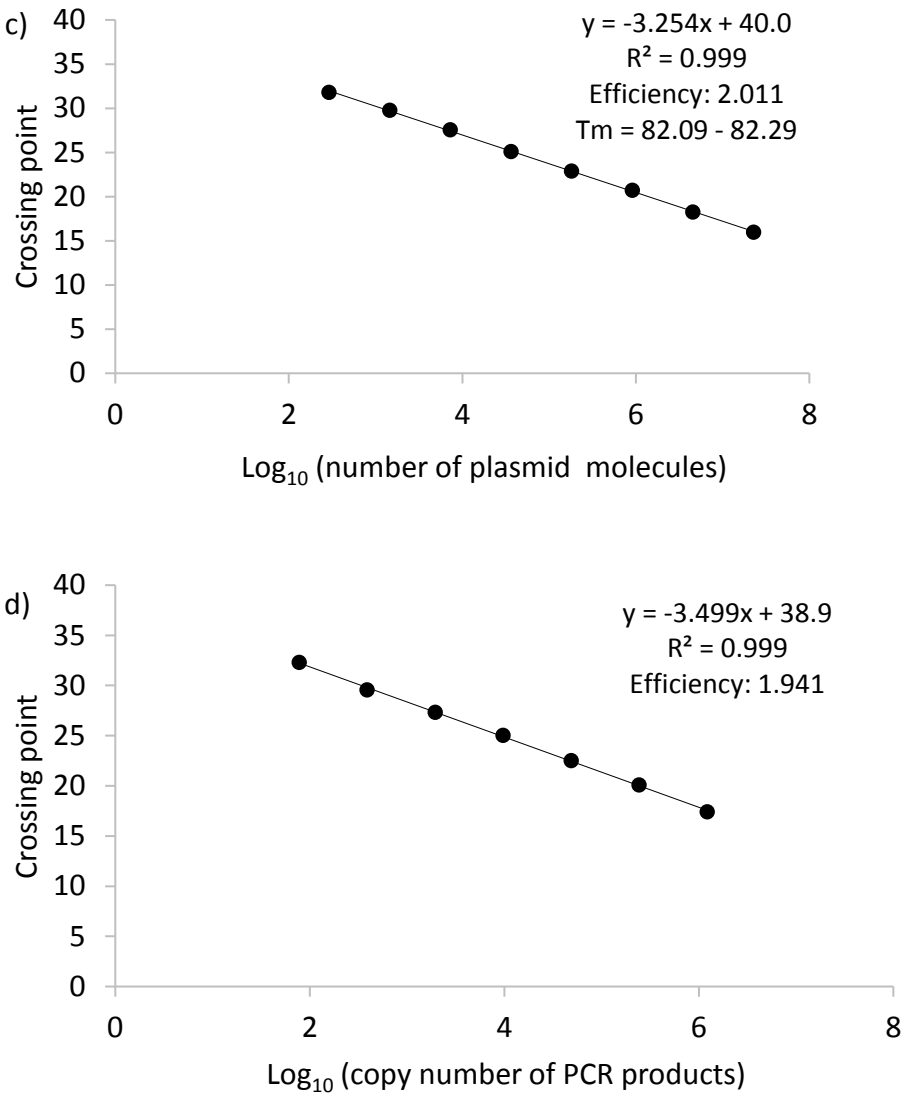
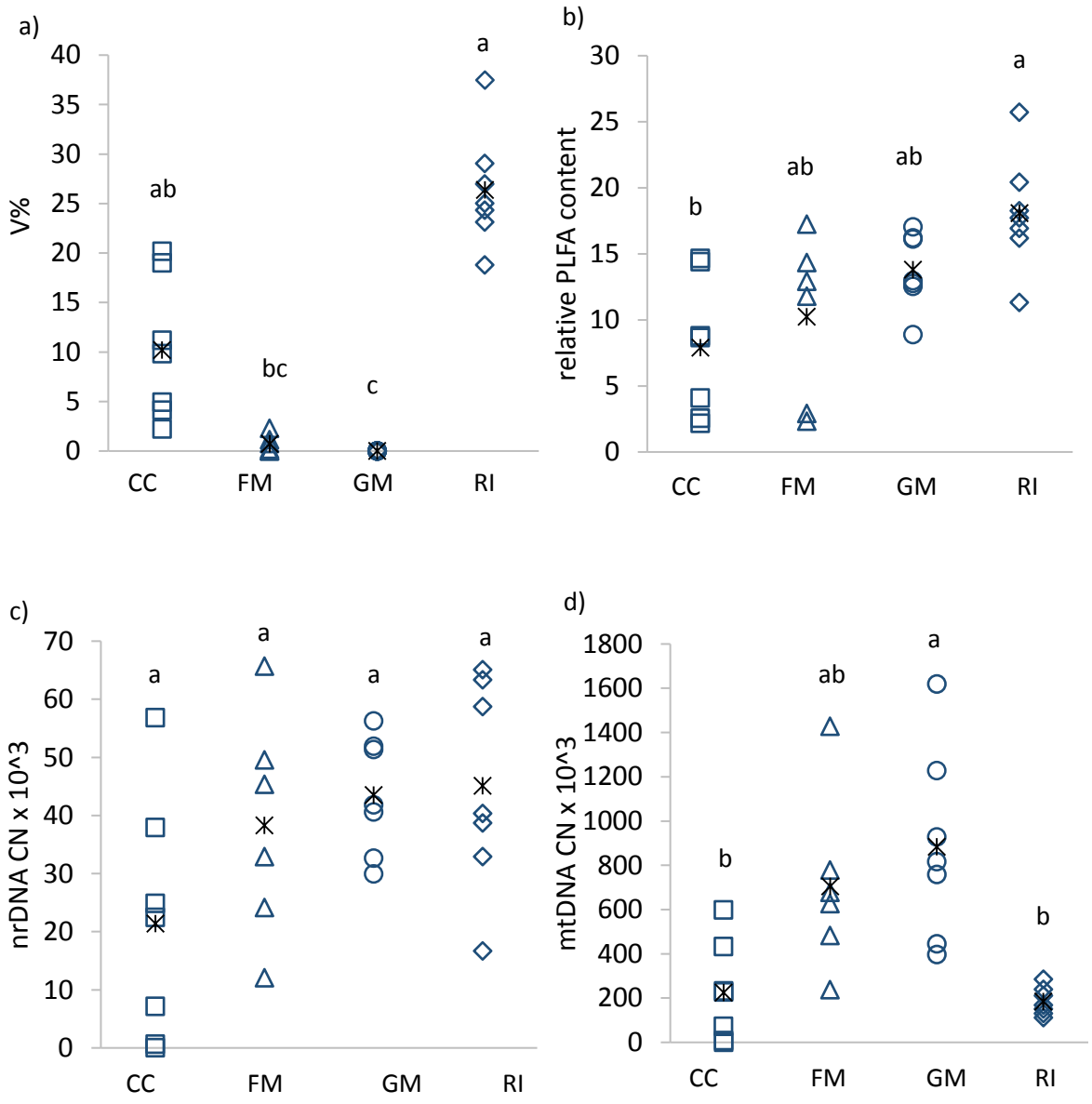


Figure S1: Representative standard curves of newly designed SYBR Green-based assays for the quantification of *Claroideoglomus claroideum* BEG96 **(a)**, *Funneliformis mosseae* BEG95 **(b)**, *Gigaspora margarita* BEG34 **(c)** and *Taq*Man-based assay for *Gigaspora margarita* BEG34 **(d)**. The linear regression lines, equations, R^2, efficiency of assays, melting temperature (Tm) of plasmid standards refer to each qPCR assay.


a)
V%
40
35
30
25
20
15
10
5
0
ab
bc
c
a
CC
FM
GM
RI
b)
relative PLFA content
30
25
20
15
10
5
0
b
ab
ab
a
CC
FM
GM
RI
c)
nrDNA CN x 10^3
70
60
50
40
30
20
10
0
a
a
a
a
CC
FM
GM
RI
d)
mtDNA CN x 10^3
1800
1600
1400
1200
1000
800
600
400
200
0
b
ab
a
b
CC
FM
GM
RI

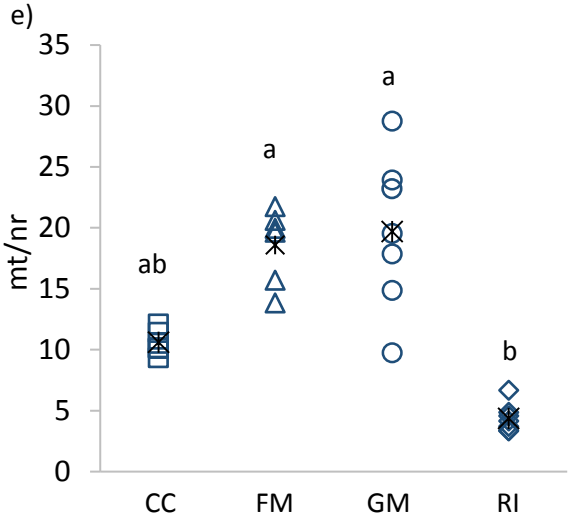
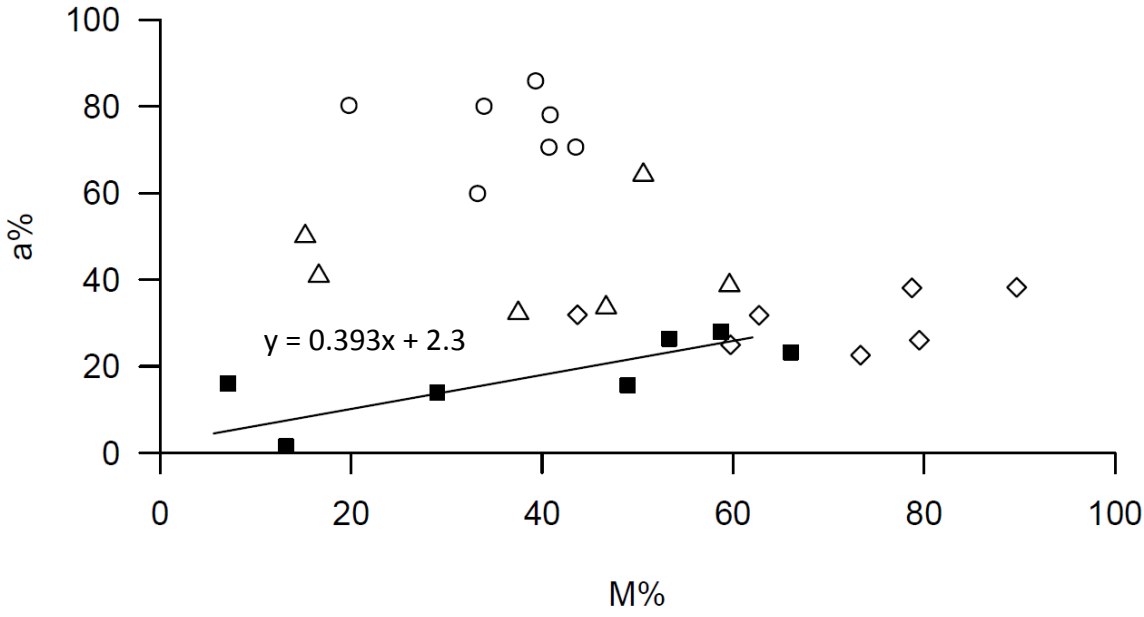
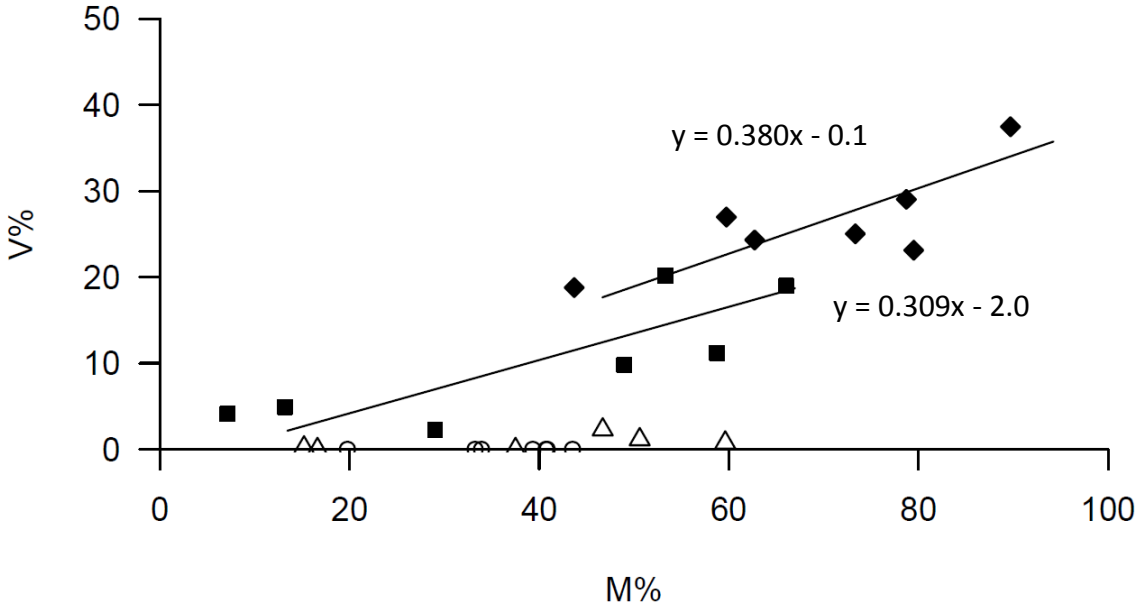
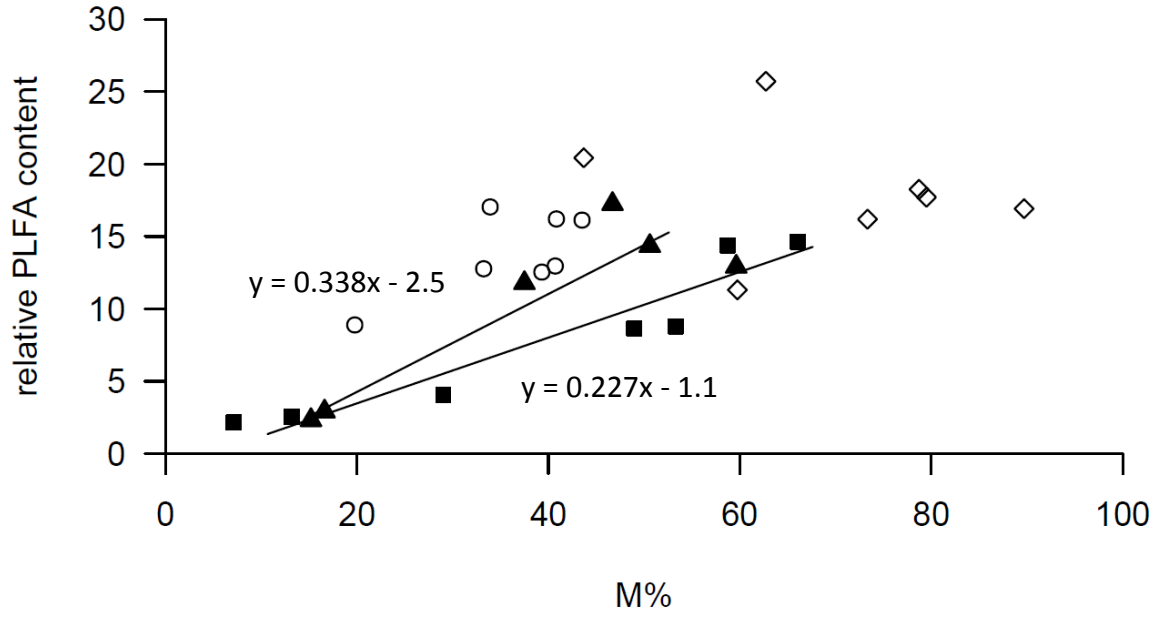
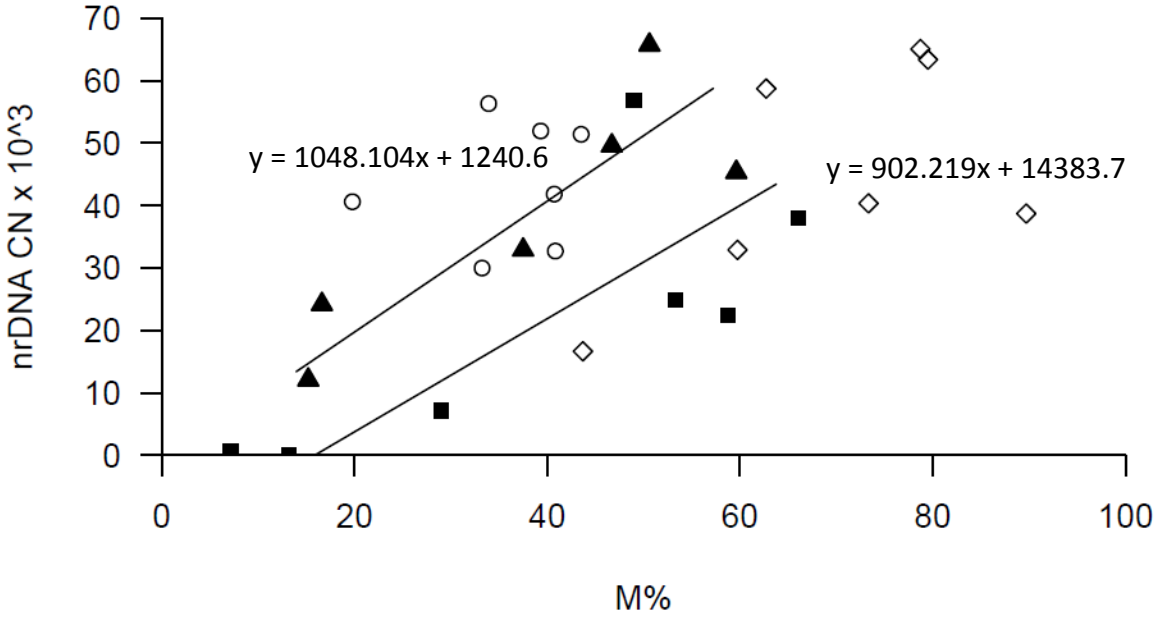


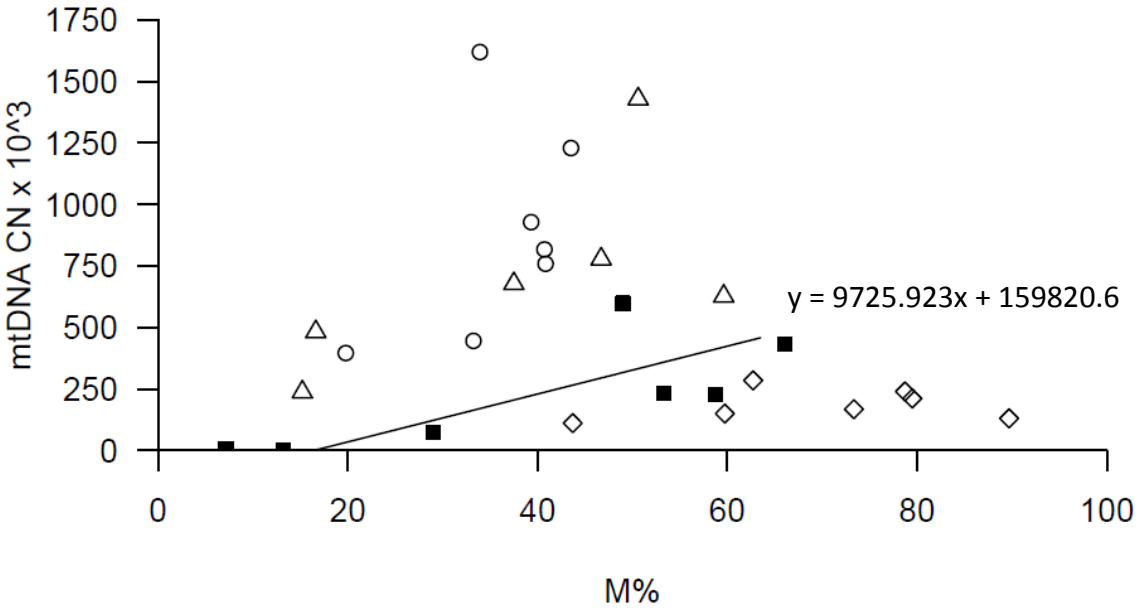
Figure S2: Ranges of the determined parameters in root samples colonized by each arbuscular mycorrhizal fungal isolate: *Claroideoglomus claroideum* BEG96 (CC, squares), *Funnelifomis mosseae* BEG95 (FM, triangles), *Gigaspora margarita* BEG34 (GI, circles), *Rhizophagus irregularis* PH5 (RI, diamonds). V% - vesicle abundance in whole root system **(a)**, PLFA - relative content of AMF-specific phospholipid fatty acids **(b)**, nrDNA CN - copy numbers of nuclear ribosomal DNA **(c)**, mtDNA CN - copy numbers of mitochondrial ribosomal DNA **(d)**, mt/nr - ratio of mtDNA CN and nrDNA CN **(e)**. Each symbol represents one root sample, asterisks show means. Different letters indicate significant differences among AMF species according to non-parametric Kruskal-Wallis test (Chi^2/P-value: V% 23.27/<0.001, PLFA 9.18/0.010, nrDNA CN 4.75/0.093, mtDNA CN 9.13/0.010, mt/nr 16.01/<0.001).

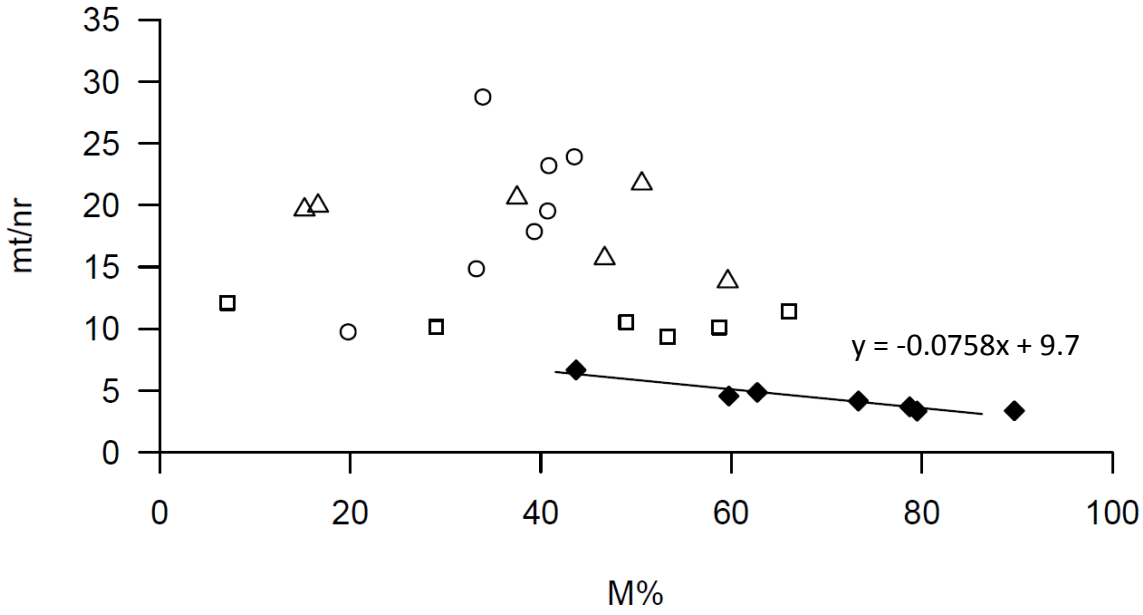

100
80
60
40
20
0
a%
y = 0.393x + 2.3
0
20
40
60
80
100
M%

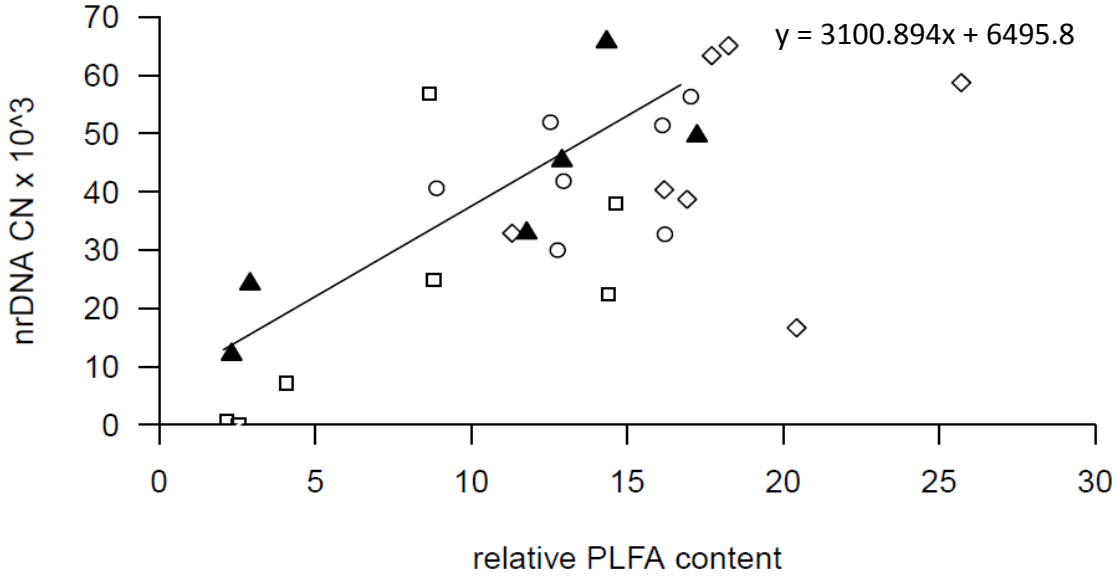

50
40
30
20
10
0
V%
y = 0.380x - 0.1
y = 0.309x - 2.0
0
20
40
60
80
100
M%

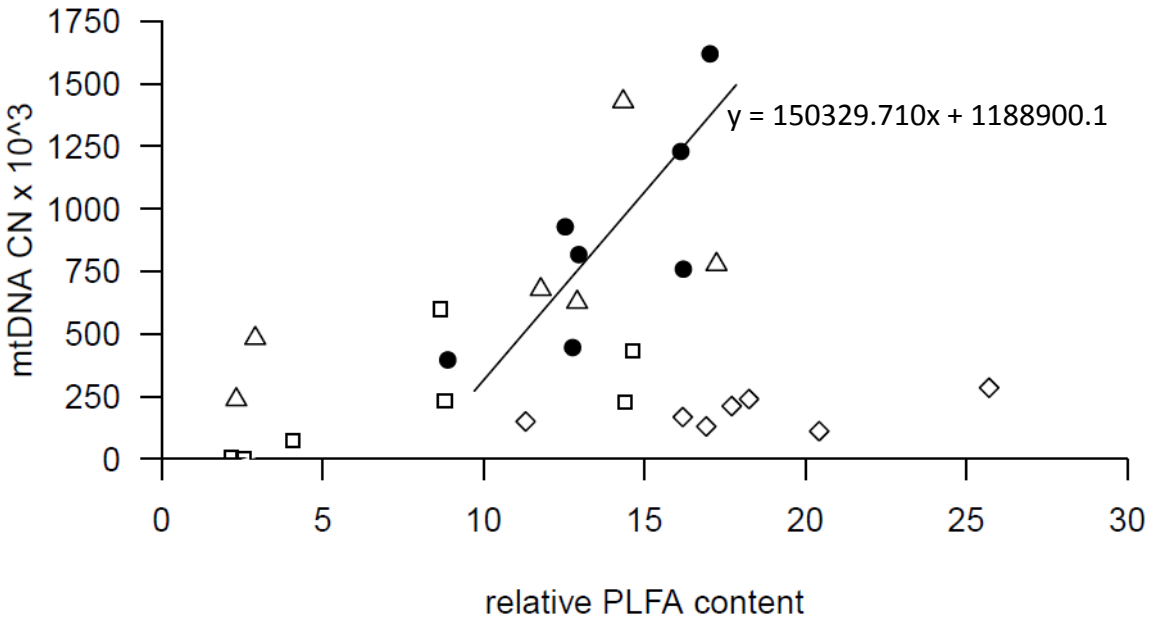

relative PLFA content
30
25
20
15
10
5
0
0
20
40
60
80
100
M%
y = 0.338x - 2.5
y = 0.227x - 1.1


nrDNA CN x 10^3
70
60
50
40
30
20
10
0
0
20
40
60
80
100
M%
y = 1048.104x + 1240.6
y = 902.219x + 14383.7


mtDNA CN x 10^3
1750
1500
1250
1000
750
500
250
0
0
20
40
60
80
100
M%
y = 9725.923x + 159820.6


mt/nr
35
30
25
20
15
10
5
0
0
20
40
60
80
100
M%
y = -0.0758x + 9.7


y = 3100.894x + 6495.8
nrDNA CN x 10^3
relative PLFA content
0
10
20
30
40
50
60
70
0
5
10
15
20
25
30


y = 150329.710x + 1188900.1
mtDNA CN x 10^3
relative PLFA content
0
250
500
750
1000
1250
1500
1750
0
5
10
15
20
25
30

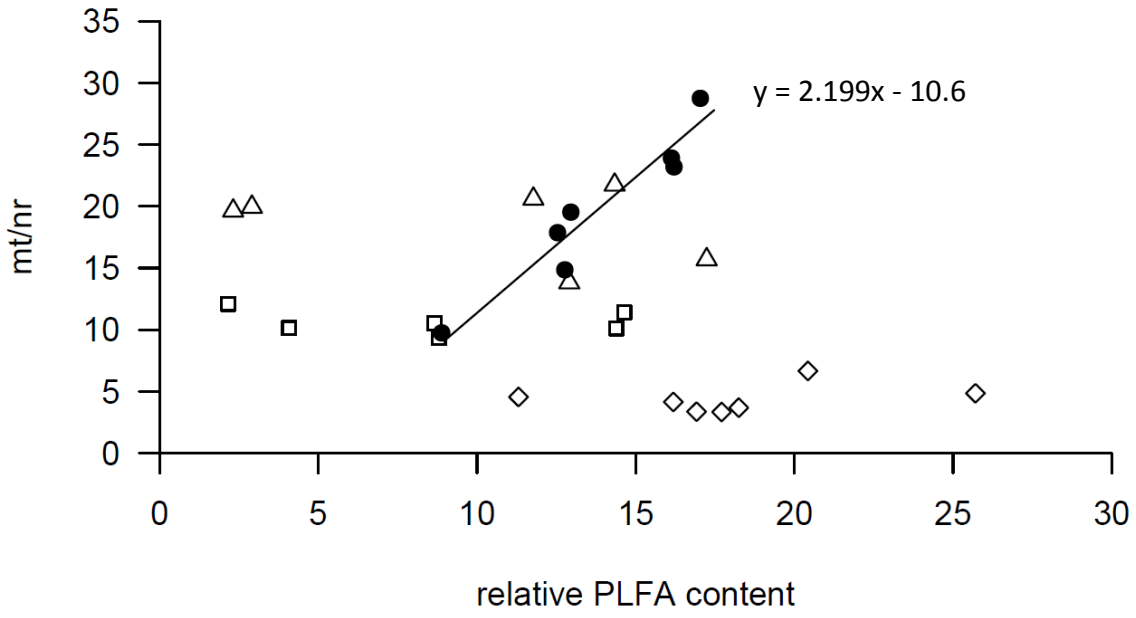


Figure S3 Pair wise relationship between quantification parameters: M% – intensity of root colonization; a% - arbuscule abundance in mycorrhizal parts of root system; V% - vesicle abundance in whole root system; relative PLFA content - content of AMF-specific phospholipid fatty acids; nrDNA CN – copy numbers of nuclear ribosomal DNA; mtDNA CN – copy numbers of mitochondrial ribosomal DNA, mt/nr - ratio of mtDNA CN to nrDNA CN. Each symbol represents one root sample colonized by *Claroideoglomus claroideum* BEG96 (square), *Funneliformis mosseae* BEG95 (triangle), *Gigaspora margarita* BEG34 (circle), or *Rhizophagus irregularis* PH5 (diamond). Regression lines of significant relationships within isolates are displayed; the corresponding points are highlighted by full symbols. For R^2 and P-values see Table S4.

Supplementary material - Paper III: Voříšková *et al.* (2019)

ONLINE RESOURCE 1

Detailed description of the quantification of arbuscular mycorrhizal fungal species in roots by qPCR

Preparation of standards for the qPCR analyses

The standards were prepared directly from the different fungal isolates used for inoculation: *Rhizophagus irregularis* PH5, *Claroideoglomus claroideum* BEG23, *Gigaspora margarita* BEG34, *Funneliformis mosseae* BEG95 and *Acaulospora tuberculata* BEG41. Template DNA was extracted from ca. 100 spores per isolate using a DNA Plant Mini kit (Qiagen). DNA extracts were then individually amplified using the primers LR1 (van Tuinen et al. 1998) and LSUmBr (Krüger et al. 2009) in 20 µl-PCR reactions with the following reagents and PCR conditions: 1x Pfu buffer (without MgSO4) (ThermoFisher), 2 mM MgSO4, 0.2 mM of each dNTP, 0.5 µM of each primer, 0.5 U Pfu DNA Polymerase (ThermoFisher) and 2 - 10 ng DNA of isolate-specific template. The thermal cycling program was set up with initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 1 min, 58 °C for 45 sec, 72 °C for 2 min and a final elongation at 72 °C for 10 min. Each DNA extract was amplified in triplicates and pooled amplicons were gel–purified using the Zymoclean Gel DNA Recovery kit (Zymo Research, Irvine, USA). DNA concentration of the amplicons was measured spectrophotometrically (BioPhotometer, Eppendorf) and the amplicons then used as standards, diluted serially with 10mM Tris (seven five-fold dilutions from 1 pg DNA μl^{-1} to 0.000064 pg DNA μl^{-1}).

Design of new qPCR assay for *Acaulospora tuberculata*

The qPCR assay targeting the nrDNA LSU region of *Acaulospora tuberculata* BEG41, was newly designed. Genomic DNA of A. tuberculata was isolated from ca. 100 spores obtained from a multi-spore pot culture using a DNA Plant Mini kit (Qiagen). The DNA extract was amplified in triplicates in the same way as a described for the preparation of the qPCR standards above. Pooled amplicons were gel-purified using a Zymoclean Gel DNA Recovery kit (Zymo Research, Irvine, USA) and cloned using a TOPO TA Cloning Kit (ThermoFisher), according to the manufacturer's protocol. Clones were sequenced using M13 primers at Macrogen Inc. Company (Amsterdam, the Netherlands). The qPCR assay with forward primer (5' GAG GAT TGCA GCG GAT G 3'), reverse primer (5' CAA TCG TTA GCA AGC TAT CG 3') and hydrolysis (TaqMan) probe (FAM – TAG TCA CCT ACC TTC TG – BHQ1) targeting A. tuberculata sequences and excluding other arbuscular mycorrhizal (AM) fungal species was designed in Allele ID version 6 software (Premier Biosoft International, Palo Alto, CA, USA), with predicted amplicon length of 79 bp. The qPCR conditions of the new assay were optimized with serial dilutions of PCR products and qPCR reactions were performed with following reagents and reaction conditions : 1 x LightCycler 480 Probes Master (Roche, Penzberg, Germany), 300 nM of each primers, 50 nM of TaqMan probe and 2.5 µl of 1:9 diluted DNA extract or the corresponding dilution of a standard. The thermal cycling program was the following: pre-incubation at 95 °C for 10 min, 40 cycles of 95 °C for 10 s, annealing at 58 °C for

30 s and elongation at 72 °C for 1 s. Cross-amplification test with DNA extracts from root samples colonized by single AM fungal species verified the specificity of the new qPCR assay within the set of the five AM fungal isolates used in this study.

Literature Cited

Krüger M, Stockinger H, Krüger C, Schüßler A (2009) DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol 183:212–223. https://doi.org/10.1111/j.1469-8137.2009.02835.x

Thonar C, Erb A, Jansa J (2012) Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities--marker design, verification, calibration and field validation. Mol Ecol Resour 12:219–232. https://doi.org/10.1111/j.1755-0998.2011.03086.x

van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Mol Ecol 7:879–887. https://doi.org/10.1046/j.1365-294x.1998.00410.x

Voříšková A, Jansa J, Püschel D, Krüger M, Cajthaml T, Vosátka M, Janoušková M (2017) Real-time PCR quantification of arbuscular mycorrhizal fungi: does the use of nuclear or mitochondrial markers make a difference? Mycorrhiza 27:577–585. https://doi.org/10.1007/s00572-017-0777-9

Table S1 Real-time PCR assays and reaction conditions used for the quantification of four AM fungal isolates, based on primers and hydrolysis probes targeted to the large subunit of nuclear ribosomal DNA (nrLSU)

target isolate	type	sequence 5'-3'	References	Primers conc.	Annealing temperature (°C)	Size of amplicon (bp)
Rhizophagus irregularis PH5	primerF	TTCGGGTAATCAGCCTTTCG	Thonar et al. 2012	400 nM	58	249
	primerR	TCAGAGATCAGACAGGTAGCC				
	hydrolysis probe	FAM-TTAACCAACCACACGGGCAAGTACA-BHQ1				
Funneliformis mosseae BEG95	primerF	GGAAACGATTGAAGTCAGTCATACCAA	Thonar et al. 2012	400 nM	58	121
	primerR	CGAAAAAGTACACCAAGAGATCCCAAT				
	hydrolysis probe	FAM-AGAGTTTCAAAGCCTTCGGATTCGC-BHQ1				
Gigaspora margarita BEG34	primerF	GAGGAAAAGAAACTAACAAGG	Voříšková et al. 2017	500 nM	60	75
	primerR	AAACCAGGTAGATTTTAAATTTG				
	hydrolysis probe	FAM-CCGCTTCACTCGCCGTTACT-BHQ1				
Claroideoglomus claroideum BEG23	primerF	GCGAGTGAAGAGGGAAGAG	Thonar et al. 2012	300 nM	52	178
	primerR	TTGAAAGCGTATCGTAGATGAAC				
	hydrolysis probe	FAM-AACAGGACATCATAGAGGGTGACAATCCC-BHQ1				
Acaulospora tuberculata BEG41	primerF	GAGGATTGCAGCGGATG	newly designed	300 nM	58	79
	primerR	CAATCGTTAGCAAGCTATCG				
	hydrolysis probe	FAM-TAGTCACCTACCTTCTG-BHQ1				

ONLINE RESOURCE 2

Supplementary Tables and Figures with additional statistical results

Table S1 The effects of inoculum history on SDW (shoot dry weight), RDW (root dry weight), phosphorus (P) content and nitrogen-to-phosphorus (N:P) ratio in shoots in the second stage of the experiment, as determined within each abiotic conditions (Conditions). Values are means (± SE), n = 6. Asterisks indicate significant differences (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$) from the corresponding non-inoculated plants (NI-control), i.e. non-mycorrhizal plants grown in the same abiotic conditions. Significant effects of inoculum history were found only for P content and N:P ratio within the low water conditions according to one-way ANOVA at $p \leq 0.05$. Means of P content and N:P ratio followed by the same letter do not differ significantly at $p \leq 0.05$ according to Tukey's test.

Conditions	Inoculum history	SDW [g]	RDW [g]	P content [mg]	N : P ratio
standard	AM fungi - standard	1.80 (± 0.03)	0.91 (± 0.03) **	7.40 (± 0.18) ***	2.14 (± 0.06) ***
standard	AM fungi - high P	1.81 (± 0.09)	0.86 (± 0.05) *	7.29 (± 0.22) ***	2.25 (± 0.19) ***
standard	AM fungi - low light	1.80 (± 0.09)	0.87 (± 0.01) *	7.30 (± 0.26) ***	2.14 (± 0.08) ***
standard	AM fungi - low water	1.70 (± 0.12)	0.78 (± 0.02)	7.14 (± 0.22) ***	2.04 (± 0.14) ***
standard	NI-control	1.65 (± 0.07)	0.74 (± 0.03)	2.59 (± 0.05)	6.35 (± 0.30)
high P	AM fungi - standard	1.98 (± 0.10)	1.00 (± 0.05)	8.27 (± 0.16) ***	2.27 (± 0.10) ***
high P	AM fungi - high P	1.94 (± 0.06) *	0.99 (± 0.05)	8.35 (± 0.32) ***	2.13 (± 0.10) ***
high P	AM fungi - low light	1.99 (± 0.10)	1.02 (± 0.04)	8.84 (± 0.28) ***	2.08 (± 0.12) ***
high P	AM fungi - low water	1.74 (± 0.08) **	0.88 (± 0.06)	7.64 (± 0.28) ***	2.19 (± 0.11) ***
high P	NI-control	2.21 (± 0.10)	0.94 (± 0.05)	4.98 (± 0.26)	4.10 (± 0.10)
low light	AM fungi - standard	1.13 (± 0.07)	0.34 (± 0.03)	3.92 (± 0.25) ***	6.26 (± 0.38) ***
low light	AM fungi - high P	1.12 (± 0.11)	0.30 (± 0.05)	3.87 (± 0.36) ***	6.21 (± 0.20) ***
low light	AM fungi - low light	1.20 (± 0.15)	0.39 (± 0.05)	4.01 (± 0.43) ***	6.22 (± 0.44) ***
low light	AM fungi - low water	1.31 (± 0.11)	0.39 (± 0.04)	4.23 (± 0.33) ***	6.42 (± 0.25) ***
low light	NI-control	1.10 (± 0.06)	0.28 (± 0.03)	2.04 (± 0.08)	10.24 (± 0.45)
low water	AM fungi - standard	0.91 (± 0.06) ***	0.61 (± 0.03) ***	3.41 (± 0.22) *** a	3.32 (± 0.23) *** b
low water	AM fungi - high P	0.84 (± 0.09) ***	0.54 (± 0.04) ***	3.01 (± 0.30) *** ab	3.70 (± 0.06) *** ab
low water	AM fungi - low light	0.75 (± 0.06) ***	0.58 (± 0.03) ***	2.30 (± 0.25) *** b	4.56 (± 0.32) *** a
low water	AM fungi - low water	0.75 (± 0.04) ***	0.57 (± 0.04) ***	2.26 (± 0.23) *** b	4.67 (± 0.57) *** a
low water	NI-control	0.35(± 0.03)	0.24 (± 0.04)	0.23 (± 0.03)	27.50 (± 1.76)

Table S2 The effects of abiotic conditions, inoculum history and their interaction on the copy numbers of the dominant arbuscular mycorrhizal fungus *Rhizophagus irregularis* in the roots of *M. sativa* in both stages of the experiment. Significant *p* value is in bold. Degrees of freedom were: (*3*,*20*) for conditions (the first stage); (*3*,*80*) for conditions, (*3*,*80*) for inoculum history and (*9*,*80*) for the interaction (the second stage). Values are means (± SE), n = 6 for the first stage, n = 24 for the second stage. Asterisks indicate significant differences (***$p < 0.001$, **$p < 0.01$, *$p \leq 0.05$) in the copy numbers of *Rhizophagus irregularis* from standard conditions separately for each stage of the experiment.

	First stage		Second stage	
	F value	*p* value	*F* value	*p* value
Conditions (A)	0.19	0.899	**6.61**	**< 0.001**
Inoculum history (B)	n.d.	n.d.	0.2	0.894
A x B	n.d.	n.d.	0.92	0.509
standard	52405 (± 2137)		109468 (± 5182)	
high P	56637 (± 5284)		140989 (± 6688) ***	
low light	54460 (± 6135)		150668 (± 7980) ***	
low water	56668 (± 3963)		133142 (± 7921) *	

Table S3 The effects of abiotic conditions on the abundances of the subdominant AM fungal species in the first stage of the experiment. Significant *p* values are in bold. Degrees of freedom were: (*3*,*20*) for GM, (*3*,*18*) for FM, (*3*,*19*) for CC and AT. CC – *Claroideoglomus claroideum*, FM – *Funneliformis mosseae*, AT – *Acaulospora tuberculata*, GM – *Gigaspora margarita*

	Conditions	
	F value	*p* value
CC	**3.16**	**0.048**
FM	**7.68**	**0.002**
AT	**8.71**	**0.001**
GM	**7.05**	**0.002**

Table S4 The effects of abiotic conditions and inoculum history on the abundances and frequencies of occurrence of CC (*Claroideoglomus claroideum*) and FM (*Funneliformis mosseae*) in the second stage of the experiment using zero-inflated models. Significant *p* values are in bold. Degrees of freedom for conditions and inoculum history were *3* in all the analyses.

		Conditions		Inoculum history	
		Chi^2	*p* value	Chi^2	*p* value
CC	count model	6.55	0.088	3.26	0.353
	binary model	**9.25**	**0.026**	6.26	0.100
FM	count model	2.81	0.422	3.39	0.335
	binary model	**31.39**	**< 0.001**	2.96	0.398

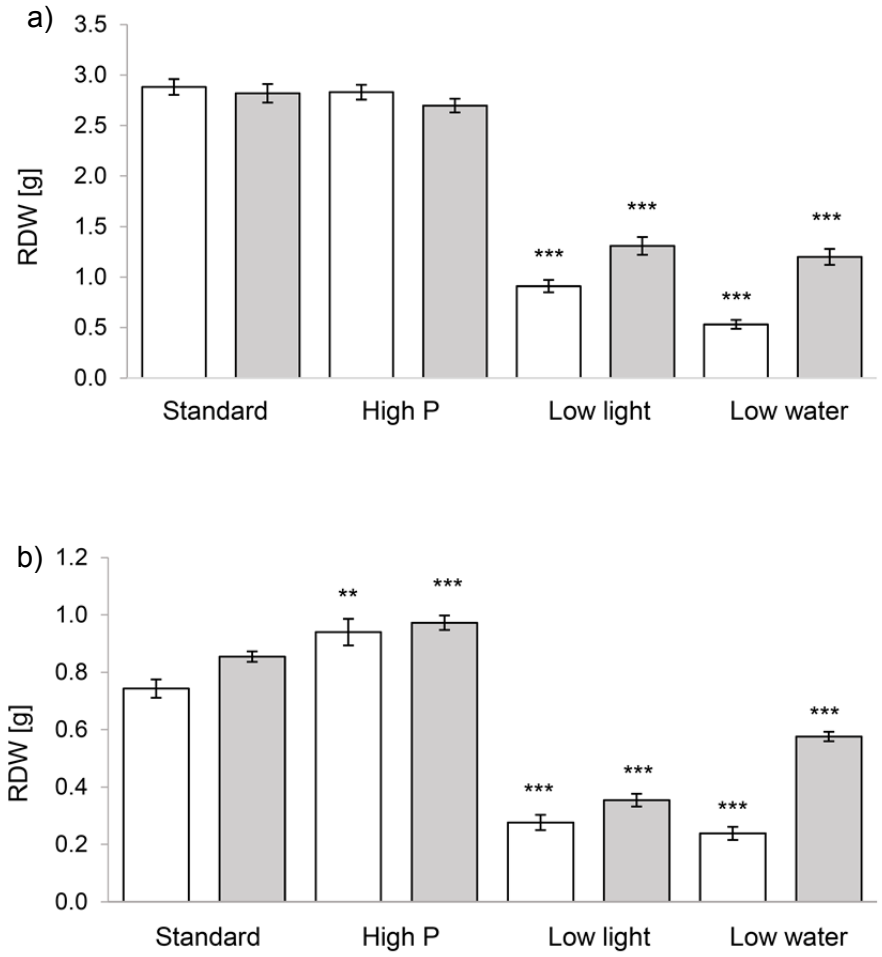


Fig. S1 Means (± standard errors) of RDW (root dry weight) of non-inoculated plants (white bars) and inoculated plants (grey bars) under different abiotic conditions a) in the first stage and b) in the second stage. In both stages, RDW values were significantly affected by conditions ($F_{3,40}$ = 402.93, $p < 0.001$; $F_{3,112}$ = 247.89, $p < 0.001$, respectively), inoculation ($F_{1,40}$ = 17.33, $p < 0.001$; $F_{1,112}$ = 37.81, $p < 0.001$, respectively) and the interaction of both factors ($F_{3,40}$ = 13.21, $p < 0.001$; $F_{3,112}$ = 8.77, $p < 0.001$). Asterisks indicate significant differences (***$p < 0.001$, **$p < 0.01$) in RDW from standard conditions

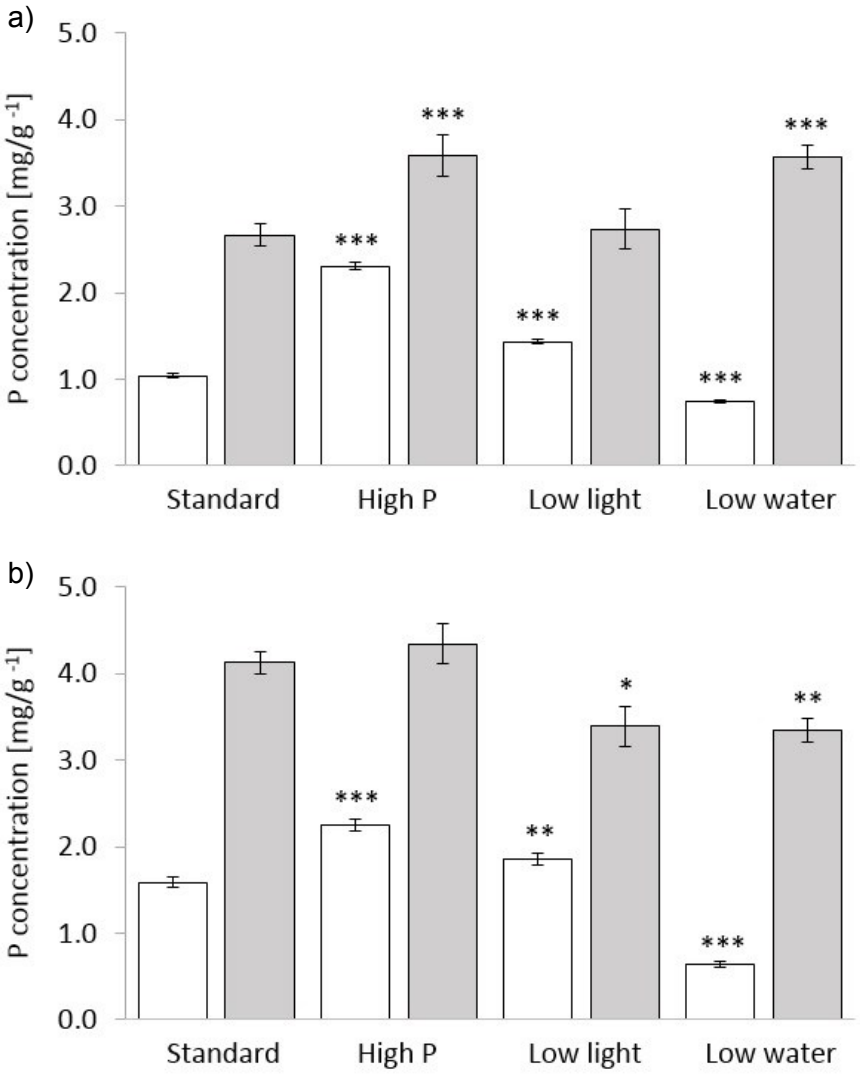


Fig. S2 (a, b) Means (± standard errors) of P concentration in shoot biomass of non-mycorrhizal plants (white bars) and mycorrhizal plants (grey bars) under different abiotic conditions a) in the first stage and b) in the second stage. In both stages, the P concentrations were significantly affected by conditions ($F = 98.56$, $p < 0.001$; $F = 99.12$, $p < 0.001$, respectively), by inoculation ($F = 303.14$, $p < 0.001$; $F = 1354.18$, $p < 0.001$, respectively) and the interaction of both factors ($F = 7.32$, $p < 0.001$; $F = 58.94$, $p < 0.001$). Asterisks indicate significant differences (***$p < 0.001$, **$p < 0.01$, *$p \leq 0.05$) from standard conditions at $p \leq 0.05$, as determined by planned treatment contrasts.

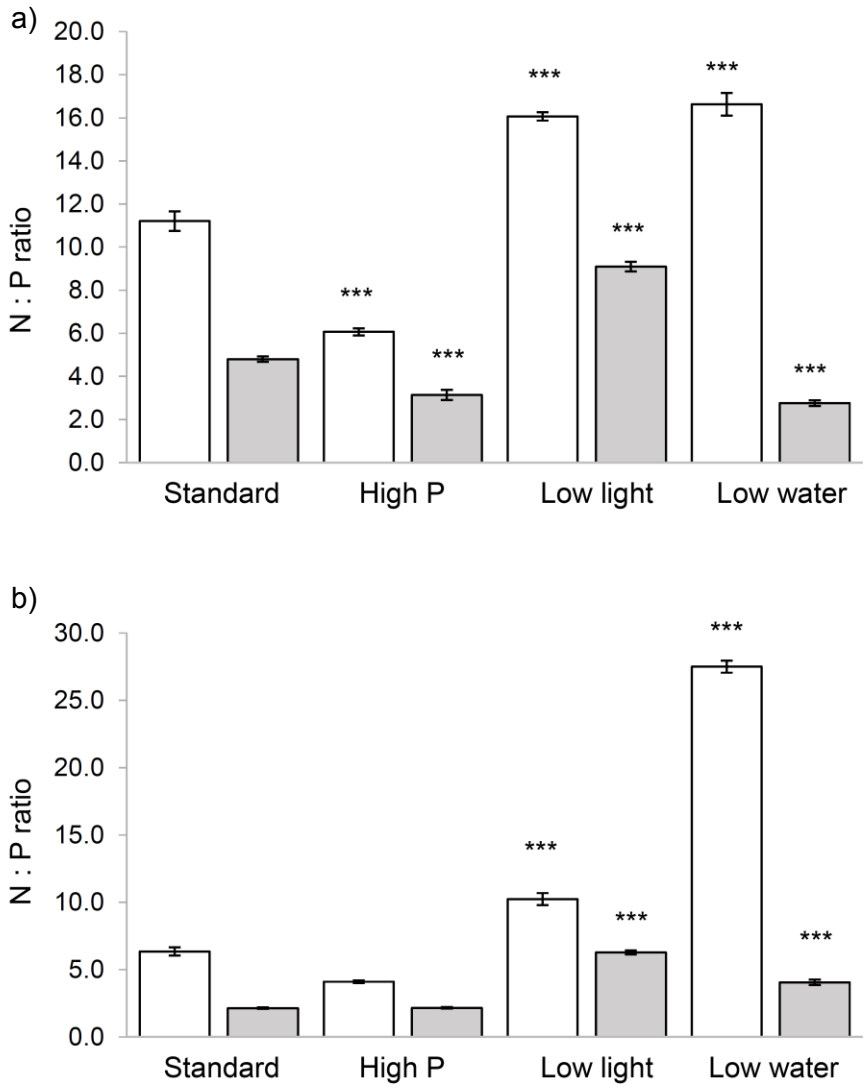


Fig. S3 Means (± standard errors) of N:P ratio of non-inoculated plants (white bars) and inoculated plants (grey bars) under different abiotic conditions a) in the first stage and b) in the second stage. In both stages, the N:P ratios were significantly affected by conditions ($F_{3,40} = 259.69$, $p < 0.001$; $F_{3,112} = 222.57$, $p < 0.001$, respectively), inoculation ($F_{1,40} = 1331.55$, $p < 0.001$; $F_{1,112} = 1075.96$, $p < 0.001$, respectively) and the interaction of both factors ($F_{3,40} = 122.76$, $p < 0.001$; $F_{3,112} = 389.01$, $p < 0.001$). Asterisks indicate significant differences (***$p < 0.001$) from standard conditions at $p \leq 0.05$, as determined by planned treatment contrasts

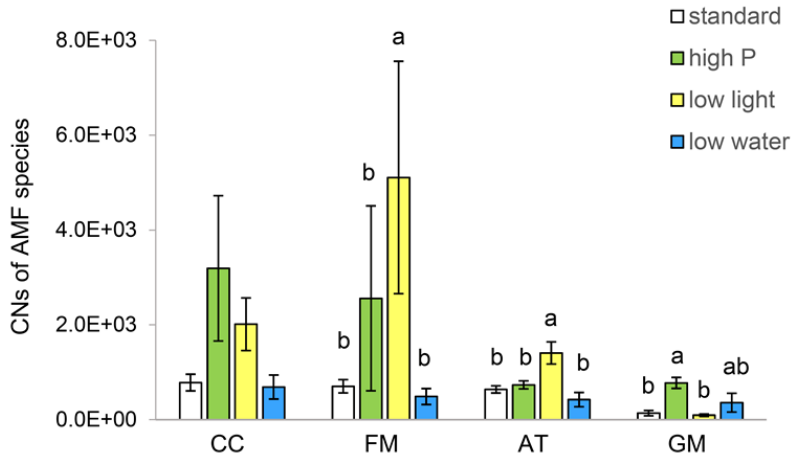


Fig. S4 Means (± standard errors) of copy numbers per ng DNA (CNs) of four AM fungal species under different abiotic conditions (in different colors) in the first stage of the experiment. Means marked by the same letter do not significantly differ at $p \leq 0.05$ according to Tukey's test. CC – *Claroideoglomus claroideum*, FM – *Funneliformis mosseae*, AT – *Acaulospora tuberculata*, GM – *Gigaspora margarita*.

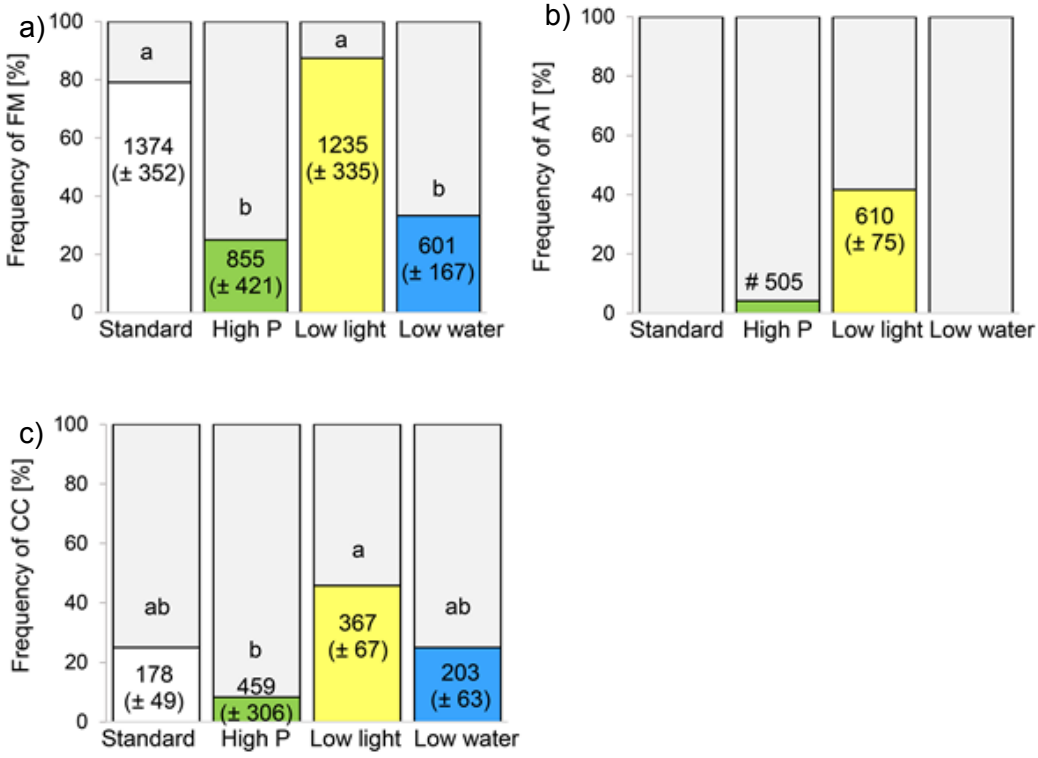


Fig. S5 Frequencies of a) FM (*Funneliformis mosseae*), b) AT (*Acaulospora tuberculata*), c) CC (*Claroideoglomus claroideum*) in root samples as determined by quantitative PCR. Values are means (± standard errors) of copy numbers per ng DNA in the samples where the fungus was detected. Means followed by the same letter do not significantly differ among abiotic conditions at $p \leq 0.05$ according to Tukey's test.. The # symbol indicates occurrence only in one sample

Supplementary material - Paper IV: Voříšková *et al*. (manuscript)

Table S1 Means of three replicates of copy numbers (CNs) of each AM fungal species in the cultures used for the preparation of the inocula.

		AT	GM	FM	CC	RI	Mixed culture	Blank culture
CNs of	AT	2226	0	0	0	0	634	0
	GM	0	15125	0	0	0	132	0
	FM	0	0	4420	0	0	911	0
	CC	0	0	0	3484	0	723	0
	RI	0	0	0	0	28030	36868	0

RI – Rhizophagus irregularis, *FM – Funneliformis mosseae*, *CC – Claroideoglomus claroideum*, *AC - Acaulospora tuberculata*, *GM – Gigaspora margarita*

Table S2 The amount (in g) of the substrates from cultures of single AM fungal species, mixed culture of all the five species and blank culture without AM fungi used to establish the inoculation treatments of the experiment (as given in the first column).

	AT	GM	FM	CC	RI	Mixed culture	Blank culture
AT+	160	10	10	10	2.5	X	X
GM+	10	160	10	10	2.5	X	X
FM+	10	10	160	10	2.5	X	X
CC+	10	10	10	160	2.5	X	X
RI+	10	10	10	10	40	X	X
MIX	X	X	X	X	X	200	X
blank	X	X	X	X	X	X	200

RI – Rhizophagus irregularis, *FM – Funneliformis mosseae*, *CC – Claroideoglomus claroideum*, *AC - Acaulospora tuberculata*, *GM – Gigaspora margarita*.

Table S3 The effect of inoculation treatment on the copy numbers (CNs) of three AM fungal isolates (*FM*, *CC*, *RI*) within each cultivation time (Time) tested by one-way ANOVA. Values are means (standard errors). Means followed by the same letter do not significantly differ at $p \leq 0.05$ according to Tukey's test. The CNs of the *AT* and *GM* isolates were not statistically analyzed (n.d.). Bold letter highlight values in those treatments, where the corresponding isolate was promoted by a higher inoculum dose.

Time (weeks)	Isolate	AT+	GM+	FM+	CC+	RI+	MIX	*F* value *
3	*AT*	**190 (90)**	46 (46)	0	0	0	0	n.d.
	GM	0	**466 (288)**	0	0	0	0	n.d.
	FM	3213 (1271)ab	1598 (965) b	**67730 (7920)** a	6771 (3488) ab	1082 (942) b	3544 (1100) ab	4.38 *
	CC	8174 (2329) ab	3562 (1071) abc	1937 (1104) bc	**112133 (21838)** a	2838 (2217) bc	499 (172) c	6.44 *
	RI	102548 (22500) bc	54951 (17316) bc	25370 (13549) c	57074 (23752) bc	**127513 (4497)** ab	202015 (20456) a	11.79 *
6	*AT*	**305 (79)**	75 (75)	93 (59)	0	0	0	n.d.
	GM	299 (202)	**608 (268)**	0	0	110 (77)	79 (79)	n.d.
	FM	5874 (1960) b	4214 (1126) b	**46455 (11080)** a	4506 (1836) b	2046 (911) b	1724 (1046) b	17.90 *
	CC	6991 (2210) ab	5458 (2043) ab	3559 (1305) ab	**25286 (7264)** a	1443 (467) bc	703 (313) c	7.39 *
	RI	54405 (3835) c	82662 (7557) bc	42370 (11458) bc	50078 (8630) b	**121280 (9911)** a	120819 (6250) a	18.32 *
9	*AT*	**2375 (378)**	238 (111)	476 (121)	217 (98)	213 (61)	135 (66)	n.d.
	GM	0	**52 (52)**	0	0	0	0	n.d.
	FM	2646 (1351) b	1740 (665) b	**11817 (2301)** a	1524 (824) b	404 (119) b	1489 (618) b	9.95 *
	CC	3643 (802) ab	2146 (833) b	1035 (228) b	**10142 (3149)** a	743 (279) b	1074 (316) b	8.95 *
	RI	50204 (4731) b	44709 (1512) b	37883 (8444) b	41369 (7618) ab	**51182 (5119)** ab	71470 (4443) a	4.26 *

Degrees of freedom were (*5*,*29*) for three weeks, (*5*,*30*) for six weeks and (*5*,*30*) for nine weeks.

Table S4 Effects of inoculation treatments on ^{13}C allocation to AM fungal lipids (^{13}C allocation), shoot biomass (SDW), P concentration, mycorrhizal P response calculated from P content (MPR – content) and from P concentration (MPR – concentration) in shoot biomass for each cultivation time separately as determined by one-way ANOVA.

	Time (weeks)					
	3		6		9	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value
^{13}C allocation [‰]	**9.82**	**< 0.001**	**3.21**	**0.0302**	**7.07**	**< 0.001**
SDW [g]	**9.25**	**< 0.001**	**10.95**	**< 0.001**	**11.21**	**< 0.001**
P concentration [mg/g]	**38.72**	**< 0.001**	**48.40**	**< 0.001**	**51.33**	**< 0.001**
MPR - content	**18.68**	**< 0.001**	**7.72**	**< 0.001**	**6.86**	**< 0.001**
MPR - concentration	**28.72**	**< 0.001**	**3.94**	**0.007**	**5.92**	**< 0.001**

Significant *p* values are in bold. Degrees of freedom were (*5*,*17*) for three weeks and (*5*,*18*) for six and nine weeks in ^{13}C allocation. Degrees of freedom were: (*6*,*34*) for three weeks and (*6*,*35*) for the six and nine weeks in SDW and P concentration. Degrees of freedom were: (*5*,*29*) for three weeks and (*5*,*30*) for six and nine weeks in both MPRs.

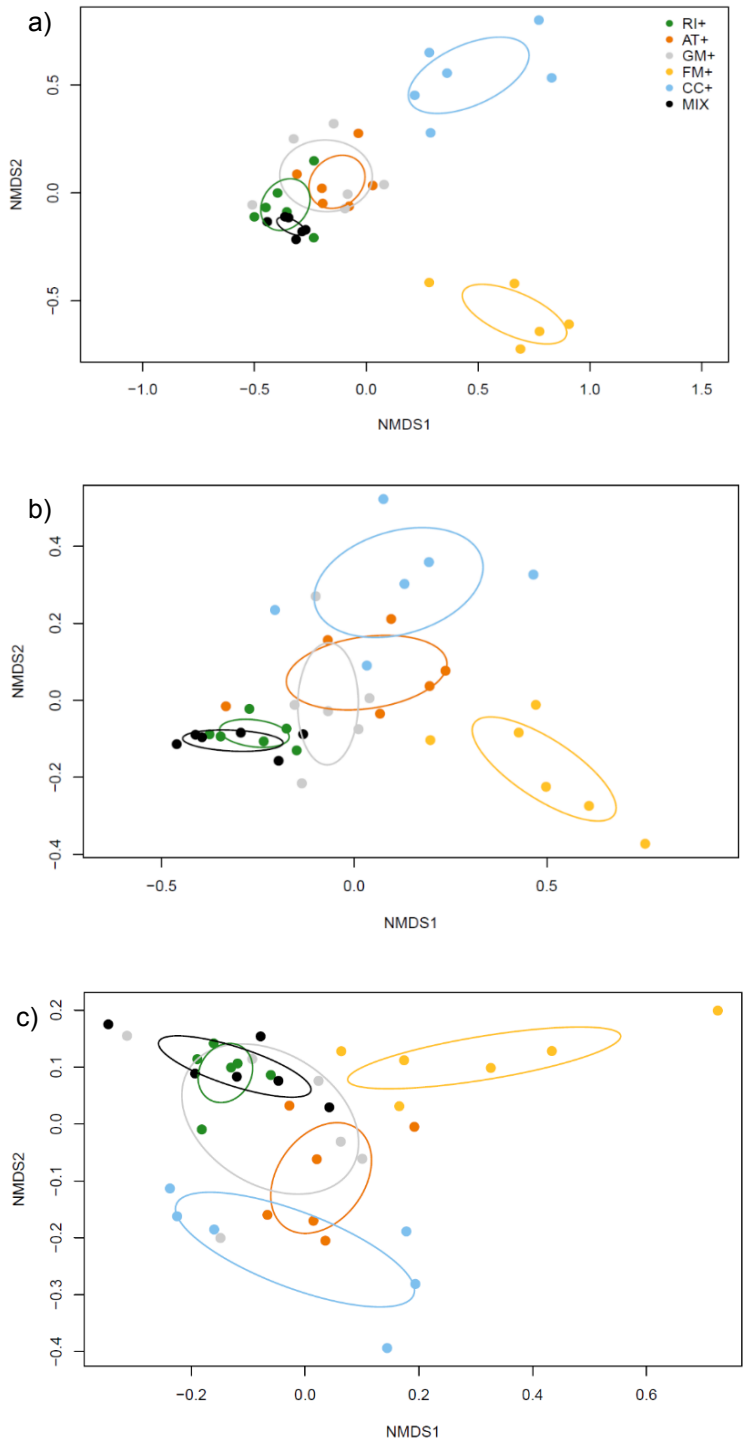


Fig. S1 Non-metrical multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities of the AM fungal communities in the different inoculation treatments after a) three weeks (stress value – 0.04) , b) six weeks (stress value – 0.06) and c) nine weeks (stress value – 0.09). Color dots and elipses indicate different inoculation treatments: blue – RI+ treatment, red – AT+ treatment, green – GM+ treatment, black – FM+ treatment, brown – CC+ treatment and yellow – MIX treatment.

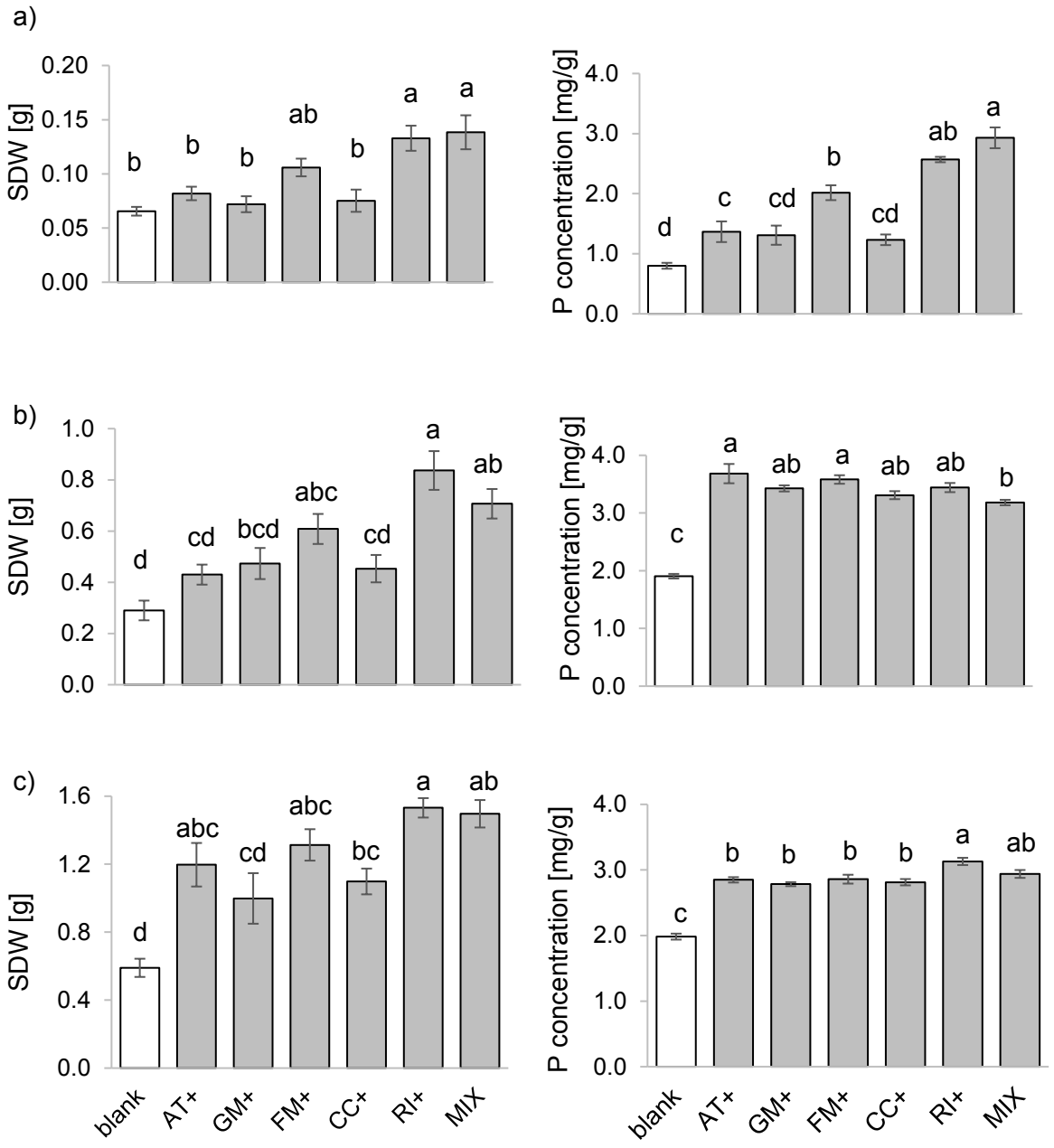


Fig. S2 Shoot dry weights (SDW) and P concentrations in the shoot biomass of plants in the different inoculation treatments after a) three, b) six, and c) 9 weeks of cultivation. Bars are means (± standard errors) of SDW or P concentration of the non-inoculated plants (blank, white bars) and inoculated plants (grey bars). Means followed by the same letter do not differ significantly at $p \leq 0.05$ by Tukey's test.)