Supplementary Material

Arbuscular mycorrhizal fungi originated from soils with a fertility gradient highlight a strong intraspecies functional variability

Valentina Marrassini[a], Laura Ercoli[a], Eiko E. Kuramae[b,c], George Kowalchuk[c], Elisa Pellegrino[a,*]

[a]Crop Science Research Center, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy

[b]Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), 6708 PB Wageningen, The Netherlands

[c]Ecology and Biodiversity Group, Department of Biology, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

Corresponding author at: Crop Science Research Center, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy. E-mail address: elisa.pellegrino@santannapisa.it (E. Pellegrino)

Supplementary Materials and Methods

1. Characterization of soil of origin

Soil samples were analyzed for the following parameters by Kuramae et al. (2010): pH, ammonium (NH_4^+), nitrate (NO_3^-), soil organic matter (SOM) (g kg^{-1}) and available phosphorus (P_{avail}). Organic matter content (kg ha^{-1}) in the surface layer 0-10 cm depth was also calculated considering a soil density of 1.3 g g^{-1}. All these analyses were carried out in five replicates. Soil pH was measured in KCl (1:2.5) (McLean, 1982), while NO_3^- and NH_4^+ concentrations were measured in KCl extracts using a Traacs 800 autoanalyzer (Novozamsky et al., 1984). Soil organic matter was measured using the modified Walkley-Black wet combustion method (Nelson and Sommers, 1982), while P_{avail} was calorimetrically determined in 0.01 M $CaCl_2$ suspensions using the Molybdenum blue method (Novozamsky et al., 1984). Soil pH was significantly different among sites, varying from neutral in W1-W2 (mean: 7.14 ± 0.03) to slightly alkaline (7.57 ± 0.03) in W0. Similarly, SOM concentration varied among sites and ranged from good (W0) to vary good content (the other sites), while P_{avail} ranged from high (W0) to very low (the other sites) and NH_4^+ from 2.0 mg kg^{-1} ± 0.1 in W0 to 8.7 mg kg^{-1} ± 0.6 in the other sites (Table S1). The other soil chemical parameters did not change among sites (SOM content: 100.31 kg ha^{-1} ± 2.39; NO_3^-: 0.80 mg kg^{-1} ± 0.32). Plant species richness (S) and Shannon-Weaver Index (*H'*) [*H'*=-SUM(P_i*Log(P_i)) where P_i=proportion of each species in the sample] were higher in W3 than in W1-W2 (S_{plant}: 23.3 *vs* 12.1; $H'_{plant:}$ 2.60 *vs* 1.96) (Kuramae et al., 2011) (Table S2). Soil prokaryotic diversity was measured as species richness, Shannon-Weaver Index (H'_{prok}) and Simpson Index (λ_{prok}) [λ=1-SUM(Ni*(Ni-1)/(N*(N-1)), where Ni = number of entities belonging to the i^{th} species and N = total number of entities in the sample]. H'_{prok} was higher in W3 than the other sites (Table S3).

2. PCR conditions

PCR were generated by 0.5 µL of DNA in volumes of 25 µL with 0.25 U of 2× Phusion® High-Fidelity DNA polymerase Master Mix HF (NEB, Frankfurt, Germany). The PCR were run using a S1000 Thermal CyclerTM (BIORAD, Hercules, CA, USA). The thermal cycler program was as follows: 98 °C for 3 min, 35 and 30 cycles at 98 °C for 10 sec, 60 and 63 °C for 30 s, 72 °C for 1 min and a final extension step at 72 °C for 10 min. Reaction yields were estimated using a 1% agarose gel containing Sybr Safe (Invitrogen, Carlsbad, CA).

3. Physical and chemical characteristics of the soil used for evaluation of AM fungal infectivity and effectiveness

Physical and chemical and characteristics of the soil used in the pot experiment were: 15.6% clay, 27.5% silt, 56.9% sand (sandy loam texture), 30.6 g kg^{-1} organic matter (Walkley-Black method; Nelson and Sommers, 1982); 24.5 mg kg^{-1} extractable iron (Fe), 1.23 mg kg^{-1} extractable zinc (Zn) (Lindsay-Norwell, 1978); 19.2 mg kg^{-1} total Fe, and 57 mg kg^{-1} total Zn (Isaac et al., 1998). The soil had a good availability of Fe and very low availability of Zn (Lindsay-Norwell, 1978).

4. Nutrient analyses

Approximately 300 mg of grounded plant samples (shoots and roots) were digested by microwave heating after the addition of 8 mL of nitric acid (HNO_3; 65%), according to the following temperature program: (a) 5 min ramp to 120°C, hold for 2 min; (b) 2 min ramp to 150°C, hold for 4 min; (c) 2 min ramp to 180°C, hold for 4 min. Blanks were run with each batch of samples for quality control. The digested solutions were diluted with Milli-Q water before the analysis in the MP-AES (Liberato et al. 2020).

Supplementary Tables

Table S1
Chemical parameters of soil under three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and a conventional arable field as reference (W0) from which arbuscular mycorrhizal fungi (AMF) were sampled, trap cultured, and single-spore trap cultures of AMF were set up.

Successional stages	pH	NH_4^+	NO_3^-	Soil organic matter	Soil organic matter	Available P
	(KCl)	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})	(kg ha^{-1})	(mg kg^{-1})
W0	7.57 ± 0.03 c †	2.00 ± 0.12 a	1.55 ± 0.16 a	33.98 ± 1.7 a	44.17 ± 2.24 a	115.35 ± 11.21 b
W1	7.11 ± 0.01 a	8.56 ± 0.37 b	0.51 ± 0.18 a	92.06 ± 0.6 b	119.68 ± 0.73 a	6.76 ± 0.94 a
W2	7.17 ± 0.04 a	8.67 ± 0.57 b	0.50 ± 0.24 a	91.60 ± 2.9 b	119.08 ± 3.88 a	5.85 ± 0.63 a
W3	7.34 ± 0.07 b	8.84 ± 0.77 b	0.63 ± 0.55 a	91.00 ± 2.0 b	118.30 ± 2.57 a	7.80 ± 0.63 a

† Values are mean of five replicates for W1, W2 and W3 and four replicates for W0 (mean ± SE) (see Fig. S1). Within each soil parameter, values followed by the different letters are statistically different according to one-way ANOVA (Tukey-B-test: $P < 0.001$)

Table S2
Plant diversity of three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from which the arbuscular mycorrhizal fungi (AMF) were sampled, trap cultured, and single-spore trap cultures were set up.

Successional stages †	Species richness	Shannon-Weaver Index
	(S_{plant} m^{-2})	(H'_{plant} m^{-2})
W1	12.80 ± 0.74 a ‡	1.96 ± 0.08 a
W2	11.40 ± 1.57 a	1.95 ± 0.24 a
W3	23.25 ± 0.95 b	2.60 ± 0.06 b

† The conventional arable field used as reference (W0) was cultivated with winter wheat (*Triticum aestivum* L.)

‡ Values are mean of five replicates for W1, W2 and W3 (mean ± ES) (see Fig. S1). Within each parameter, values followed by the different letters are statistically different according to one-way ANOVA (Tukey-B-test: $P < 0.001$)

Table S3
Soil prokaryotic diversity of three grasslands that were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and of a conventional arable field cultivated with winter wheat (*Triticum aestivum* L.) as reference (W0). Soils were used to sample and trap culture the arbuscular mycorrhizal fungi (AMF), and single-spore trap cultures were set up.

Successional stages	Species richness (S_{prok})	Shannon-Weaver Index (H'_{prok})	Simpson Index (λ_{prok})
W0	15.80 ± 0.20 a †	2.18 ± 0.00 a	0.87 ± 0.00 a
W1	15.40 ± 0.25 a	2.19 ± 0.01 a	0.87 ± 0.00 a
W2	15.00 ± 0.32 a	2.17 ± 0.01 a	0.87 ± 0.00 a
W3	15.60 ± 0.25 a	2.18 ± 0.02 b	0.87 ± 0.00 a

† Values are mean of five replicates for W1, W2 and W3 (mean ± ES) (see Fig. S1). Within each parameter, values followed by different letters are statistically different according to one-way ANOVA (Tukey-B-test: $P < 0.05$)

Table S4
Spore density and arbuscular mycorrhizal (AM) fungal colonization of the 19 AM fungal single-spore inocula (crude inoculum). The AM fungi were isolated from three grasslands where soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from a conventional arable field used as reference (W0).

AM fungal isolates	Spore density	AM fungal colonization
	n of spores g^{-1}	%
W0-4‡	12.0 ± 0.7†	54.7 ± 3.5
W0-6	13.4 ± 1.3	56.0 ± 2.6
W0-12	11.9 ± 1.7	54.3 ± 3.5
W1-18	11.7 ± 1.4	56.3 ± 6.8
W1-20	14.3 ± 0.6	69.0 ± 2.1
W1-21	12.1 ± 0.6	56.0 ± 4.9
W1-22	11.5 ± 0.7	67.7 ± 3.9
W1-24	10.7 ± 0.6	76.7 ± 0.8
W1-25	13.0 ± 0.7	68.0 ± 4.7
W1-27	10.5 ± 0.5	64.0 ± 4.2
W1-28	11.0 ± 0.9	64.7 ± 5.0
W2-32	10.4 ± 0.4	65.0 ± 3.5
W2-35	13.2 ± 1.3	68.3 ± 1.8
W2-36	11.6 ± 0.4	56.7 ± 1.8
W3-37	10.8 ± 0.9	58.0 ± 2.5
W3-52	12.7 ± 0.1	58.7 ± 4.2
W3-63	10.9 ± 0.5	65.3 ± 4.3
W3-69	10.7 ± 0.0	65.0 ± 4.7
W3-73	11.8 ± 1.5	61.7 ± 2.8

† Values are mean of five replicates for W1, W2 and W3 (mean ± ES)
‡ AMF fungal isolate code i.e. W0-4, W0 refers to the site and 4 to the number of the AMF isolate

Table S5

List of newly generated sequences belonging to Glomeromycota phylum (1.5-kb-long SSU-ITS-LSU gene fragment flanked by the PCR primers SSUmCf and LSUmBr; Krüger et al., 2009) and list of the corresponding highly similar sequences obtained from BLAST in the NCBI database.

Newly generated sequence	Other newly generated sequences	Accession number of the highly similar sequence	Description of the highly similar sequence	Minimum/ maximum percentage of identity	Minimum/ maximum percentage of query cover
seq1 isolate W0-4	seq2 isolate W0-4, seq3 isolate W0-4, seq1 isolate W3-73, seq2 isolate W3-73, seq3 isolate W3-73	FR750059	Entrophosporaceae - *Entrophospora claroidea* †	87.0 - 88.9	78 - 100
seq1 isolate W0-6	seq2 isolate W0-6, seq3 isolate W0-6	MK521694	Entrophosporaceae - *E. claroidea*	97.5 - 97.9	99 - 100
seq1 isolate W0-12	seq2 isolate W0-12, seq3 isolate W0-12, seq1 isolate W1-27, seq2 isolate W1-27, seq3 isolate W1-27, seq1 isolate W1-28, seq2 isolate W1-28, seq3 isolate W1-28,	OP455135	Glomeraceae - *Funneliformis mosseae*	86.6 - 90.7	74 - 100
seq1 isolate W1-18	seq2 isolate W1-18, seq3 isolate W1-18, seq1 isolate W1-20, seq2 isolate W1-20, seq3 isolate W1-20, seq1 isolate W1-24, seq2 isolate W1-24, seq3 isolate W1-24	LR739040	Glomeraceae - *F. mosseae*	87.5 - 96.0	96 - 100
seq1 isolate W1-21	seq2 isolate W1-21, seq3 isolate W1-21, seq1 isolate W2-35, seq2 isolate W2-35, seq3 isolate W2-35, seq1 isolate W3-37, seq1 isolate W3-37, seq1 isolate W3-37seq1 isolate W3-52, seq2 isolate W3-52, seq3 isolate W3-52, seq1 isolate W3-63, seq2 isolate W3-63, seq3 isolate W3-63, seq1 isolate W3-69, seq2 isolate W3-69, seq3 isolate W3-69,	MH590059	Glomeraceae - *F. mosseae*	87.0 - 98.4	85 - 100
seq1 isolate W1-25	seq2 isolate W1-25, seq3 isolate W1-25	MK521679	Glomeraceae - *F. mosseae*	87.6 - 89.2	81 - 96
seq1 isolate W1-22	seq2 isolate W1-22, seq3 isolate W1-22, seq1 isolate W2-36, seq2 isolate W2-36, seq3 isolate W2-36	AM494586	Archaeosporaceae - *Archaeospora* sp.	86.6 - 97.1	78 - 85
seq1 isolate W2-32	seq2 isolate W2-32, seq3 isolate W2-32	FR750037	Archaeosporaceae - *Archaeospora trappei*	92.6 - 93.5	63 - 67

† Previously known as Claroideoglomeraceae - *Claroideoglomus claroideum* (Redecker et al., 2013)

Table S6

P-values of nested two-way ANOVA on the effect of arbuscular mycorrhizal fungal (AMF) species and isolates on fungal and plant functional diversity (inter- and intra-species diversity, respectively). AMF colonization traits were: percentage of AMF root colonization, percentage of root length containing arbuscules (Arb) and percentage of root length containing vesicles (Ves). Plant traits were: shoot and root dry weight, length, and mineral concentrations. The host plant is leek (*Allium porrum* L.). Nineteen AMF isolates were propagated from three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from a conventional arable field as reference (W0).

	AMF col			Dry weight		Length		Shoot							Root						
	Col	Arb	Ves	Shoot	Root	Shoot	Root	Zn	Cu	Mn	K	Ca	Mg	Fe	Zn	Cu	Mn	K	Ca	Mg	Fe
Species[†]	0.125 [‡]	**0.044**	0.574	0.271	0.458	0.280	0.081	0.973	**0.029**	0.874	0.054	0.338	0.285	0.377	0.927	**0.028**	0.903	**0.014**	**0.036**	0.093	0.989
Isolate[§]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.117	**0.006**	0.499	0.567	**0.004**	**<0.001**	**0.041**	**0.001**	**0.007**	0.070	0.064	**<0.001**	**<0.001**	**0.046**	0.137	**0.008**	0.527

[†] AMF species used as fixed factor: *Entrophospora claroidea*, *Funneliformis mosseae* and *Archaespora trappei*. Three AMF species belonging to three orders

[‡] In bold statistically significant values ($P \leq 0.05$). Three replicates for AMF col and five replicates for all the other parameters

[§] AMF isolate used as random factor (nested factor within AMF species): three isolates of *E. claroidea*; 13 isolates of *F. mosseae* and three isolates of *A. trappei*

Table S7

Effect of arbuscular mycorrhizal fungal (AMF) species and isolates on mineral concentration in shoots (inter- and intra-species diversity, respectively). The host plant was leek (*Allium porrum* L.). Twenty AMF isolates were propagated from three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from a conventional arable field as reference (W0).

Treatment	Shoots					
	Zn	Mn	K	Ca	Mg	Fe
	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}
Non-mycorrhizal control	8.1±2.8 †	- ‡	-	-	-	68.2±11.2
Species §						
E. claoidea	15.2±0.8	74.73±8.5	41010.51±2215	9233.5±313	3643.8±131	95.7±7.2
F. mosseae	15.1±0.4	72.48±3.1	34402.07±905	8502.3±147	3332.0±74	91.7±5.0
A. trappei	15.0±0.4	78.59±6.9	35396.48±1270	8491.9±227	3429.0±98	75.0±4.1
Isolate #						
E. claroidea						
W0-4 ††	14.8±1.4	-	-	-	-	75.0±4.6
W0-6	17.2±1.1	-	-	-	-	105.2±6.3
W3-73	13.7±1.1	-	-	-	-	107.1±17.7
F. mosseae						
W0-12	14.4±0.8	-	-	-	-	90.1±14.0
W1-18	15.0±0.9	-	-	-	-	65.9±2.5
W1-20	15.8±0.7	-	-	-	-	70.2±5.2
W1-21	14.4±1.0	-	-	-	-	62.9±5.6
W1-24	13.7±0.7	-	-	-	-	92.1±16.6
W1-25	13.3±1.0	-	-	-	-	94.0±5.3
W1-27	14.1±0.5	-	-	-	-	76.1±8.2
W1-28	15.6±2.1	-	-	-	-	82.5±6.2
W2-35	14.7±0.8	-	-	-	-	93.0±8.3
W3-37	15.9±01.2	-	-	-	-	93.8±21.0
W3-52	15.2±0.6	-	-	-	-	124.6±42.1
W3-63	15.9±1.4	-	-	-	-	122.0±24.0
W3-69	17.7±3.3	-	-	-	-	124.6±18.8
A. trappei						
W1-22	14.5±1.0	-	-	-	-	84.0±9.8
W2-32	14.9±0.6	-	-	-	-	65.3±2.3
W2-36	15.6±0.4	-	-	-	-	75.8±5.8

† $P > 0.05$, see Table S5. Five replicates for all parameters

‡ Hyphen refer to significant values $P \leq 0.05$, see Table S5

§ AMF species used as fixed factor: *Entrophospora claroidea*, *Funneliformis mosseae* and *Archaespora trappei*. Three AMF species belonging to three orders

AMF isolate used as random factor (nested factor within AMF species): three isolates of *E. claroidea*; 13 isolates of *F. mosseae* and three isolates of *A. trappei*.

†† AMF fungal isolate code i.e. W0-4, W0 refers to the site and 4 to the number of the AMF isolate

Table S8
Effect of arbuscular mycorrhizal fungal (AMF) species and isolates on mineral concentration in shoot (inter- and intra-species diversity, respectively). The host plant was leek (*Allium porrum* L.). Twenty AMF isolates were propagated from three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from a conventional arable field as reference (W0).

Treatment	Root				
	Zn	Mn	Ca	Mg	Fe
	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}
Non-mycorrhizal control	31.1±7.4 †	132.4±18.2	- ‡	6161.6±340.1	316.1±43.9
Species §					
E. claoidea	33.5±1.6	314.5±64	-	8273.1±352	479.7±36.8
F. mosseae	33.9±1.2	328.3±71	-	7346.2±139	473.8±18.0
A. trappei	34.8±1.3	256.8±43	-	7076.3±196	476.7±41.4
Isolate #					
E. claroidea					
W0-4 ††	35.2	-	4748.8	-	493.1
W0-6	34.0	-	5237.0	-	494.5
W3-73	31.1	-	4622.2	-	451.6
F. mosseae					
W0-12	33.0	-	4236.9	-	523.8
W1-18	28.2	-	4406.7	-	572.8
W1-20	42.2	-	4413.8	-	529.8
W1-21	31.3	-	3911.6	-	454.8
W1-24	34.3	-	4599.2	-	481.6
W1-25	28.7	-	4368.0	-	517.8
W1-27	33.7	-	3896.7	-	414.0
W1-28	31.5	-	4527.6	-	417.2
W2-35	28.4	-	4643.6	-	333.6
W3-37	41.7	-	4263.2	-	508.9
W3-52	36.4	-	3937.3	-	480.2
W3-63	29.8	-	4488.8	-	385.6
W3-69	33.6	-	5015.7	-	539.5
A. trappei					
W1-22	33.2	-	4224.7	-	484.0
W2-32	34.7	-	4020.8	-	533.1
W2-36	36.6	-	4380.6	-	412.9

† $P > 0.05$, see Table S5. Five replicates for all parameters

‡ Hyphen refer to significant values $P \leq 0.05$, see Table S5

§ AMF species used as fixed factor: *Entrophospora claroidea*, *Funneliformis mosseae* and *Archaeospora trappei.* Three AMF species belonging to three orders

AMF isolate used as random factor (nested factor within AMF species): three isolates of *E. claroidea*; 13 isolates of *F. mosseae* and three isolates of *A. trappei*

†† AMF fungal isolate code i.e. W0-4, W0 refers to the site and 4 to the number of the AMF isolate

Table S9

Permutational analyses of variance (PERMANOVAs) on the effect of arbuscular mycorrhizal fungal (AMF) species and isolates on fungal and plant functional diversity (inter- and intra-species diversity, respectively). AMF colonization traits are: percentage of AMF root colonization, percentage of root length containing arbuscules (Arb) and percentage of root length containing vesicles (Ves). Plant traits are: shoot and root dry weight and length, and mineral concentrations. The host plant is leek (*Allium porrum* L.). Nineteen isolates were propagated from three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from a conventional arable field as reference (W0).

	df	Pseudo-F	*P*(perm)
Species [†]	2	1.74	0.054 [‡]
Isolate [§]	16	2.1	**0.001**
PERMDISP			
Species	-	-	**0.010**
Isolate	-	-	**0.008**

[†] AMF species used as fixed factor: *Entrophospora claroidea*, *Funneliformis mosseae* and *A. trappei*. Three AMF species belonging to three orders

[‡] In bold statistically significant values ($P \leq 0.05$). Three replicates for AMF col and five replicates for all the other parameters

[§] AMF isolate used as random factor (nested factor within AMF species): three isolates of *E. claroidea*; 13 isolates of *F. mosseae* and three isolates of *A. trappei*.

Table S10

Pearson correlation values (r) among plant traits [shoot dry weight, SDW; root dry weight, RDW; shoot length, SL; root length, RL; mineral concentrations in shoot (s) and root (r)] and arbuscular mycorrhizal fungal (AMF) traits (percentage of AMF root colonization, Col; percentage of root length containing arbuscules, Arb; percentage of root length containing vesicles, Ves.). In bold statistically significant correlations: sig. (2-tailed) *P*-values ≤ 0.05 (for the *P* values see Table S11). The data input was square root transformed and normalized. The host plant is leek (*Allium porrum* L.). Nineteen AMF isolates were propagated from three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from a conventional arable field as reference (W0). Three replicates (pot) for all parameters.

	SDW	RDW	SL	RL	[Zn]r	[Cu]r	[Mn]r	[K]r	[Ca]r	[Mg]r	[Fe]r	[Zn]s	[Cu]s	[Mn]s	[K]s	[Ca]s	[Mg]s	[Fe]s	Arb	Ves	Col
SDW																					
RDW	**0.747** †																				
SL	**0.771**	**0.639**																			
RL	**0.396**	**0.334**	0.232																		
[Zn]r	0.037	0.013	-0.102	0.021																	
[Cu]r	-0.001	0.101	0.017	-0.054	**0.286**																
[Mn]r	**-0.443**	**-0.315**	**-0.488**	-0.244	0.198	**-0.121**															
[K]r	**-0.71**	**-0.545**	**-0.576**	**-0.265**	-0.01	**0.174**	**0.449**														
[Ca]r	**-0.519**	**-0.382**	**-0.49**	**-0.303**	0.179	**0.301**	**0.491**	**0.521**													
[Mg]r	**-0.659**	**-0.522**	**-0.583**	-0.256	0.100	0.084	**0.537**	**0.692**	**0.769**												
[Fe]r	0.256	**0.225**	**0.340**	0.082	0.143	0.201	-0.118	-0.242	0.046	-0.112											
[Zn]s	**-0.465**	-0.212	**-0.484**	-0.177	**0.343**	0.137	**0.522**	**0.278**	**0.455**	**0.447**	0.001										
[Cu]s	**-0.277**	-0.079	**-0.308**	-0.212	-0.018	**0.503**	0.032	0.173	0.244	0.021	-0.074	**0.418**									
[Mn]s	**-0.418**	**-0.303**	**-0.481**	-0.051	0.113	-0.220	**0.767**	**0.326**	**0.292**	**0.377**	-0.074	**0.516**	-0.032								
[K]s	**-0.768**	**-0.529**	**-0.433**	**-0.279**	0.089	0.023	**0.401**	**0.578**	**0.46**	**0.576**	-0.072	**0.375**	0.149	**0.353**							
[Ca]s	**-0.663**	**-0.474**	**-0.577**	-0.226	-0.069	-0.034	**0.440**	**0.618**	**0.509**	**0.566**	-0.221	**0.326**	**0.271**	**0.457**	**0.621**						
[Mg]s	**-0.438**	**-0.279**	**-0.472**	-0.132	-0.023	0.029	**0.528**	**0.419**	**0.493**	**0.399**	-0.103	**0.276**	0.207	**0.481**	**0.434**	**0.568**					
[Fe]s	**-0.591**	**-0.403**	**-0.557**	-0.215	0.154	0.004	**0.479**	**0.446**	**0.377**	**0.437**	-0.177	**0.471**	**0.316**	**0.519**	**0.109**	**0.667**	**0.519**				
Arb	-0.17	-0.12	-0.150	**-0.416**	-0.056	0.124	-0.186	0.164	0.166	**0.273**	-0.039	-0.087	0.165	**-0.365**	0.109	0.121	-0.224	-0.071			
Ves	0.149	0.184	0.001	0.134	0.076	-0.022	0.045	-0.167	-0.071	-0.144	-0.061	-0.090	-0.047	0.032	-0.023	0.020	0.087	0.039	0.150		
Col	0.026	0.072	-0.097	-0.243	0.066	0.075	-0.143	-0.119	0.058	0.021	-0.076	-0.039	0.135	-0.247	-0.065	-0.062	-0.067	-0.071	**0.681**	**0.500**	

† In bold statistically significant correlations: green values correspond to *P*-values ≤ 0.05; red values correspond to *P*-values ≤ 0.01; black values correspond to *P*-values ≤ 0.001 (for *P* values see Table S11).

Table S11

Sig. (2-tailed) *P*-values of Pearson correlations among plant traits [shoot dry weight, SDW; root dry weight, RDW; shoot length, SL; root length, RL; mineral concentrations in shoots (s) and roots (r)] and arbuscular mycorrhizal fungal (AMF) traits (percentage of AMF root colonization, Col; percentage of root length containing arbuscules, Arb; percentage of root length containing vesicles, Ves.) (for the r values see Table S9). Data input was square root transformed and normalized. The host plant is leek (*Allium porrum* L.). Nineteen AMF isolates were propagated from three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from a conventional arable field as reference (W0). Three replicates (pot) for all parameters.

	SDW	RDW	SL	RL	[Zn]r	[Cu]r	[Mn]r	[K]r	[Ca]r	[Mg]r	[Fe]r	[Zn]s	[Cu]s	[Mn]s	[K]s	[Ca]s	[Mg]s	[Fe]s	Arb	Ves	Col
SDW																					
RDW	**<0.001**																				
SL	**<0.001**	**<0.001**																			
RL	**0.002**	**0.011**	0.082																		
[Zn]r	0.793	0.926	0.448	0.876																	
[Cu]r	0.989	0.470	0.898	0.689	**0.031**																
[Mn]r	**0.001**	**0.017**	**<0.001**	0.068	0.140	0.371															
[K]r	**<0.001**	**<0.001**	**<0.001**	**0.047**	0.941	0.196	**<0.001**														
[Ca]r	**<0.001**	**0.003**	**<0.001**	**0.022**	0.182	**0.023**	**<0.001**	**<0.001**													
[Mg]r	**<0.001**	**<0.001**	**<0.001**	0.054	0.458	0.532	**<0.001**	**<0.001**	**<0.001**												
[Fe]r	0.055	0.090	**0.010**	0.545	0.290	0.133	0.384	0.070	0.732	0.407											
[Zn]s	**<0.001**	0.109	**<0.001**	0.187	**0.009**	0.309	**<0.001**	0.037	**<0.001**	**<0.001**	0.997										
[Cu]s	**0.035**	0.552	**0.020**	0.113	0.894	**<0.001**	0.811	0.197	0.068	0.875	0.585	**0.001**									
[Mn]s	**0.001**	**0.022**	**<0.001**	0.707	0.405	0.101	**<0.001**	0.013	**0.027**	**0.004**	0.584	**<0.001**	0.812								
[K]s	**<0.001**	**<0.001**	**0.001**	**0.036**	0.511	0.864	**0.002**	**<0.001**	**<0.001**	**<0.001**	0.594	**0.004**	0.270	**0.007**							
[Ca]s	**<0.001**	**<0.001**	**<0.001**	0.090	0.612	0.805	**0.001**	**<0.001**	**<0.001**	**<0.001**	0.098	**0.013**	**0.042**	**<0.001**	**<0.001**						
[Mg]s	**<0.001**	**0.036**	**<0.001**	0.327	0.863	0.831	**<0.001**	**0.001**	**<0.001**	**0.002**	0.446	**0.037**	0.121	**<0.001**	**0.001**	**<0.001**					
[Fe]s	**<0.001**	**0.002**	**<0.001**	0.109	0.254	0.976	**<0.001**	**0.001**	**0.004**	**0.001**	0.188	**<0.001**	**0.017**	**<0.001**	**<0.001**	**<0.001**	**<0.001**				
Arb	0.207	0.370	0.265	**0.001**	0.677	0.359	0.167	0.224	0.216	**0.04**	0.772	0.521	0.221	**0.005**	0.421	0.369	0.095	0.602			
Ves	0.259	0.171	0.995	0.322	0.576	0.873	0.742	0.214	0.599	0.285	0.651	0.508	0.731	0.813	0.863	0.885	0.520	0.774	0.266		
Col	0.849	0.594	0.472	0.068	0.624	0.581	0.289	0.377	0.666	0.875	0.574	0.775	0.318	0.064	0.633	0.645	0.623	0.598	**<0.001**	**<0.001**	

Supplementary Figures

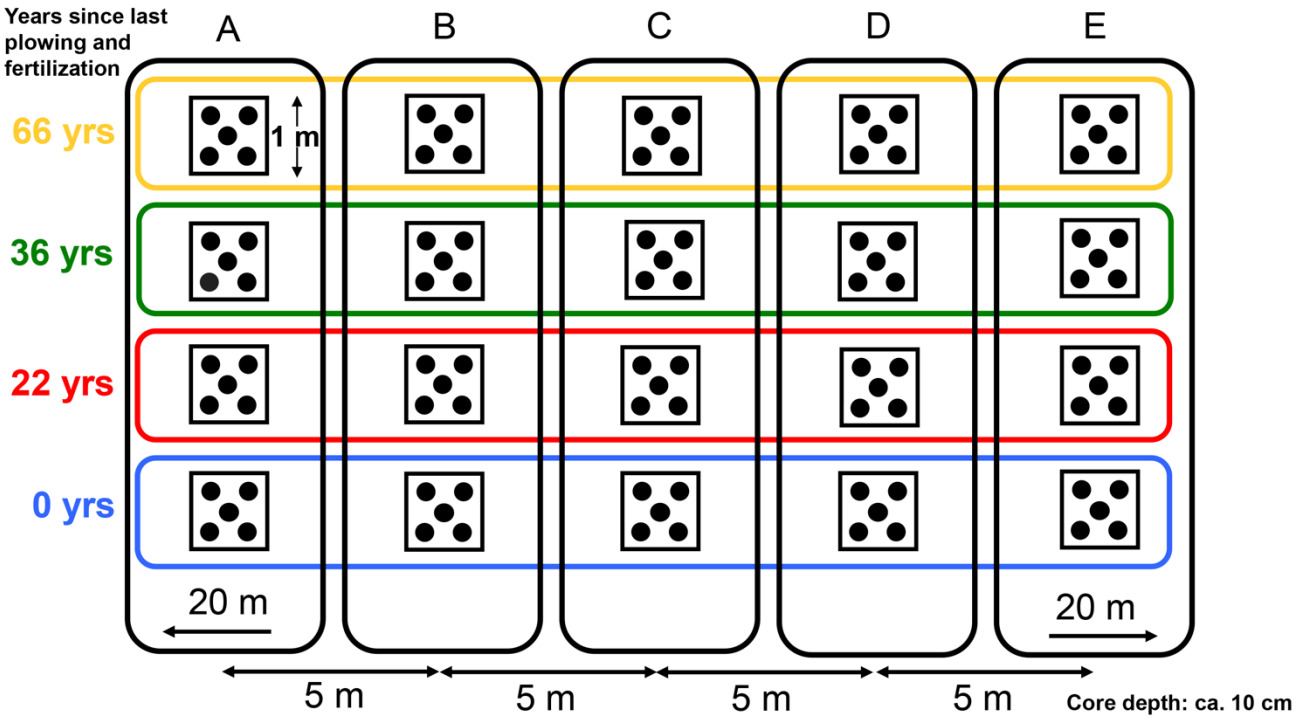

Fig. S1. Scheme of the original soil sample collection performed in a nature reserve at Wrakelberg (Netherland) in February 2007 (Kuramae et al., 2010, 2011). Soil samples were taken at four sites along linear transects of 20 m: W0, a conventional arable field with winter wheat (*Triticum aestivum* L.) (i.e., reference site); W1, W2 and W3, three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (i.e., chronosequences of secondary succession of chalk grasslands with a neutral pH). At each of the four sites, five (A, B, C, D and E) within-field replicate composite soil samples were made by combining two cores (10-cm depth, 2 cm diameter) taken less than 10 cm apart. This yielded a total of 20 soil composite samples. Soil samples were sieved at 3 mm, thus excluding larger root fragments and pieces of chalk.

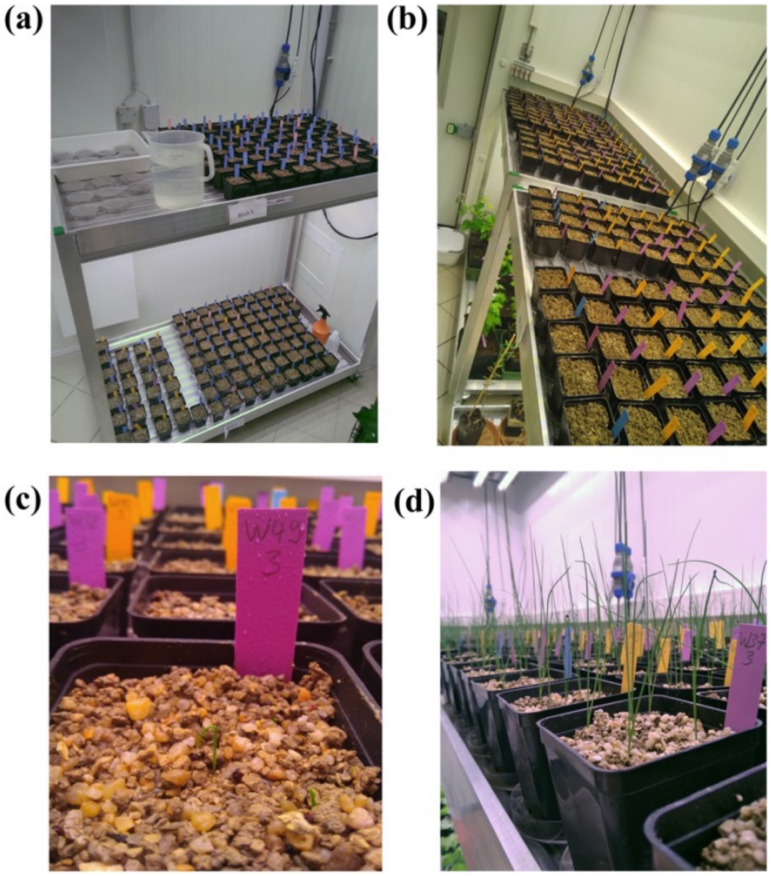

Fig. S2. Growth chamber pot experiment for the evaluation of the infectivity and effectiveness of the arbuscular mycorrhizal (AM) fungal single-spore isolates using the model plant leek (*Allium porrum* L.). Experimental units (pots) arranged in a completely randomized design with five replicates (a, b); early germination of leek (c), and development of *A. porrum* plants one month after emergence (d).

Fig. S3. Arrangement of the growth chamber pot experiment for the evaluation of the infectivity and effectiveness of the arbuscular mycorrhizal (AM) fungal single-spore isolates using the model plant leek (*Allium porrum* L.). The arrangement was a completely randomized design with 19 isolates of arbuscular mycorrhizal fungi (AMF) and the non-mycorrhizal controls (gray) and five replicates for each level of the treatment. The AM fungal isolates were propagated from four sites: W0, a conventional arable field with winter wheat (*Triticum aestivum* L.) (i.e., reference site); W1, W2 and W3, three grasslands whose soils were last plowed and fertilized 22, 36 and 66 years ago (i.e., chronosequences of secondary succession of chalk grasslands with a neutral pH). In the image the colors correspond to the sites: W0=blue; W1=red; W2=green; W3=yellow. Three AM fungal AM fungal isolates were evaluated for W0, while eight, three and five isolates were evaluated for W1, W2 and W3, respectively. The code "W1-18-r2" in the figure refers to the site "W1", to the number of the AM fungal isolate "18" and to the replicate "r2".

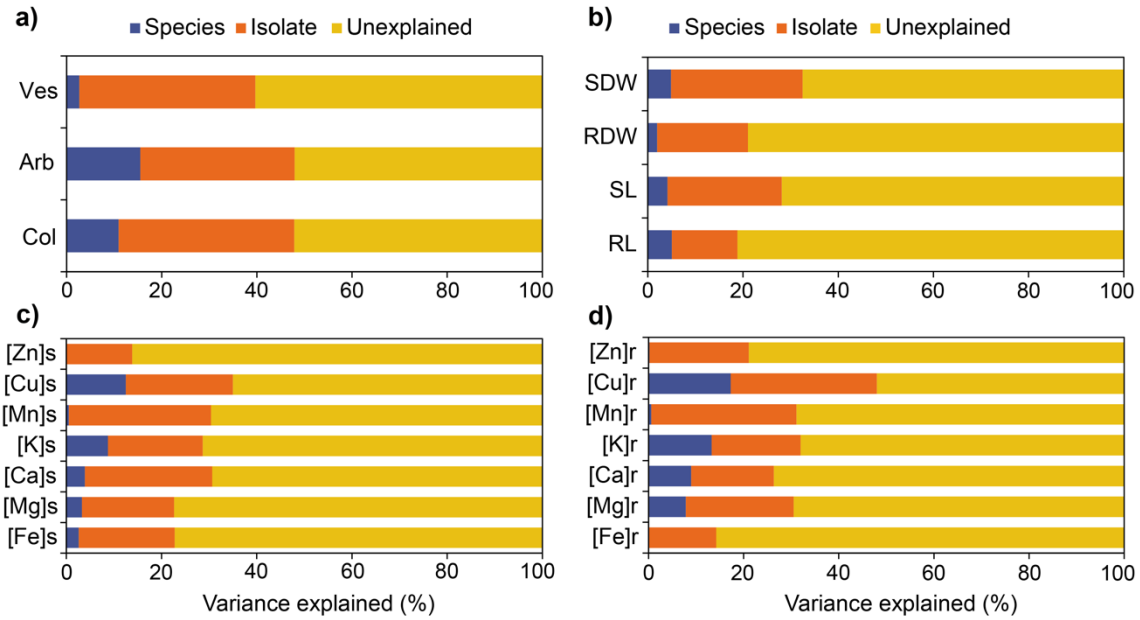

Fig. S4. Partitioning of total variance of arbuscular mycorrhizal fungal (AMF) and plant traits (AMF infectivity and effectiveness) into factors "Species" and "Isolate". Arbuscular mycorrhizal fungal traits are: percentage of AMF root colonization, Col; percentage of root length containing arbuscules, Arb; percentage of root length containing vesicles, Ves. Plant traits are: shoot dry weight, SDW; root dry weight, RDW; shoot length, SL; root length, RL; mineral concentrations in shoots (s) and roots (r). AMF species used as fixed factor: *Entrophospora claroidea*, *Funneliformis mosseae* and *Archaespora trappei*. Three AMF species belonging to three orders. AMF isolate used as random factor (nested factor within AMF species): three isolates of *E. claroidea*; 13 isolates of *F. mosseae* and three isolates of *A. trappei*. See Table S6 for the *P* values of the nested two-way ANOVAs.

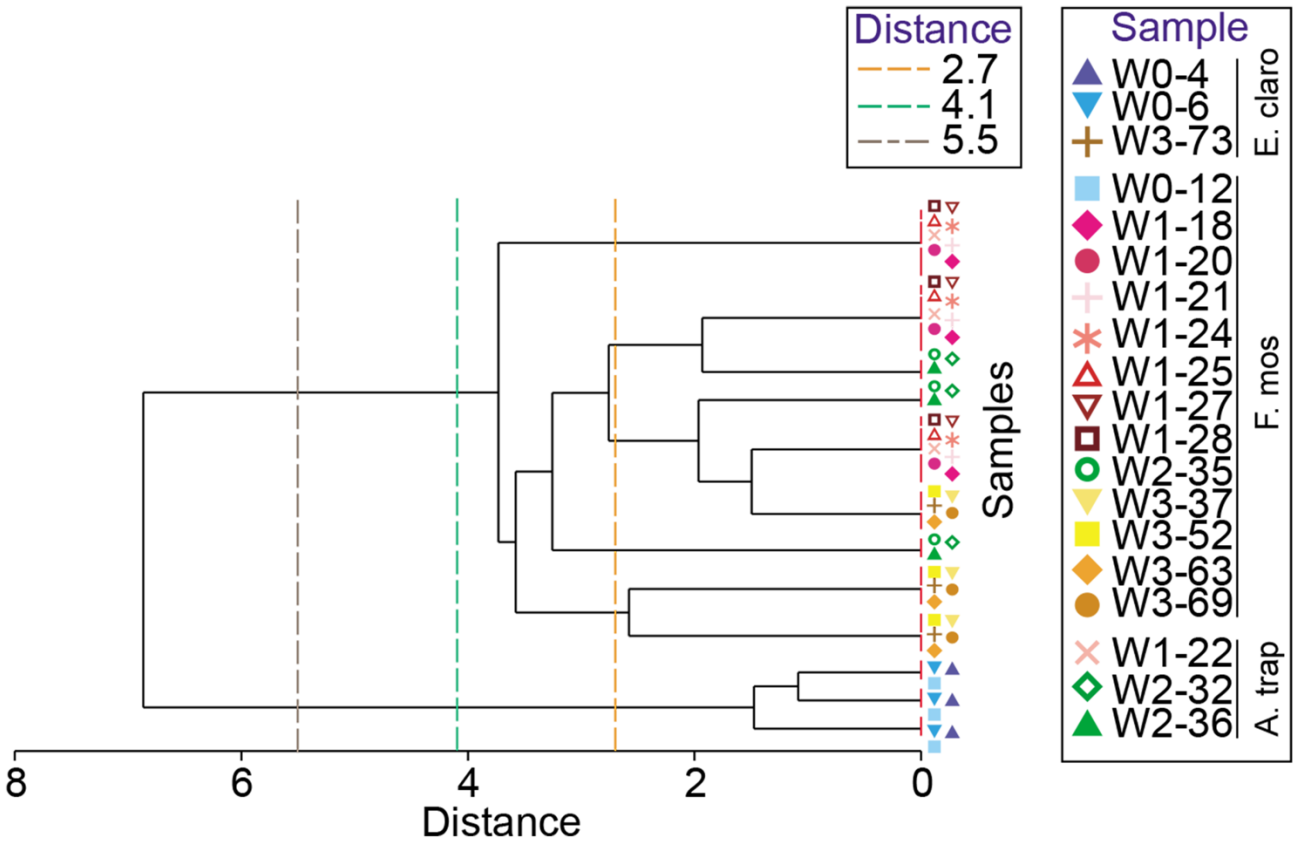

Fig. S5. Dendrogram of Similarity Profile (SIMPROF) cluster analysis (horizontal dendrogram) grouping the different samples of the soils of origin from which the arbuscular mycorrhizal fungal (AMF) isolates were propagated. The cluster analysis is based on the following parameters: soil pH, total N, ammonium (NH_4^+), nitrate (NO_3^-), organic matter, available P; plant species richness (S_{plant}) and Shannon-Weaver Index (H'_{plant}); prokaryotic species richness (S_{prok}), Shannon-Weaver Index (H'_{prok}) and Simpson Index (λ_{prok}). Soils belong to three grasslands that were last plowed and fertilized 22, 36 and 66 years ago (W1, W2 and W3: 22, 36, 66 years) and from a conventional arable field as reference (W0). Replicates are three per each type of soil (W0, W1, W2 and W3). Samples are grouped into clusters based on their similarity/homogeneity of parameters. Red clusters are supported by the SIMPROF analysis. Supported distance thresholds are showed. Data input was squared root transformed and normalized, and the Euclidean matrix of similarity was calculated.

References

Isaac, R.A., Johnson, W.C., Kalra, Y., 1998. Elemental determination by inductively coupled plasma atomic emission spectrometry, in: Kalra, Y., (Ed.), Handbook and Reference Methods for Plant Analysis. CRC Press, Boca Raton, Florida, pp. 165–170.

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.

Kuramae, E., Gamper, H., van Veen, J., Kowalchuk, G., 2011. Soil and plant factors driving the community of soil-borne microorganisms across chronosequences of secondary succession of chalk grasslands with a neutral pH. FEMS Microbiol. Ecol. 77, 285-294. https://doi.org/10.1111/j.1574-6941.2011.01110.x.

Kuramae, E., Gamper, H., Yergeau, E., Piceno, Y.M., Brodie, E.L., DeSantis, T.Z., Andersen G.L., van Veen J., Kowalchuk, G., 2010. Microbial secondary succession in a chronosequence of chalk grasslands. ISME J. 4, 711-715. https://doi.org/10.1038/ismej.2010.11.

Liberato, C.G., Juan, A.V.A., Barros, A.V., Raquel, C., Machado, A.R.A., Nóbrega, J.A., Schiavo, D., 2020. Determination of macro and micronutrients in plants using the Agilent 4200 MP AES. Agilent Technologies.

Lindsay, W.L., Norvell, W., 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil. Sci. Soc. Am. J. 42, 421-428. https://doi.org/10.2136/sssaj1978.03615995004200030009x.

Mclean, E.O., 1982. Soil pH and Lime Requirement, in: Page, A.L. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, American Society of Agronomy, Soil Science Society of America, Madison, pp. 199-224.

Nelson, D.W., Sommers, L., 1983. Total carbon, organic carbon, and organic matter, in: Page, A.L. (Eds.), Methods of soil analysis. Part 2. Chemical and Microbiological Properties, American Society of Agronomy, Soil Science Society of America, Madison, pp. 539-579.

Novozamsky, I, Houba, V.J.G., Temminghoff, E., Van der Lee, J.J., 1984. Determination of 'total' N and 'total' P in single soil digest. NJAS-Wagen. J. Life Sc. 32, 322-324. https://doi.org/10.18174/njas.v32i4.16889.

Redecker, D., Schüßler, A., Stockinger, H., Stürmer, S. L., Morton, J.B., Walker, C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23, 515-531. https://doi.org/10.1007/s00572-013-0486-y.