Supplementary Material

Plant genotype and inoculation with indigenous arbuscular mycorrhizal (AM) fungi modulate wheat productivity and quality of processed products through changes in the frequency of root AM fungal taxa

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Supplementary Methods

Supplementary Methods S1. *Nutrient determinations*

Grain samples were ground and oven dried at 60°C for four days. Approximately 0.3 g of grain samples were grounded and digested by a microwave-assisted acid digestion system (COOLPEX Smart Microwave Reaction System, Yiyao Instrument Technology Development Co., Ltd., Shanghai, China) and a Microwave Plasma Atomic Emission Spectroscopy (4210 MP-AES, Agilent Technologies, Santa Clara, CA, USA). In detail the digestion was performed by microwave heating after the addition of 8 mL of nitric acid (HNO3; 65%) with the following temperature program: 5 min ramp to 120°C, hold for 2 min; 2 min ramp to 150°C, hold for 4 min; 2 min ramp to 180°C, hold for 4 min. Blanks were run with each batch of samples for quality control. Then, the digested solutions were diluted with Milli-Q water before the analysis in the MP-AES (Liberato et al. 2020).

Supplementary Tables

Table S1

Physical and chemical parameters of soil of the field sites located in Pienza, Siena (Italy). The field experiment was conducted in 2020 and 2021 (from January to July).

Year	Clay	Silty	Sand	pH	Totale N	C/N	Soil organic content	Available P	Available Fe	Available Zn	CEC [†]
	$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	H_2O	$mg\ kg^{-1}$		$g\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$meq\ 100\ g^{-1}$
2020	464.0 ± 11.5 [‡]	472.0 ± 14.8	64.0 ± 3.5	8.4 ± 0.0	1.1 ± 0.1	7.5 ± 0.2	13.9 ± 0.0	8.0 ± 0.7	10.0 ± 0.6	1.9 ± 0.1	23.1 ± 0.4
2021	436.7 ± 9.4	399.7 ± 13.8	163.5 ± 22.3	8.2 ±0.0	1.2 ± 0.0	9.5 ± 0.3	19.1 ± 0.0	17.7 ± 1.5	15.6 ± 0.6	0.6 ± 0.1	22.5 ± 0.0

[†] CEC: cation exchange capacity.

[‡] Values are mean of five soil replicates (mean ± SE).

Table S2

List of the genetic material of wheat used in the study: group, genotype, year of release (YR), pedigree and area of origin. The field experiments were conducted on-farm in 2020 and 2021 (from January to July).

Group	Genotype	YR	Pedigree	Area of origin/breeder
Old	Bianco Nostrale [†]	-	Landrace	Tuscany/mountains
	Andriolo	1945	Landrace	Tuscany/mountains
	Abbondanza	1950	Autonomia/Fontarronco	Tuscany/hills/mountains
	Sieve	1960	EST Mottin 72/Bellevue II	Tuscany/mountains
Modern	Bologna	1999	H89092/H89136/Soissons	SIS (Emilia-Romagna), C.C. Benoist (France)

[†] Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank (http://germoplasma.regione.toscana.it) and in the Italian Register of Agricultural and Food Interest Biodiversity (https://rica.crea.gov.it/APP/anb/search.php), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System (SIAN; https://www.sian.it/mivmPubb/autenticazione.do) (Ercoli et al., 2018).

Table S3

P-values of three-way ANOVAs on the effect of year of cultivation (Y), wheat genotype (G) and arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) and their interactions on: AMF root colonization (Col), percentage of root length containing arbuscules (Arb), percentage of root length containing vesicles (Ves) at the two-leaves unfolded stage (GS12; Zadoks et al., 1974) and at the physiological maturity (GS90); grain yield and nutrient concentration in grain. In bold statistically significant *P* values according to the postdoc Tukey-B test.

Factor	GS12			GS90			Grain yield	Grain nutrient concentration								
	Col	Arb	Ves	Col	Arb	Ves	q ha^{-1}	Zn	Cu	Mn	K	Ca	Mg	Fe	N	P
Y [†]	**0.002** [‡]	**<0.001**	0.556	**<0.001**	**<0.001**	**0.040**	**<0.001**	0.395	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
G [§]	0.833	0.910	0.707	**0.006**	0.203	**0.019**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
Inoc [#]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.062	**0.021**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Y x G	0.180	0.773	**0.016**	**<0.001**	**0.014**	**0.003**	**<0.001**	**<0.001**	**0.004**	**0.017**	**<0.001**	**0.011**	**0.017**	**<0.001**	**<0.001**	**<0.001**
Y x Inoc	0.279	1.000	0.592	**0.025**	0.472	0.338	0.889	0.001	**<0.001**	0.925	**0.003**	**<0.001**	**0.001**	0.726	0.131	0.706
G x Inoc	**0.023**	**0.040**	0.606	**0.026**	**0.042**	0.497	0.144	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.205	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Y x G x Inoc	0.175	0.517	0.842	0.767	**0.046**	0.340	**<0.001**	**0.045**	**0.001**	**0.001**	**0.001**	**0.024**	**<0.001**	**<0.001**	**0.006**	**0.001**

[†] Year of cultivation: 2020 and 2021. Growing season from January to July.

[‡] Three replicate plots per treatment.

[§] Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2).

[#] Inoculum consisting of 14 AMF species originating from a local agricultural site (Pellegrino and Bedini, 2014).

Table S4

Results of permutational analyses of variance (PERMANOVAs) and variation partitioning of the effect of year of cultivation (Y), wheat genotype (G), arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) and their interactions on the plant and AMF functional parameters. Measures of the AMF functional parameters were performed at GS12 (two leaves unfolded stage; Zadoks et al., 1974) and GS90 (physiological maturity). Results of the distance-based test for homogeneity of multivariate dispersions (PERMDIP) to check the dispersion among groups (β-diversity).

Factor [†]	df	Pseudo-*F*	*P*(perm)	Variance explained
Y [‡]	1	35.02	**0.001** [§]	**3.81** [§]
G [#]	4	2.56	**0.002**	**16.66**
Inoc	1	18.45	**0.001**	**32.50**
Y x G	4	3.28	**0.001**	**5.19**
Y x Inoc	1	1.69	0.145	10.88
G x Inoc	4	2.09	**0.017**	**1.32**
Y x G x Inoc	4	1.11	0.345	1.06
PERMDISP [††]				
Y			**0.014**	
G			0.898	
Inoc			0.785	

[†] PERMANOVA was performed following a permutation method based on an unrestricted permutation of raw data (Monte Carlo test based on 999 permutations) with Y, G and Inoc used as fixed factor factors and three replicate plots per treatment.

[‡] Year of cultivation: 2020 and 2021. Growing season from January to July.

[§] In bold statistically significant values ($P \leq 0.05$) and the corresponding explained variance.

[#] Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System) (Table S2).

[††] Inoculum consisting of 14 AMF species originating from a local agricultural site (Pellegrino and Bedini, 2014).

[‡‡] PERMDISP based on 999 permutations.

Table S5

Results of permutational multivariate analysis of variance (PERMANOVA) pairwise comparisons of the effect of wheat genotype (G) and arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) on plant and AMF functional parameters.

Pairs (Genotype & Inoc) [†]		
G: -M	Pseudo-*F*	*P*(perm) [‡]
Bianco Nostrale [§] *vs* Andriolo	1.19	0.215
Bianco Nostrale *vs* Abbondanza	1.92	**0.012**
Bianco Nostrale *vs* Sieve	1.76	**0.039**
Bianco Nostrale *vs* Bologna	1.73	**0.042**
Andriolo vs Abbondanza	1.42	0.108
Andriolo vs Sieve	1.17	0.224
Andriolo vs Bologna	1.37	0.147
Abbondanza vs Sieve	1.61	0.067
Abbondanza vs Bologna	1.89	**0.029**
Sieve vs Bologna	1.74	**0.031**
G: +M		
Bianco Nostrale *vs* Andriolo	0.92	0.539
Bianco Nostrale *vs* Abbondanza	1.93	**0.005**
Bianco Nostrale *vs* Sieve	1.44	0.114
Bianco Nostrale *vs* Bologna	1.82	**0.011**
Andriolo vs Abbondanza	1.26	0.215
Andriolo vs Sieve	1.38	0.096
Andriolo vs Bologna	1.45	0.105
Abbondanza vs Sieve	1.74	**0.011**
Abbondanza vs Bologna	1.31	0.17
Sieve vs Bologna	1.27	0.174
Inoc [#]		
Bianco Nostrale +M *vs* -M	2.48	**0.004**
Andriolo +M *vs* -M	2.17	**0.013**
Abbondanza +M *vs* -M	1.48	**0.023**
Sieve +M *vs* -M	2.94	**0.001**
Bologna +M *vs* -M	2.16	**0.006**

[†] Pairwise comparisons between genotypes in inoculated and not inoculated treatments (+M and -M, respectively) and between +M and -M in each genotype.

[‡] *P* values based on Monte-Carlo permutational test with 999 permutations.

[§] Genotype: Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of agricultural and food interest biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System) (see TableS2).

[#] Inoculun consisting of 14 AMF species originating from a local agricultural site (Pellegrino and Bedini, 2014).

Table S6

Results of permutational multivariate analysis of variance (PERMANOVA) pairwise comparisons of the effect of year of cultivation (Y) and wheat genotype (G) on plant and arbuscular mycorrhizal fungal functional parameters.

Pairs (Year & G) †		
Y: 2020	Pseudo-*F*	*P*(perm) ‡
Bianco Nostrale § *vs* Andriolo	1.73	**0.037**
Bianco Nostrale *vs* Abbondanza	2.97	**0.001**
Bianco Nostrale *vs* Sieve	1.79	**0.025**
Bianco Nostrale *vs* Bologna	2.15	**0.005**
Andriolo vs Abbondanza	1.40	0.134
Andriolo vs Sieve	1.25	0.203
Andriolo vs Bologna	1.29	0.169
Abbondanza vs Sieve	1.88	**0.027**
Abbondanza vs Bologna	1.72	**0.048**
Sieve vs Bologna	1.38	0.125
Y: 2021		
Bianco Nostrale *vs* Andriolo	1.21	0.224
Bianco Nostrale *vs* Abbondanza	1.43	0.104
Bianco Nostrale *vs* Sieve	2.16	**0.015**
Bianco Nostrale *vs* Bologna	1.44	0.101
Andriolo vs Abbondanza	0.59	0.848
Andriolo vs Sieve	2.10	**0.027**
Andriolo vs Bologna	1.55	0.096
Abbondanza vs Sieve	2.18	**0.011**
Abbondanza vs Bologna	1.55	**0.048**
Sieve vs Bologna	1.60	0.082
Y #		
Bianco Nostrale 2020 *vs* 2021	3.70	**0.001**
Andriolo 2020 *vs* 2021	3.28	**0.001**
Abbondanza 2020 *vs* 2021	4.07	**0.002**
Sieve 2020 *vs* 2021	2.73	**0.001**
Bologna 2020 *vs* 2021	1.90	**0.012**

† Pairwise comparisons between genotypes in 2020 and 2021 and between year of cultivation in each genotype.

‡ *P* values based on Monte-Carlo permutational test with 999 permutations.

§ Genotype: Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System) (Table S2).

Year of cultivation: 2020 and 2021. Growing season from January to July.

Table S7

Representative sequences of arbuscular mycorrhizal fungi (AMF) found in the roots of the five wheat genotypes inoculated and not inoculated with a local AMF consortium. The representaive sequences were obtained from two plant growth stages: two-leaves unfolded stage (GS12; Zadoks et al., 1974) and physiological maturity (GS90). Accession number of the retrieved AM fungal representative sequences, with the correponding virtual taxon (VTX) (following the BLAST against the MaarjAM database; Öpik et al., 2010), AM fungal family, taxon and the accession number of the representative sequence of the VTX.

Representative sequence (RS)	Accession number	VTX	Family	Taxon	Representative sequence in MaarjAM database
OTU ABN1-3.9999 [†]	OR978408	VTX00009	Archaeosporaceae	*Archaeospora* sp.	AJ854098
OTU ABM1-1.177	OR978407	VTX00030	Acaulosporaceae	*Acaulospora* sp.	AF074346
OTU ABM2-3.7559	OR978418	VTX00052	Gigasporaceae	*Scutellospora* sp./*Gigaspora* sp. [‡]	AJ496115
OTU SIN1-3.56268	OR978411	VTX00062	Diversisporaceae	*Diversispora* sp.	AM849266
OTU ADM1-2.15105	OR978438	VTX00063	Glomeraceae	*Septoglomus viscosum / Septoglomus* sp.	AJ505812
OTU ABM1-2.983	OR978436	VTX00064	Glomeraceae	*Glomus* sp. / *Septoglomus* sp.	AM849308
OTU ABM1-1.3	OR978416	VTX00065	Glomeraceae	*Funneliformis caledonium* / *Funneliformis* sp.	Y17635
OTU ABM2-2.4548	OR978417	VTX00067	Glomeraceae	*Funneliformis mosseae*/ *Funneliformis* sp.	AJ306438
OTU ABM1-3.1702	OR978432	VTX00072	Glomeraceae	*Glomus* sp. / *Rhizophagus* sp.	AM849312
OTU ABN2-1.11065	OR978423	VTX00098	Glomeraceae	*Glomus* sp.	AB325992
OTU ABN1-1.7706	OR978422	VTX00103	Glomeraceae	*Glomus* sp.	EU350053
OTU ABM1-1.209	OR978431	VTX00105	Glomeraceae	*Rhizophagus irregularis / Rizhophagus* sp.	AJ505617
OTU BIN2-1.35057	OR978434	VTX00113	Glomeraceae	*Glomus* sp. / *Rhizophagus* sp.	AJ418876
OTU ABM2-1.3129	OR978420	VTX00143	Glomeraceae	*Glomus* sp.	AM849290
OTU ADN2-3.24761	OR978425	VTX00153	Glomeraceae	*Glomus* sp.	AJ496108
OTU SIN2-1.57526	OR978427	VTX00154	Glomeraceae	*Glomus* sp.	DQ396751
OTU ADN2-2.23781	OR978414	VTX00191	Glomeraceae	*Glomus* sp. / *Entrophospora* sp.	AM849300
OTU ABM1-1.12	OR978412	VTX00193	Entrophosporaceae	*Entrophospora lamellosum* / *Entrophospora* sp.	AJ276087
OTU SIN1-3.56251	OR978426	VTX00199	Glomeraceae	*Glomus* sp.	AM849311
OTU ABN2-1.11207	OR978424	VTX00202	Glomeraceae	*Glomus* sp.	AJ563889
OTU ABM1-2.677	OR978430	VTX00214	Glomeraceae	*Glomus* sp. / *Rhizophagus* sp.	AF074370
OTU ABM1-1.25	OR978413	VTX00056	Entrophosporaceae	*Entrophospora* sp.	AY916419
OTU BIM1-3.28760	OR978429	VTX00238	Paraglomeraceae	*Paraglomus occultum*	AJ276081
OTU ABM1-1.96	OR978419	VTX00342	Glomeraceae	*Glomus* sp.	FN429114
OTU ADM1-3.16371	OR978409	VTX00354	Diversisporaceae	*Diversispora* sp.	GU238355
OTU BIN2-3.37753	OR978415	VTX00357	Entrophosporaceae	*Entrophospora* sp.	HE576881
OTU ABN1-1.7697	OR978433	VTX00363	Glomeraceae	*Glomus* sp. / *Rhizophagus* sp.	FR821540
OTU ABN1-3.10107	OR978439	VTX00365	Glomeraceae	*Glomus* sp.	HM215924
OTU SIM1-2.48796	OR978410	VTX00380	Diversisporaceae	*Diversispora* sp.	FN869704
OTU ABM1-1.58	OR978435	VTX00392	Glomeraceae	*Glomus* sp. / *Septoglomus* sp.	HM215654
OTU ABM2-1.2631	OR978437	VTX00409	Glomeraceae	*Glomus* sp. / *Septoglomus* sp.	HE615074
OTU ABM2-3.6941	OR978421	VTX00419	Glomeraceae	*Glomus* sp.	EU340305
OTU ABM1-2.576	OR978428	VTX00446	Paraglomeraceae	*Paraglomus* sp.	LT981603

[†] Unique code identifier e.g. OTU ABN1-3.9999 (OTU: operational taxonomic unit; AB: Abbondanza wheat genotype; N: mock inoculated, control; 1: GS12; 3: replicate plot; 9999: read number). Wheat genotypes: Bianco Nostrale, BI; Andriolo, AN,; Abbondanza AB; Sieve, SI (old not dwarf genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity). Inoculation treatments: N, mock inoculated, control; M, inoculated with 14 AMF species originating from a local agricultural site (Pellegrino and Bedini, 2014).

[‡] 1st taxon assigned following to the BLAST against the MaarjAM database; 2nd taxon: refined using the Neighbor Joining tree built with the newly generated RS and the most similar sequences obtained from the BLAST in the National Center for Biotechnology Information (NCBI).

Table S8

P-values of three-way ANOVAs on the effect of wheat genotype (G), arbuscular mycorrhizal fungal (AMF) inoculation (Inoc), growth stage (GS) and their interactions on the richness, Shannon index (*H'*) and Simpson index (λ) of AM fungi in the roots of wheat sampled in 2021.

Factor [†]	Richness	*H'*	**λ**
G [‡]	**0.012** [§]	0.170	0.344
Inoc [#]	0.363	0.180	0.126
GS [††]	1,000	0.103	0.303
G x Inoc	0.882	0.555	0.444
G x GS	0.387	0.995	0.991
Inoc x GS	**0.011**	**0.027**	**0.016**
G x Inoc xGS	0.313	0.261	0.465

[†] G, Inoc and GS used as fixed factor and three replicate plots per treatment.

[‡] Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System) (Table S2).

[§] In bold statistically significant values ($P \leq 0.05$).

[#] Inoculum consisting of 14 AMF species originating from a local agricultural site (Pellegrino and Bedini, 2014).

[††] GS: two-leaves unfolded stage (GS12; Zadoks et al., 1974) and physiological maturity (GS90).

Table S9

Results of permutational analyses of variance (PERMANOVAs) and variation partitioning of the effect of wheat genotype (G), arbuscular mycorrhizal fungal (AMF) inoculation (Inoc), growth stage (GS) and their interactions on the AMF community structure in the roots of wheat sampled in 2021. Results of the distance-based test for homogeneity of multivariate dispersions (PERMDIP) to check the dispersion among groups (β-diversity).

Factor †	df	Pseudo-*F*	*P*(perm)	Variance explained
G ‡	4	1.89	**0.004** §	**3.81** §
Inoc #	1	4.60	**0.001**	**6.16**
GS ††	1	14.83	**0.001**	**23.68**
G x Inoc	4	1.90	**0.007**	**7.71**
G x GS	4	1.72	**0.010**	**6.13**
Inoc x GS	1	1.48	0.166	1.64
G x Inoc x GS	4	0.97	0.493	-0.50
PERMDISP ‡‡				
G			0.232	
Inoc			**0.001**	
GS			**0.001**	

† PERMANOVA was performed following a permutation method based on an unrestricted permutation of raw data (Monte Carlo test based on 999 permutations) with G, Inoc and GS used as fixed factor factors and three replicate plots per treatment.

‡ Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System) (Table S2).

§ In bold statistically significant values ($P \leq 0.05$) and the corresponding explained variance.

Inoculum consisting of 14 AMF species originating from a local agricultural site (Pellegrino and Bedini, 2014).

†† GS: two-leaves unfolded stage (GS12; Zadoks et al., 1974) and physiological maturity (GS90).

‡‡ PERMDISP based on 999 permutations.

Table S10

Results of permutational multivariate analysis of variance (PERMANOVA) pairwise comparisons of the significant effect of wheat genotype (G) and arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) on the AMF community structure within the roots sampled in 2021.

Pairs (Genotype & Inoc) [†]		
G: -M	Pseudo-*F*	*P*(perm) [‡]
Bianco Nostrale [§] *vs* Andriolo	1.26	0.124
Bianco Nostrale *vs* Abbondanza	1.29	0.145
Bianco Nostrale *vs* Sieve	1.59	**0.034**
Bianco Nostrale *vs* Bologna	1.13	0.274
Andriolo vs Abbondanza	1.23	0.180
Andriolo vs Sieve	1.71	**0.019**
Andriolo vs Bologna	1.58	**0.012**
Abbondanza vs Sieve	1.15	0.254
Abbondanza vs Bologna	1.59	**0.021**
Sieve vs Bologna	1.99	**0.001**
G: +M		
Bianco Nostrale *vs* Andriolo	1.24	0.167
Bianco Nostrale *vs* Abbondanza	1.48	**0.030**
Bianco Nostrale *vs* Sieve	1.25	0.128
Bianco Nostrale *vs* Bologna	1.58	**0.037**
Andriolo vs Abbondanza	1.19	0.180
Andriolo vs Sieve	1.14	0.188
Andriolo vs Bologna	1.33	0.080
Abbondanza vs Sieve	0.78	0.794
Abbondanza vs Bologna	1.08	0.326
Sieve vs Bologna	0.94	0.520
Inoc [#]		
Bianco Nostrale +M *vs* -M	1.11	0.306
Andriolo +M *vs* -M	1.29	0.120
Abbondanza +M *vs* -M	1.76	**0.007**
Sieve +M *vs* -M	1.67	**0.022**
Bologna +M *vs* -M	1.79	**0.004**

[†] Pairwise comparisons between genotypes in inoculated and not inoculated treatments (+M and -M, respectively) and between +M and -M in each genotype.

[‡] *P* values based on Monte-Carlo permutational test with 999 permutations.

[§] Genotype: Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of agricultural and food interest biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System) (see TableS2).

[#] Inoculun consisting of 14 AMF species originating from a local agricultural site (Pellegrino and Bedini, 2014).

Table S11

Results of permutational multivariate analysis of variance (PERMANOVA) pairwise comparisons of the significant effect of wheat genotype (G) and growth stage (GS) on the AMF community structure within the roots sampled in 2021.

Pairs (G & GS) [†]		
G: GS12	Pseudo-*F*	*P*(perm) [‡]
Bianco Nostrale [§] *vs* Andriolo	1.31	0.124
Bianco Nostrale *vs* Abbondanza	1.25	0.176
Bianco Nostrale *vs* Sieve	1.63	**0.021**
Bianco Nostrale *vs* Bologna	1.35	0.086
Andriolo vs Abbondanza	1.58	**0.003**
Andriolo vs Sieve	1.95	**0.001**
Andriolo vs Bologna	1.63	**0.013**
Abbondanza vs Sieve	1.26	0.143
Abbondanza vs Bologna	1.34	0.077
Sieve vs Bologna	1.86	**0.002**
G: GS90		
Bianco Nostrale *vs* Andriolo	1.32	0.084
Bianco Nostrale *vs* Abbondanza	0.99	0.436
Bianco Nostrale *vs* Sieve	0.82	0.701
Bianco Nostrale *vs* Bologna	1.39	**0.030**
Andriolo vs Abbondanza	0.51	0.996
Andriolo vs Sieve	0.79	0.840
Andriolo vs Bologna	1.27	0.072
Abbondanza vs Sieve	0.69	0.861
Abbondanza vs Bologna	1.08	0.292
Sieve vs Bologna	0.98	0.481
GS [#]		
Bianco Nostrale GS12 *vs* GS90	2.09	**0.008**
Andriolo GS12 vs GS90	2.17	**0.001**
Abbondanza GS12 vs GS90	2.09	**0.001**
Sieve GS12 vs GS90	2.25	**0.001**
Bologna GS12 vs GS90	1.74	**0.004**

[†] Pairwise comparisons between genotypes in GS12 and GS90 and between growth stage in each genotype.

[‡] *P* values based on Monte-Carlo permutational test with 999 permutations.

[§] Genotype: Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System) (see Table S2).

[#] GS: two-leaves unfolded stage (GS12; Zadoks et al., 1974) and physiological maturity (GS90).

Supplementary Figures

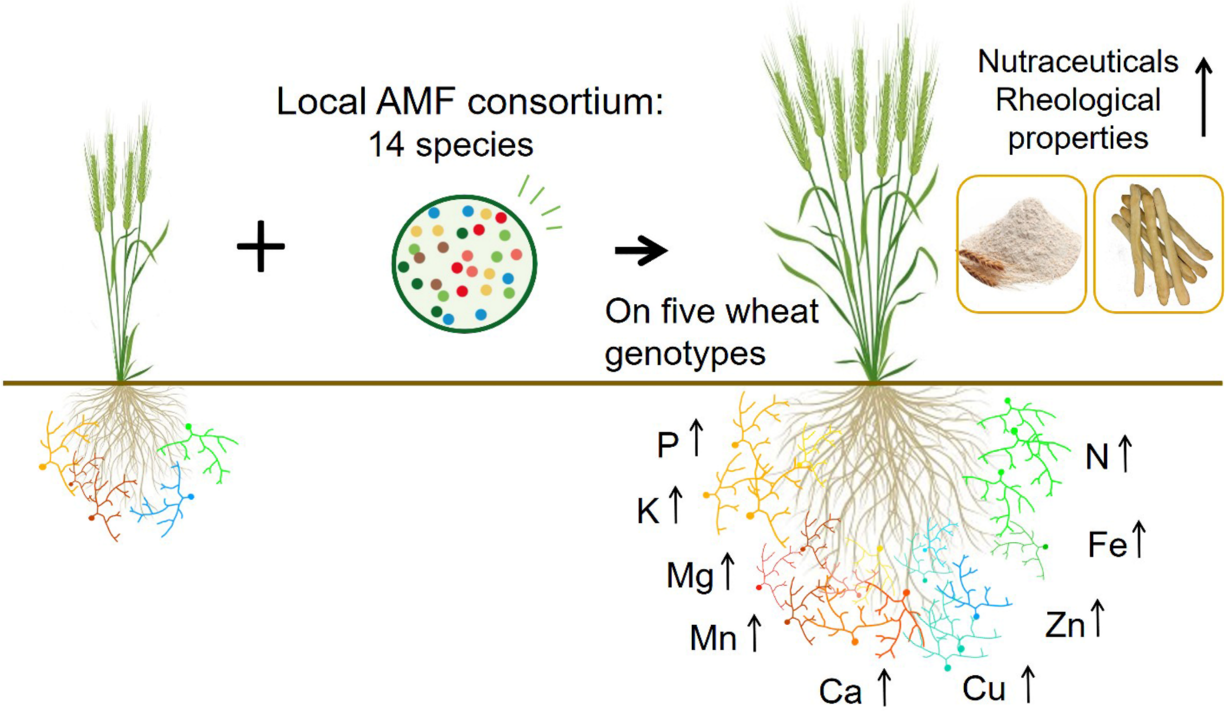


Fig. S1. Graphical scheme of the study we aimed to elucidate the effect of field inoculation of wheat with a indigenous arbuscular mycorrhizal fungal (AMF) consortium on grain yield, nutrient concentration and quality of processed products (i.e., flour and breadsticks). We hypothesized that AM fungal inoculation will not modify root AM fungal community composition, whereas it will highly modify the AMF root abundance and community structure with subsequent multiple beneficial effects for the host and for processed products. Field inoculation was carried out in two years of cultivations (2020 and 2021). Five bread wheat genotypes were inoculated with the AMF consortium: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site, and mock-inoculum as control.

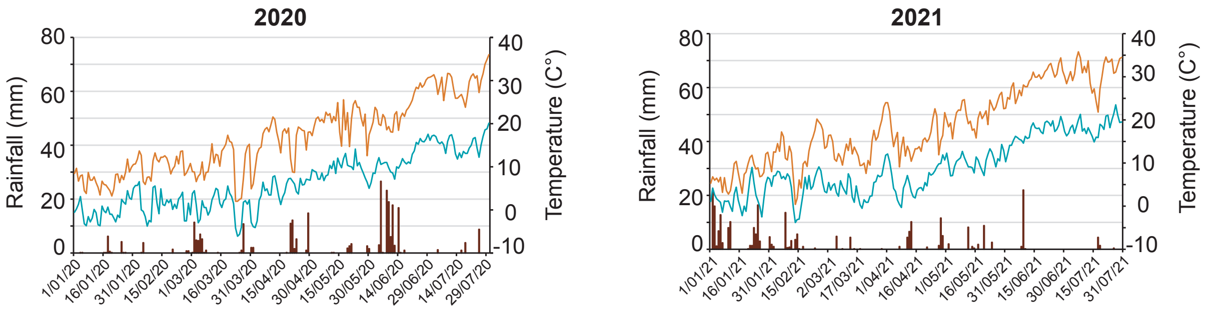


Fig. S2. Daily rainfall data and air maximum and minimum temperature during the growth cycle of wheat in 2020 and 2021 at the experimental site in the farm "Azienda Agricola Grappi Luchino", Pienza, Siena, Tuscany. The data were collected from a nearby weather station in Monticchiello, Pienza, Siena (43°03'57.6"N 11°43'30.0"E, 484 m above sea level).

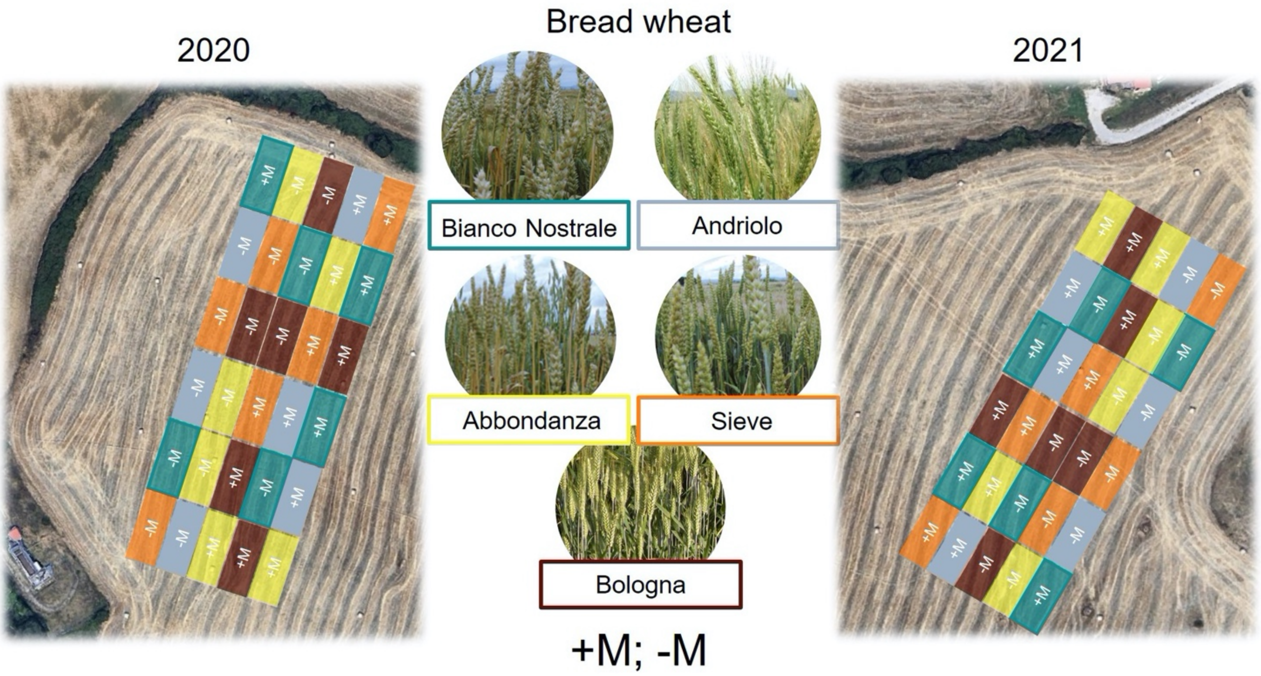


Fig. S3. Schematic overview of the experimental design testing the effect of year of cultivation (Y), wheat genotype (G) and arbuscular mycorrhizal fungal inoculation (Inoc). The experiment was a completely randomized design. The field experiment was conducted in 2020 and 2021 (from January to July), with three replicate plots (300 m^2 each). The field sites were located in Pienza, Siena (Italy). Five bread wheat genotypes were studied: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M) (Pellegrino and Bedini, 2014), and mock-inoculum as control (-M).

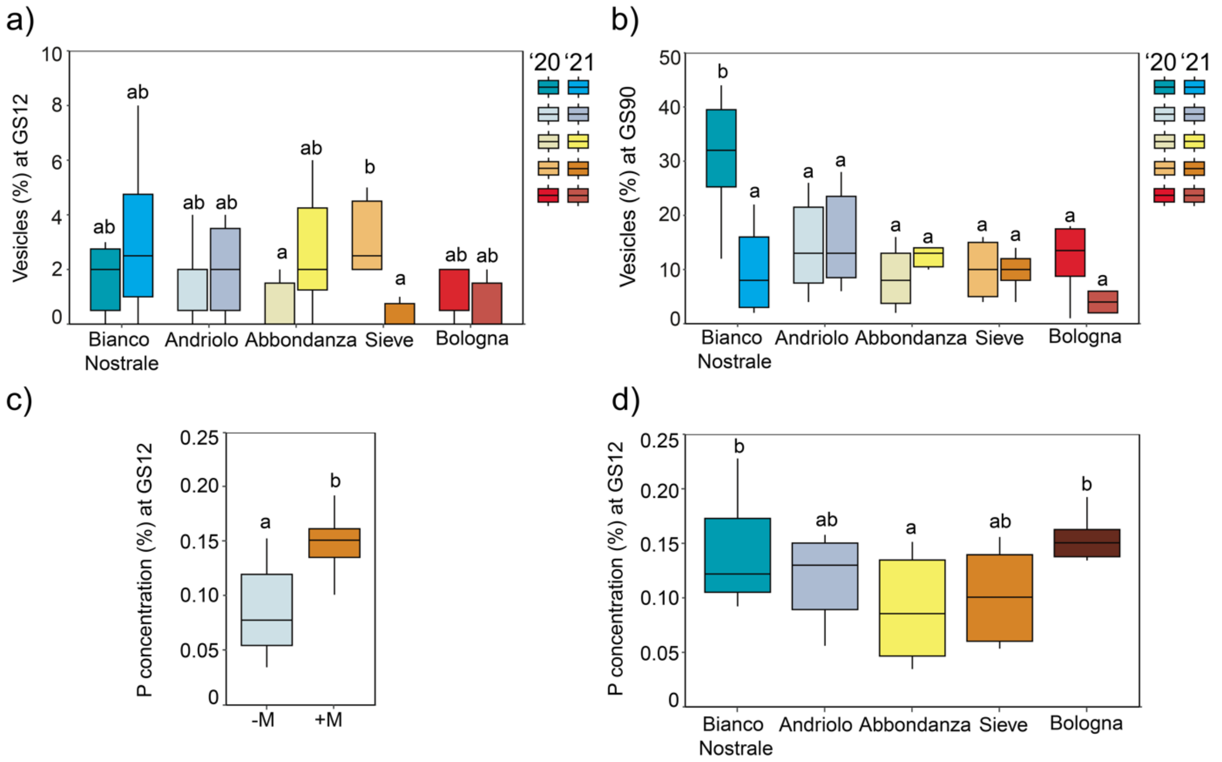


Fig. S4. Effect of the interactions between year of cultivation (Y) and wheat genotype (G) on the percentage of root length containing vesicles at two-leaves unfolded stage (GS12; Zadoks et al., 1974) (a) and at physiological maturity (GS90) (b) (Table S3). Effect of the arbuscular mycorrhizal fungal (AMF) inoculation and effect of G on phosphorus (P) concentration in the shoots of wheat at the GS12 (c,d) ($P<0.001$ and $P=0.015$, respectively). The years of cultivation were: 2020 ('20) and 2021 (21'). Five bread wheat genotypes were tested: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M), and mock-inoculum as control (-M). The output of the boxplots is based on three replicate plots for each treatment. Different letters highlight statistically significant differences according to Tukey-B-test.

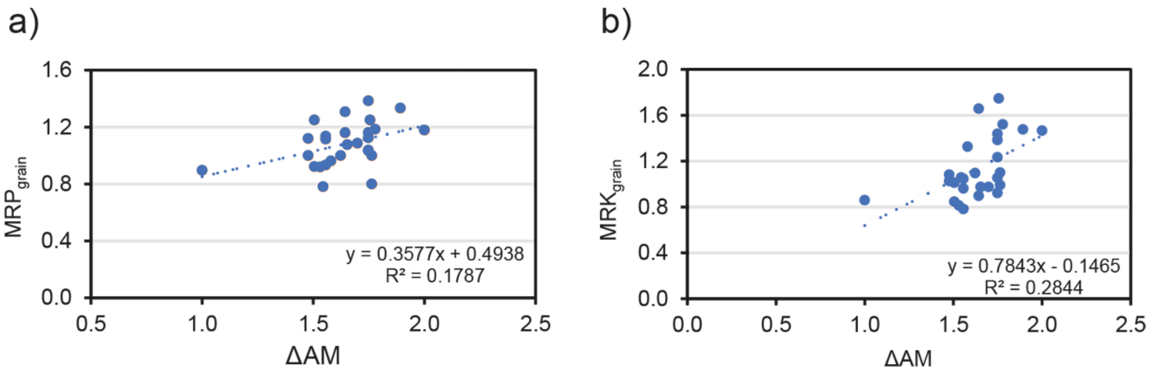


Fig. S5. Relationship between the change in mycorrhizal colonization (ΔAM) (ΔAM = AM_{+AMF} − $AM_{control}$ expressed as log_{10}) and the mycorrhizal response ratio for phosphorous (P) and potassium (K) concentration in grain (a and b, respectively) (MRP_{grain}= P Concentration in $grain_{+AMF}$/P Concentration in $grain_{control}$; MRK_{grain}= K Concentration in $grain_{+AMF}$/K Concentration in $grain_{control}$) (P: R^2=0.179, *P*=0.028; K: R^2=0.284, *P*=0.004). The equation of the relationships is reported in the figures.

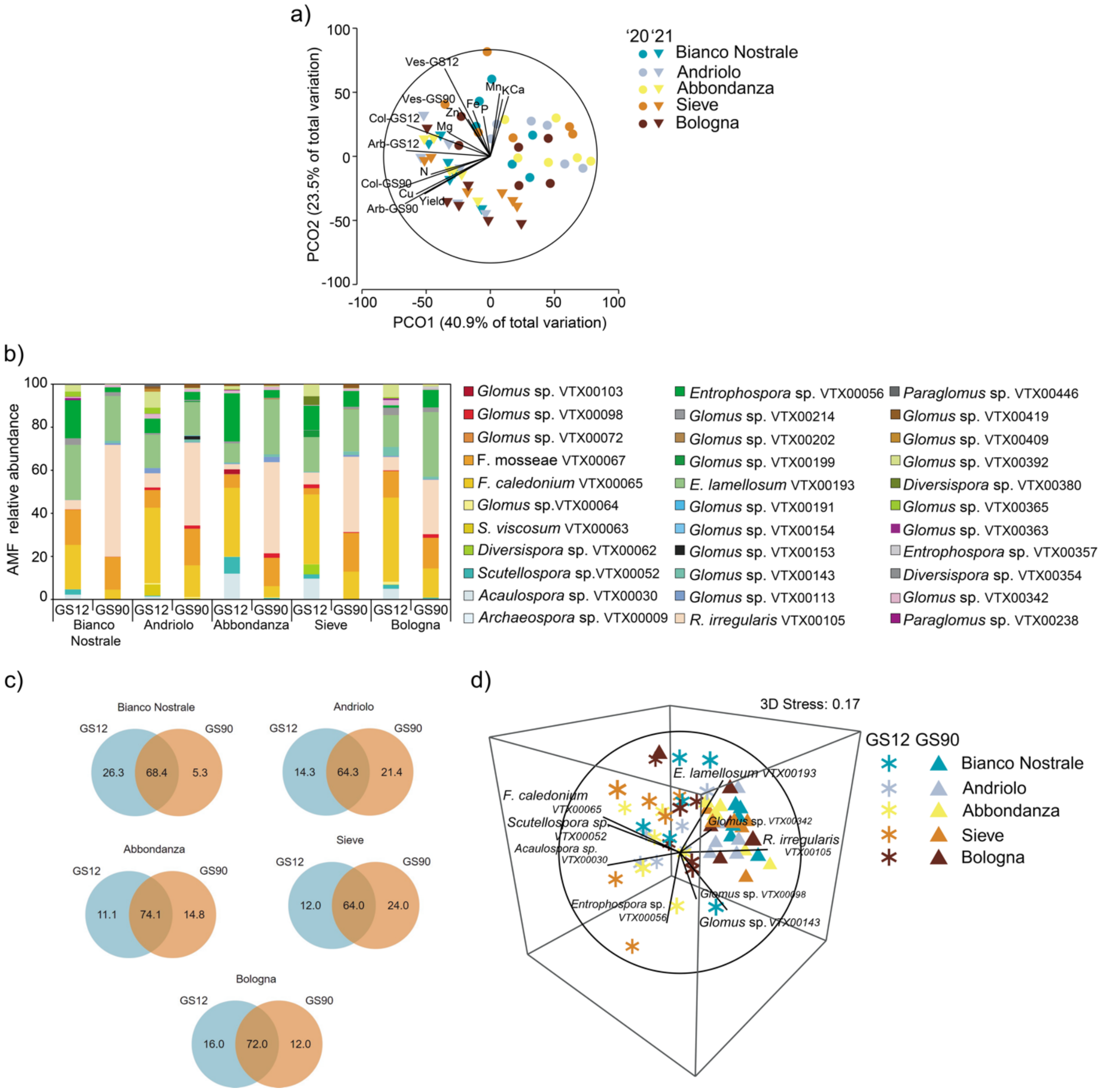
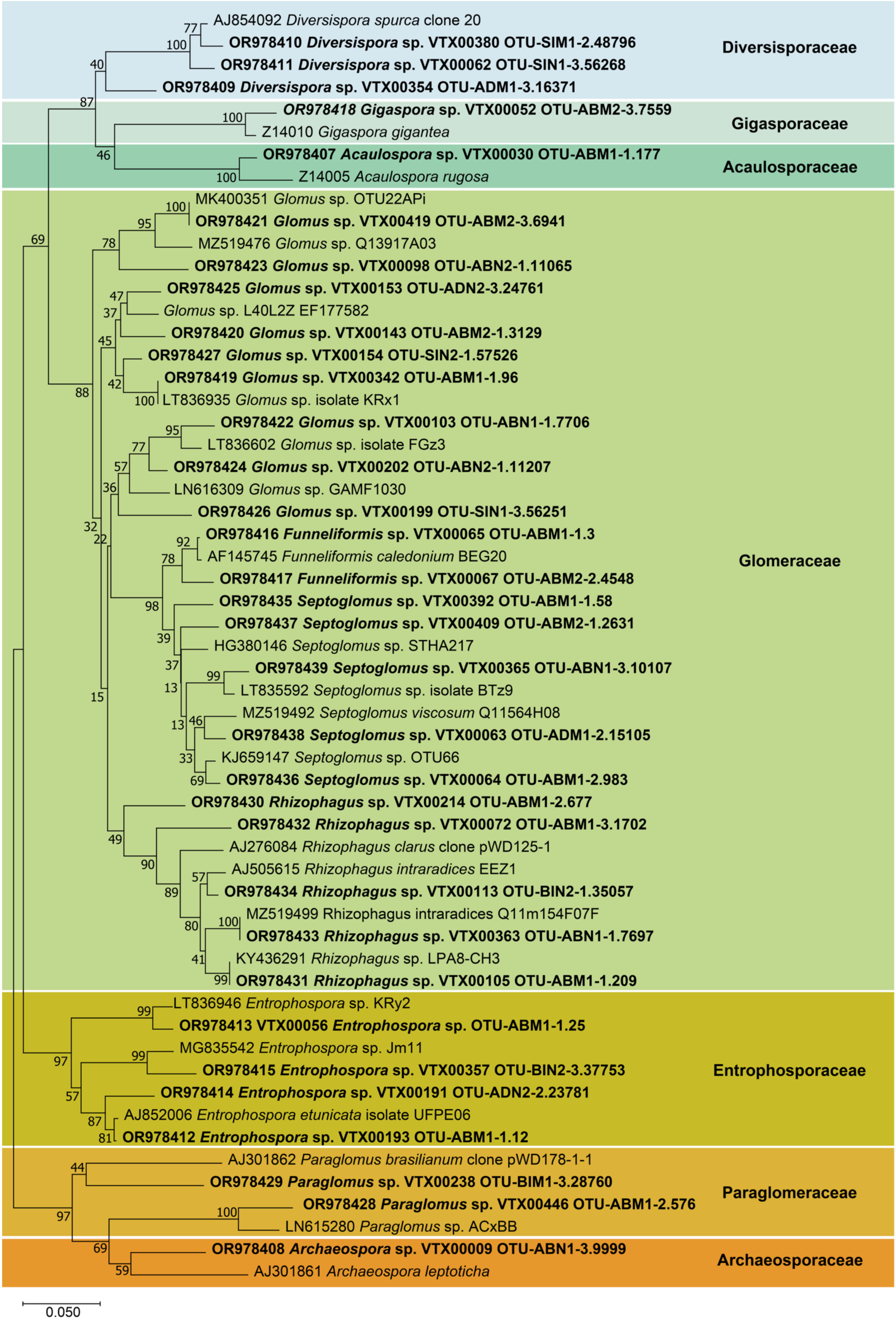


Fig. S6. Principal coordinate analysis (PCO) biplot (a) on the significant effect of year of cultivation and wheat genotype on plant and arbuscular mycorrhizal fungal (AMF) functional parameters (Table S4). The plant parameters were: yield and nutrient concentration in grain (i.e., N, P, K, Mg, Ca, Zn, Fe, Mn, Cu). The AMF parameters were: AMF root colonization (Col), percentage of root length containing arbuscules (Arb) and percentage of root length containing vesicles (Ves) at the two-leaves unfolded stage (GS12; Zadoks et al., 1974); Col, Arb and Ves at the physiological maturity (GS90). Data were obtained from two years of cultivation: 2020 and 2021. Five bread wheat genotypes were tested: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany

Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M), and mock-inoculum as control (-M). The PCO is based on the Euclidian distance matrix of similarity calculated on the square-root and standardized parameters of three replicate plots for each treatment. Relative abundance (b) of the AM virtual taxa (VTX) found in the roots of the five wheat genotypes at GS12 and GS90. Venn diagrams (c) of the AM fungi (i.e., VTX) uniquely retrieved and shared between GS12 and GS90 in each wheat genotype (data are expressed as percentages). The relative abundances used in (b) and (c) are means of +M and -M obtained in the 2021 year of cultivation. Information about VTX is reported in Table S7. Three-dimensional non-metric multidimensional scaling (nMDS) plot (d) on the significant effect of the interaction between wheat genotype and growth stage on the AMF community structure in the roots of wheat (Table S9). The nMDS is based on the Bray-Curtis similarity matrix calculated on the fourth-root of the relative abundances of the AM fungi (i.e., VTX). In the plot the overlay of vectors is reported and only the AMF taxa with a strong correlation ($r > 0.6$) are displayed. The data matrix is built using the AMF abundances found in 2021.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AJ854092 Diversispora spurca clone 20
100
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40
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OR978409 Diversispora sp. VTX00354 OTU-ADM1-3.16371
Diversisporaceae
87
100
OR978418 Gigaspora sp. VTX00052 OTU-ABM2-3.7559
Z14010 Gigaspora gigantea
Gigasporaceae
46
OR978407 Acaulospora sp. VTX00030 OTU-ABM1-1.177
100
Z14005 Acaulospora rugosa
Acaulosporaceae
MK400351 Glomus sp. OTU22APi
100
OR978421 Glomus sp. VTX00419 OTU-ABM2-3.6941
95
69
78
MZ519476 Glomus sp. Q13917A03
OR978423 Glomus sp. VTX00098 OTU-ABN2-1.11065
OR978425 Glomus sp. VTX00153 OTU-ADN2-3.24761
47
37
Glomus sp. L40L2Z EF177582
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45
OR978427 Glomus sp. VTX00154 OTU-SIN2-1.57526
42
OR978419 Glomus sp. VTX00342 OTU-ABM1-1.96
88
100
LT836935 Glomus sp. isolate KRx1
OR978422 Glomus sp. VTX00103 OTU-ABN1-1.7706
95
77
LT836602 Glomus sp. isolate FGz3
57
OR978424 Glomus sp. VTX00202 OTU-ABN2-1.11207
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LN616309 Glomus sp. GAMF1030
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32
22
OR978416 Funneliformis sp. VTX00065 OTU-ABM1-1.3
92
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AF145745 Funneliformis caledonium BEG20
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Glomeraceae
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13
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13
46
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KJ659147 Septoglomus sp. OTU66
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OR978436 Septoglomus sp. VTX00064 OTU-ABM1-2.983
OR978430 Rhizophagus sp. VTX00214 OTU-ABM1-2.677
OR978432 Rhizophagus sp. VTX00072 OTU-ABM1-3.1702
49
AJ276084 Rhizophagus clarus clone pWD125-1
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AJ505615 Rhizophagus intraradices EEZ1
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OR978413 VTX00056 Entrophospora sp. OTU-ABM1-1.25
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97
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OR978428 Paraglomus sp. VTX00446 OTU-ABM1-2.576
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Paraglomeraceae
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LN615280 Paraglomus sp. ACxBB
OR978408 Archaeospora sp. VTX00009 OTU-ABN1-3.9999
69
Archaeosporaceae
59
AJ301861 Archaeospora leptoticha
0.050

Fig. S7. Neighbor-Joining (NJ) (Saitou and Nei, 1987) tree of the representative sequences of arbuscular mycorrhizal fungi (AMF; Glomeromycota) (operational taxonomic units, OTUs) retrieved within the roots of five wheat genotypes inoculated in 2021 by a indigenous consortium of AM fungi and mock inoculated. Roots were sampled at two growth stages (two-leaves unfolded stage, GS12 and physiological maturity GS90; Zadoks et al., 1974). The tree includes also the most similar AM fungal sequences from NCBI. The representative sequences were assigned to Virtual Taxa (VTX) by BLAST against the MaarjAM database (https://maarjam.botany.ut.ee) (a total of 33 AM fungal VTX belonging to seven families). The analysis involved 57 sequences and a total of 274 positions. The analysis was performed by 1000 bootstrap replicates (Felsenstein 1985) and the Kimura 2-parameter method (Kimura, 1980). Bootstrap values are shown. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021). The newly generated representative AM fungal sequences are in bold and the VTX code is reported together with the accession number and the ID code of the OTU (Table S7). All newly generated sequences were deposited in the NCBI Sequence Read (SRA) database as SUB14096345 (accession numbers from OR978407 to OR978439). The NJ tree is based on the sequences obtained from the amplification of the small subunit ribosomal RNA (SSU) fragments amplified using a nested PCR approach with two primer pairs (AML1/AML2 and WANDA-ill/AML2- ill) (Lee et al., 2008; Dumbrell et al., 2011).

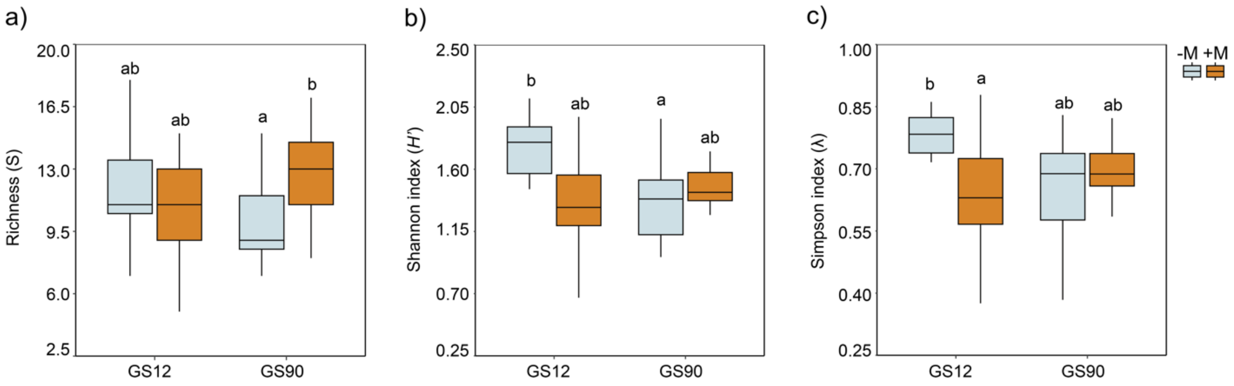

Fig. S8 Significant effect of the interaction between arbuscular mycorrhizal fungal (AMF) inoculation and growth stage on the richness, Shannon and Simpson indices of the AM fungal virtual taxa retrieved in roots of wheat (Table S8). Information about VTX is reported in Table S7. The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M), and mock-inoculum as control (-M). The plant growth stages are: at the two-leaves unfolded stage (GS12; Zadoks et al., 1974) and at the physiological maturity (GS90). The output of the boxplots is based on three replicate plots for each treatment. Different letters highlight statistically significant differences according to Tukey-B-test.

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