Title:

Organic agricultural practice enhances arbuscular mycorrhizal symbiosis in correspondence to soil warming and altered precipitation patterns

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Running title: AM fungi in agroecosystems under future climate change

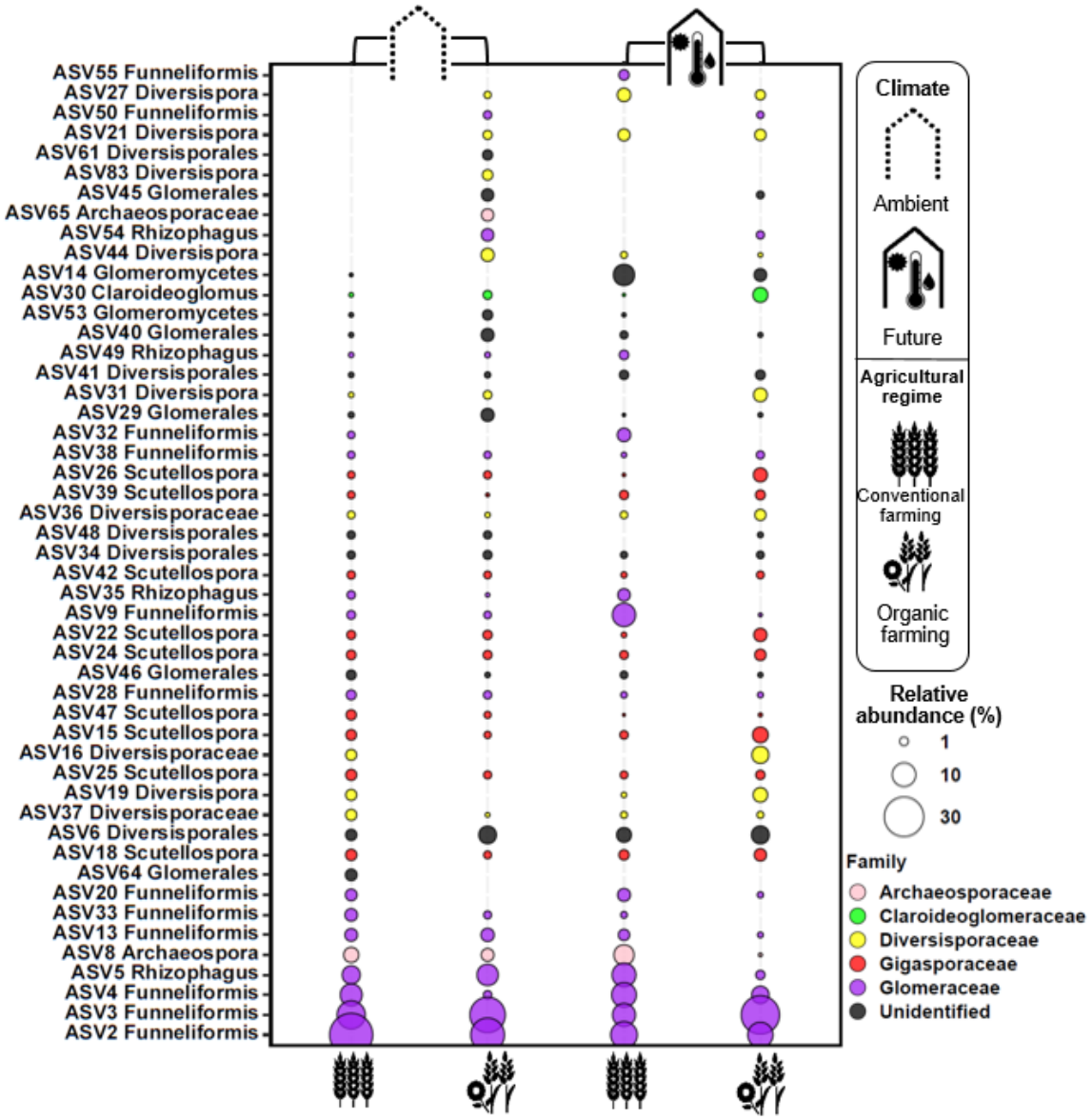

Figure S1. The most abundant amplicon sequence variants (ASVs) detected in conventional and organic farming plots under ambient and future climate scenarios. ASVs were selected if their relative abundance was greater than 0.01% in one sample at least.

Table S1. Results of split-plot-ANOVA of the impacts of climate, agricultural practice and their interactions on edaphic factors of the experimental plots. Significant effects are indicated in **bold** font (*P < 0.05, **P < 0.01, ***P <0.001). MOI = soil moisture in % (w/w), N-i = mineral inorganic-N (mg/Kg) (nitrate + ammonium), TOC = total organic carbon in %, TN = Total nitrogen (%) is the sum of NO_3-N, NO_2-N, NH_4-N and organically bound nitrogen, C = Hot water extractable organic carbon (mg/kg), N = Hot water extractable nitrogen (mg/kg), P = plant available phosphorous in mg/100g soil, K = plant available potassium in mg/100g soil.

Factors	MOI		temp		NH_4		NO_3	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Climate regime	**36.78**	**0.003 ****	0.22	0.660	0.09	0.770	7.13	0.05571
Agricultural practice	0.68	0.433	0.01	0.893	**35.82**	**0.0003 *****	**56.80**	**6.692e-05 *****
Climate regime X Agricultural practice	1.05	0.335	0.003	0.954	0.12	0.730	0.13	0.72103
Factors	**TOC**		**TN**		**TOC/TN**		**C**	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Climate regime	2.82	0.167	0.0031	0.958	3.73	0.125	0.0001	0.993
Agricultural practice	**21.98**	**0.001 ****	**12.01**	**0.008 ****	3.15	0.113	**40.61**	**0.0002 *****
Climate regime X Agricultural practice	0.75	0.408	2.11	0.184	0.03	0.866	5.008	0.055
Factors	**N**		**C/N**		**pH**		**P**	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Climate regime	0.06	0.805	0.60	0.481	6.39	0.064	**9.72**	**0.035 ***
Agricultural practice	**30.15**	**0.0005*****	**19.70**	**0.002 ****	0.94	0.358	0.91	0.367
Climate regime X Agricultural practice	2.35	0.163	1.03	0.339	1.35	0.277	1.15	0.313
Factors	**K**		**N-i**		**TC/P**		**TN/P**	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Climate regime	0.005	0.944	1.38	0.305	4.519	0.100	3.748	0.124
Agricultural practice	**26.87**	**0.0008 *****	**143.29**	**2.184e-06 *****	1.060	0.333	1.531	0.251
Climate regime X Agricultural practice	0.005	0.944	0.44	0.525	0.739	0.414	0.185	0.678

Table S2. Soil physicochemical variables. Mean values ± standard deviation. Based on the results of split-plot-ANOVA, different lower-case letters indicate significant differences according to Fisher's Least Significant Difference. MOI = soil moisture in % (w/w), Temp = soil temperature, N-i = mineral inorganic-N (mg/Kg) (nitrate + ammonium), TOC = total organic carbon in %, TN = Total nitrogen (%) is the sum of NO_3-N, NO_2-N, NH_4-N and organically bound nitrogen, C = Hot water extractable organic carbon (mg/kg), N = Hot water extractable nitrogen (mg/kg), P = plant available phosphorous in mg/100g soil, K = plant available potassium in mg/100g soil. A= ambient climate, F= future climate, CF= conventional farming, OF= organic farming. Analysis of the physicochemical properties of conventional and organic farming plots' soil revealed that all forms of inorganic (NH_4, NO_3) and organic N, total C, and plant-available K were significantly higher in conventionally managed soil. There were no detectable differences in plant-available soil P concentration between the two agricultural systems. Soil moisture, as a climate influenced factor, was significantly lower under future climate, while soil temperature did not differ significantly between the two climate regimes during our study period.

Climate	**CF**	**CF**	**OF**	**OF**
Agricultural practice	**A**	**F**	**A**	**F**
MOI	12.16±2.36 a	10.92±2.48 b	12.12±2.24 a	11.3±2 b
Temp	14.10±1.54 a	14.47±1.54 a	14.27±1.49 a	14.18±1.30 a
NH_4	9.42±7.17 a	8.46±9.19 a	2.23±0.79 b	2.15±0.64 b
NO_3	8.2±4.59 a	6.95±6.14 a	3.38±1.69 b	2.5±0.93 b
N-i	17.62±10.25 a	15.41±13.77 a	5.61±1.5 b	4.64±1.14 b
TOC	1.97±0.14 ab	2±0.14 a	1.83±0.14 c	1.89±0.14 bc
TN	0.16±0.02 a	0.15±0.02 ab	0.14±0.02 c	0.15±0.03 bc
TOC/TN	12.62±1.16	13.1±1.35	12.91±1.26	13.34±1.61
C	576.24±65.93 a	548.05±75.38 a	467.16±50.06 b	495.67±55.1 b
TC/P	0.365±0.15	0.286±0.12	0.402±0.22	0.310±0.181
TN/P	0.028±0.01	0.021±0.009	0.031±0.01	0.023±0.01
N	61.7±10.38 a	58.72±12.51 a	44.8±7.11 b	49.21±8.19 b
C/N	9.42±0.59 b	9.5±0.87 b	10.53±0.84 a	10.19±0.88 a
pH	6.77±0.48	6.84±0.53	6.54±0.6	6.85±0.52
P	6.61±3.49 b	8.44±3.61 a	5.88±3.11 b	8.49±4.09 a
K	15.8±8.84 a	16.29±4.96 a	7.02±2.38 b	6.19±1.42 b

Table S3. Impact of edaphic factors on variation in AMF community and richness. PERMANOVA for the correlation between AMF community composition (Bray–Curtis dissimilarity distance) and each edaphic variable. Spearman's rank correlation between AMF richness and each edaphic variable. MOI = soil moisture in % (w/w), Temp = soil temperature, N-i = mineral inorganic-N (mg/Kg) (nitrate + ammonium), TOC = total organic carbon in %, TN = Total nitrogen (%) is the sum of NO_3–N, NO_2–N, NH_4–N, and organically bound nitrogen, C = Hot water extractable organic carbon (mg/kg), N = Hot water extractable nitrogen (mg/kg), C.N = C/N, P = plant available phosphorous in mg/100 g soil, K = plant available potassium in mg/100 g soil.

Edaphic variables	Total arbuscular mycorrhizal community		Total ASV richness		Glomeraceae richness		Gigasporaceae richness		Diversisporaceae richness	
	r^2	*P*-value	Rho value	*P*-value	Rho value	*P*-value	Rho value	*P*-value	Rho value	*P*-value
MOI	**0.131**	**0.021**	-0.137	0.294	0.284	0.052	-0.279	0.057	-0.065	0.621
Temp	0.020	0.556	0.09	0.64	0.30	0.07	-0.14	0.42	0.12	0.51
NH_4	**0.202**	**0.003**	-0.224	0.084	**0.335**	**0.008**	**-0.387**	**0.002**	-0.270	0.072
NO_3	**0.102**	**0.047**	-0.156	0.231	0.245	0.059	-0.156	0.232	-0.187	0.152
N-i	**0.185**	**0.003**	-0.242	0.062	0.275	0.062	**-0.314**	**0.014**	-0.275	0.067
TOC	0.017	0.641	**-0.348**	**0.006**	-0.010	0.941	-0.157	0.229	-0.139	0.289
TN	0.019	0.563	**-0.296**	**0.021**	0.023	0.863	-0.077	0.560	-0.118	0.370
TOC/TN	0.016	0.609	**0.289**	**0.025**	0.055	0.676	-0.016	0.905	0.165	0.207
TC/P	**0.157**	**0.012**	**-0.29**	**0.03**	-0.01	0.87	**-0.38**	**0.01**	-0.04	0.86
N/P	**0.198**	**0.004**	-0.33	0.06	-0.03	0.78	-0.40	0.92	-0.03	0.32
C	0.019	0.586	-0.071	0.587	0.174	0.183	0.066	0.614	-0.117	0.374
N	0.016	0.612	-0.007	0.957	0.141	0.283	0.174	0.183	-0.164	0.209
C/N	0.029	0.423	-0.086	0.510	-0.083	0.528	**-0.299**	**0.020**	0.235	0.070
pH	**0.108**	**0.029**	0.222	0.087	-0.036	0.784	**0.444**	**0.0003**	-0.023	0.862
P	**0.192**	**0.003**	0.260	0.076	0.012	0.924	**0.375**	**0.003**	0.054	0.682
K	**0.249**	**0.001**	-0.217	0.094	0.243	0.061	-0.246	0.058	**-0.316**	**0.013**

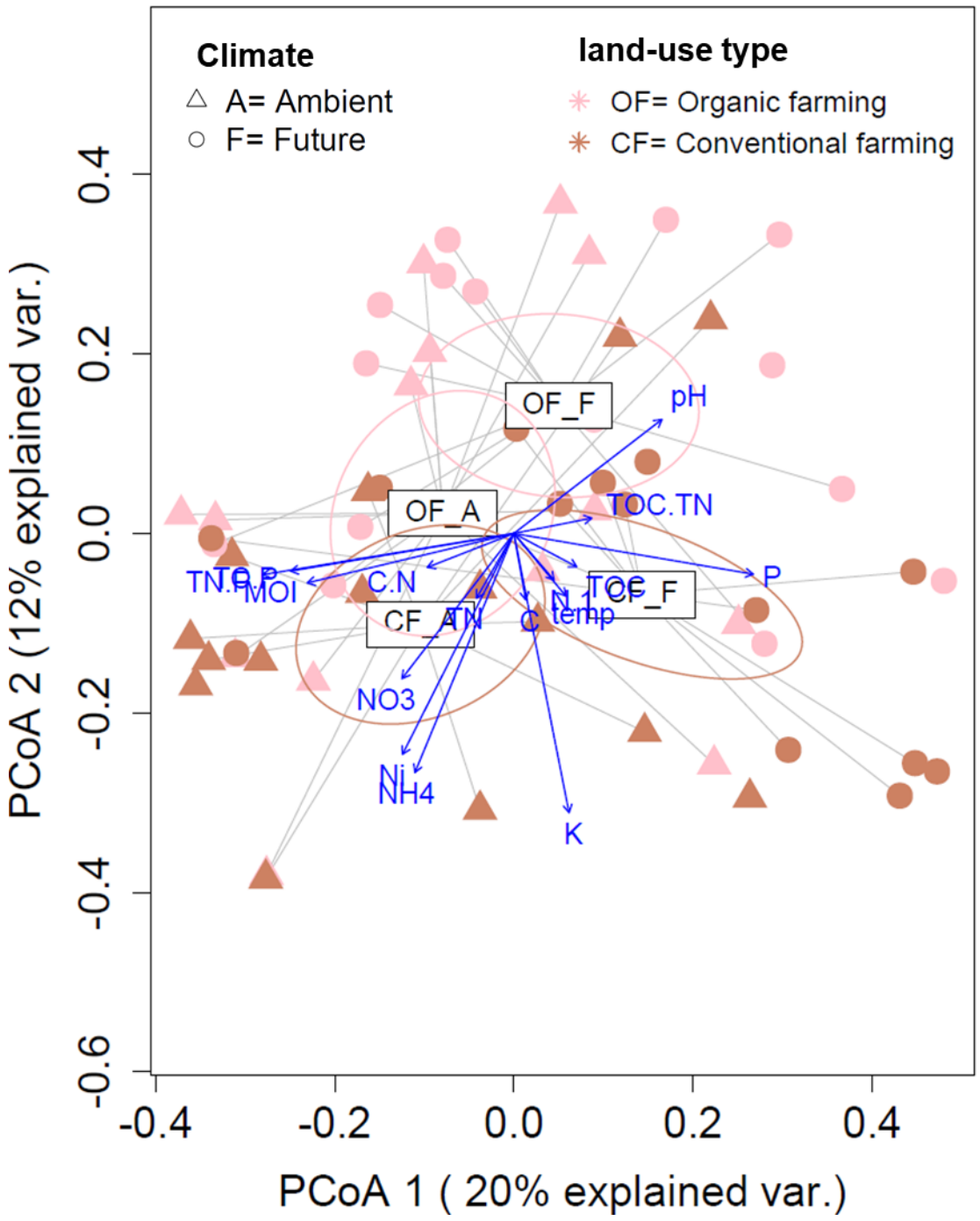

Figure S2. Principal Coordinate Analysis (PCoA) dissimilarity matrix (Bray–Curtis dissimilarity matrix, permutations = 999) of the AMF community of wheat roots and vector fitting of the edaphic variables of plots subjected to conventional and organic farming practices under ambient and future climate regimes.

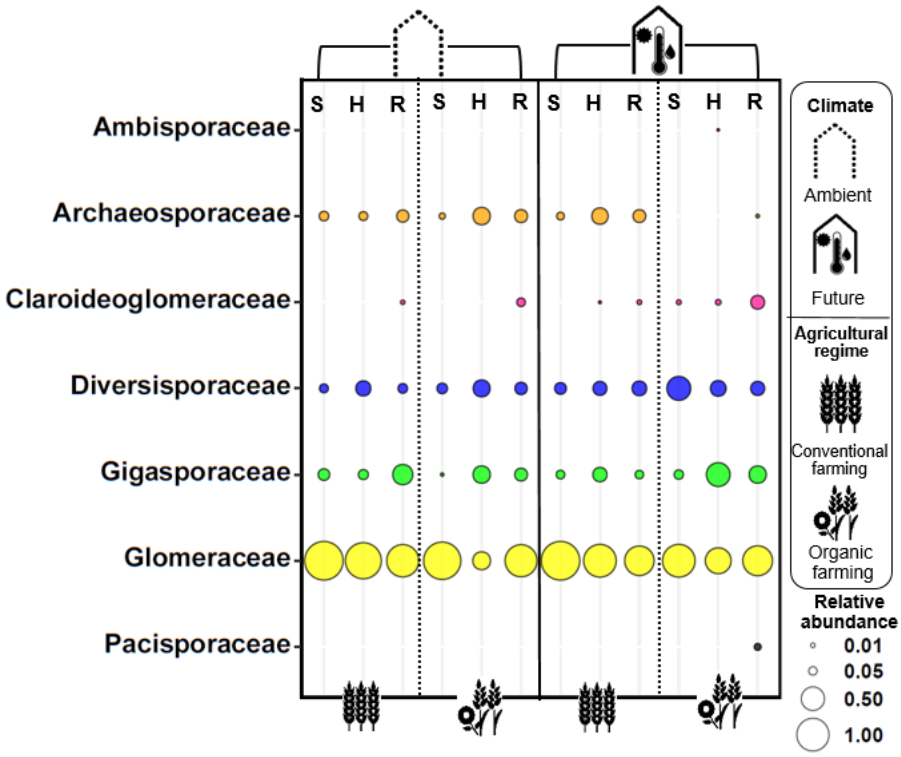

Figure S3. The abundance of all families detected in conventional and organic farming plots under ambient and future climate scenarios. Plant growth stages; S= rosette, H= heading, R= ripening.

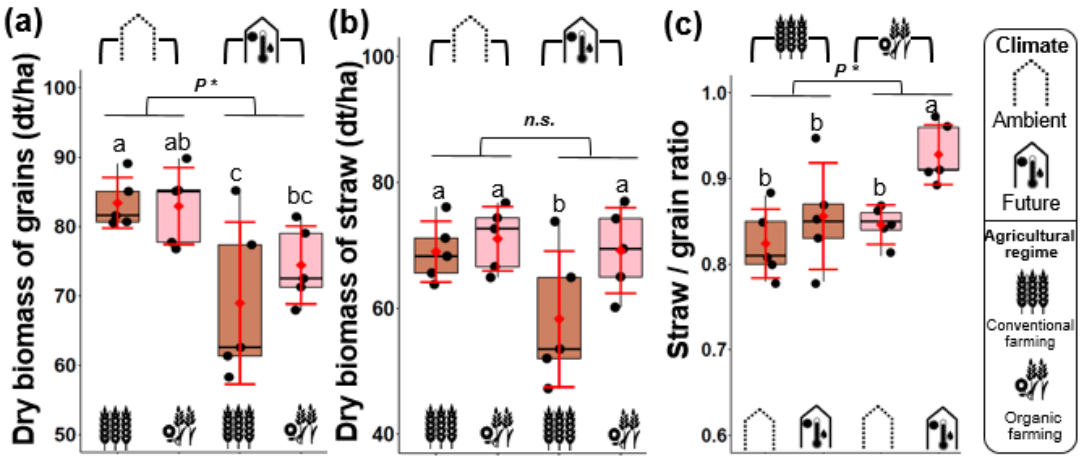

Figure S4. Wheat yield parameters **a** grain dry biomass, **b** straw dry biomass and **c** straw/ grain ratio in conventional and organic farming systems under ambient and future climate conditions. Different lower-case letters indicate significant differences according to Fisher's Least Significant Difference. Error bars are representing standard deviation, ♦ represent mean values. Grain yield was significantly reduced under the future climate scenario (~72 dt/ha) compared to the ambient climate (~83 dt/ha). This reduction under future climate was mainly caused by grain yield reduction under conventional farming, in which the yield significantly dropped by ~17 % as compared to the ambient climate. In contrast, the grain yield from organic farming plots did not differ significantly between ambient and future climate. Although the mean dry matter of wheat straw was not influenced by the agricultural practice or climate manipulation, organic farming significantly increased the straw/grain ratio.

Table S4. Results of split-plot-ANOVA of the impacts of climate, agricultural practice and their interactions on wheat yield. Significant effects are indicated in **bold** font (*P < 0.05, **P < 0.01, ***P <0.001).

Factors	Grain		Straw		Straw/grain ratio	
	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Climate regime	**8.70**	**0.041***	2.24	0.208	5.64	0.076
Agricultural practice	0.87	0.376	5.31	*0.0501*	**7.83**	**0.023***
Climate regime X Agricultural practice	1.22	0.301	2.46	0.155	1.50	0.254

Table S5. Results of split-plot-ANOVA of the impacts of climate, agricultural practice and their interactions on nutrient concentrations of harvested wheat grains. Significant effects are indicated in **bold** font (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Factors	C		N		P	
	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Climate regime	2.03	0.226	1.74	0.257	0.31	0.603
Agricultural practice	**11.02**	**0.010 ***	**35.51**	**0.0003 *****	**6.51**	**0.034 ***
Climate regime X Agricultural practice	1.25	0.294	0.52	0.491	**8.75**	**0.018 ***

Factors	K		Mg		Ca	
	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Climate regime	0.74	0.438	2.58	0.183	2.10	0.220
Agricultural practice	**35.97**	**0.0003 *****	0.60	0.459	4.58	0.064
Climate regime X Agricultural practice	**7.72**	**0.023 ***	5.22	0.051	0.17	0.687

Factors	S		Na		Mn		Fe	
	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Climate regime	3.19	0.148	0.08	0.782	1.31	0.314	**48.13**	**0.002 ****
Agricultural practice	**10.04**	**0.013***	0.001	0.974	**9.23**	**0.016***	0.01	0.906
Climate regime X Agricultural practice	0.57	0.468	0.86	0.380	0.88	0.373	0.05	0.818

Table S6. Nutrients concentrations of wheat grains. Mean values (mg^kg^{-1}) ± standard deviation. Based on the results of split-plot-ANOVA, different lower-case letters indicate significant differences according to Fisher's Least Significant Difference. A= ambient climate, F= future climate, CF= conventional farming, OF= organic farming. Only Fe concentration was significantly higher under future climate than under ambient climate. Significantly higher concentrations of C, N, S, and Mn in wheat grains were detected under conventional farming as compared to organic farming. Interactive effects of both climate and agricultural practice were detected in case of P and K. The highest concentrations of P were detected only in organic farming plots under future climate. Additionally, application of organic farming under future climate conditions increased the concentration of K to resemble that in the conventional farming system.

Climate	Ambient		Future	
Agricultural practice	CF	OF	CF	OF
C (mg^g-1)	436.7±0.8 ab	436±0.5 b	437.5±1.1 a	436.2±0.4 b
N (mg^g-1)	23.4±0.4 a	21.5±0.9 b	24.1±1.1 a	21.6±0.9 b
P (mg^kg-1)	3215.3±205.8 b	3197.9±128 b	3134.7±230.7 b	3370.7±227.3 a
K (mg^kg-1)	4432.5±163 a	4026±73.4 c	4396±138.2 ab	4246.9±205.5 b
Mg (mg^kg-1)	1082.7±39 ab	1056.7±59.4 b	1077.8±38.2 ab	1130.6±58.3 a
Ca (mg^kg-1)	448.4±15.5 a	417.7±56 ab	425.5±12.8 ab	404.8±14.6 b
S (mg^kg-1)	1522.6±11.7 ab	1467.7±71.1 b	1586.4±29.3 a	1496.8±89 b
Na (mg^kg-1)	16.4±5.9 a	14.6±5.2 a	13.5±3.8 a	15.1±6.9 a
Mn (mg^kg-1)	33.2±0.8 a	30.1±3.2 b	33.9±3.1 a	32.2±3 ab
Fe (mg^kg-1)	38.6±1.3 bc	38.4±2.7 c	41.9±2.1 ab	42.3±3.9 a

Figure S5. Aerial view for the *Global Change Experimental Facility* (*GCEF*) field research station of the Helmholtz Centre for Environmental Research in Bad Lauchstädt, Saxony-Anhalt, Germany, photo taken by Tricklabor Berlin/Service Drone.

Figure S6. Closed shelters and panels of the future climate plots of the *GCEF*, photo taken by UFZ/ André Künzelmann

DNA extraction and amplification, Illumina library preparation, and MiSeq sequencing

Fine roots were homogenised with the aid of liquid nitrogen and 0.1 g of the ground material was used for DNA extraction using a DNeasy Plant Mini kit (QIAGEN-MO BIO, Carlsbad, California, USA) according to the manufacturer's instructions. The quantity and quality of the DNA extracts were assessed using NanoDrop ND-8000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany). Genomic DNA was amplified using nested polymerase chain reaction (PCR). The first reaction was performed with GlomerWT0 (5′-CGAGDWTCATTCAAATTTCTGCCC-3′) (Wubet et al., 2006) and Glomer1536 (5′-AATARTTGCAATGCTCTATCCCCA-3′) primer pair (Wubet et al., 2006; Morgan and Egerton-Warburton, 2017). The second reaction [amplification of a 560 bp fragment of the small subunit (SSU/18S) of the rRNA gene region] was performed using NS31 (5′-TTGGAGGGCAAGTCTGGTGCC-3′) (Simon et al., 1992; Morgan and Egerton-Warburton, 2017) and AML2 (5′-GAACCCAAACACTTTGGTTTCC-3′) primers (Lee et al., 2008). All PCRs were conducted using the proofreading Kapa HiFi polymerase (2X KAPA HiFi HotStart ReadyMix, Kapa Biosystems, Boston, MA, United States). The first reaction was carried out in a final volume of 15 μL with 7.5 μL 2X KAPA HiFi HotStart ReadyMix, 0.3 μL of each primer (10 μM), and 1 μL DNA template (10 ng/μL). The PCR program was as follows: 95°C for 3 min, then 5 cycles of 98°C for 20 sec, 60°C (decreased by 1°C in each cycle) for 20 sec, 72°C for 40 sec, then 22 cycles of 98°C for 20 sec, 55°C for 20 sec, 72°C for 40 sec followed by one cycle of 72°C for 5 min and 10°C hold. Resultant products of the first amplification were diluted 1:10 and then used as a template in the second PCR that was carried out in a final volume of 15 μL with 7.5 μL 2X KAPA HiFi HotStart ReadyMix, 0.3 μL of each primer (10 μM), and 1 μL PCR product as template. The reaction conditions were as follows: 95°C for 3 min, then 30 cycles of 98°C for 20 sec, 63°C for 15 sec, 72°C for 20 sec followed by one cycle of 72°C for 5 min and 10°C hold. The PCR product was purified using Agencourt AMPure® XP beads (Beckman Coulter Inc., Indianapolis, IN, USA), followed by indexing of the purified amplicons using Nextera index kit (Illumina, San Diego, CA, USA). The indexed PCR products were purified again using the same beads. The amplicon libraries were quantified by PicoGreen assays (Molecular Probes, Eugene, OR, United States) and pooled in one tube to give equimolar representation of each. Paired-end sequencing of 2 × 300 bp was performed using MiSeq Reagent kit v3 on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, United States) at the Department of Soil Ecology, Helmholtz Centre of Environmental Research [UFZ, Halle (Saale), Germany]. The raw 18S rRNA gene amplicon sequences have been deposited in the Sequence Read Archive (SRA) operated by the National Center for Biotechnology Information (NCBI) under BioProject accession number: PRJNA678852