

Supplemental Data

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Table S1 The effect of elevated O₃ and cultivars on the soil and plant properties analyzed by ANOVA.

Soil or plant variable	O ₃		Cultivar		O ₃ x Cultivar	
	F value	P value	F value	P value	F value	P value
pH	6.814	0.0311	0.033	0.8612	0.061	0.8117
Microbial biomass carbon	0.047	0.8340	1.687	0.2300	3.355	0.1040
Dissolved organic carbon	15.215	0.0045	7.189	0.0279	1.439	0.2646
Total carbon (TC)	1.054	0.3350	1.419	0.2680	1.419	0.2680
Total nitrogen (TN)	0.299	0.5990	1.166	0.3120	0.454	0.5190
C/N ratio (CNR)	0.142	0.7160	0.273	0.6150	0.486	0.5060
TC-grain	15.949	0.0040	0.108	0.75068	2.121	0.1834
TN-grain	0.267	0.6190	1.902	0.2050	2.484	0.1540
CNR-grain	0.498	0.5000	3.159	0.1130	1.933	0.2020
TC-straw	6.211	0.0374	3.169	0.1129	0.014	0.9085
TN-straw	3.691	0.091	0.923	0.3650	2.700	0.1390
CNR-straw	3.032	0.1200	0.430	0.5300	2.810	0.1320
Panicle length	0.617	0.4550	0.137	0.7210	3.238	0.1100
Grain weight	12.110	0.0083	7.672	0.0243	0.000	0.9958
Plant weight	1.645	0.2360	0.888	0.3740	0.023	0.8840
Straw-root weight	2.740	0.1360	0.383	0.5530	1.567	0.2460

Table S2 The number of detected genes and their α -diversity, and evenness of rhizosphere microbial communities from Yannong 19 and Yangmai 16 wheat cultivars at aO₃ and eO₃ conditions.

	C-Y19	C-Y16	O-Y19	O-Y16
No. of detected genes	1901±224.5	2120±369.2	1998±203.3	2061±170.8
Archaea	70.1±10.1	76.3±17.1	76.7±11.4	76.3±6.7
Bacteria	1498±298.2	1674±324.1	1573±280.7	1612±219.7
Fungi	101.7±35.1	111±25.9	88.7±13.6	100.7±21.0
Shannon index (H')	7.26±0.235	7.35±0.197	7.24±0.149	7.32±0.132
Simpson's reciprocal index (1 / D)	1074±313.5	1177±199.5	985±155.9	1087±148.4
Simpson evenness	0.718±0.148 ^a	0.686±0.241 ^a	0.614±0.147 ^c	0.652±0.173 ^b

C-Y19: O₃-sensitive wheat cultivar at aO₃; C-Y16: O₃-relatively sensitive wheat cultivar at aO₃; O-Y19: O₃-sensitive wheat cultivar at eO₃; O-Y16: O₃-relatively sensitive at eO₃. The significance was tested by the Student t-test at $p < 0.05$.

Table S3 Significantly changed genes with O₃ and wheat cultivar analyzed by ANOVA.

Gene or enzyme	Functional process	O ₃		Cultivar		O ₃ :Cultivar	
		F value	P value	F value	P value	F value	P value
SMR	Antibiotic resistance	0.3109	0.5773	7.1749	0.0075	2.1681	0.1412
<i>fhs</i> /FTHFS	Acetogenesis	7.8243	0.0054	0.0052	0.9426	0.0508	0.8218
Exoglucanase	C degradation	0.1699	0.6806	5.2054	0.0236	0.0972	0.7555
<i>limEH</i>		3.3607	0.0774	4.7885	0.0372	0.5504	0.4643
<i>lip</i>		0.0275	0.8685	0.0317	0.8591	3.9740	0.0486
<i>aclB</i>	C fixation	0.7601	0.3962	4.5182	0.0495	0.1080	0.7467
CODH		0.2341	0.6287	7.2823	0.0073	3.5637	0.0598
<i>chrA</i>	Metal resistance	0.0548	0.8150	5.5815	0.0183	1.3816	0.2401
<i>corC</i>		7.6562	0.0071	0.0619	0.8042	0.1088	0.7425
<i>copA</i>		0.2675	0.6051	6.3048	0.0121	1.6811	0.1949
<i>nifH</i>	Nitrogen fixation	0.4097	0.5222	4.0418	0.0445	2.5435	0.1109
<i>pimF</i>	Organic remediation	0.2207	0.6386	4.4700	0.0347	3.7838	0.0520
<i>pobA</i>		1.5717	0.2108	9.3416	0.0024	1.7187	0.1907
<i>alkH</i>		0.0026	0.9960	0.7324	0.3936	5.0641	0.0260
<i>chnB</i>		0.0377	0.8463	1.6780	0.1976	4.4957	0.0360
ALN		2.6850	0.1399	5.8526	0.0419	0.5333	0.4860
<i>dsrA</i>		Sulfite reduction	0.6436	0.4226	3.9130	0.0482	2.8688

Table S4 Effects of elevated O₃ and wheat cultivars on the abundances of key functional genes involved in N cycling analyzed by ANOVA. Only genes with > 3 probes detected were shown.

Gene or enzyme	Functional process	O ₃ (%) ^a		Cult. (%) ^b		p-value ^c		
		Y19	Y16	aO ₃	eO ₃	O ₃	Cult.	O ₃ :cult.
<i>nifH</i>	Nitrogen fixation	18.58	-6.11	29.97	2.91	0.5222	0.0445	0.1109
<i>amoA</i>	Nitrification	-20.32	-26.84	36.34	25.20	0.3613	0.3654	0.7940
<i>narG</i>	Denitrification	20.73	-8.53	43.99	9.09	0.8238	0.1480	0.3845
<i>nirK</i>		5.86	-5.38	7.17	-4.21	0.9972	0.9164	0.6531
<i>nirS</i>		18.14	-14.20	46.49	6.39	0.9453	0.1635	0.3151
<i>norB</i>		25.64	-1.39	36.24	6.93	0.7196	0.4973	0.6773
<i>nosZ</i>		24.47	-22.88	40.47	-12.96	0.9093	0.7176	0.4008
<i>nirA</i>		Assimilatory N reduction	35.04	-22.75	44.51	-17.33	0.9709	0.7225
<i>nirB</i>	8.87		-4.07	20.30	6.01	0.9405	0.6138	0.7957
<i>nasA</i>	56.40		3.30	54.19	1.83	0.3710	0.4064	0.4551
<i>napA</i>	Dissimilatory N reduction	-61.67	-33.00	-33.16	16.82	0.0989	0.5972	0.4336
<i>nrfA</i>		3.52	-18.71	36.10	6.87	0.6576	0.3831	0.5583
<i>gdh</i>	Ammonification	177.15	-42.73	-25.52	-84.61	0.8592	0.1662	0.6703
<i>ureC</i>		-7.94	-22.71	19.24	0.11	0.1637	0.4413	0.4462
<i>hzo</i>	Anammox	15.76	-8.44	38.80	9.78	0.9301	0.2789	0.5516

a: Percent change by elevated O₃ was calculated using the following formula: $(eO_3 - aO_3) * 100 / aO_3$, where aO₃ and eO₃ were the average signal intensities of genes detected by GeoChip 3.0 at ambient O₃ or elevated O₃, respectively. b: Percentage change by cultivar is calculated using the following formula: $(cult_{Y16} - cult_{Y19}) * 100 / cult_{Y19}$ under aO₃ or eO₃ conditions; c: p values based on ANOVA analysis using the model: $Y \sim O_3 + cultivar + O_3:cultivar$.

Table S5 Effects of elevated O₃ and wheat cultivars on the abundances of key functional genes involved in S and P cycling analyzed by ANOVA. Only genes with > 3 probes detected were shown.

Gene or enzyme	Functional process	O ₃ (%) ^a		Cult. (%) ^b		p-value ^c		
		Y19	Y16	aO ₃	eO ₃	O ₃	Cult.	O ₃ :cult.
Phytase	Phosphorus utilization	-1.87	-27.46	74.29	28.85	0.9326	0.6415	0.5178
<i>ppk</i>		29.38	-11.05	56.08	7.31	0.8320	0.2531	0.4158
<i>ppx</i>		24.66	-15.65	43.90	-2.63	0.9417	0.1634	0.1056
<i>aprA</i>	Sulfur metabolism	15.86	0.43	24.22	7.68	0.7463	0.5136	0.7624
<i>dsrA</i>	Sulfite reduction	25.85	-6.69	38.03	2.34	0.4226	0.0482	0.0906
<i>dsrB</i>		9.86	-0.26	19.32	8.32	0.8365	0.5388	0.8259
<i>sox</i>	Sulfur oxidation	9.96	-3.01	0.69	-11.19	0.9118	0.8528	0.8354

a: Percent change by elevated O₃ was calculated using the following formula: $(eO_3 - aO_3) * 100 / aO_3$, where aO₃ and eO₃ were the average signal intensities of genes detected by GeoChip 3.0 at ambient O₃ or elevated O₃, respectively. b: Percentage change by cultivar is calculated using the following formula: $(cult_{Y16} - cult_{Y19}) * 100 / cult_{Y19}$ under aO₃ or eO₃ conditions; c: p values based on ANOVA analysis using the model: $Y \sim O_3 + cultivar + O_3: cultivar$.

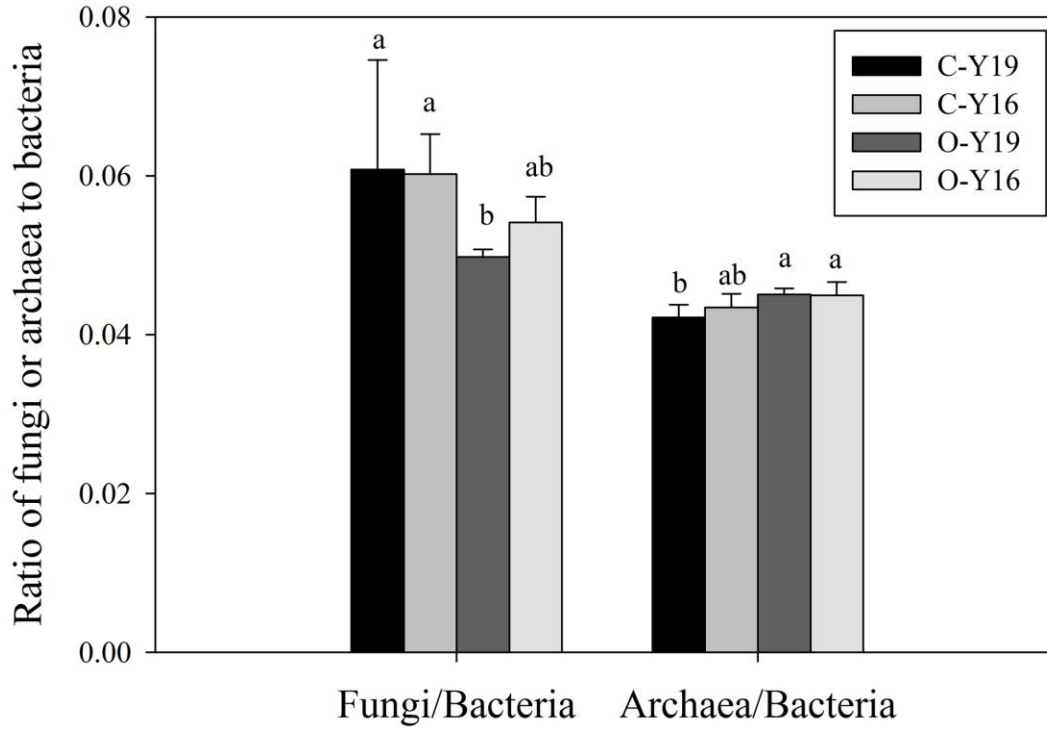


Fig. S1 The ratios of fungi to bacteria and archaea to bacteria based on the sum of the signal intensities detected by GeoChip 3.0. The significance was tested by the Student t-test at $p < 0.05$. from O_3 -sensitive and O_3 -tolerant wheat cultivars at aO_3 and eO_3 conditions. C-Y19: O_3 -sensitive wheat cultivar at aO_3 ; C-Y16: O_3 -relatively sensitive wheat cultivar at aO_3 ; O-Y19: O_3 -sensitive wheat cultivar at eO_3 ; O-Y16: O_3 -relatively sensitive at eO_3 .

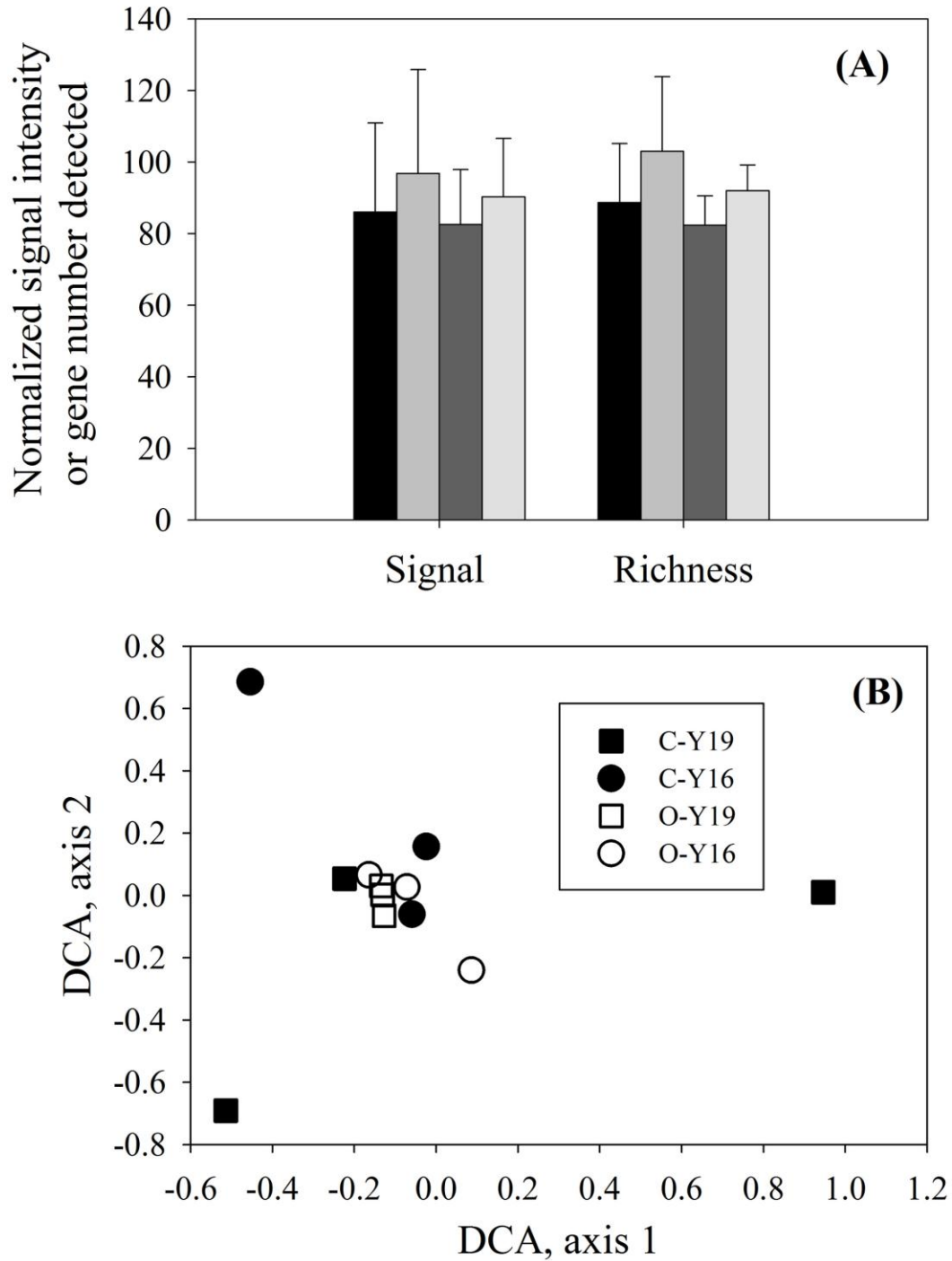


Fig. S2 The number and abundance of detected *gyrB* genes (A) and DCA analysis of phylogenetic structure (B) of microbial communities of Yannong Y19 and Yangmai 16 wheat cultivars under aO₃ and eO₃. C-Y19: O₃-sensitive wheat cultivar at aO₃; C-Y16: O₃-relatively sensitive wheat cultivar at aO₃; O-Y19: O₃-sensitive wheat cultivar at eO₃; O-Y16: O₃-relatively sensitive at eO₃.