

Additional file 1

Supplemental Table S1. Primers used for quantitative PCR.

Primer name	Primer sequence (5'→3')	Amplicon size (bp)	Master mix used for qPCR	Thermal profile of qPCR	Reference
nosZ-1826Fm	GSC AGR TSK KSA AGT GGA A	186	Power SYBR MasterMix	95°C for 10 min, 40 cycles at 95°C for 15 s, 62°C for 1 m, 95°C for 10s (+0.3)	[1]
nosZ-2012R	CGG TCC TTS GAG AAC TT				
16S-331F	TCC TAC GGG AGG CAG CAG T	195	FAST SYBR MasterMix	95°C for 5 min, 40 cycles at 95°C for 5 s, 60°C for 30 s, 95°C for 10s (+0.3)	[2, 3]
16S-518R	ATT ACC GCG GCT GCTG G				
TEF_F	ACT GTG CAG TAG TAC TTG GTG	155	FAST SYBR MasterMix	95°C for 5 min, 40 cycles at 95°C for 5 s, 60°C for 30 s, 95°C for 10s (+0.3)	[4]
TEF_R	AAG CTA GGA GGT ATT GAC AAG				

1. Usyskin-Tonne A, Hadar Y, Yermiyahu U, Minz D. Elevated CO₂ has a significant impact on denitrifying bacterial community in wheat roots. *Soil Biol Biochem* 2020; **142**: 107697.
2. Hunter N, Nadkarni MA, Jacques NA, Martin FE. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology* 2002; **148**: 257–266.
3. Lopez I, Ruiz-Larrea F, Cocolin L, Orr E, Phister T, Marshall M, et al. Design and evaluation of PCR primers for analysis of bacterial populations in wine by denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 2003; **69**: 6801–6807.
4. Ruppel S, Rühlmann J, Merbach W. Quantification and localization of bacteria in plant tissues using quantitative real-time PCR and online emission fingerprinting. *Plant Soil* 2006; **286**: 21–35.

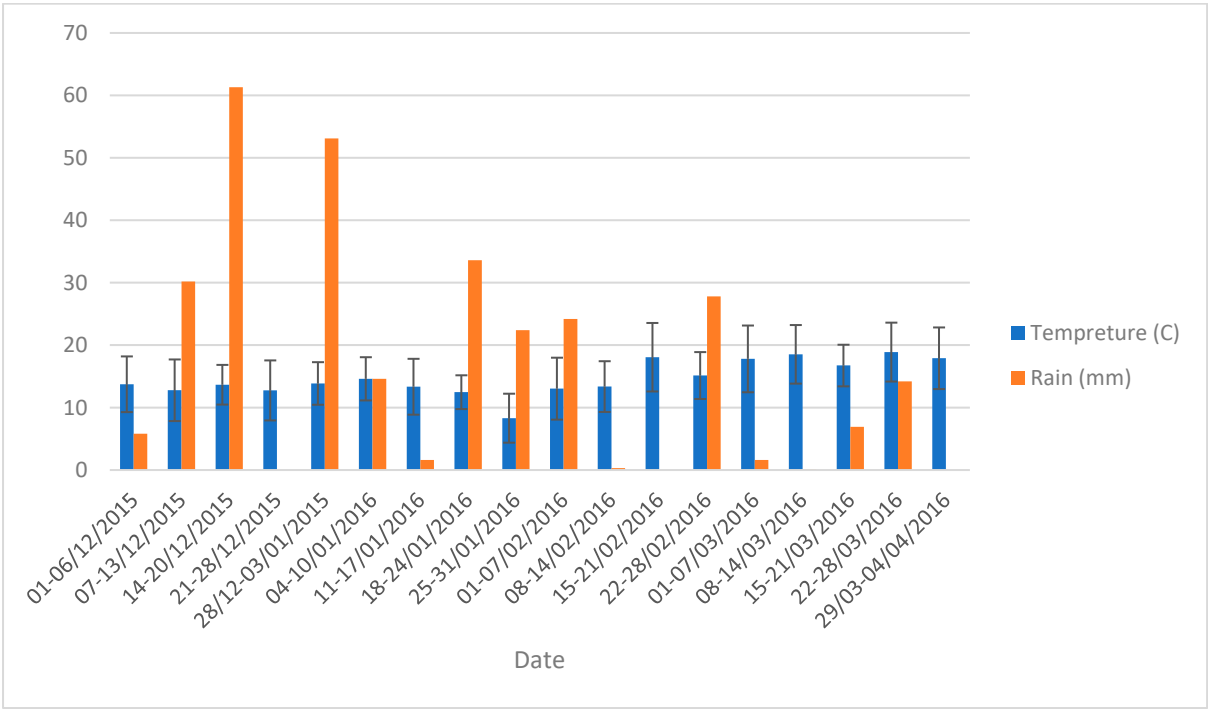
Supplemental Table S2. Root-associated and rhizosphere contigs divided into catalogues

Niche	Catalogue name	Number of contigs	N50 (bp)
Roots	week 1	233,475	700
	week 2	295,394	780
	week 5	349,966	691
	week 9	265,158	702
	week 12	439,827	736
	week 17a	371,640	759
	week 17b	567,046	680
Rhizosphere	week 1	470,984	701
	week 2	485,480	706
	week 5	557,525	726
	week 9	458,671	722
	week 12a	266,674	694
	week 12b	294,897	692
	week 17a	274,544	689
	week 17b	264,367	685

Supplemental Table S3. List of normalized gene counts per cell for *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*

Bacteria phyla	Function group	Function group type	Relative abundance (%)	Number of genes before spike formation	Number of genes after spike formation	Functional genes significantly abundant before or after spike formation	Number of genes per cell
Actinobacteria	Amino acid	ABC transporters	27	809	715	before	30
Actinobacteria	Amino acid	ABC transporters	37	7381	12003	after	325
Actinobacteria	Amino acid	Biosynthesis	27	3	3	before	0
Actinobacteria	Amino acid	Biosynthesis	37	5457	9522	after	258
Actinobacteria	Amino acid	Degradation	27	1532	2327	before	57
Actinobacteria	Amino acid	Metabolism	27	18848	28884	before	702
Actinobacteria	Amino acid	Metabolism	37	3400	5278	after	143
Actinobacteria	Antibiotics synthesis and resistance	ABC transporters	27	56	22	before	2
Actinobacteria	Antibiotics synthesis and resistance	ABC transporters	37	1068	1848	after	50
Actinobacteria	Antibiotics synthesis and resistance	Biosynthesis	37	10672	19366	after	524
Actinobacteria	Antibiotics synthesis and resistance	Resistance	27	4063	6187	before	151
Actinobacteria	Antibiotics synthesis and resistance	Resistance	27	279	557	before	10
Actinobacteria	Antibiotics synthesis and resistance	Transporters	27	133	236	before	5
Actinobacteria	Chemotaxis		27	3332	6834	before	124
Actinobacteria	Quorum sensing		27	16740	24655	before	624
Actinobacteria	Secretion system		27	2142	3301	before	80
Actinobacteria	Biofilm formation		27	7643	12537	before	285
Actinobacteria	Carbon	ABC transporters	27	828	1429	before	31
Actinobacteria	Carbon	ABC transporters	37	27467	46799	after	1267
Actinobacteria	Carbon	Biosynthesis	27	5617	6387	before	209
Actinobacteria	Carbon	Biosynthesis	37	6121	9364	after	253
Actinobacteria	Carbon	Degradation	37	10924	17979	after	487
Actinobacteria	Carbon	Metabolism	27	2854	4229	before	106
Actinobacteria	Carbon	Metabolism	37	42138	73426	after	1988
Actinobacteria	Carbon	Transporters	27	42	16	before	2
Actinobacteria	Ion	ABC transporters	27	8	8	before	0
Actinobacteria	Ion	Transporters	27	44	28	before	2
Actinobacteria	Metal	ABC transporters	27	2561	4684	before	95
Actinobacteria	Metal	ABC transporters	37	325	621	after	17
Actinobacteria	Nitrogen	Denitrification	27	501	780	before	19
Bacteroidetes	Amino acid	ABC transporters	5	29	17	before	6
Bacteroidetes	Amino acid	ABC transporters	5	8	34	after	6
Bacteroidetes	Amino acid	Biosynthesis	5	416	695	after	132
Bacteroidetes	Amino acid	Degradation	5	128	156	before	24
Bacteroidetes	Amino acid	Metabolism	5	5114	6912	before	975
Bacteroidetes	Amino acid	Metabolism	5	491	604	after	115
Bacteroidetes	Antibiotics synthesis and resistance	ABC transporters	5	7	5	before	1
Bacteroidetes	Antibiotics synthesis and resistance	ABC transporters	5	37	59	after	11
Bacteroidetes	Antibiotics synthesis and resistance	Biosynthesis	5	1001	1298	after	247
Bacteroidetes	Antibiotics synthesis and resistance	Resistance	5	2264	2751	before	453
Bacteroidetes	Antibiotics synthesis and resistance	Transporters	5	251	284	before	48
Bacteroidetes	Chemotaxis		5	254	480	before	48
Bacteroidetes	Quorum sensing		5	1314	1721	before	251
Bacteroidetes	Secretion system		5	74	47	before	14
Bacteroidetes	Biofilm formation		5	545	793	before	104
Bacteroidetes	Carbon	ABC transporters	5	780	1003	before	149
Bacteroidetes	Carbon	ABC transporters	5	17	20	after	4
Bacteroidetes	Carbon	Biosynthesis	5	1760	1786	before	335
Bacteroidetes	Carbon	Biosynthesis	5	822	1317	after	251
Bacteroidetes	Carbon	Degradation	5	4939	7264	after	1382
Bacteroidetes	Carbon	Metabolism	5	18	20	before	3
Bacteroidetes	Carbon	Metabolism	5	8230	11833	after	2251
Bacteroidetes	Ion	ABC transporters	5	78	51	before	15
Bacteroidetes	Ion	Transporters	5	94	123	before	18
Bacteroidetes	Ion	Transporters	5	5	2	before	1
Bacteroidetes	Metal	ABC transporters	5	73	62	before	14
Bacteroidetes	Nitrogen	Denitrification	5	41	25	before	8
Proteobacteria	Amino acid	ABC transporters	62	27986	23593	before	450
Proteobacteria	Amino acid	ABC transporters	52	4231	4492	after	87
Proteobacteria	Amino acid	Biosynthesis	62	7	17	before	0
Proteobacteria	Amino acid	Biosynthesis	52	3341	4203	after	82
Proteobacteria	Amino acid	Degradation	62	7584	6383	before	122
Proteobacteria	Amino acid	Metabolism	62	78019	70650	before	1253
Proteobacteria	Amino acid	Metabolism	52	6463	7226	after	140
Proteobacteria	Antibiotics synthesis and resistance	ABC transporters	62	11196	9291	before	180
Proteobacteria	Antibiotics synthesis and resistance	ABC transporters	52	51	46	after	1
Proteobacteria	Antibiotics synthesis and resistance	Biosynthesis	52	6975	6541	after	127
Proteobacteria	Antibiotics synthesis and resistance	Resistance	62	36956	36280	before	594
Proteobacteria	Antibiotics synthesis and resistance	Resistance	62	17899	15325	before	289
Proteobacteria	Antibiotics synthesis and resistance	Transporters	62	36841	30887	before	592
Proteobacteria	Chemotaxis		62	47886	39568	before	769
Proteobacteria	Quorum sensing		62	53297	52493	before	856
Proteobacteria	Secretion system		62	86817	67617	before	1395
Proteobacteria	Biofilm formation		62	41563	37687	before	668
Proteobacteria	Carbon	ABC transporters	62	30072	25734	before	483
Proteobacteria	Carbon	ABC transporters	52	9950	10446	after	203
Proteobacteria	Carbon	Biosynthesis	62	15081	13209	before	242
Proteobacteria	Carbon	Biosynthesis	52	12626	13861	after	269
Proteobacteria	Carbon	Degradation	52	7271	8049	after	156
Proteobacteria	Carbon	Metabolism	62	13902	12629	before	223
Proteobacteria	Carbon	Metabolism	52	55133	53344	after	1035
Proteobacteria	Carbon	Transporters	62	2101	1498	before	34
Proteobacteria	Ion	ABC transporters	62	4067	3169	before	65
Proteobacteria	Ion	Transporters	62	290	89	before	5
Proteobacteria	Ion	Transporters	62	8365	6147	before	134
Proteobacteria	Metal	ABC transporters	62	25229	22403	before	405
Proteobacteria	Nitrogen	Denitrification	62	7715	5879	before	124

Supplemental Figure S1. Weather conditions during wheat growth



Error bars indicate average temperature during a day (maximum) and average temperature during a night (minimum).

Supplemental Figure S2. Green (A) and yellow (B) spikes at the final stages of wheat growth (17 week)

