

**Wheat genome architecture influences interactions with phytobeneficial microbial functional groups in the rhizosphere**

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**Supplementary figures and tables**

**Fig. S1 Rarefaction curves of bacterial communities in bulk soil and wheat species at 40 days.**

*nifH* amplicons obtained from samples were sequenced (MiSeq metabarcoding) and sequences were rarefied to obtain species richness (number of OTUs) according to the number of sequences considered.

**Fig. S2 Between-Class Analyses (BCA) of the total bacterial community (A) and free nitrogen-fixers (B) in bulk soil and wheat species at 40 days, based on *rrs* data and the Euclidian distance.** OTUs most associated with the statistical classes are indicated, as well as the corresponding phylum (using a colour code). The six classes (corresponding to the five wheat species and bulk soil) were significantly different from one another (Monte-Carlo tests,  $P < 0.05$ ).

**Fig. S3 Relative abundance of *nifH*, *acdS* and *ppdC* genes in bulk soil and wheat species at 14 and 40 days of growth.** (A and B) Relative abundance of *nifH* genes at 14 (A) and 40 (B) days. (C and D) Relative abundance of *acdS* genes at 14 (C) and 40 (D) days. (E and F) Relative abundance of *ppdC* genes at 14 (E) and 40 (F) days. Relative abundance was calculated by dividing the number of *nifH*, *acdS* and *ppdC* copies by the number of *rrs* copies. Wild wheats are portrayed in purple and domesticated wheats in blue. Lines are (from left to right) TU2, TU7, TU8 for *T. urartu*, AS1, AS3, AS7, AS8 for *Ae. speltooides*, TD0 (not sampled at day 14 because of insufficient development), TD1, TD3, TD9 for *T. dicoccon*, AT0, AT1, AT5, AT9 for *Ae. tauschii*, and AE2, AE4, AE5, AE6 for *T. aestivum*. Differences between species were assessed by a nested ANOVA and Tukey's HSD tests, while a one-way ANOVA followed by Tukey's HSD tests was

performed to compare all wheat species with bulk soil ( $P < 0.05$ ; letters  $\alpha$ ,  $\beta$  and  $\gamma$  are used to show statistical differences). Differences between lines of a same species were studied by a nested ANOVA and Tukey's HSD tests ( $P < 0.05$ ; letters a, b and c are used to show statistical differences).

**Fig. S4  $\text{Log}_{10}$  number of *phlD* copies in bulk soil and in the rhizosphere of wheat species at 14 days of growth.** Wild wheats are portrayed in purple and domesticated wheats in blue. Each point corresponds to one individual plant (replicate) of a given wheat line or one bulk soil sample, and different symbols are used for the different wheat lines in a same species. The dashed line represents the quantification limit *i.e.* the lowest standard concentration at which the method is able to provide a quantification ( $1.2 \times 10^3$  copies). Points at the bottom correspond to samples that were below the detection limit. The other samples were either measurable (over the detection limit) or at the detection limit. At day 40, all samples were below the detection limit.

**Fig. S5 Rarefaction curves of diazotroph communities in bulk soil and wheat species at 40 days.** *nifH* amplicons obtained from samples were sequenced (MiSeq metabarcoding) and sequences were rarefied to obtain species richness (number of OTUs) according to the number of sequences considered.

**Fig. S6 Relative abundance of phyla (A) and genera (B) of the nitrogen fixer community in bulk soil and wheats, based on *nifH* analysis.**



Fig. S1

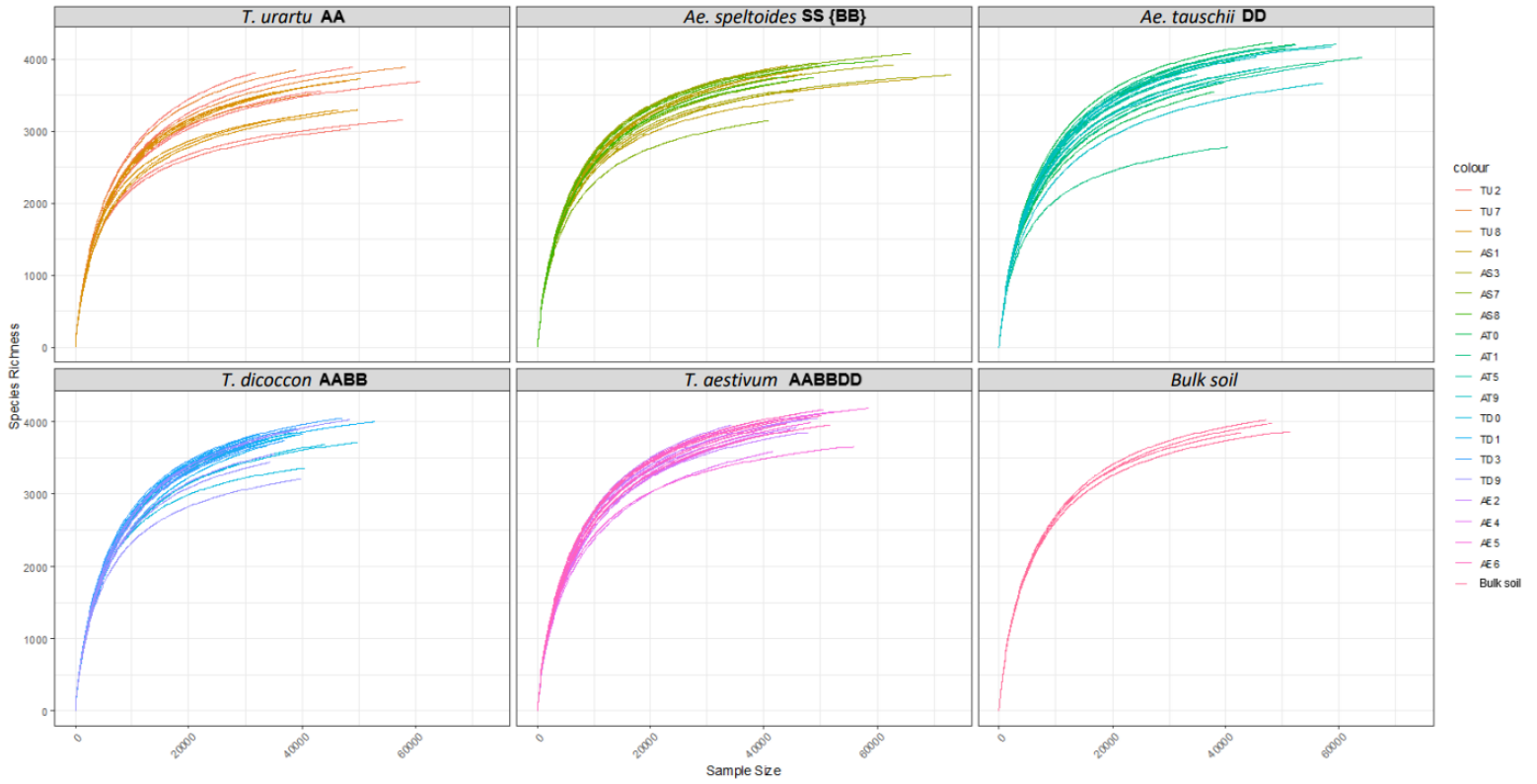
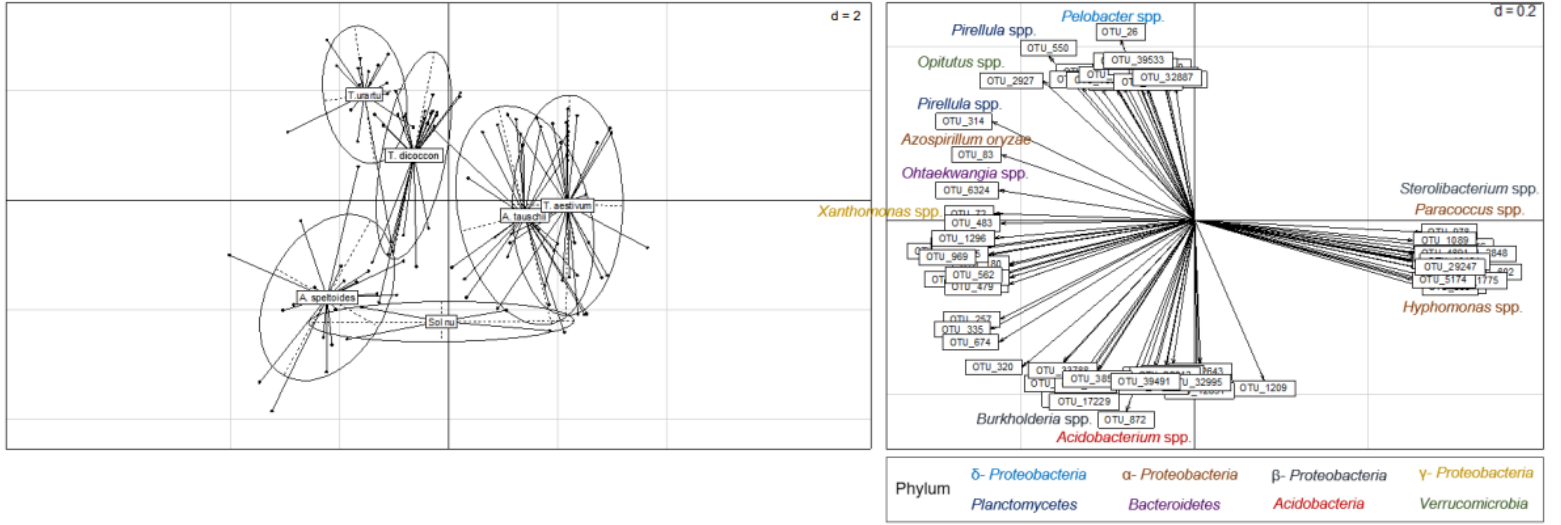


Fig. S2

A



B

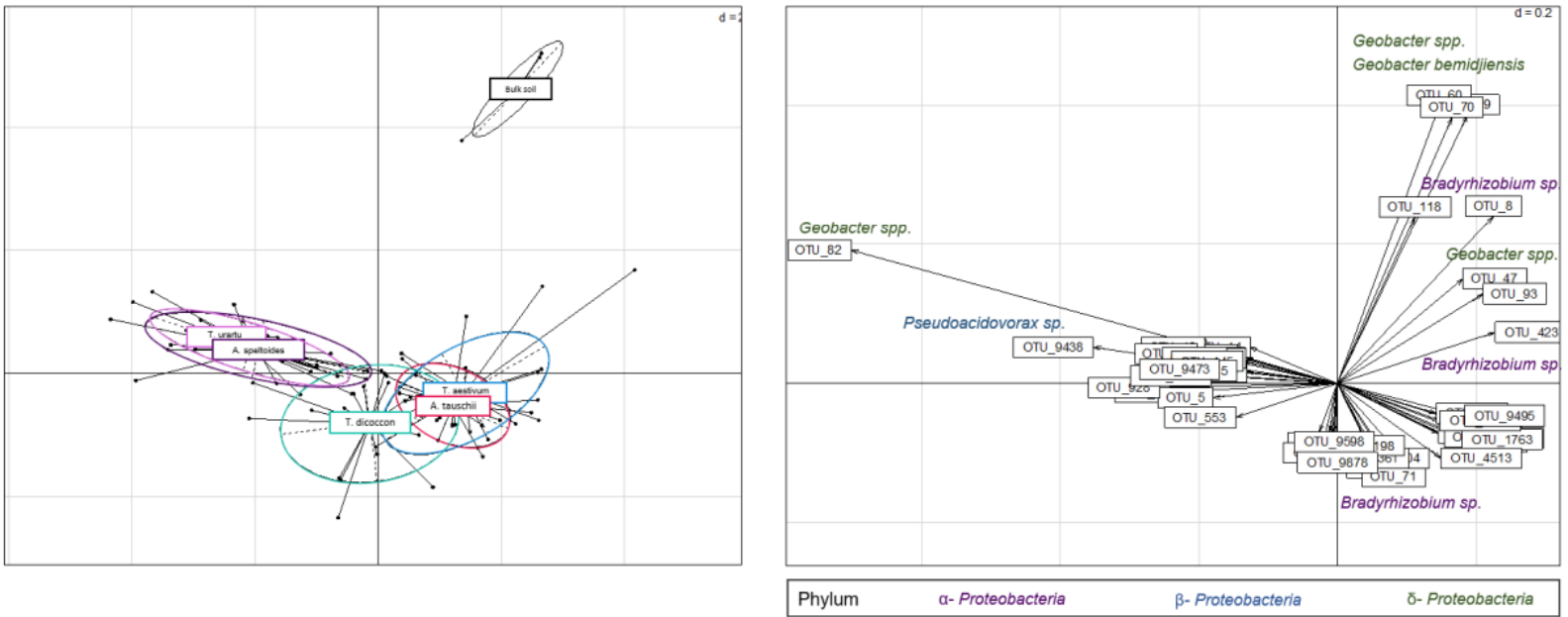


Fig. S3

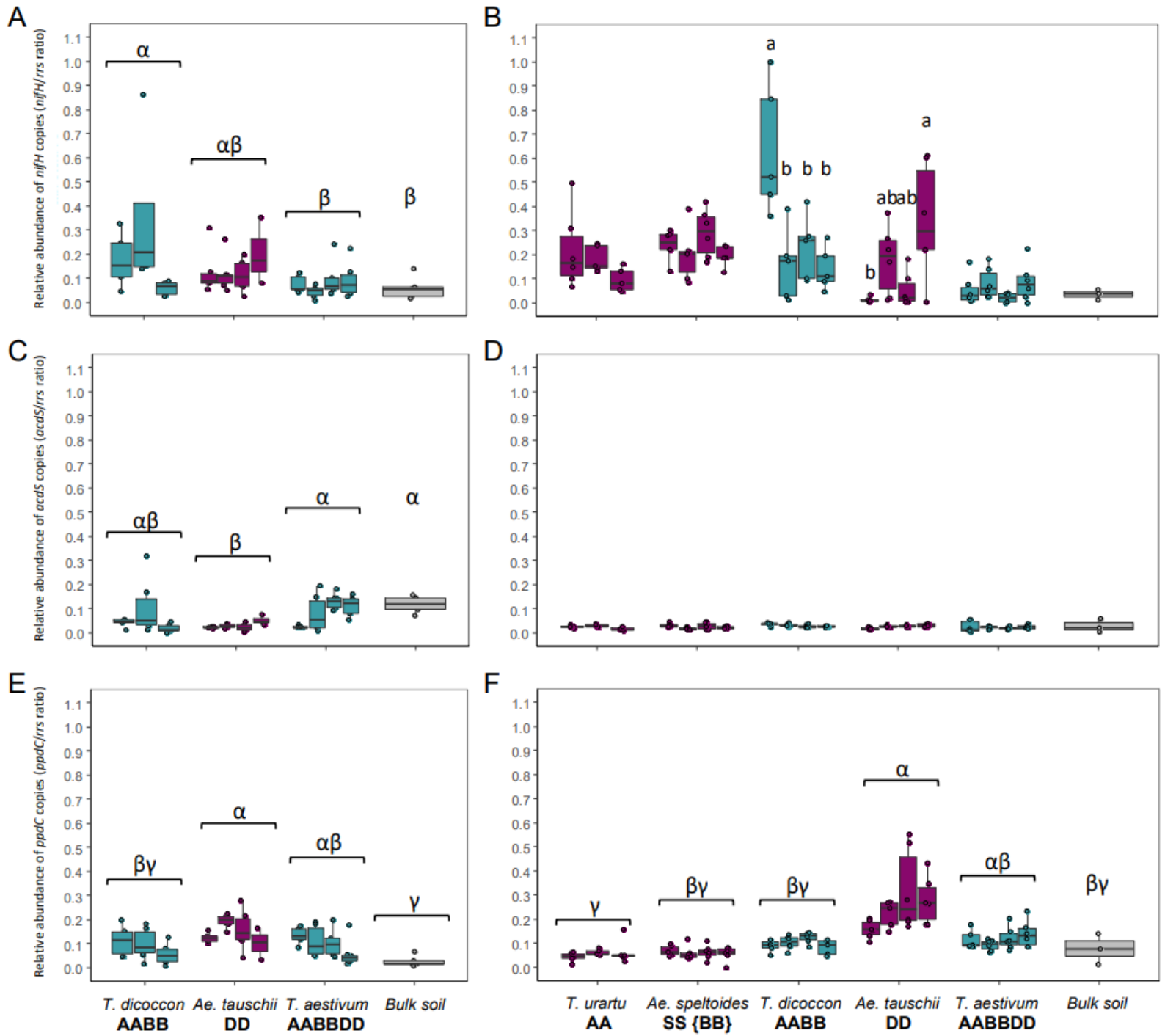


Fig. S4

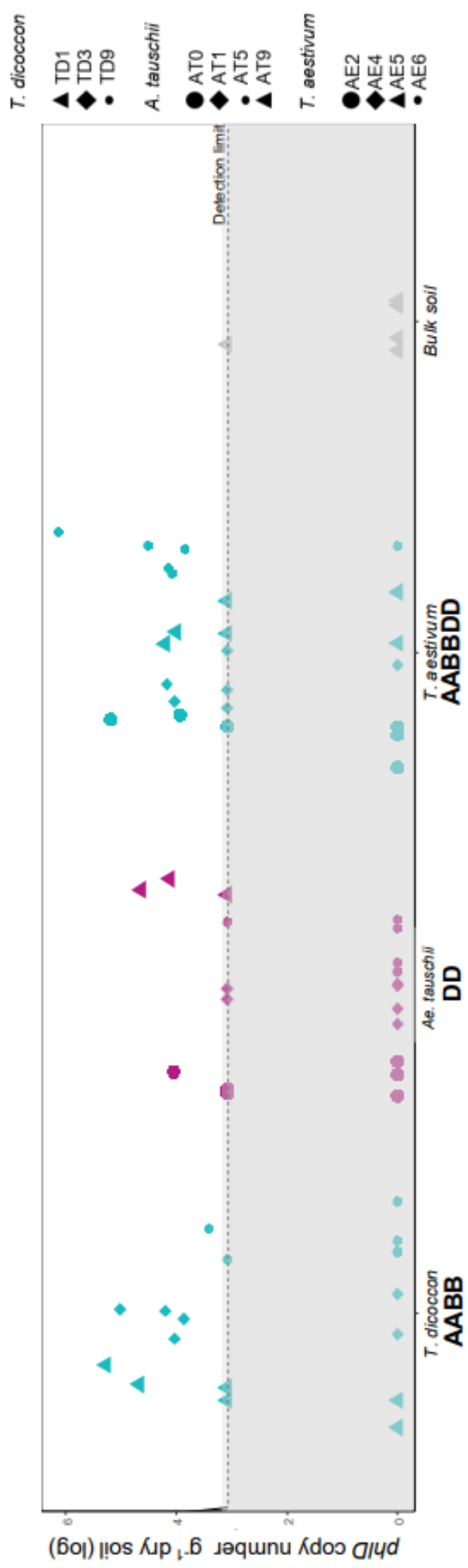




Fig. S5

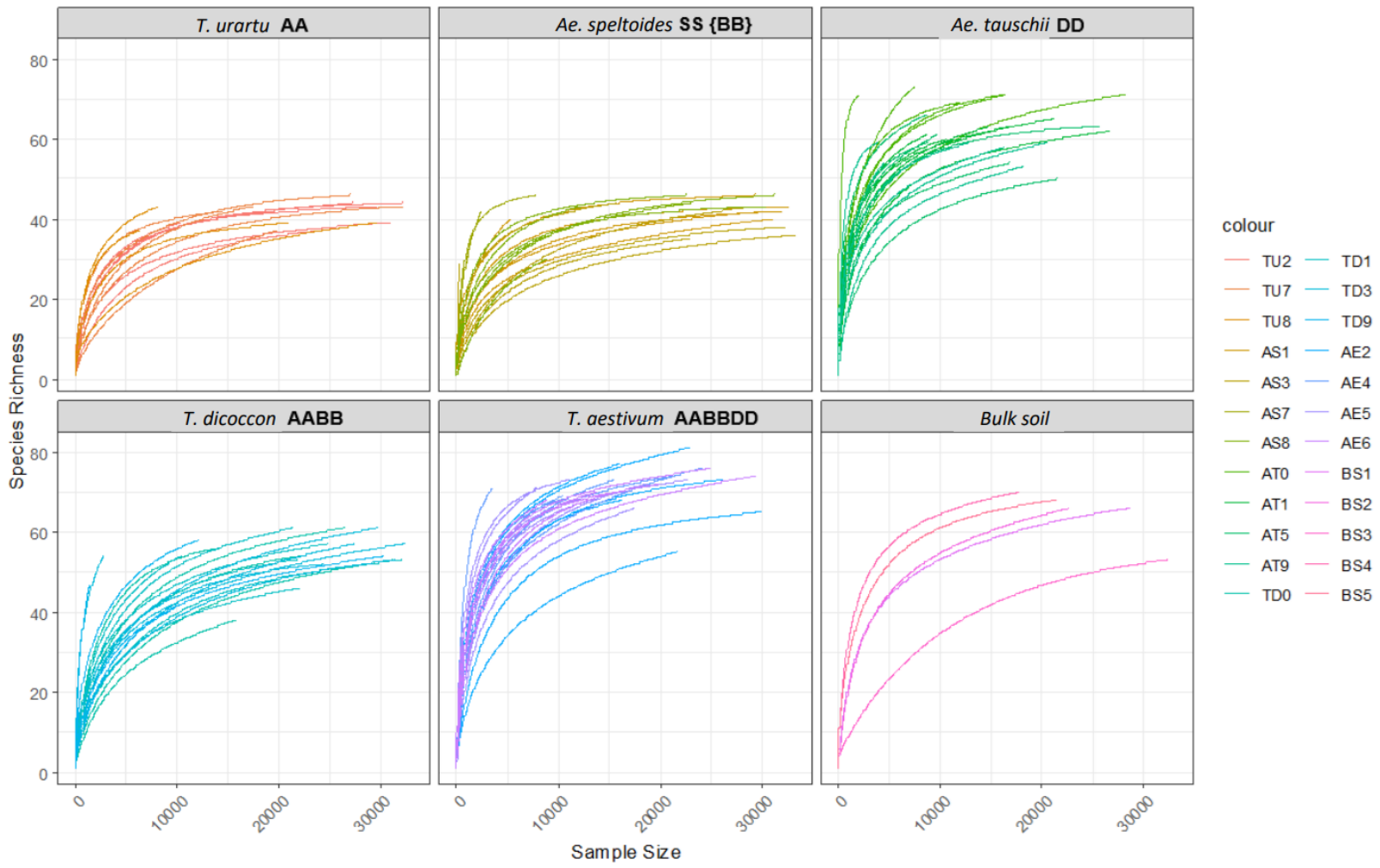
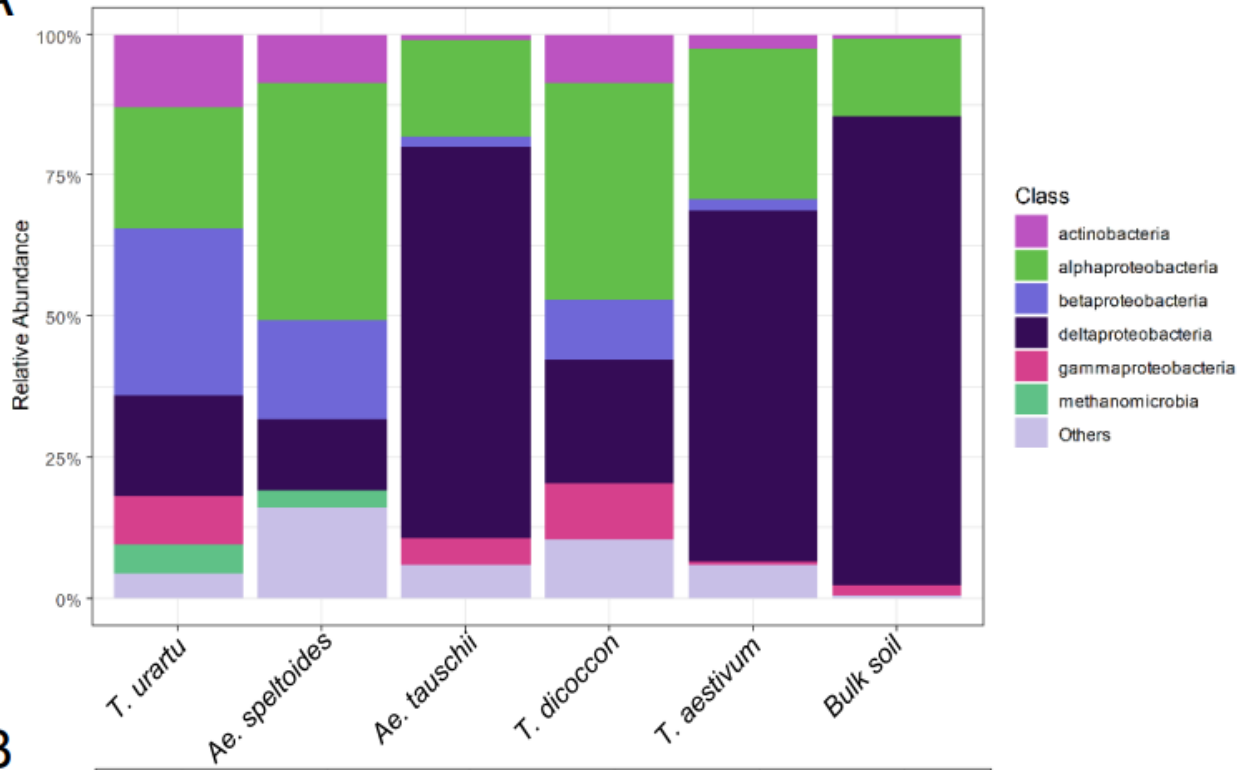
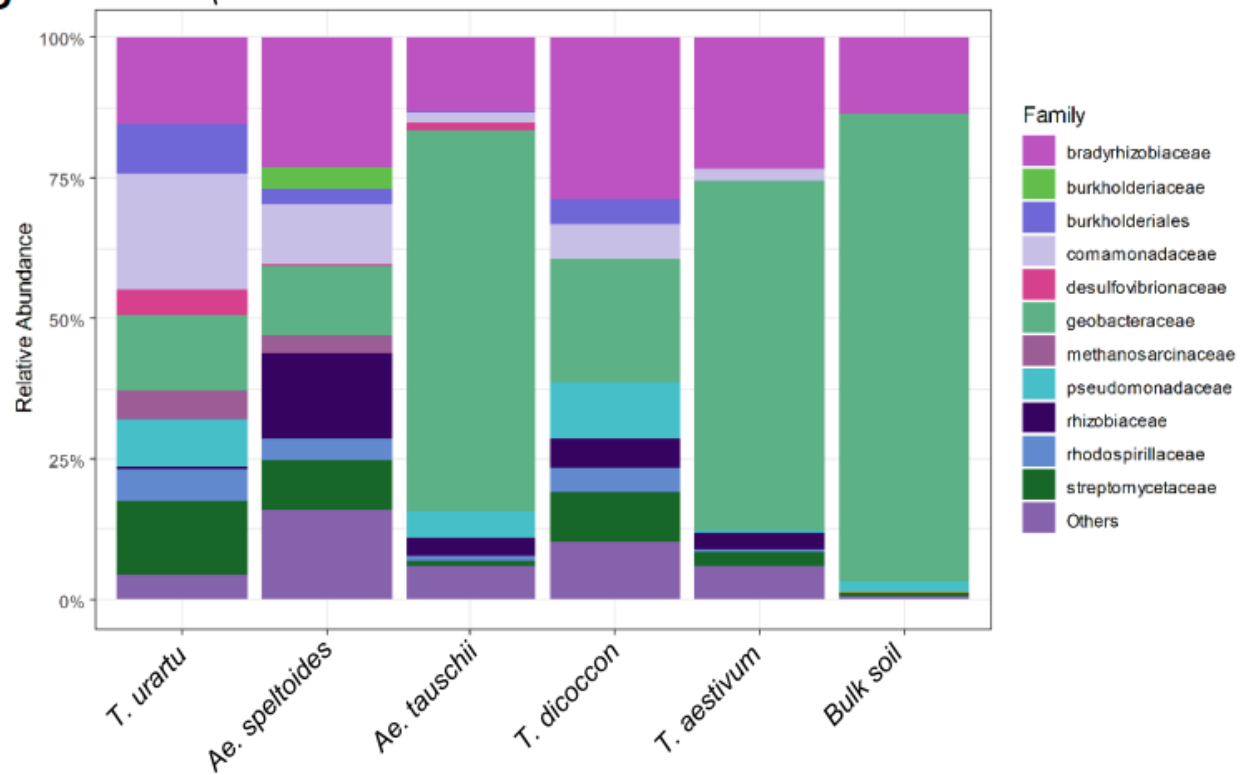


Fig. S6

A



B



**Table S1. List of wheat accessions.** Seeds were produced at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany). subsp.: sub-species, convar. : between sub-species and varieties, var. : variety/line.

Genome	Species	Sub taxa	Accession number	Line number
AA	<i>Triticum urartu</i>	var. <i>binartulutriru</i>	TRI 11496	TU8
		var. <i>albonigricans</i>	TRI17142	TU9
		var. <i>albonigricans</i>	TRI 18402	TU2
		var. <i>spontaneoabum</i>	TRI 18407	TU7
BB{SS}	<i>Aegilops speltoides</i>	subsp. <i>speltoides</i> var. <i>speltoides</i>	AE 104	AS7
		subsp. <i>speltoides</i> var. <i>speltoides</i>	AE 1064	AS3
		subsp. <i>speltoides</i> var. <i>speltoides</i>	AE 379	AS1
		subsp. <i>speltoides</i> var. <i>speltoides</i>	AE 419	AS8
AABB	<i>Triticum dicoccon</i>	subsp. <i>abyssinicum</i> var. <i>arras</i>	TRI 15032	TD9
		subsp. <i>dicoccon</i> convar. <i>dicoccon</i>	TRI 17809	TD0
		var. <i>dicoccon</i>		
		subsp. <i>dicoccon</i> convar. <i>dicoccon</i>	TRI 4303	TD3
		var. <i>dicoccon</i>		
DD	<i>Aegilops tauschii</i>	subsp. <i>tauschii</i> var. <i>meyeri</i>	AE 1069	AT5
		subsp. <i>tauschii</i> var. <i>tauschii</i>	AE 1650	AT0
		subsp. <i>tauschii</i> var. <i>meyeri</i>	AE 235	AT9
		subsp. <i>tauschii</i> var. <i>meyeri</i>	AE 278	AT1
AABBDD	<i>Triticum aestivum</i>	var. <i>greacum</i>	TRI 17732	AE4
		var. <i>aestivum</i>	TRI 2442	AE2
		var. <i>lutescens</i>	TRI 29475	AE3
		var. <i>lutescens</i>	TRI 29513	AE6

**Table S2. List of primer pairs used for qPCR amplification of *rrs*, *nifH*, *acdS*, *ppdC* and *phlD*.**

Targeted gene	Primer name	Sequence	Amplicon size	Reference
<i>rrs</i>	519F	5' CAGCMGCCGCGGTAANWC 3'	300 bp	Lane, 1991
	907R	5' CCGTCAATTCMTTTRAGTT 3'		
<i>nifH</i>	polF	5' ATSGCCATCATYTCRCCGGA 3'	360 bp	Poly <i>et al.</i> , 2001
	polR	5' TGCGAYCCSAARGCBGACTC 3'		
<i>acdS</i>	accF5	5' GGCAACAAGMYSCGCAAGCT 3'	153 bp	Bouffaud <i>et al.</i> , 2018
	accR8	5' CTGCACSAGSACGCACTTCA 3'		
<i>ppdC</i>	3F <i>ppdC</i>	5' GGCGACTGCCTGTTACCC 3'	236 bp	Gruet <i>et al.</i> , 2022b
	3R <i>ppdC</i>	5' CCAGCTGGCGTTGTTGAAC 3'		
<i>phlD</i>	B2BF	5' ACCCACC GCAGCATCGTTTATGAGC 3'	319 bp	Almario <i>et al.</i> , 2013
	B2BR3	5'AGCAGAGCGACGAGA ACTCCAGGGA 3'		

**Table S3. Plant shoot parameters at 40 days of growth (means  $\pm$  standard deviations ; n = 6).** Letters  $\alpha$ ,  $\beta$  and  $\gamma$  are used to show statistical differences between species, and letters a, b and c for differences between lines of a same species (ANOVA and Tukey's HSD)

tests at  $P < 0.05$ ). All plants of each line were at the same growth stage.

Genotype	Dry shoot biomass (mg)	Number of leaves
<b><i>Triticum urartu</i> <math>\gamma</math></b>		
TU2	291 $\pm$ 32 a	6 (end of tillering)
TU7	160 $\pm$ 27 ab	6 (end of tillering)
TU8	94 $\pm$ 28 b	6 (end of tillering)
<b><i>Triticum dicoccon</i> <math>\beta</math></b>		
TD0	186 $\pm$ 17 b	6 (end of tillering)
TD1	290 $\pm$ 56 ab	6 (end of tillering)
TD3	465 $\pm$ 72 a	6 (end of tillering)
TD9	467 $\pm$ 36 a	6 (end of tillering)
<b><i>Triticum aestivum</i> <math>\alpha</math></b>		
AE2	491 $\pm$ 63 a	6 (end of tillering)
AE4	553 $\pm$ 52 a	6 (end of tillering)
AE5	488 $\pm$ 20 a	6 (end of tillering)
AE6	658 $\pm$ 61 a	6 (end of tillering)
<b><i>Aegilops speltoides</i> <math>\gamma</math></b>		
AS1	346 $\pm$ 47 a	6 (end of tillering)
AS3	214 $\pm$ 29 bc	6 (end of tillering)
AS7	124 $\pm$ 16 c	6 (end of tillering)
AS8	338 $\pm$ 24 b	6 (end of tillering)
<b><i>Aegilops tauschii</i> <math>\alpha</math></b>		
AT0	452 $\pm$ 33 a	6 (end of tillering)
AT1	602 $\pm$ 30 a	6 (end of tillering)
AT5	538 $\pm$ 48 a	6 (end of tillering)
AT9	515 $\pm$ 58 a	6 (end of tillering)

**Table S4. Statistical tests of PERMANOVA/adonis performed on *rrs* and *nifH* sequence data from Illumina MiSeq metabarcoding (based on Bray Curtis distances, 10<sup>6</sup> permutations). (A) Results of PERMANOVA for *rrs* sequences, (B) Results for *rrs* diversity pairwise comparisons (*P* values are displayed), (C) Results of PERMANOVA for *nifH* sequences, (D) Results for *nifH* diversity pairwise comparisons (*P* values are displayed).**

<b>A) Nested model : Species / Line (<i>rrs</i>)</b>		
R <sup>2</sup>		0.3
Species	F	4.3
	<i>P</i>	< 0.001
Species / Line	F	1.8
	<i>P</i>	< 0.001

<b>B) Pairwise/adonis (<i>rrs</i>)</b>					
	<i>T. urartu</i>	<i>Ae. speltoides</i>	<i>T. dicoccon</i>	<i>Ae. tauschii</i>	<i>T. aestivum</i>
<i>T. urartu</i>		< 0.001	0.03	< 0.001	< 0.001
<i>Ae. speltoides</i>			0.01	< 0.001	< 0.001
<i>T. dicoccon</i>				0.01	< 0.001
<i>Ae. tauschii</i>					0.08
<i>T. aestivum</i>					

<b>C) Nested model : Species / Line (<i>nifH</i>)</b>		
R <sup>2</sup>		0.2
Species	F	1.8
	<i>P</i>	< 0.001
Species / Line	F	0.9
	<i>P</i>	0.9

<b>D) Pairwise/adonis (<i>nifH</i>)</b>					
	<i>T. urartu</i>	<i>Ae. speltoides</i>	<i>T. dicoccon</i>	<i>Ae. tauschii</i>	<i>T. aestivum</i>
<i>T. urartu</i>		0.04	0.49	< 0.001	< 0.001
<i>Ae. speltoides</i>			0.07	< 0.001	< 0.001
<i>T. dicoccon</i>				0.001	< 0.001
<i>Ae. tauschii</i>					0.07
<i>T. aestivum</i>					

**Table S5. Dry root biomass at 40 days of growth (means  $\pm$  standard deviations ; n = 6).** The differences between genotypes (i.e. between different species and between lines of a same species) were not statistically significant (ANOVA and Tukey's HSD tests at  $P < 0.05$ ).

<b>Genotype</b>	<b>Dry root biomass (mg)</b>
<b><i>Triticum Urartu</i></b>	
TU2	103.0 $\pm$ 13.6
TU7	86.8 $\pm$ 13.4
TU8	90.6 $\pm$ 20.8
<b><i>Triticum dicoccon</i></b>	
TD0	93.1 $\pm$ 15.1
TD1	98.6 $\pm$ 23.7
TD3	112.2 $\pm$ 16.1
TD9	119.0 $\pm$ 16.1
<b><i>Triticum aestivum</i></b>	
AE2	120.1 $\pm$ 16.2
AE4	122.4 $\pm$ 23.9
AE5	122.1 $\pm$ 17.8
AE6	110.8 $\pm$ 10.6
<b><i>Aegilops speltoides</i></b>	
AS1	107.4 $\pm$ 13.4
AS3	100.2 $\pm$ 10.2
AS7	91.7 $\pm$ 19.2
AS8	101.5 $\pm$ 10.2
<b><i>Aegilops tauschii</i></b>	
AT0	113.8 $\pm$ 15.2
AT1	122.5 $\pm$ 7.6
AT5	102.8 $\pm$ 8.0
AT9	110.5 $\pm$ 9.8