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

3

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24 **Running title:** Wheat genome and microbial functional groups

25

26 **Abstract**

27

28 Wheat has undergone a complex evolutionary history, which led to allopolyploidization and
29 the hexaploid bread wheat *Triticum aestivum*. However, the significance of wheat genomic
30 architecture for beneficial plant-microbe interactions is poorly understood, especially from a
31 functional standpoint. In this study, we tested the hypothesis that wheat genomic architecture
32 was an overriding factor determining root recruitment of microorganisms with particular
33 plant-beneficial traits. We chose five wheat species representing genomic profiles AA
34 (*Triticum urartu*), BB {SS} (*Aegilops speltoides*), DD (*Aegilops tauschii*), AABB (*Triticum*
35 *dicoccon*) and AABBDD (*Triticum aestivum*) and assessed by qPCR their ability to interact
36 with free-nitrogen fixers, 1-aminocyclopropane-1-carboxylate deaminase producers, 2,4-
37 diacetylphloroglucinol producers and auxin producers via the phenylpyruvate decarboxylase
38 pathway, in combination with Illumina MiSeq metabarcoding analysis of N fixers (and of the
39 total bacterial community). We found that the abundance of the microbial functional groups
40 could fluctuate according to wheat genomic profile, as did the total bacterial abundance. N
41 fixer diversity and total bacterial diversity were also influenced significantly by wheat
42 genomic profile. Often, rather similar results were obtained for genomes DD (*Ae. tauschii*)
43 and AABBDD (*T. aestivum*), pointing for the first time that the D genome could be
44 particularly important for wheat-bacteria interactions.

45

46 **KEYWORDS**

47 ACC deaminase producers, auxin producers, diazotrophs, holobiont, microbial functional
48 groups, plant-bacteria interactions.

49

50 1. INTRODUCTION

51

52 Along with rice and maize, durum wheat and especially bread wheat are major crops for
53 human nutrition. The importance of wheat stems from its capacity to be cultivated in various
54 parts of the world, across a wide range of soil and climatic conditions (Willcox, 2005; Haas *et*
55 *al.*, 2019). Accordingly, wheat is exposed to contrasted microbial communities, as the soil
56 microbiome displays strong biogeographic patterns (Nemergut *et al.*, 2011; Bru *et al.*, 2011;
57 Karimi *et al.*, 2018; Bay *et al.*, 2020).

58 Wheat has undergone a complex evolutionary history, which involved several events
59 of genomic hybridization and subsequent domestication (Feldman & Kislev, 2007; Asouti &
60 Fuller, 2012; Marcussen *et al.*, 2014; Haberer *et al.*, 2016; Baidouri *et al.*, 2017; Pont *et al.*,
61 2019; Glémin *et al.*, 2019). These multiple hybridizations took place between different
62 *Triticum* and *Aegilops* species (Figure 1), and led to allopolyploidization (*i.e.* the addition of
63 parental genomes) (Soltis & Soltis, 2000). Thus, *Triticum urartu* (AA genome) and an
64 unknown BB-genome *Aegilops* having *Aegilops speltoides* (SS genome) as ancestor
65 hybridized and yielded wild emmer *Triticum dicoccoides* (AABB genome), from which
66 *Triticum dicoccon* and then durum wheat *Triticum durum* (both with AABB genome) derived
67 (Özkan *et al.*, 2011). In addition, *T. dicoccon* and *Aegilops tauschii* (DD genome) hybridized
68 to form an unknown AABBDD-genome wild wheat, which gave bread wheat *Triticum*
69 *aestivum* (AABBDD genome) after domestication (Salamini *et al.*, 2002). Genomic
70 hybridization and polyploidization are extensively documented in plants, albeit not to such
71 extent in the other *Poaceae* (Paterson *et al.*, 2004; Wei *et al.*, 2007; Messing, 2009).

72 Genomic hybridization and polyploidization may have strong impacts on (i) plant
73 adaptation, e.g. through the possibility to evolve new functions (Soltis & Soltis, 2000; Soltis
74 *et al.*, 2015), and (ii) plant physiology via, in particular, epigenetic effects (Jackson, 2017).

75 Since each gene is present in several copies, it can possibly lead to sub-functionalization (all
76 copies evolve to become complementary), neo-functionalization (one copy gains a new
77 function) or non-functionalization (one copy is not functional anymore) (Soltis *et al.*, 2015).
78 Polyploidization is thought to confer ecological benefits (Soltis & Soltis, 2000; Soltis *et al.*,
79 2015). Polyploids display a patchwork of parental and new characteristics, including for traits
80 influencing plant-microbe interactions such as root exudation, root uptake patterns, etc. (Saia
81 *et al.*, 2019; Iannucci *et al.*, 2021; Gruet *et al.*, 2022a).

82 In soil, plant roots interact with a myriad of microorganisms (Moëgne-Loccoz *et al.*,
83 2015), many of them being beneficial or deleterious to the plant (Vacheron *et al.*, 2013;
84 Parnell *et al.*, 2016; Nowell *et al.*, 2016). Therefore, microbial interactions are important
85 factors modulating wheat performance (Narula *et al.*, 2000; Glick, 2014; Majeed *et al.*, 2015;
86 Pérez-de-Luque *et al.*, 2017; Bernardo *et al.*, 2019; Dellagi *et al.*, 2020), and it is likely that
87 these interactions could have influenced the selection of wheat genotypes at the different
88 stages of its evolutionary history (Gruet *et al.*, 2022a). However, data available on this issue is
89 scarce. Wheat species of different genomic architectures have been compared, with a focus on
90 bacterial taxonomic diversity (Ito *et al.*, 2020), which limited the possibility to consider plant-
91 beneficial traits harbored by rhizosphere bacteria. Functional aspects of the bacterial
92 microbiome, also, might depend on the genomic traits of wheat, and this hypothesis was
93 investigated here.

94 The objective of this study was to assess whether wheat genotypes of contrasting
95 genomic architectures differ in their ability to interact with functional microbial groups
96 important for plant growth. To this end, wheat species of genomic profiles AA (*T. urartu*,
97 wild), SS (*A. speltoides*, wild; SS is the closest genome to BB that is currently available), DD
98 (*Ae. tauschii*, wild), AABB (*T. dicoccon*, domesticated) and AABBDD (*T. aestivum*,
99 domesticated) (Figure 1) were grown in a same soil. Their ability to recruit key plant-

100 beneficial functional groups (*i.e.* all microorganisms performing a same function) was
101 assessed based on qPCR and/or MiSeq sequencing. The focus was put on four prominent
102 groups, *i.e.* (i) free nitrogen fixers (by targeting the nitrogenase gene *nifH* involved in N₂
103 fixation) (Poly *et al.*, 2001), (ii) 1-aminocyclopropane-1-carboxylate (ACC) deaminase
104 producers (*acdS*; cleavage of the ethylene precursor ACC) (Glick, 2005, 2014), (iii) producers
105 of the auxinic-type root-branching signal 2,4-diacetylphloroglucinol (DAPG) (polyketide
106 synthase gene *phlD*) (Brazelton *et al.*, 2008), and (iv) *ppdC*⁺ 3-indole acetic acid (IAA)
107 producers (phenylpyruvate decarboxylase gene *ppdC*) (Spaepen *et al.*, 2007b; Zhao, 2014;
108 Gruet *et al.*, 2022b), based on their importance for plant performance and the availability of
109 molecular tools. In addition, the total bacterial community was also monitored, using the 16S
110 rRNA gene *rrs*, to assess whether trends at the level of individual functional groups were also
111 relevant at the scale of the entire community.

112

113 **2. MATERIALS AND METHODS**

114

115 **2.1. Plant material**

116 For each of the five species *T. urartu*, *T. dicoccon*, *T. aestivum*, *Ae. speltoides* and *Ae.*
117 *tauschii*, four contrasted genotypes (Table S1; *i.e.* : TU8, TU9, TU2, TU7 for *T. urartu*, AS7,
118 AS3, AS1, AS8 for *Ae. speltoides*, TD9, TD0, TD3, TD1 for *T. dicoccon*, AT5, AT0, AT9,
119 AT1 for *Ae. tauschii*, and AE4, AE2, AE3, AE6 for *T. aestivum*) were selected from the
120 wheat collection of the Leibniz Institute of Plant Genetics and Crop Plant Research
121 (Gatersleben, Germany), based on passport data available. All seeds used in the experiment
122 were produced in the same fields at Gatersleben.

123

124 **2.2. Greenhouse experiment and plant sampling**

125 Wheat lines were cultivated in soil originating from the loamy surface horizon (clay 16%, silt
126 44%, sand 40%; 2.1% organic matter, pH 7, cation exchange capacity 8.2 cmol.kg⁻¹) of a
127 luvisol located at La Côte Saint-André near Lyon (France) and kept for decades as a
128 permanent meadow. The soil was sieved at 6 mm and evenly mixed before preparing pots (2.5
129 kg soil per 3-dm³ pot).

130 Before sowing, wheat seeds were surface-disinfected by soaking 30 min in 1.2% sodium
131 hypochlorite solution, rinsed five times with sterile demineralized water, and soaked in sterile
132 water for one hour. Six replicates (i.e. six pots) were used for each wheat line, with four seeds
133 per pot (thinned to two seedlings on day 10). Six non-planted pots were also included as
134 controls. The pots were placed following a randomized block design (6 blocks) in a
135 greenhouse with 16 h of light (25°C) and 8 h of darkness (18°C). Blocks and pots within the
136 blocks were regularly moved around to minimise greenhouse heterogeneity effects. Every two
137 days, water was poured to the surface of the soil to adjust water content to soil retention
138 capacity (21% w/w).

139 *Ae. tauschii*, *T. dicoccon* (except genotype TD0) and *T. aestivum* were sampled at 14
140 and 40 days of growth, whereas *T. dicoccon* TD0, *T. urartu* and *Ae. speltooides* were only
141 sampled at 40 days because their growth at 14 days was still limited. In addition, *T. urartu*
142 genotype TU9 underwent wilting and could not be included in the study. Whole root systems
143 were recovered to consider possible growth fluctuations between genotypes, as recommended
144 (Simmons *et al.*, 2018), and they were shaken to remove loosely-adhering soil. Roots and
145 tightly-adhering soil were frozen in liquid nitrogen and lyophilized for 48 h. After
146 lyophilisation, root-adhering soil was separated from roots (by removing every root piece with
147 tweezers) and placed at -80°C until DNA extraction. Shoots were also sampled and dried at
148 50°C for 4 days, and shoot dry weight was measured. Soil from non-planted pots was also
149 frozen in liquid nitrogen, lyophilized and kept at -80°C.

151 **2.3. DNA extraction from soil**

152 Nucleic acid extraction was done as described by Bouffaud *et al.* (2014) from 0.5 g of
153 rhizosphere or bulk soil. Briefly, frozen soil was homogenized using a vortex and 0.5 g (for
154 standardization) of bulk or rhizosphere soil were taken from the total amount of soil. Then,
155 0.5 mL of zirconium beads (VWR, Fontenay-sous-Bois, France), 0.5 mL of extraction buffer
156 (5% hexadecyltrimethylammonium bromide and 1 mM 1,4-dithio-DL-threitol in a 0.12 M
157 phosphate buffer at pH 8.0) and 0.5 mL of phenol-chloroform-isoamyl alcohol (24:24:1 v/v/v)
158 were added to soil, and they were mixed in a bead beater (TissueLyser II Retsch;
159 Courtaboeuf, France) for 90 s at 30 m s⁻¹ and centrifuged for 10 min at 16,000 g. Supernatants
160 were extracted a second time in phenol-chloroform-isoamyl alcohol and once in chloroform-
161 isoamyl alcohol (24:1 v/v). Nucleic acids were precipitated overnight with a 3 M potassium
162 acetate solution at pH 4.8 and absolute ethanol at -20°C. After centrifugation for 30 min at
163 16,000 g, pellets were washed in 70% cold ethanol and dissolved in 60 µL RNase-free water.
164 Extracted nucleic acids were quantified using a Nanophotometer NP80 (Implen GmbH;
165 Munich, Germany) and results were expressed in ng µL⁻¹. Very similar DNA yields were
166 obtained from every sample.

167

168 **2.4. qPCR analyses of *rrs* and functional genes *nifH*, *acdS*, *ppdC* and *phlD***

169 qPCR is a method of reference for quantify root-associated microorganisms (Hou *et al.*, 2021;
170 Wippel *et al.*, 2021; Liu *et al.*, 2022; Cadot *et al.*, 2021). The size of the total bacterial
171 community and of four functional groups (free nitrogen fixers, ACC deaminase producers,
172 *ppdC*⁺ IAA producers and DAPG producers) was estimated by quantitative PCR, at 14 days
173 of growth for *T. dicoccon*, *Ae. tauschii* and *T. aestivum*, and 40 days for bulk soil and for all
174 five wheat species. qPCR reactions for the corresponding genes (*rrs*, *nifH*, *acdS*, *ppdC* and
175 *phlD*, respectively) were performed in 20 µL containing 10 µL of LightCycler-DNA Master

176 SYBR Green I master mix (Roche Applied Science, Penzberg, Germany), 2 μL of sample
177 DNA (10 ng DNA), and primers 519F/907R for *rrs* (0.6 μL , to reach a final concentration of
178 0.6 μM), 3F*ppdC*/3R*ppdC* for *ppdC* (0.6 μL , final concentration 0.6 μM), *polF/polR* for *nifH*
179 (2 μL , final concentration 0.5 μM), *accF5/accR8* for *acdS* (1 μL , final concentration 1 μM) or
180 B2BR3/B2BF for *phlD* (1 μL , final concentration 1 μM). PCR-grade water was added to
181 reach 20 μL . All primers pairs and references are shown in Table S2. The *nifH* primers
182 *polF/polR* are recommended to quantify diazotrophs (Gaby and Buckley, 2017). Primers
183 amplify *nifH*, *ppdC* and *phlD* from prokaryotes, whereas for *acdS* a minority of fungal
184 sequences may also be recovered. Testing of *rrs* primers 519F/907R by Illumina MiSeq
185 sequencing (see 2.5 below for procedures), using one replicate of *T. aestivum* line AE6, *T.*
186 *dicoccon* line TD3 and *T. urartu* line TU2 at 40 days identified only 1.77% (*T. aestivum*) to
187 5.74% (*T. urartu*) of chloroplast or mitochondria sequences (mean 4.64%), leading to a
188 negligible overestimate of 0.02 \log_{10} units on average (and 0.03 \log_{10} units for *T. urartu*) for
189 qPCR values that ranged from 6.3 to 8.7 \log_{10} bacteria per g of rhizosphere soil (see 3.1
190 below).

191 For each targeted gene, the cycling program included an initial activation step of 10 min
192 at 95°C, and the specificity of the amplification was verified by melting curve analysis,
193 assessed by increasing the temperature from 65°C to 95°C, at a rate of 0.1°C s⁻¹. For *rrs*, 40
194 cycles of amplification were done (10 s at 95°C, 50 s at 63°C, 30 s at 72°C) and the standard
195 curve was generated using a plasmid containing one copy of *rrs*, after dilution from 10⁶ to 10
196 copies. For *nifH*, 50 cycles of amplification were done (10 s at 95°C, 15 s at 64°C, 15 s at
197 72°C) and the standard curve was generated using genomic DNA of *Azospirillum* (ex
198 *brasiliense*) *baldaniorum* Sp245, after dilution from 5 × 10⁻⁹ to 5 × 10⁻¹⁴ g DNA μL^{-1}
199 (corresponding to 1.5 × 10⁶ to 15 copies, respectively). For *acdS*, 50 cycles of amplification
200 were done (15 s at 95°C, 15 s at 67°C, 10 s at 72°C) and the standard curve was generated

201 using genomic DNA of *Burkholderia cenocepacia* J2315, after dilution from 5×10^{-9} to $5 \times$
202 10^{-14} g DNA μL^{-1} (corresponding to 1×10^6 to 10 copies, respectively). For *ppdC*, 50 cycles
203 of amplification were done (15 s at 95°C, 15 s at 63°C, 10 s at 72°C) and the standard curve
204 was generated using genomic DNA of *Ae. baldaniorum* Sp245, after dilution from 5×10^{-9} to
205 5×10^{-14} g DNA μL^{-1} (corresponding to 1.5×10^6 to 15 copies, respectively). Lastly, for *phlD*,
206 50 cycles of amplification were done (30 s at 95°C, 7 s at 67°C, 15 s at 72°C) and the
207 standard curve was generated using genomic DNA of *Pseudomonas (ex fluorescens, ex*
208 *kilonensis) ogarae* F113, after dilution from 3×10^{-9} to 3×10^{-14} g DNA μL^{-1} (corresponding
209 to 9×10^5 to 9 copies, respectively).

210 In order to verify possible bias linked to the presence of qPCR inhibitors, two samples
211 for each targeted gene were diluted at 1/50, 1/100, 1/150, and 1/200. A curve was drawn for
212 each sample to determine the dilution range in which the efficiency of amplification was
213 between 80 and 100% (cycle threshold according to amplicon concentration).

214 Results expressed in g μL^{-1} were converted into numbers of copies using the formula
215 $[\text{DNA (g)} \times \text{Avogadro's number (molecules mol}^{-1})]/[\text{number of DNA bp in amplified}$
216 $\text{fragments} \times 660 \text{ (g mol}^{-1})]$, based on an average of 660 g mol^{-1} per base pair. They were
217 normalized to the total DNA quantity extracted from 0.5 g of soil, and expressed into number
218 of copies g^{-1} dry soil as previously done (Bouffaud *et al.* 2016, 2018; Ke *et al.*, 2019; Simonin
219 *et al.*, 2015, 2016, 2018; Renoud *et al.*, 2020). The size of the bacterial rhizosphere
220 community was calculated taking into account that a given individual bacterium may contain
221 several copies of *rrs*, ranging usually from 2 to 6 copies (Klappenbach *et al.*, 2001; Větrovský
222 & Baldrian, 2013). An intermediate value of 4 copies was chosen to estimate community size,
223 by dividing qPCR data by 4. To estimate the relative abundance of the four functional groups,
224 the corresponding qPCR data were divided by the number of *rrs* copies for the same sample.
225 All results were \log_{10} -transformed for subsequent analysis.

227 **2.5. *rrs* and *nifH* sequencing from bulk soil and rhizosphere DNA**

228 The diversity of the total bacterial community and of the free nitrogen fixers in rhizosphere
229 and bulk soil sampled at 40 days was assessed by Illumina MiSeq sequencing (2×300 bp) of
230 *rrs* and *nifH* amplicons, respectively. DNA extracts were sent to MolecularResearch (MR)
231 DNA laboratory (www.mrdnalab.com; Shallowater, Texas, USA) for sequencing. Primers
232 515F/806R (targeting the V4 region of *rrs* gene) (Caporaso *et al.*, 2011) and Zehr-nifHf/Zehr-
233 nifHr (for *nifH*) (Zehr & McReynolds, 1989) were used for the sequencing library, the
234 forward primer carrying a barcode. These *nifH* primers were not used for qPCR (see above) as
235 they are too degenerated (Gaby and Buckley, 2017). For each gene, a 30-cycles PCR was
236 achieved using the HotStarTaq Plus Master mix (Qiagen; Valencia, California, USA) with 3
237 min at 94°C, followed by 30 cycles of 30 s at 94°C, 40 s at 53°C and 1 min at 72°C, with a
238 final elongation step of 5 min at 72°C. Samples were pooled together in the same proportions
239 and purified using calibrated Ampure XP beads (Beckman Coulter; Brea, California, USA).
240 The DNA libraries were obtained with Illumina TruSeq DNA library protocol. Sequencing
241 was carried out on a MiSeq following manufacturer's instructions.

242 Sequencing data were treated with UPARSE pipeline (Edgar, 2013). Sequences were
243 depleted of barcodes, and sequences < 150 bp or with ambiguous base calls were removed.
244 The remaining sequences were denoised, operational taxonomic units (OTUs) were generated
245 (using UCLUST in standard default; Edgar, 2010) and chimeras were removed using
246 UCHIME (Edgar *et al.*, 2011). Final OTUs were classified using BLASTn comparing OTUs
247 to a database derived from RDPII and NCBI for both *rrs* and *nifH* sequencing
248 (rdp.cme.msu.edu, ncbi.nlm.nih.gov). For *rrs*, OTUs (*i.e.* genus to species level) were
249 defined at 97% similarity, genera at 94.5%, families at 86.5%, orders at 82%, classes at 78.5%
250 and phyla at 75% (Yarza *et al.*, 2014). For *nifH* also, the gene is highly conserved and carries
251 taxonomic information (Achouak *et al.*, 1999; Gaby & Buckley, 2014; Kapili *et al.*, 2020),

252 and *nifH*-based OTUs were defined at 97% similarity, as done previously (Collavino *et al.*,
253 2014; Gaby *et al.*, 2017; Renoud *et al.*, 2020). 80% of *nifH*-based OTUs could be affiliated
254 down to the species level, 13.5% to the genus level, and the rest could not. Results were
255 generated as contingency tables used for subsequent analysis. Taxonomy bar plots were
256 generated at the most informative taxonomic levels for *rrs* (*i.e.* phylum and genus) and *nifH*
257 (*i.e.* class, family, genus and species) sequences, using the same similarity thresholds for both
258 genes (Gaby *et al.*, 2017).

259

260 **2.6. Statistical analysis**

261 All analyses were done at $P < 0.05$, using R 4.0.3 software (<https://www.r-project.org>).

262 For qPCR results and sequence data, outliers were detected using the Grubbs' test
263 (Grubbs, 1969; Burns *et al.*, 2005), based on number of gene copies and Bray-Curtis distance,
264 respectively. For qPCR, at 14 days, this sorting removed one replicate of *Ae. tauschii*
265 genotype AT0 for *rrs*, one replicate each of *T. dicoccon* TD1, *Ae. tauschii* AT0 and *T.*
266 *aestivum* AE6 for *acdS*, and one replicate of *Ae. tauschii* AT0 and one replicate of *T. aestivum*
267 AE2 for *ppdC*. At 40 days, one replicate each of *T. urartu* TU2 and TU7, *Ae. tauschii* AT0
268 and *T. aestivum* AE6 for *rrs*, one replicate each of *T. urartu* TU8, *Ae. speltooides* AS8, *T.*
269 *dicoccon* TD9, TD3, TD1 and *Ae. tauschii* AT0 for *nifH*, one replicate each of *T. dicoccon*
270 TD9, *Ae. tauschii* AT5, AT0 and *T. aestivum* AE5 for *acdS*, and one replicate each of *T.*
271 *dicoccon* TD9, *Ae. tauschii* AT1 and *T. aestivum* AE2 for *ppdC*, were removed. For sequence
272 data, two replicates of *Ae. speltooides* AS7 and of *T. dicoccon* TD9 were removed for *nifH*
273 analysis.

274 For qPCR results, first an ANOVA approach with a nested fixed effects model,
275 followed by Tukey's HSD tests, was used to compare wheat species, taking into account the
276 variability between lines of a same species. Second, this approach was also carried out to

277 compare wheat lines belonging to a same species. Third, one-way ANOVA, followed by
278 Tukey's HSD tests, was performed to compare all wheat species with bulk soil, by
279 considering each wheat line as a replicate (*i.e.* using the means) for the corresponding species.
280 All data and residuals were checked, which showed normal distribution and homogeneity of
281 variance.

282 Sequence data were treated and filtered with the R package phyloseq, using a workflow
283 described previously (McMurdie & Holmes, 2013; Callahan *et al.*, 2015). For each wheat
284 species and line, rarefaction curves were generated to characterize sequencing depth. OTUs
285 retrieved (i) in less than 20% of the samples (*i.e.* less than 24 of the 122 samples) and (ii)
286 represented by no more than three sequences in the study were removed from the dataset. In
287 each sample, the sequence count for each OTU was divided by the total number of sequences
288 in that sample, and this normalization was done for the *nifH* and *rrs* datasets. α -Diversity was
289 investigated based on Shannon index, inverse Simpson index and observed richness. For these
290 indices, the same statistical approaches as for qPCR (*i.e.* nested ANOVA with two series of
291 Tukey's HSD tests, and one-way ANOVA with Tukey's HSD tests) were followed to
292 compare wheat species, wheat lines within species, as well as wheat and bulk soil.
293 Compositions of the total bacterial community and free nitrogen fixers were assessed for the
294 five wheat species, and bacterial taxa abundances were compared between species and bulk
295 soil by a one-way ANOVA followed by Tukey's HSD tests. The differences of structure and
296 taxonomic composition of the total rhizosphere bacterial community and of free nitrogen
297 fixers associated with the different wheat species and bulk soil were assessed using a
298 PERMANOVA/adonis ('vegan' package) on the same nested model as before (based on
299 variance comparison of Bray Curtis distances for the structure [Bray & Curtis, 1957], and on
300 differential taxa abundance for taxonomic composition), which was done with 10,000
301 permutations. Dissimilarities were visually represented using Non-metric MultiDimensional

302 Scaling (NMDS), where each point corresponds to one individual plant (replicate) of one
303 wheat line or one bulk soil replicate, and statistical ellipses (i.e. confidence interval of 95%
304 based on multivariate normal distribution) were calculated for bulk soil and each wheat
305 species. A Between-Class Analysis (BCA) was also performed, following a factorial design
306 where the inertia between lines of a same species is minimized and the inertia between lines
307 of different species is maximized. After calculation of the contribution of OTUs to each axis
308 of the factorial design, this allowed to identify OTUs most associated with each wheat
309 species.

310

311 **3. RESULTS**

312

313 **3.1. Abundance of the total bacterial community at 14 and 40 days**

314 The different wheat genotypes grew well in the sieved soil chosen, and the level of variability
315 for plant root biomass at 40 days was small (Table S5). At 14 days of growth of *Ae. tauschii*,
316 *T. dicoccon* and *T. aestivum*, the size of the rhizobacterial community of individual plants
317 ranged from 7.4 to 8.7 log₁₀ bacteria per g of rhizosphere soil (Figure 2A). For each wheat
318 species, the differences between genotypes were not significant. Bacterial community size in
319 the rhizosphere of *T. dicoccon* was higher than in bulk soil, but the differences between wheat
320 species were not significant.

321 At 40 days of growth, the size of the bacterial rhizosphere community varied from 6.3
322 to 8.1 log₁₀ bacteria per g of soil (Figure 2B). Within the five wheat species, *Ae. tauschii* AT0
323 displayed higher levels than the three other lines of this species, whereas the other differences
324 were not significant. Bacterial community size in the rhizosphere was higher for *T. urartu*
325 than for *Ae. speltoides*, and for *Ae. tauschii* and *T. aestivum* than for the three other wheat

326 species. In bulk soil, bacterial community size was higher than in the rhizosphere of *Ae.*
327 *speltoides* but lower than in the rhizosphere of *Ae. tauschii* and *T. aestivum*.

328

329 **3.2. Diversity of the total bacterial community at 40 days**

330 The *rrs* sequences obtained were grouped in 42,329 OTUs. Rarefaction curves indicated that
331 the asymptote was reached for 99 of the 110 samples (Figure S1).

332 For α -diversity, the only intra-species difference was between *T. dicoccon* lines TD3
333 and TD9 when using the inverse Simpson index (Figure 3C). The differences between wheat
334 species and bulk soil were not statistically significant based on Shannon or inverse Simpson
335 indices, whereas the Chao 1 index was higher for bulk soil, *Ae. tauschii* and *T. aestivum* than
336 for *T. urartu*, and for *T. aestivum* than for *Ae. speltoides* (Figure 3ABC).

337 Averaged profile of bacterial taxa abundance for each species is shown in Figure 4, as
338 rather similar profiles were obtained for each line belonging to a same species (not shown).
339 *Actinobacteria* and *Proteobacteria* were the most abundant phyla in the rhizosphere of all five
340 species and in bulk soil, followed with *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes* and
341 *Gemmatimonadetes* (Figure 4A). There were significantly more *Actinobacteria* with *T.*
342 *aestivum* in comparison to *T. urartu* and *Ae. speltoides*. The relative abundance of
343 *Bacteroidetes* was higher in the rhizosphere of *T. urartu*, *Ae. speltoides* and *T. dicoccon*
344 compared with *Ae. tauschii* and *T. aestivum*. The 20 most abundant genera represented about
345 half the sequences (Figure 4B), and among them the relative abundance of *Acidobacterium*
346 was higher in bulk soil compared with the rhizosphere of the five wheat species, and that of
347 *Conexibacter* was higher with *T. aestivum* than *T. urartu*, *Ae. speltoides* and *T. dicoccon*.

348 NMDS and PERMANOVA/adonis analyses showed that the bacterial communities
349 associated with *T. urartu*, *Ae. speltoides*, *T. dicoccon*, *T. aestivum* and bulk soil were all
350 statistically different from each other ($P < 0.05$; Table S4), whereas *Ae. tauschii* did not differ

351 statistically from *T. aestivum* and from bulk soil (the explanatory variable ‘wheat species’
352 accounting for 38% of the total data variation; Figure 5A; Table S4).

353 BCA indicated that OTUs 26 (the *Proteobacteria* genus *Pelobacter*), 83 (the
354 *Proteobacteria* species *Azospirillum oryzae*), 550 (the *Planctomycetes* genus *Pirelulla*) and
355 2927 (the *Verrucomicrobia* genus *Opitutus*) were most associated with *T. dicoccon* and *T.*
356 *urartu* (Figure S2A). The *Proteobacteria* OTUs 29247 (genus *Hyphomonas*), 602 (genus
357 *Paracoccus*) and 928 (genus *Sterolibacterium*) were most associated with *Ae. tauschii* and *T.*
358 *aestivum*. OTUs 872 (the *Acidobacteria* genus *Acidobacterium*) and 17229 (the
359 *Proteobacteria* genus *Burkholderia*) were most associated with bulk soil.

360

361 **3.3. Abundance of *nifH*, *acdS*, *ppdC* and *phlD*-based functional groups at 14 and 40 days**

362 At day 14, the number of diazotrophs ranged from 5.9 to 7.9 log₁₀ bacteria per g of
363 rhizosphere soil when considering plant individuals (Figure 2C), which corresponded to a
364 relative abundance (based on *nifH/rrs* ratio) of 0.0094 to 0.86 (Figure S3A). The *nifH* copy
365 number (log₁₀) was significantly higher in the rhizosphere of *T. dicoccon* than in the
366 rhizosphere of *Ae. tauschii* and *T. aestivum* and in bulk soil (Figure 2C), whereas the *nifH/rrs*
367 ratio was significantly lower in the rhizosphere of *T. aestivum* and in bulk soil than in the
368 rhizosphere of *T. durum*. At 40 days of growth, the number of diazotrophs ranged from 4.7 to
369 7.4 log₁₀ bacteria per g of rhizosphere soil (Figure 2D), which gave a *nifH/rrs* ratio from
370 0.0011 to 1 (Figure S3B). The number of *nifH* copies (log₁₀) was not significantly different
371 between the various wheat species, or between wheat species and bulk soil (Figure 2D), but
372 differences between lines within *A. tauschii* and *T. aestivum* species were observed.
373 Differences in *nifH/rrs* ratio between wheat species or with bulk soil were not significant
374 either, whereas differences were found between lines, within *Ae. tauschii* and *T. dicoccon*
375 (Figure S3B).

376 At day 14, the number of ACC deaminase microorganisms ranged from 5.8 to 7.4
377 \log_{10} *acdS*⁺ bacteria per g of rhizosphere soil (Figure 2E), which gave a relative abundance
378 (*acdS/rrs* ratio) from 0.0014 to 0.32 (Figure S3C). The number of *acdS* copies (\log_{10}) was
379 statistically different between *T. dicoccon*, *Ae. tauschii* and *T. aestivum*, and between
380 individual lines within *T. dicoccon* and *T. aestivum* (Figure 2E). The *acdS/rrs* ratio was
381 statistically lower in the rhizosphere of *Ae. tauschii* than in the rhizosphere of *T. aestivum* and
382 in bulk soil, and intra-species differences between lines were observed for *T. dicoccon* and *Ae.*
383 *tauschii* (Figure S3C). At 40 days of growth, the number of ACC deaminase microorganisms
384 varied between 4.7 and 6.5 \log_{10} *acdS*⁺ microorganisms per g of rhizosphere soil (Figure 2F),
385 which corresponded to an *acdS/rrs* ratio between 0.0042 and 0.059 (Figure S3D). The number
386 of *acdS* copies (\log_{10}) was significantly higher in the rhizosphere of *Ae. tauschii* and *T.*
387 *aestivum* compared with the rhizosphere of *T. urartu*, *Ae. speltoides*, *T. dicoccon* and in bulk
388 soil (Figure 2F), whereas differences between wheat species were not significant in terms of
389 relative abundance (Figure S4B).

390 At day 14, the number of *ppdC*⁺ IAA producers ranged from 5.6 to 7.3 \log_{10} *ppdC*⁺
391 bacteria per g of rhizosphere soil (Figure 2G), corresponding to a relative abundance (based
392 on *ppdC/rrs* ratio) from 0.0074 to 0.28 (Figure S3G). *ppdC* copy number (\log_{10}) was not
393 significantly different between the rhizosphere of *T. dicoccon*, *Ae. tauschii* and *T. aestivum*
394 (Figure 2G), but the *ppdC/rrs* ratio was significantly higher in the rhizosphere for *Ae. tauschii*
395 compared with *T. dicoccon* and in the rhizosphere of *Ae. tauschii* and *T. aestivum* than in bulk
396 soil (Figure S3E). At day 40, the number of *ppdC*⁺ IAA producers was between 4.9 and 7.1
397 \log_{10} *ppdC*⁺ bacteria per g of rhizosphere soil (Figure 2H), giving a *ppdC/rrs* ratio from
398 0.00108 to 0.55 (Figure S3F). The number of *ppdC* copies (\log_{10}) was higher in the
399 rhizosphere of *Ae. tauschii* and *T. aestivum* than in the rhizosphere of *T. urartu*, *Ae.*
400 *speltoides*, *T. dicoccon* and in bulk soil (Figure 2H). The relative abundance of *ppdC*⁺ IAA

401 producers was significantly higher in the rhizosphere of *Ae. tauschii* compared with the other
402 wheats (except *T. aestivum*) and bulk soil, as well as in *T. aestivum* rhizosphere than in *T.*
403 *urartu* rhizosphere. Intra-species differences were observed between lines of *Ae. tauschii*
404 (Figure S3F).

405 At day 14, the number of *phlD*⁺ bacteria was at the quantification limit (defined here
406 as the lowest concentration at which the method is able to provide quantification, *i.e.* the
407 lowest standard concentration giving a qPCR efficacy between 80 and 100%) of 1.20×10^3
408 *phlD* copies g⁻¹ dry soil or below this limit for respectively 3 and 7 of 17 *T. durum* samples, 6
409 and 10 of 19 *Ae. tauschii* samples, 6 and 7 of 24 *T. aestivum* samples and 1 and 5 of 6 bulk
410 soil samples, which means these bacteria could be quantified in only 7 samples of *T.*
411 *dicoccon*, 3 samples of *Ae. tauschii* and 11 samples of *T. aestivum*. For each of the three
412 species, the number of *phlD*⁺ bacteria in these samples ranged from 10⁴ to 10⁶ cells g⁻¹ dry
413 soil. Quantification of *phlD*⁺ bacteria in bulk soil was not possible (Figure S4). At day 40,
414 *phlD*⁺ bacteria were below detection limit for all samples.

415

416 **3.4. Diversity of free nitrogen fixers at 40 days**

417 *nifH* sequences obtained at 40 days were grouped in 9908 OTUs. Rarefaction curves indicated
418 that the asymptote was reached for 97 of the 115 samples (Figure S5). α -diversity analysis of
419 the free nitrogen fixers indicated that Chao 1 index was lower for *T. urartu* and *Ae. speltoides*
420 than for bulk soil and *T. dicoccon*, *Ae. tauschii* and *T. aestivum*, whereas the latter three
421 differed from one another (Figure 3D). Shannon index was lower for *T. urartu*, *Ae. speltoides*,
422 *T. dicoccon* compared with *Ae. tauschii*, as well as *T. urartu* and *T. dicoccon* compared with
423 *T. aestivum* (Figure 3E). Inverse Simpson index was higher for *T. urartu* and *Ae. speltoides*
424 than *Ae. tauschii* (Figure 3F). For all three indices, intra-species differences were not
425 significant.

426 Based on *nifH* data, six major classes of diazotrophs were found in the rhizosphere of
427 the five wheat species, *i.e.* *Actinobacteria*, *α-Proteobacteria*, *β-Proteobacteria*, *γ-*
428 *Proteobacteria*, *δ-Proteobacteria* and *Methanosarcina* (Figure S6A). The most abundant
429 genera were *Geobacter*, *Bradyrhizobium*, *Pelomonas*, *Rhizobium* and *Streptomyces* (Figure
430 4C). The relative abundance profiles (based on *nifH*⁺ genera) of *Ae. tauschii* and *T. aestivum*
431 were similar to that of bulk soil but different from the profile of *T. urartu*, *Ae. speltoides* and
432 *T. dicoccon*. The principal difference was a higher relative abundance of *nifH*⁺ *Geobacter* in
433 the rhizosphere of *Ae. tauschii* and *T. aestivum*, as well as in bulk soil compared with the
434 rhizosphere of the three other wheats (Figure 4C). There were more *nifH*⁺ *Streptomyces* and
435 *Pelomonas* in the rhizosphere of *T. urartu*, *Ae. speltoides* and *T. dicoccon* than in the
436 rhizosphere of *T. aestivum* and *Ae. tauschii*.

437 NMDS (Figure 5B) and PERMANOVA/adonis analyses showed that the diazotroph
438 communities associated with *T. urartu*, *Ae. speltoides* and *T. dicoccon* did not differ ($P >$
439 0.05 ; Table S4). Bray-Curtis distances indicated that *T. urartu* and *Ae. speltoides* were the
440 closest. *Ae. tauschii* and *T. aestivum* displayed similar diazotroph communities and differed
441 significantly from *T. urartu*, *Ae. speltoides* and *T. dicoccon* ($P < 0.05$; Figure 5B; Table S4).
442 *Ae. tauschii* line AT0 was very close to *T. aestivum* lines. Only *T. urartu* and *Ae. speltoides*
443 exhibited a diazotroph community significantly different from that of bulk soil. The ‘wheat
444 species’ variable explained 24% of the total variation observed.

445 BCA showed that the two *nifH*⁺ OTUs 9438 (the *β-Proteobacteria* genus
446 *Pseudoacidovorax*) and 82 (the *δ-Proteobacteria* genus *Geobacter*) were most associated with
447 *Ae. speltoides* and *T. urartu*. The *δ-Proteobacteria* OTUs 60, 70 and 47 (corresponding also
448 to the genus *Geobacter*) and the *α-Proteobacteria* OTUs 8 and 423 (genus *Bradyrhizobium*)
449 were most associated with *Ae. tauschii* as well as *T. aestivum*. The OTU 71 (corresponding
450 also to the genus *Bradyrhizobium*) was most associated with *T. dicoccon* (Figure S2B).

451

452 **4. DISCUSSION**

453

454 Due to several hybridization and polyploidization events, wheat genome architecture can vary
455 drastically between wheat species, which may have an impact on root properties (Waines &
456 Ehdaie, 2007; Pérez-Jaramillo *et al.*, 2016; Iannucci *et al.*, 2017; Roucou *et al.*, 2018; Golan
457 *et al.*, 2018; Ahmadi *et al.*, 2018; Gruet *et al.*, 2022a). Consequently, it is expected to affect
458 rhizosphere microbiota as well, a hypothesis that was tested in the current work. This was
459 made possible by the availability of current representatives with AA, BB, AABB, DD and
460 AABBDD genomic architectures, as we do not have access to the exact same wheat species or
461 genotypes that participated in the hybridization events (Gruet *et al.*, 2022a). In addition,
462 environmental conditions prevailing at the times of these hybridizations are not well
463 documented, and here we chose a single set of soil and greenhouse features, based on their
464 ability to sustain growth of the different wheat species tested, so as to focus comparisons on
465 the genomic effects. Indeed, in the experiment the different wheats followed similar
466 development patterns despite contrasted plant biomass (Table S3). The analysis of a large
467 range of genotypes meant that only one soil type could be used, as done when comparing
468 different wheat species (Ito *et al.*, 2020; Kinnunen-Grubb *et al.*, 2020; Spor *et al.*, 2020;
469 Tkacz *et al.*, 2020). Likewise, we used seeds produced under the same field growth conditions
470 to avoid potential bias. Substantial variability in microbial data was observed between
471 different lines of a same species, which could be expected since contrasted genotypes were
472 used to consider some of the diversity existing in each species. Against this background, we
473 sought to identify overriding effects on wheat-microorganisms interactions related to wheat
474 genome architecture.

475 The first approach focused on the size of microbial functional groups important for
476 plant growth. This was done by qPCR, as metagenomics at current sequencing depths is not as
477 effective for genes that are not highly abundant in the community. qPCR did not prove
478 effective for DAPG producers, which were often below the detection limit. In other
479 rhizosphere conditions and with other counting methods, the abundance of DAPG producers
480 was plant genotype-dependant (Mazzola *et al.*, 2004; Meyer *et al.*, 2010; Latz *et al.*, 2015).
481 Under soilless conditions, root colonization by DAPG⁺ *P. ogarae* F113 was higher for ancient
482 than modern genotypes of *T. aestivum* (Valente *et al.*, 2020). *phlD* gene is only present in few
483 species among the *Pseudomonas* (Almario *et al.*, 2017), and here only 254 of 41,165 *rrs*-
484 based OTUs (0.62%) were affiliated to this genus. For the other functional groups, the
485 comparison between AA (*T. urartu*), BB {SS} (*Ae. speltoides*) and AABB (*T. dicoccon*)
486 genome representatives did not evidence any significant difference in the total amounts of
487 *nifH*, *acdS* and *ppdC* genes at 40 days, suggesting a lack of specific impact of genome A vs B
488 on the size of the corresponding functional groups (Figure 2). For the comparison between
489 AABB (*T. dicoccon*), DD (*Ae. tauschii*) and AABBDD (*T. aestivum*) representatives, the total
490 amount of *nifH* genes did not differ at 40 days and was lower (but by a small extent) for *T.*
491 *aestivum* than for *Ae. tauschii* at 14 days. However, higher amounts of *acdS* and *ppdC* genes
492 were found at 40 days with *Ae. tauschii* and *T. aestivum* compared with *T. dicoccon*, which
493 suggests a particular effect of genome D. Similar effects were not observed at 14 days, even
494 though *acdS* levels for *T. aestivum* (but not *Ae. tauschii*) exceeded those for *T. dicoccon*. This
495 type of finding was made possible by the development of *acdS* and *ppdC* qPCR primers in
496 recent years (Bouffaud *et al.*, 2018; Gruet *et al.*, 2022b). In parallel, higher levels with *Ae.*
497 *tauschii* and *T. aestivum* compared with *T. dicoccon* (and bulk soil) were also observed for the
498 size of the total bacterial community at 40 days (while levels in bulk and rhizosphere soils did

499 not differ at 14 days). This suggests that genome D effects may materialize at a wider scale in
500 the root-associated microbiota, i.e. beyond the breadth of particular functional groups.

501 The second approach focused on the diversity of a key microbial functional group (N
502 fixers), which has the potential to contribute to nitrogen nutrition of wheat (Majeed *et al.*,
503 2015; Dellagi *et al.*, 2020). The diversity of N fixers can vary with *Poaceae* genotype, as
504 found in oat (Soares *et al.*, 2006), maize (Bouffaud *et al.*, 2016; Favela *et al.*, 2021) and rice
505 (Engelhard *et al.*, 2000), but effects potentially related to plant genomic architecture are not
506 documented. Here, the comparison between AA, BB {SS} and AABB representatives did not
507 show any particular effect of wheat genomic profile on the diversity of this functional group,
508 whereas *Ae. tauschii* (DD) and *T. aestivum* (AABBDD) recruited a different array of N fixers
509 compared with *T. dicoccon* (AABB), pointing again to a particular role for genome D (Figure
510 5). When considering *nifH*-based OTUs in the rhizosphere, δ -*Proteobacteria* of the
511 *Geobacteraceae* family were more prevalent with *Ae. tauschii* and *T. aestivum* than *T.*
512 *dicoccon*, and especially OTUs from the genera *Geobacter* and the species *Geobacter*
513 *uraniireducens*.

514 When considering the wider scale of the global rhizobacterial community, differences
515 between the AA, BB {SS} and AABB representatives (*T. urartu*, *Ae. speltooides* and *T.*
516 *dicoccon*, respectively) were statistically significant, unlike in Tkacz *et al.* (2020) where the
517 comparison focused on synthetic hybrids (rather than natural lines). Here, differences were
518 not significant for N fixers, meaning that selection of diazotrophs was a trait conserved during
519 the early stages of wheat evolution. Since the total bacterial community of *T. aestivum*
520 (AABBDD) did not differ from the one of *Ae. tauschii* (DD) while being distinct from that of
521 *T. dicoccon* (AABB), it also means that the diazotroph selection pattern of *Ae. tauschii*
522 (shared with its descendant *T. aestivum*) paralleled selection traits that operated also at the
523 scale of the whole community. Differences in whole bacterial community were also evidenced

524 in previous work, but with only one (Ito *et al.*, 2020) or two representatives (Wipf &
525 Coleman-Derr, 2021) for genomes BB {SS} and DD, which limited the possibility of inter-
526 species comparisons.

527 The fungal rhizosphere community is influenced by wheat genome architecture
528 (Özkurt *et al.*, 2019; Tkacz *et al.*, 2020). Thus, the presence of the D genome leads to
529 preferential selection of *Glomeromycota* fungi, as found for mycorrhizal colonization of
530 hexaploid wheat (Kapulnik & Kushnir, 1991; Hetrick *et al.*, 1992; Tkacz *et al.*, 2020). In
531 addition, several differences in stress tolerance and pathogen resistance between durum and
532 bread wheats are due to the D genome, not present in durum wheat (Saia *et al.*, 2019). For
533 example, resistance metabolites against *Fusarium* head blight involves expression of genome
534 D genes (Kage *et al.*, 2017). Here, the focus was on functional groups, and results point to
535 positive effects of the D genome on selection by hexaploid wheat of ACC deaminase
536 producers and IAA *ppdC*⁺ producers, two plant-beneficial functional groups. ACC deaminase
537 producers act as an ACC sink and can lower the availability of ACC, which is the precursor of
538 ethylene in plants. High concentration of ethylene, produced in case of stress, has deleterious
539 effects on plant growth, while lower ethylene levels stimulate root elongation (Glick, 2014).
540 ACC deaminase producers can improve the growth and yield of wheat and other plants under
541 salt stress and drought conditions (Zahir *et al.*, 2009; Shakir *et al.*, 2012; Hassan *et al.*, 2014).
542 Exogenous IAA affects various plant processes, leading to cell proliferation, root system
543 branching and root elongation (Scarpella *et al.*, 2010; Overvoorde *et al.*, 2010).
544 Microorganisms producing IAA via the phenylpyruvate decarboxylase pathway have plant-
545 beneficial effects (Spaepen *et al.*, 2007a), as shown upon inoculation of wheat with the *ppdC*⁺
546 PGPR *A. baldaniorum* Sp245 vs a *ppdC*⁻ derivative (Spaepen *et al.*, 2007b).

547 Even if genes in hexaploid wheat are highly conserved in each of the sub-genomes A,
548 B and D, without high global dominance of transcriptional activity (Haberer *et al.*, 2016), the

549 genes of each genome display different expression patterns depending on plant tissue or
550 growth stage (Soltis *et al.*, 2015; Ramirez-Gonzales *et al.*, 2018). Among transcripts
551 recovered from 11 different wheat tissues, those of homeolog genes of the D sub-genome
552 were more abundant than transcripts of the A and B sub-genomes, including in roots
553 (Ramirez-Gonzales *et al.*, 2018). It is conceivable that genes or alleles of the D genome could
554 lead to particular root properties and root exudation patterns likely to explain similarities with
555 *Ae. tauschii* in the recruitment of specific microbial populations.

556

557 **5. CONCLUSION**

558

559 This work extends previous findings on the impact of wheat genome architecture on the
560 taxonomic profile of the rhizobacterial community, by using a wider range of individual
561 genotypes for each type of genomic architecture and complementing total bacteria community
562 assessments with qPCR data. More importantly, we show for the first time that this impact is
563 also significant when considering specific microbial functional groups important for plant
564 growth, thereby validating our hypothesis. Our findings point to a significant role of the D
565 genome originating from *Ae. tauschii*, which means that the importance of genome D would
566 not be restricted to the case of mycorrhizal fungi (Tkacz *et al.*, 2020). Rather, it may be of
567 prime relevance for the functioning of beneficial plant-microbe interactions at large, which
568 opens new perspective for microbiota-targeted breeding.

569

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574

575 **CONFLICT OF INTEREST**

576 The authors declare no conflict of interest.

577

578 **DATA AVAILABILITY STATEMENT**

579 Sequencing data for *rrs* and *nifH* are available on the SRA database under the BioProject

580 numbers PRJNA798370 and PRJNA802348 respectively.

581

582 **SUPPLEMENTARY MATERIAL**

583 The Supplementary Material for this article can be found online.

584

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878

879 **Legends**

880

881 **FIGURE 1.** Simplified outline of wheat evolutionary history and place of the wheat species
882 studied. Coloured wheats (purple for wild species and blue for domesticated species) were
883 used as representatives of genomic architectures AA (*i.e.* *Triticum urartu*), SS (which is close
884 to BB; Pont et al. 2019) (*Aegilops speltoides*), DD (*Aegilops tauschii*), AABB (*Triticum*
885 *dicoccon*), and AABBDD (*Triticum aestivum*). A hybridization event took place 500 000
886 years ago between *T. urartu* (A genome) and the B genome donor, leading to the wild
887 tetraploid wheat *T. dicoccoides* and later to the cultivated emmer *T. dicoccon* and to *T. durum*.
888 Another hybridization took place around 10 000 years ago between *T. dicoccon* and *Ae.*
889 *tauschii* (D genome), leading subsequently to the hexaploid wheat *T. aestivum*. Wheat
890 genomes are composed of 14 (AA, BB or DD), 28 (AABB) or 42 chromosomes (AABBDD).

891

892 **FIGURE 2.** Size of the total bacterial community and of microbial functional groups in bulk
893 soil and the rhizosphere of wheat species at 14 and 40 days of growth. (A and B) Size of the
894 bacterial community at 14 (A) and 40 (B) days of growth. Raw copy numbers of *rrs* were
895 divided by 4 (*i.e.* corrected *rrs* numbers) to estimate bacterial numbers. Plant sequences
896 representing in the order of $0.02 \log_{10}$ only, their impact on qPCR was negligible. (C and D)
897 Size of the diazotroph community at 14 (C) and 40 (D) days. (E and F) Size of the ACC
898 deaminase community at 14 (E) and 40 (F) days. (G and H) Size of the *ppdC*⁺ IAA producer
899 community at 14 (G) and 40 (H) days. Wild wheats are portrayed in purple and domesticated
900 wheats in blue. Lines are (from left to right) TU2, TU7, TU8 for *T. urartu*, AS1, AS3, AS7,
901 AS8 for *Ae. speltoides*, TD0 (not sampled at day 14 because of insufficient development),
902 TD1, TD3, TD9 for *T. dicoccon*, AT0, AT1, AT5, AT9 for *Ae. tauschii*, and AE2, AE4, AE5,
903 AE6 for *T. aestivum*. Differences between species were assessed by a nested ANOVA and

904 Tukey's HSD tests, while a one-way ANOVA followed by Tukey's HSD tests was performed
905 to compare all wheat species with bulk soil ($P < 0.05$; letters α , β and γ are used to show
906 statistical differences). Differences between lines of a same species were studied by a nested
907 ANOVA and Tukey's HSD tests ($P < 0.05$; letters a, b and c are used to show statistical
908 differences).

909

910 **FIGURE 3.** α -Diversity of total bacterial community and free nitrogen-fixers in bulk soil and
911 the rhizosphere of wheat species at 40 days of growth, based on *rrs* and *nifH* analysis. Chao 1
912 (A and D), Shannon (B and E) and inverse Simpson (InvSimpson; C and F) diversity indices
913 were computed. Wild wheats are portrayed in purple and domesticated wheats in blue. Lines
914 are (from left to right) TU2, TU7, TU8 for *T. urartu*, AS1, AS3, AS7, AS8 for *Ae. speltoides*,
915 TD0, TD1, TD3, TD9 for *T. dicoccon* AT0, AT1, AT5, AT9 for *Ae. tauschii*, and AE2, AE4,
916 AE5, AE6 for *T. aestivum*. Differences between species were assessed by a nested ANOVA
917 and Tukey's HSD tests, while a one-way ANOVA followed by Tukey's HSD tests was
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919 show statistical differences). Differences between lines of a same species were studied by a
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921 statistical differences).

922

923 **FIGURE 4.** Relative abundance of bacterial taxa of the total bacterial community and free
924 nitrogen-fixers in bulk soil and the rhizosphere of wheats, based on *rrs* and *nifH* analysis.
925 Relative abundance of all phyla (A) and the 20 most abundant genera (B) based on *rrs*
926 analysis. Relative abundance of genera (C) and species (D) based on *nifH* analysis.

927

928 **FIGURE 5.** Non-metric MultiDimensional Scaling (NMDS) of the total bacterial community
929 and the free nitrogen-fixers in bulk soil and the rhizosphere of wheats at 40 days, based on *rrs*
930 and *nifH* data and Bray-Curtis distances. (A) NMDS of the rhizobacterial community. (B)
931 NMDS of the free nitrogen-fixers (B). Each point corresponds to one individual plant
932 (replicate) of a given wheat line or one bulk soil sample, and different symbols are used for
933 the different wheat lines. Distinct colours are used for each of the wheat species and bulk soil.
934 Statistical ellipses (confidence interval of 95% based on assuming multivariate normal
935 distribution) are shown for each wheat species and for bulk soil. Letters a, b, c, d and e are
936 used to show statistical relations between wheat species and bulk soil
937 (PERMANOVA/Adonis, $P < 0.05$).

938

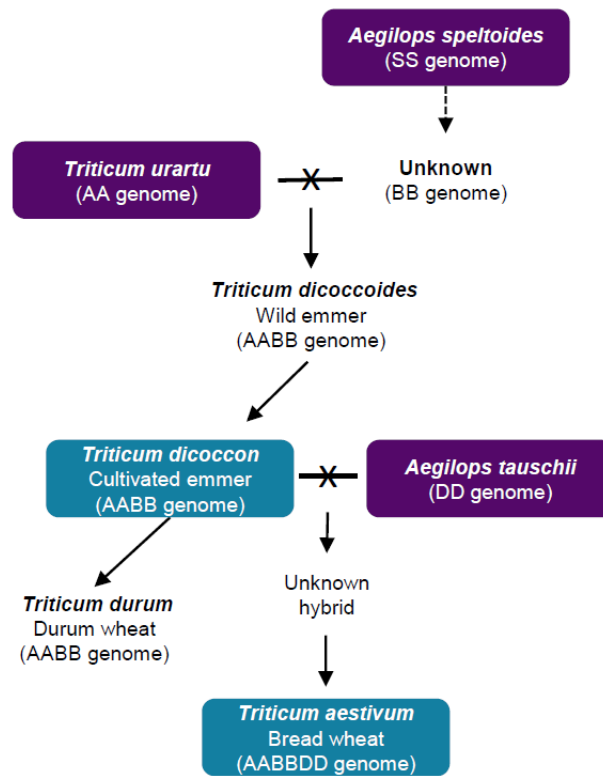


Figure 1. Simplified outline of wheat evolutionary history and place of the wheat species studied. Coloured wheats (purple for wild species and blue for domesticated species) were used as representatives of genomic architectures AA (*i.e.* *Triticum urartu*), SS (which is close to BB; Pont et al. 2019) (*Aegilops speltoides*), DD (*Aegilops tauschii*), AABB (*Triticum dicoccon*), and AABBDD (*Triticum aestivum*). A hybridization event took place 500 000 years ago between *T. urartu* (A genome) and the B genome donor, leading to the wild tetraploid wheat *T. dicoccoides* and later to the cultivated emmer *T. dicoccon* and to *T. durum*. Another hybridization took place around 10 000 years ago between *T. dicoccon* and *Ae. tauschii* (D genome), leading subsequently to the hexaploid wheat *T. aestivum*. Wheat genomes are composed of 14 (AA, BB or DD), 28 (AABB) or 42 chromosomes (AABBDD).

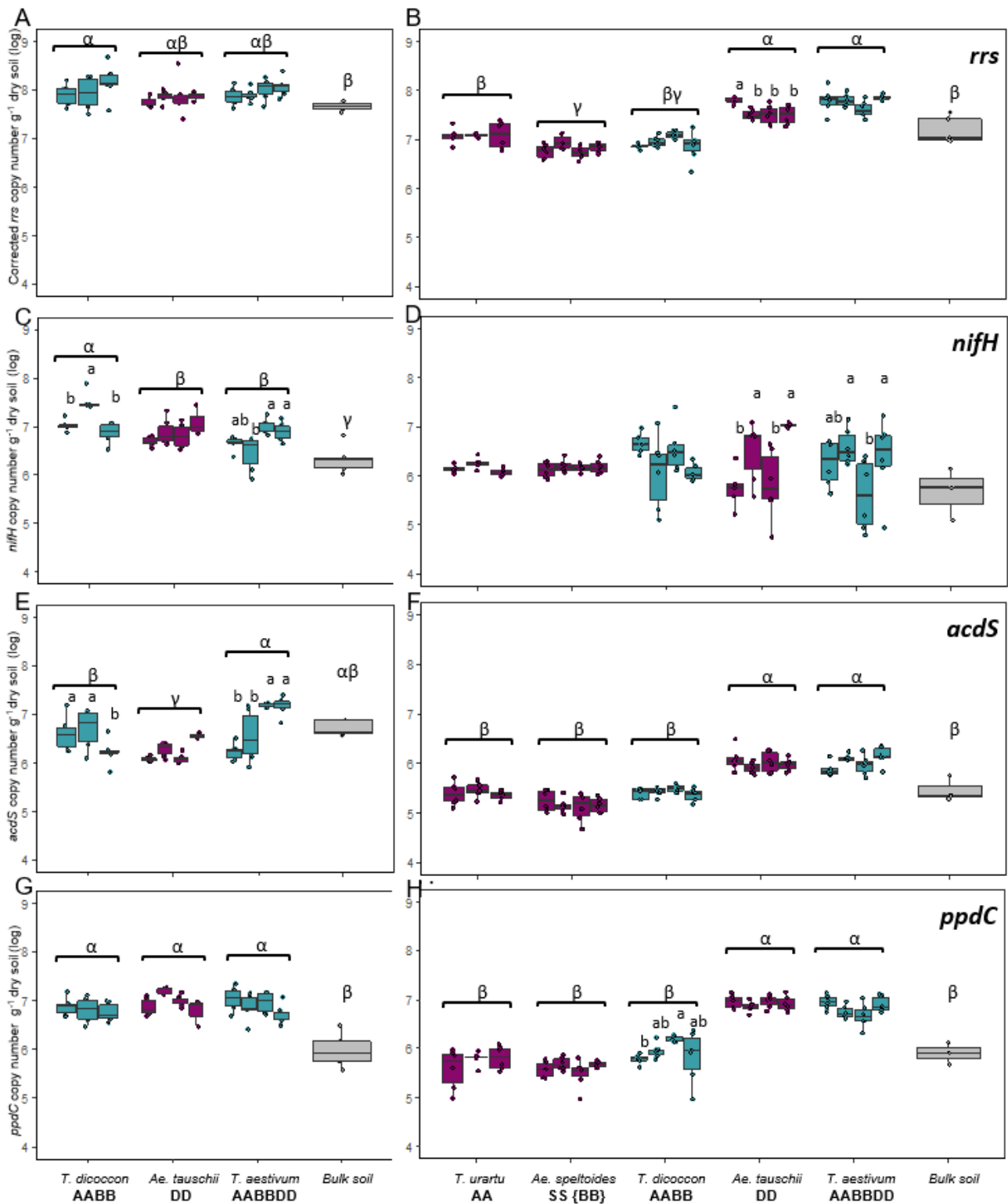


Figure 2. Size of the total bacterial community and of microbial functional groups in bulk soil and the rhizosphere of wheat species at 14 and 40 days of growth. (A and B) Size of the bacterial community at 14 (A) and 40 (B) days of growth. Raw copy numbers of *rrs* were divided by 4 (i.e. corrected *rrs* numbers) to estimate bacterial numbers. Plant sequences representing in the order of 0.02 \log_{10} only, their impact on qPCR was negligible. (C and D) Size of the diazotroph community at 14 (C) and 40 (D) days. (E and F) Size of the ACC deaminase community at 14 (E) and 40 (F) days. (G and H) Size of the *ppdC*⁺ IAA producer community at 14 (G) and 40 (H) days. 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. speltoides*, 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 α , β and γ 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).

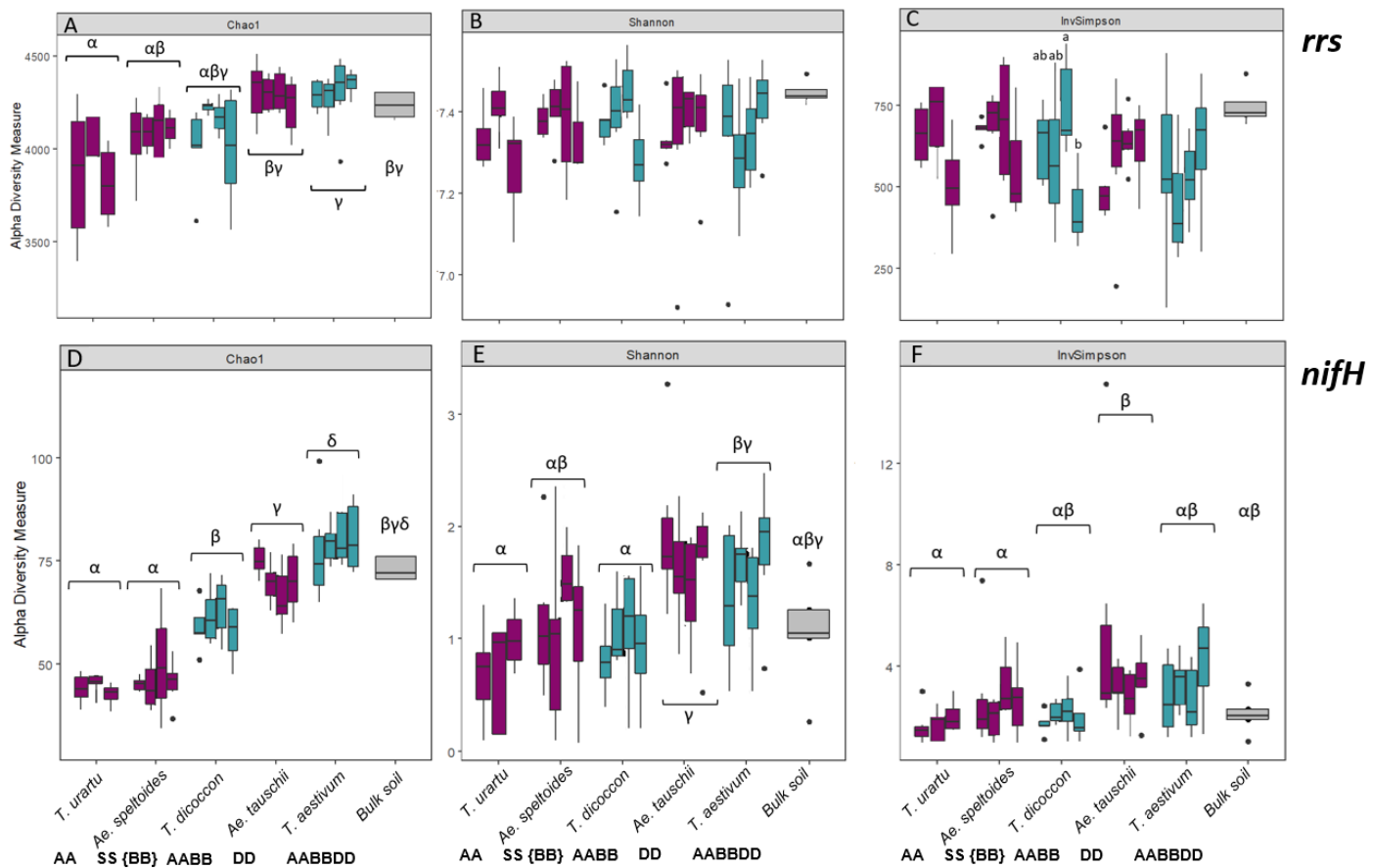


Figure 3. α -Diversity of total bacterial community and free nitrogen-fixers in bulk soil and the rhizosphere of wheat species at 40 days of growth, based on *rrs* and *nifH* analysis. Chao 1 (A and D), Shannon (B and E) and inverse Simpson (InvSimpson; C and F) diversity indices were computed. Wild 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. speltoides*, TD0, 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 α , β and γ 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).

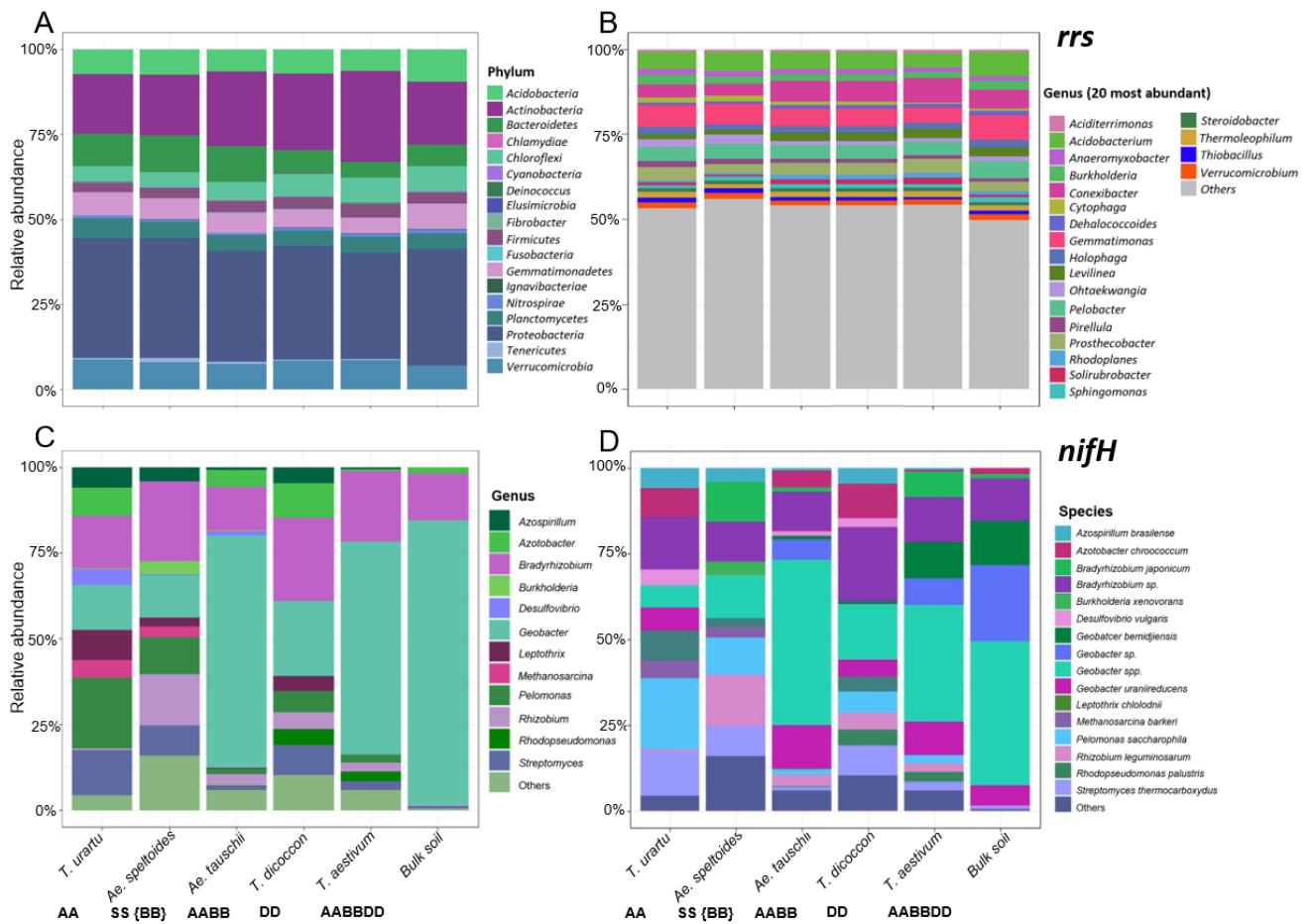


Figure 4. Relative abundance of bacterial taxa of the total bacterial community and free nitrogen-fixers in bulk soil and the rhizosphere of wheats, based on *rrs* and *nifH* analysis. Relative abundance of all phyla (A) and the 20 most abundant genera (B) based on *rrs* analysis. Relative abundance of genera (C) and species (D) based on *nifH* analysis.

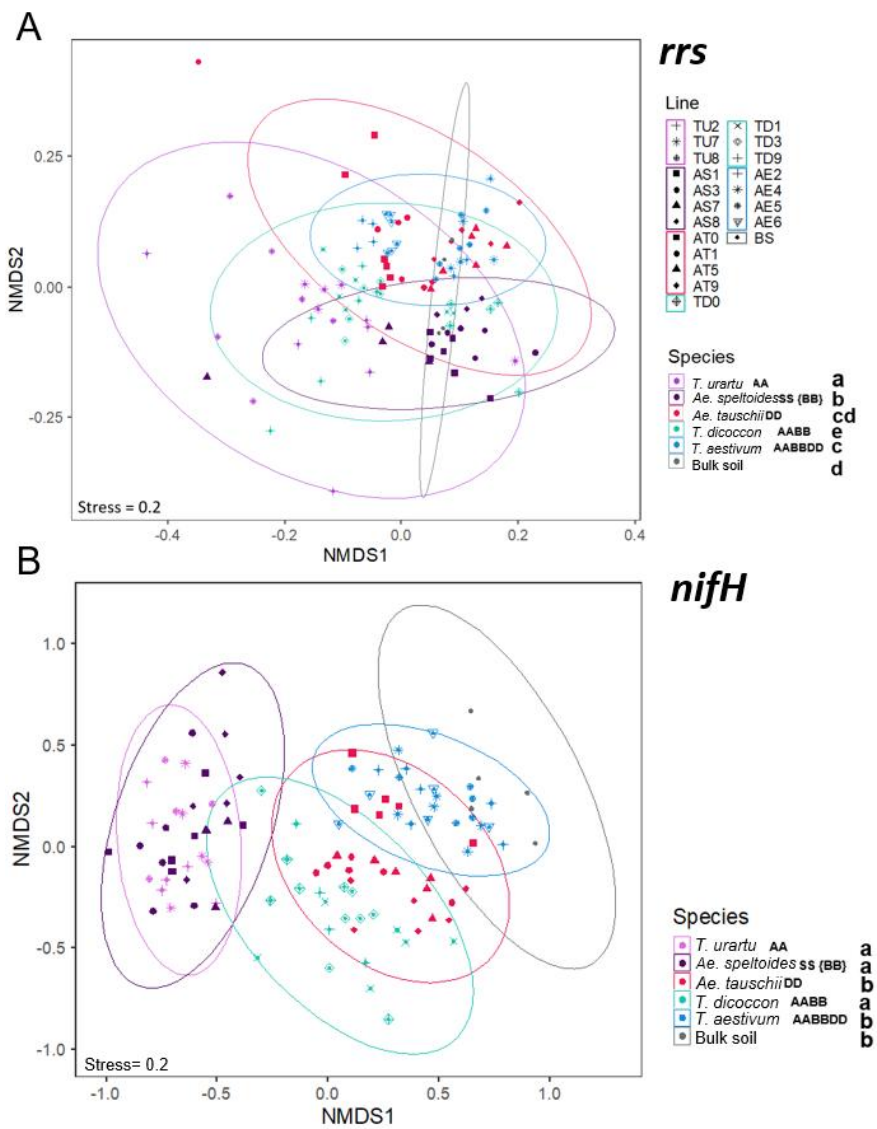


Figure 5. Non-metric MultiDimensional Scaling (NMDS) of the total bacterial community and the free nitrogen-fixers in bulk soil and the rhizosphere of wheats at 40 days, based on *rrs* and *nifH* data and Bray-Curtis distances. (A) NMDS of the rhizobacterial community. (B) NMDS of the free nitrogen-fixers (B). 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. Distinct colours are used for each of the wheat species and bulk soil. Statistical ellipses (assuming multivariate normal distribution) are shown for each wheat species and for bulk soil. Letters a, b, c, d and e are used to show statistical relations between wheat species and bulk soil (PERMANOVA/Adonis, $P < 0.05$).