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

3

4

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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 N<sub>2</sub>  
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*<sup>+</sup> 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<sup>-1</sup>) 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-dm<sup>3</sup> 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. speltoides* 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<sup>-1</sup> 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<sup>-1</sup>. 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*<sup>+</sup> 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  $\mu\text{L}$  of sample  
177 DNA (10 ng DNA), and primers 519F/907R for *rrs* (0.6  $\mu\text{L}$ , to reach a final concentration of  
178 0.6  $\mu\text{M}$ ), 3F*ppdC*/3R*ppdC* for *ppdC* (0.6  $\mu\text{L}$ , final concentration 0.6  $\mu\text{M}$ ), *polF/polR* for *nifH*  
179 (2  $\mu\text{L}$ , final concentration 0.5  $\mu\text{M}$ ), *accF5/accR8* for *acdS* (1  $\mu\text{L}$ , final concentration 1  $\mu\text{M}$ ) or  
180 B2BR3/B2BF for *phlD* (1  $\mu\text{L}$ , final concentration 1  $\mu\text{M}$ ). PCR-grade water was added to  
181 reach 20  $\mu\text{L}$ . 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<sup>-1</sup>. 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 10<sup>6</sup> 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<sup>-9</sup> to 5 × 10<sup>-14</sup> g DNA  $\mu\text{L}^{-1}$   
199 (corresponding to 1.5 × 10<sup>6</sup> 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  $5 \times 10^{-9}$  to  $5 \times$   
202  $10^{-14}$  g DNA  $\mu\text{L}^{-1}$  (corresponding to  $1 \times 10^6$  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  $5 \times 10^{-9}$  to  
205  $5 \times 10^{-14}$  g DNA  $\mu\text{L}^{-1}$  (corresponding to  $1.5 \times 10^6$  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  $3 \times 10^{-9}$  to  $3 \times 10^{-14}$  g DNA  $\mu\text{L}^{-1}$  (corresponding  
209 to  $9 \times 10^5$  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  $\mu\text{L}^{-1}$  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  $\text{mol}^{-1}$  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  $\text{g}^{-1}$  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](http://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](http://rdp.cme.msu.edu), [ncbi.nlm.nih.gov](http://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.  $\alpha$ -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 log<sub>10</sub> 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 log<sub>10</sub> 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  $\alpha$ -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 log<sub>10</sub> 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 (log<sub>10</sub>) 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 log<sub>10</sub> 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 (log<sub>10</sub>) 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*<sup>+</sup> 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*<sup>+</sup> 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*<sup>+</sup> IAA producers ranged from 5.6 to 7.3  $\log_{10}$  *ppdC*<sup>+</sup>  
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*<sup>+</sup> IAA producers was between 4.9 and 7.1  
397  $\log_{10}$  *ppdC*<sup>+</sup> 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*<sup>+</sup> 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*<sup>+</sup> 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  $1.20 \times 10^3$   
408 *phlD* copies g<sup>-1</sup> 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*<sup>+</sup> bacteria in these samples ranged from 10<sup>4</sup> to 10<sup>6</sup> cells g<sup>-1</sup> dry  
413 soil. Quantification of *phlD*<sup>+</sup> bacteria in bulk soil was not possible (Figure S4). At day 40,  
414 *phlD*<sup>+</sup> 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).  $\alpha$ -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*<sup>+</sup> 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*<sup>+</sup> *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*<sup>+</sup> *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*<sup>+</sup> 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<sup>+</sup> *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,  $\delta$ -*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. speltoides* 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*<sup>+</sup> 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*<sup>+</sup>  
546 PGPR *A. baldaniorum* Sp245 vs a *ppdC*<sup>-</sup> 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

585 **REFERENCES**

586

587 Achouak W, Normand P, Heulin T. 1999. Comparative phylogeny of *rrs* and *nifH* genes in  
588 the *Bacillaceae*. *International Journal of Systematic and Evolutionary Microbiology* 49:  
589 961–967.

590 Ahmadi J, Pour-Aboughadareh A, Fabriki-Ourang S, Mehrabi A-A, Siddique KH. 2018.  
591 Screening wheat germplasm for seedling root architectural traits under contrasting water  
592 regimes: potential sources of variability for drought adaptation. *Archives of Agronomy  
593 and Soil Science* 64: 1351–1365.

594 Almario J, Bruto M, Vacheron J, Prigent-Combaret C, Moëgne-Loccoz Y, Muller D. 2017.  
595 Distribution of 2,4-diacetylphloroglucinol biosynthetic genes among the *Pseudomonas*  
596 spp. reveals unexpected polyphyletism. *Frontiers in Microbiology* 8: 1218.

597 Asouti E, Fuller DQ. 2012. From foraging to farming in the southern Levant: the development  
598 of Epipalaeolithic and Pre-pottery Neolithic plant management strategies. *Vegetation  
599 History and Archaeobotany* 21: 149–162.

600 Baidouri ME, Murat F, Veyssiere M, Molinier M, Flores R, Burlot L, Alaux M, Quesneville  
601 H, Pont C, Salse J. 2017. Reconciling the evolutionary origin of bread wheat (*Triticum  
602 aestivum*). *New Phytologist* 213: 1477–1486.

603 Bay SK, McGeoch MA, Gillor O, Wieler N, Palmer DJ, Baker DJ, Chown SL, Greening C.  
604 2020. Soil bacterial communities exhibit strong biogeographic patterns at fine taxonomic  
605 resolution. *mSystems* 5: e00540-20

606 Bernardo L, Carletti P, Badeck FW, Rizza F, Morcia C, Ghizzoni R, Roupheal Y, Colla G,  
607 Terzi V, Lucini L. 2019. Metabolomic responses triggered by arbuscular mycorrhiza  
608 enhance tolerance to water stress in wheat cultivars. *Plant Physiology and Biochemistry*  
609 137: 203–212.

610 Bouffaud M-L, Renoud S, Dubost A, Moëgne-Loccoz Y, Muller D. 2018. 1-  
611 Aminocyclopropane-1-carboxylate deaminase producers associated to maize and other  
612 *Poaceae* species. *Microbiome* 6: 114.

613 Bouffaud M-L, Renoud S, Moëgne-Loccoz Y, Muller D. 2016. Is plant evolutionary history  
614 impacting recruitment of diazotrophs and *nifH* expression in the rhizosphere? *Scientific*  
615 *Reports* 6: 1-9.

616 Bray JR, Curtis JT. 1957. An ordination of the upland forest communities of southern  
617 Wisconsin. *Ecological Monographs* 27: 326–349.

618 Brazelton JN, Pfeufer EE, Sweat TA, McSpadden Gardener BB, Coenen C. 2008. 2,4-  
619 Diacetylphloroglucinol alters plant root development. *Molecular Plant-Microbe*  
620 *Interactions* 21: 1349–1358.

621 Bru D, Ramette A, Saby NPA, Dequiedt S, Ranjard L, Jolivet C, Arrouays D, Philippot L.  
622 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the  
623 landscape scale. *The ISME Journal* 5: 532–542.

624 Burns MJ, Nixon GJ, Foy CA, Harris N. 2005. Standardisation of data from real-time  
625 quantitative PCR methods – evaluation of outliers and comparison of calibration curves.  
626 *BMC Biotechnology* 5: 31.

627 Cadot S, Gfeller V, Hu L, Singh N, Sanchez-Vallet A, Glauser G, Croll D, Erb M, van der  
628 Heijden M, Schlaeppi K. 2021. Soil composition and plant genotype determine  
629 benzoxazinoid-mediated plant-soil feedbacks in cereals. *Plant, Cell & Environment* 44: 3732-  
630 3744.

631 Callahan B, Proctor D, Relman D, Fukuyama J, Holmes S. 2015. Reproducible research  
632 workflow in R for the analysis of personalized human microbiome data. In:  
633 Biocomputing 2016, *Proceedings in the Pacific Symposium*, 183–194.

634 Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer  
635 N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of  
636 sequences per sample. *Proceedings of the National Academy of Sciences of the United*  
637 *States of America* 108: 4516–4522.

638 Collavino MM, Tripp HJ, Frank IE, Vidoz ML, Calderoli PA, Donato M, Zehr JP, Aguilar  
639 OM. 2014. *nifH* pyrosequencing reveals the potential for location-specific soil chemistry  
640 to influence N<sub>2</sub>-fixing community dynamics. *Environmental Microbiology* 16: 3211–  
641 3223.

642 Dellagi A, Quillere I, Hirel B. 2020. Beneficial soil-borne bacteria and fungi: a promising  
643 way to improve plant nitrogen acquisition. *Journal of Experimental Botany* 71: 4469-  
644 4479.

645 Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads.  
646 *Nature Methods* 10: 996-998.

647 Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity  
648 and speed of chimera detection. *Bioinformatics* 27: 2194-2200.

649 Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST.  
650 *Bioinformatics* 26: 2460-2461.

651 Engelhard M, Hurek T, Reinhold- Hurek B. 2000. Preferential occurrence of diazotrophic  
652 endophytes, *Azoarcus* spp., in wild rice species and land races of *Oryza sativa* in  
653 comparison with modern races. *Environmental Microbiology* 2: 131–141.

654 Favela A, O. Bohn M, D. Kent A. 2021. Maize germplasm chronosequence shows crop  
655 breeding history impacts recruitment of the rhizosphere microbiome. *The ISME Journal*  
656 15: 2454–2464.

657 Feldman M, Kislev ME. 2007. Domestication of emmer wheat and evolution of free-threshing  
658 tetraploid wheat. *Israel Journal of Plant Sciences* 55: 207–221.

659 Gaby JC, Buckley DH. 2014. A comprehensive aligned *nifH* gene database: a multipurpose  
660 tool for studies of nitrogen-fixing bacteria. *Database* 2014: bau001.

661 Gaby JC, Rishishwar L, Valderrama-Aguirre LC, Green SJ, Valderrama-Aguirre A, Jordan  
662 IK, Kostka JE. 2017. Diazotroph community characterization via a high-throughput *nifH*  
663 amplicon sequencing and analysis pipeline. *Applied and Environmental Microbiology* 84:  
664 e01512-17.

665 Gaby JC, Buckley DH. 2017. The use of degenerate primers in qPCR analysis of functional  
666 genes can cause dramatic quantification bias as revealed by investigation of *nifH* primer  
667 performance. *Microbial Ecology* 74: 701-708.

668 Glémin S, Scornavacca C, Dainat J, Burgarella C, Viader V, Ardisson M, Sarah G, Santoni S,  
669 David J, Ranwez V. 2019. Pervasive hybridizations in the history of wheat relatives.  
670 *Science Advances* 5: eaav9188.

671 Glick BR. 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC  
672 deaminase. *FEMS Microbiology Letters* 251: 1–7.

673 Glick BR. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the  
674 world. *Microbiological Research* 169: 30–39.

675 Golan G, Hendel E, Méndez Espitia GE, Schwartz N, Peleg Z. 2018. Activation of seminal  
676 root primordia during wheat domestication reveals underlying mechanisms of plant  
677 resilience: Root number determines drought adaptation in wheat. *Plant, Cell &*  
678 *Environment* 41: 755–766.

679 Grubbs FE. 1969. Procedures for detecting outlying observations in samples. *Technometrics*  
680 11: 1–21.

681 Gruet C, Muller D, Moëgne-Loccoz Y. 2022a. Significance of the diversification of wheat  
682 species for the assembly and functioning of the root associated-microbiome. *Frontiers in*  
683 *Microbiology* doi.org/10.3389/fmicb.2021.782135

684 Gruet C, Oudot A, Abrouk D, Moënne-Loccoz Y, Muller D. 2022b. Rhizosphere analysis of  
685 auxin producers harboring the phenylpyruvate decarboxylase pathway. *Applied Soil*  
686 *Ecology* 173: 104363.

687 Haas M, Schreiber M, Mascher M. 2019. Domestication and crop evolution of wheat and  
688 barley: Genes, genomics, and future directions. *Journal of Integrative Plant Biology* 61:  
689 204–225.

690 Haberer G, Mayer KF, Spannagl M. 2016. The big five of the monocot genomes. *Current*  
691 *Opinion in Plant Biology* 30: 33–40.

692 Hassan W, Bano R, Bashir F, David J. 2014. Comparative effectiveness of ACC-deaminase  
693 and/or nitrogen-fixing rhizobacteria in promotion of maize (*Zea mays* L.) growth under  
694 lead pollution. *Environmental Science and Pollution Research* 21: 10983–10996.

695 Hetrick BAD, Wilson GWT, Cox TS. 1992. Mycorrhizal dependence of modern wheat  
696 varieties, landraces, and ancestors. *Canadian Journal of Botany* 70: 2032–2040.

697 Hou S, Thiergart T, Vannier N, Mesny F, Ziegler J, Pickel B, Hacquard S. 2021. A  
698 microbiota-root-shoot circuit favours *Arabidopsis* growth over defence under suboptimal  
699 light. *Nature Plants* 7: 1078-1092.

700 Iannucci A, Canfora L, Nigro F, De Vita P, Beleggia R. 2021. Relationships between root  
701 morphology, root exudate compounds and rhizosphere microbial community in durum  
702 wheat. *Applied Soil Ecology* 158: 103781.

703 Iannucci A, Fragasso M, Beleggia R, Nigro F, Papa R. 2017. Evolution of the crop  
704 rhizosphere: impact of domestication on root exudates in tetraploid wheat (*Triticum*  
705 *turgidum* L.). *Frontiers in Plant Science* 8: 2124.

706 Ito N, Mori N, Miyashita NT. 2020. Rhizospheric bacterial community structure of *Triticum*  
707 and *Aegilops* revealed by pyrosequencing analysis of the 16S rRNA gene: dominance of  
708 the A genome over the B and D genomes. *Genes & Genetic Systems* 95: 249–268.



709 Jackson SA. 2017. Epigenomics: dissecting hybridization and polyploidization. *Genome*  
710 *Biology* 18: 117.

711 Kage U, Karre S, Kushalappa AC, McCartney C. 2017. Identification and characterization of  
712 a fusarium head blight resistance gene *TaACT* in wheat QTL-2DL. *Plant Biotechnology*  
713 *Journal* 15: 447–457.

714 Kapili BJ, Barnett SE, Buckley DH, Dekas AE. 2020. Evidence for phylogenetically and  
715 catabolically diverse active diazotrophs in deep-sea sediment. *The ISME Journal* 14:  
716 971–983.

717 Kapulnik Y, Kushnir U. 1991. Growth dependency of wild, primitive and modern cultivated  
718 wheat lines on vesicular-arbuscular mycorrhiza fungi. *Euphytica* 56: 27–36.

719 Karimi B, Terrat S, Dequiedt S, Saby NPA, Horrigue W, Lelièvre M, Nowak V, Jolivet C,  
720 Arrouays D, Wincker P, *et al.* 2018. Biogeography of soil bacteria and archaea across  
721 France. *Science Advances* 4: eaat1808.

722 Ke X, Feng S, Wang J, Lu W, Zhang W, Chen M, Lin M. 2019. Effect of inoculation with  
723 nitrogen-fixing bacterium *Pseudomonas stutzeri* A1501 on maize plant growth and the  
724 microbiome indigenous to the rhizosphere. *Systematic and Applied Microbiology* 42:  
725 248-260.

726 Klappenbach JA, Saxman PR, Cole JR, Schmidt TM. 2001. rrndb: the ribosomal RNA operon  
727 copy number database. *Nucleic Acids Research* 29: 181–184.

728 Kinnunen-Grubb M, Sapkota R, Vignola M, Nunes IM, Nicolaisen M. 2020. Breeding  
729 selection imposed a differential selective pressure on the wheat root-associated  
730 microbiome. *FEMS Microbiology Ecology* 96: fiae196.

731 Latz E, Eisenhauer N, Scheu S, Jousset A. 2015. Plant identity drives the expression of  
732 biocontrol factors in a rhizosphere bacterium across a plant diversity gradient. *Functional*  
733 *Ecology* 29: 1225–1234.

734 Liu W, Zhou X, Jin T, Li Y, Wu B, Yu D, Delgado-Baquerizo M. 2022. Multikingdom  
735 interactions govern the microbiome in subterranean cultural heritage sites. *Proceedings*  
736 *of the National Academy of Sciences of the United States of America* 119: e2121141119.

737 Majeed A, Abbasi MK, Hameed S, Imran A, Rahim N. 2015. Isolation and characterization of  
738 plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant  
739 growth promotion. *Frontiers in Microbiology* 6: 198.

740 Marcussen T, Sandve SR, Heier L, Spannagl M, Pfeifer M, The International Wheat Genome  
741 Sequencing Consortium, Jakobsen KS, Wulff BBH, Steuernagel B, Mayer KFX, *et al.*  
742 2014. Ancient hybridizations among the ancestral genomes of bread wheat. *Science* 345:  
743 1250092.

744 Mazzola M, Funnell DL, Raaijmakers JM. 2004. Wheat cultivar-specific selection of 2,4-  
745 diacetylphloroglucinol-producing fluorescent *Pseudomonas* species from resident soil  
746 populations. *Microbial Ecology* 48: 338–348.

747 McMurdie PJ, Holmes S. 2013. phyloseq: An R Package for reproducible interactive analysis  
748 and graphics of microbiome census data. *PLoS ONE* 8: e61217.

749 Messing J. 2009. The polyploid origin of maize. In: Bennetzen JL, Hake S, eds. Handbook of  
750 maize: genetics and genomics. New York, NY: Springer, 221–238.

751 Meyer JB, Lutz MP, Frapolli M, Péchy-Tarr M, Rochat L, Keel C, Défago G, Maurhofer M.  
752 2010. Interplay between wheat cultivars, biocontrol pseudomonads, and soil. *Applied and*  
753 *Environmental Microbiology* 76: 6196–6204.

754 Moëgne-Loccoz Y, Mavingui P, Combes C, Normand P, Steinberg C. 2015. Microorganisms  
755 and biotic interactions. In: Environmental Microbiology: Fundamentals and Applications,  
756 395–444.

757 Narula N, Kumar V, Behl RK, Deubel A, Gransee A, Merbach W. 2000. Effect of P-  
758 solubilizing *Azotobacter chroococcum* on N, P, K uptake in P-responsive wheat

759 genotypes grown under greenhouse conditions. *Journal of Plant Nutrition and Soil*  
760 *Science* 163: 393–398.

761 Nemergut DR, Costello EK, Hamady M, Lozupone C, Jiang L, Schmidt SK, Fierer N,  
762 Townsend AR, Cleveland CC, Stanish L, *et al.* 2011. Global patterns in the biogeography  
763 of bacterial taxa. *Environmental Microbiology* 13: 135–144.

764 Nowell RW, Laue BE, Sharp PM, Green S. 2016. Comparative genomics reveals genes  
765 significantly associated with woody hosts in the plant pathogen *Pseudomonas syringae*:  
766 Adaptation to woody hosts in *Pseudomonas syringae*. *Molecular Plant Pathology* 17:  
767 1409–1424.

768 Overvoorde P, Fukaki H, Beeckman T. 2010. Auxin control of root development. *Cold Spring*  
769 *Harbor Perspectives in Biology* 2: a001537.

770 Özkan H, Willcox G, Graner A, Salamini F, Kilian B. 2011. Geographic distribution and  
771 domestication of wild emmer wheat (*Triticum dicoccoides*). *Genetic Resources and Crop*  
772 *Evolution* 58: 11–53.

773 Özkurt E, Hassani MA, Sesiz U, Künzel S, Dagan T, Özkan H, Stukenbrock EH. 2019.  
774 Higher stochasticity of microbiota composition in seedlings of domesticated wheat  
775 compared to wild wheat. *bioRxiv* 685164.

776 Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM, DiLeo MV. 2016. From  
777 the lab to the farm: an industrial perspective of plant beneficial microorganisms.  
778 *Frontiers in Plant Science* 7: 1110.

779 Paterson AH, Bowers JE, Chapman BA. 2004. Ancient polyploidization predating divergence  
780 of the cereals, and its consequences for comparative genomics. *Proceedings of the*  
781 *National Academy of Sciences of the United States of America* 101: 9903–9908.

782 Pérez-de-Luque A, Tille S, Johnson I, Pascual-Pardo D, Ton J, Cameron DD. 2017. The  
783 interactive effects of arbuscular mycorrhiza and plant growth-promoting rhizobacteria

784 synergistically enhance host plant defences against pathogens. *Scientific Reports* 7:  
785 16409.

786 Pérez-Jaramillo J, Mendes R, Raaijmakers J. 2016. Impact of plant domestication on  
787 rhizosphere microbiome assembly and functions. *Plant Molecular Biology* 90: 635–644.

788 Poly F, Ranjard L, Nazaret S, Gourbière F, Jocteur Monrozier L. 2001. Comparison of *nifH*  
789 gene pools in soils and soil microenvironments with contrasting properties. *Applied and*  
790 *Environmental Microbiology* 67: 2255–2262.

791 Pont C, Leroy T, Seidel M, Tondelli A, Duchemin W, Armisen D, Lang D, Bustos-Korts D,  
792 Goué N, Balfourier F, *et al.* 2019. Tracing the ancestry of modern bread wheats. *Nature*  
793 *Genetics* 51: 905–911.

794 Ramirez-Gonzalez RH, Borrill P, Lang D, Harrington SA, Brinton J, Venturini L, Davey M,  
795 Jacobs J, ..., Uauy C. 2018. The transcriptional landscape of polyploid wheat. *Science*  
796 361: eaar6089.

797 Renoud S, Bouffaud M-L, Dubost A, Prigent-Combaret C, Legendre L, Moëgne-Loccoz Y,  
798 Muller D. 2020. Co-occurrence of rhizobacteria with nitrogen fixation and/or 1-  
799 aminocyclopropane-1-carboxylate deamination abilities in the maize rhizosphere. *FEMS*  
800 *Microbiology Ecology* 96: fiae062.

801 Roucou A, Violle C, Fort F, Roumet P, Ecartot M, Vile D. 2018. Shifts in plant functional  
802 strategies over the course of wheat domestication. *Journal of Applied Ecology* 55: 25–37.

803 Saia S, Fragasso M, De Vita P, Beleggia R. 2019. Metabolomics provides valuable insight for  
804 the study of durum wheat: a review. *Journal of Agricultural and Food Chemistry* 67:  
805 3069–3085.

806 Salamini F, Özkan H, Brandolini A, Schäfer-Pregl R, Martin W. 2002. Genetics and  
807 geography of wild cereal domestication in the near east. *Nature Reviews Genetics* 3: 429–  
808 441.

809 Scarpella E, Barkoulas M, Tsiantis M. 2010. Control of leaf and vein development by auxin.  
810 *Cold Spring Harbor Perspectives in Biology* 2: a001511.

811 Shakir MA, Bano A, Arshad M. 2012. Rhizosphere bacteria containing ACC-deaminase  
812 conferred drought tolerance in wheat grown under semi-arid climate. *Soil and*  
813 *Environment* 31: 108–112.

814 Simmons T, Caddell D, Deng S, Colemann-Derr D. 2018. Exploring the root microbiome:  
815 extracting bacterial community data from the soil, rhizosphere, and root endosphere.  
816 *Journal of Visualized Experiments* 135: e57561.

817 Simonin M, Cantarel AM, Crouzet A, Gervaix J, Martins JMF, Richaume A. 2018. Negative  
818 effects of copper oxide nanoparticles on carbon and nitrogen cycle microbial activities in  
819 contrasting agricultural soils and in presence of plants. *Frontiers in Microbiology* 9:  
820 3102.

821 Simonin M, Richaume A, Guyonnet J, Dubost A, Martins JMF, Pommier T. 2016. Titanium  
822 dioxide nanoparticles strongly impact soil microbial function by affecting archaeal  
823 nitrifiers. *Scientific reports* 6: 33643.

824 Simonin M, Guyonnet J, Martins JMF, Ginot Morgane, Richaume A. 2015. Influence of soil  
825 properties on the toxicity of TiO<sub>2</sub> nanoparticles on carbon mineralization and bacterial  
826 abundance. *Journal of Hazardous Materials* 283: 529-535.

827 Soares RA, Roesch LFW, Zanatta G, de Oliveira Camargo FA, Passaglia LMP. 2006.  
828 Occurrence and distribution of nitrogen fixing bacterial community associated with oat  
829 (*Avena sativa*) assessed by molecular and microbiological techniques. *Applied Soil*  
830 *Ecology* 33: 221–234.

831 Soltis PS, Marchant DB, Van de Peer Y, Soltis DE. 2015. Polyploidy and genome evolution  
832 in plants. *Current Opinion in Genetics & Development* 35: 119–125.

833 Soltis PS, Soltis DE. 2000. The role of genetic and genomic attributes in the success of  
834 polyploids. *Proceedings of the National Academy of Sciences of the United States of*  
835 *America* 97: 7051–7057.

836 Spaepen S, Vanderleyden J, Remans R. 2007a. Indole-3-acetic acid in microbial and  
837 microorganism-plant signaling. *FEMS Microbiology Reviews* 31: 425–448.

838 Spaepen S, Versées W, Gocke D, Pohl M, Steyaert J, Vanderleyden J. 2007b.  
839 Characterization of phenylpyruvate decarboxylase, involved in auxin production of  
840 *Azospirillum brasilense*. *Journal of Bacteriology* 189: 7626–7633.

841 Tkacz A, Pini F, Turner TR, Bestion E, Simmonds J, Howell P, Greenland A, Cheema J,  
842 Emms DM, Uauy C, *et al.* 2020. Agricultural selection of wheat has been shaped by  
843 plant-microbe interactions. *Frontiers in Microbiology* 11: 132.

844 Vacheron J, Desbrosses G, Bouffaud M-L, Touraine B, Moënné-Loccoz Y, Muller D,  
845 Legendre L, Wisniewski-Dyé F, Prigent-Combaret C. 2013. Plant growth-promoting  
846 rhizobacteria and root system functioning. *Frontiers in Plant Science* 4: 356.

847 Valente J, Gerin F, Le Gouis J, Moënné-Loccoz Y, Prigent-Combaret C. 2020. Ancient  
848 wheat varieties have a higher ability to interact with plant growth-promoting  
849 rhizobacteria. *Plant, Cell & Environment* 43: 246–260.

850 Větrovský T, Baldrian P. 2013. The variability of the 16S rRNA gene in bacterial genomes  
851 and its consequences for bacterial community analyses. *PLoS ONE* 8: e57923.

852 Waines JG, Ehdaie B. 2007. Domestication and crop physiology: roots of green-revolution  
853 wheat. *Annals of Botany* 100: 991–998.

854 Wei F, Coe E, Nelson W, Bharti AK, Engler F, Butler E, Kim H, Goicoechea JL, Chen M,  
855 Lee S, *et al.* 2007. Physical and genetic structure of the maize genome reflects its  
856 complex evolutionary history. *PLoS Genetics* 3: e123.

857 Willcox G. 2005. The distribution, natural habitats and availability of wild cereals in relation  
858 to their domestication in the Near East: multiple events, multiple centres. *Vegetation*  
859 *History and Archaeobotany* 14: 534–541.

860 Wipf HML, Coleman-Derr D. 2021. Evaluating domestication and ploidy effects on the  
861 assembly of the wheat bacterial microbiome. *PLoS ONE* 16: e0248030.

862 Wipfel K, Tao K, Niu Y, Zgadzaj R, Kiel N, Guan R, Dahms E, Zhang P, Jensen D,  
863 Logemann E, Radutoiu S, Schulze-Lefert P, Garrido-Oter, R. 2021. Host preference and  
864 invasiveness of commensal bacteria in the Lotus and *Arabidopsis* root microbiota. *Nature*  
865 *Microbiology* 6: 1150-1162.

866 Yarza P, Yilmaz P, Pruesse E, Glöckner F, Ludwig W, Schleifer K, Whitman W, Euzéby J,  
867 Amann R, Rossello-Mora R. 2014. Uniting the classification of cultured and uncultured  
868 *Bacteria* and *Archaea* using 16S rRNA gene sequences. *Nature Reviews Microbiology*  
869 12: 635–645.

870 Zahir ZA, Ghani U, Naveed M, Nadeem SM, Asghar HN. 2009. Comparative effectiveness of  
871 *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and  
872 yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. *Archives of*  
873 *Microbiology* 191: 415–424.

874 Zehr JP, McReynolds LA. 1989. Use of degenerate oligonucleotides for amplification of the  
875 *nifH* gene from the marine cyanobacterium *Trichodesmium thiebautii*. *Applied and*  
876 *Environmental Microbiology* 55: 2522-2526.

877 Zhao Y. 2014. Auxin biosynthesis. *The Arabidopsis Book* 12: e0173.

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*<sup>+</sup> 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  $\alpha$ ,  $\beta$  and  $\gamma$  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

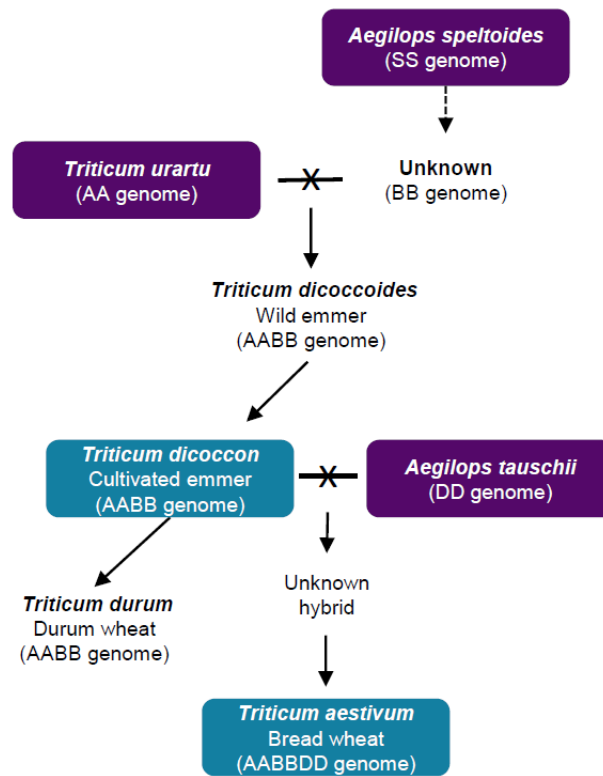
910 **FIGURE 3.**  $\alpha$ -Diversity of total bacterial community and free nitrogen-fixers in bulk soil and  
911 the rhizosphere of wheat species at 40 days of growth, based on *rrs* and *nifH* analysis. Chao 1  
912 (A and D), Shannon (B and E) and inverse Simpson (InvSimpson; C and F) diversity 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  
918 performed to compare all wheat species with bulk soil ( $P < 0.05$ ; letters  $\alpha$ ,  $\beta$  and  $\gamma$  are used to  
919 show statistical differences). Differences between lines of a same species were studied by a  
920 nested ANOVA and Tukey's HSD tests ( $P < 0.05$ ; letters a, b and c are used to show  
921 statistical differences).

922

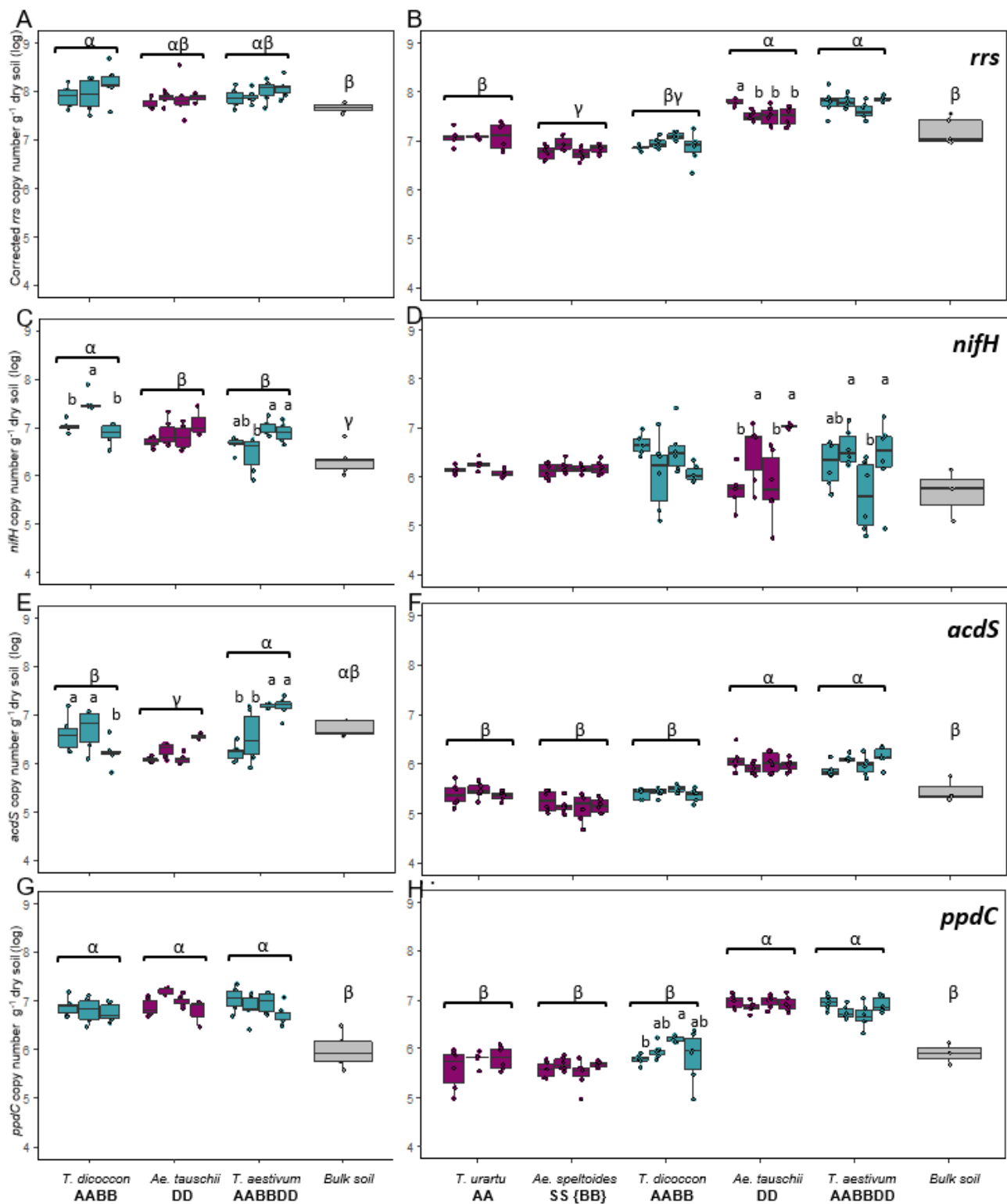
923 **FIGURE 4.** Relative abundance of bacterial taxa of the total bacterial community and free  
924 nitrogen-fixers in bulk soil and the rhizosphere of wheats, based on *rrs* and *nifH* analysis.  
925 Relative abundance of all phyla (A) and the 20 most abundant genera (B) based on *rrs*  
926 analysis.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

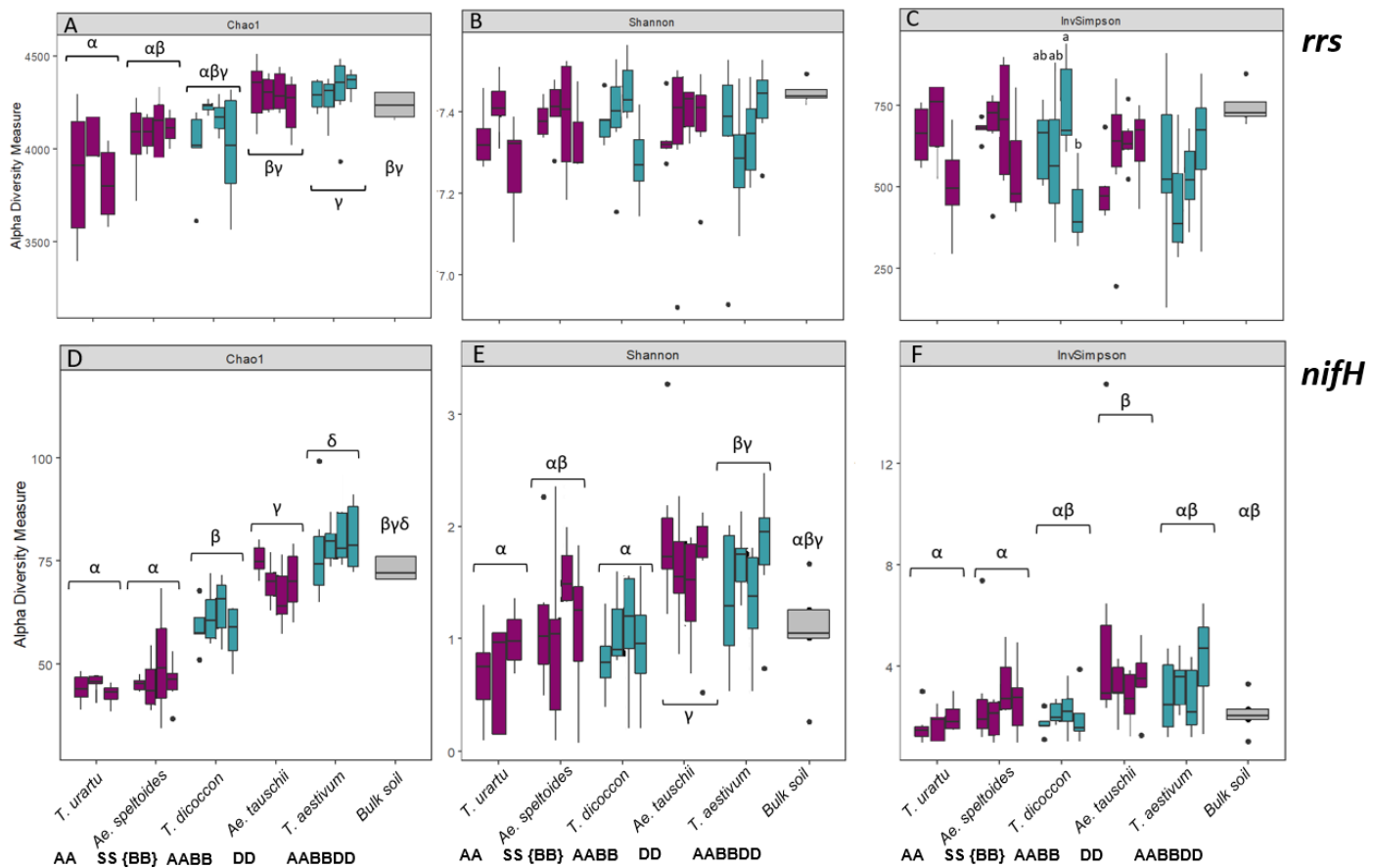


**Figure 1. Simplified outline of wheat evolutionary history and place of the wheat species studied.** Coloured wheats (purple for wild species and blue for domesticated species) were used as representatives of genomic architectures AA (*i.e.* *Triticum urartu*), SS (which is close to BB; Pont et al. 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. dicocoides* 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).

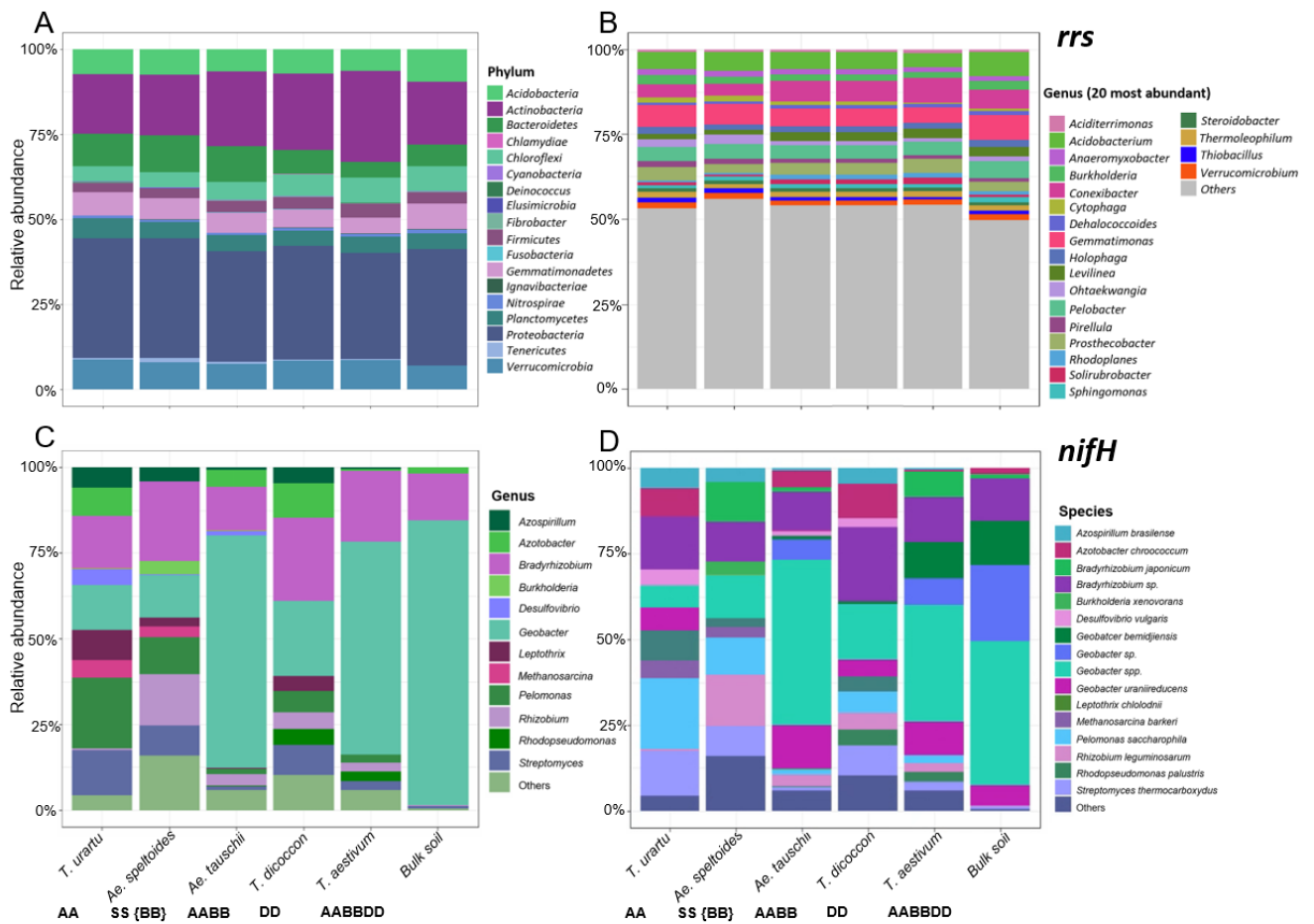


**Figure 2. Size of the total bacterial community and of microbial functional groups in bulk soil and the rhizosphere of wheat species at 14 and 40 days of growth.** (A and B) Size of the bacterial community at 14 (A) and 40 (B) days of growth. Raw copy numbers of *rrs* were divided by 4 (i.e. corrected *rrs* numbers) to estimate bacterial numbers. Plant sequences representing in the order of 0.02 log<sub>10</sub> 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*<sup>+</sup> 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  $\alpha$ ,  $\beta$  and  $\gamma$  are used to show statistical differences). Differences between lines of a same

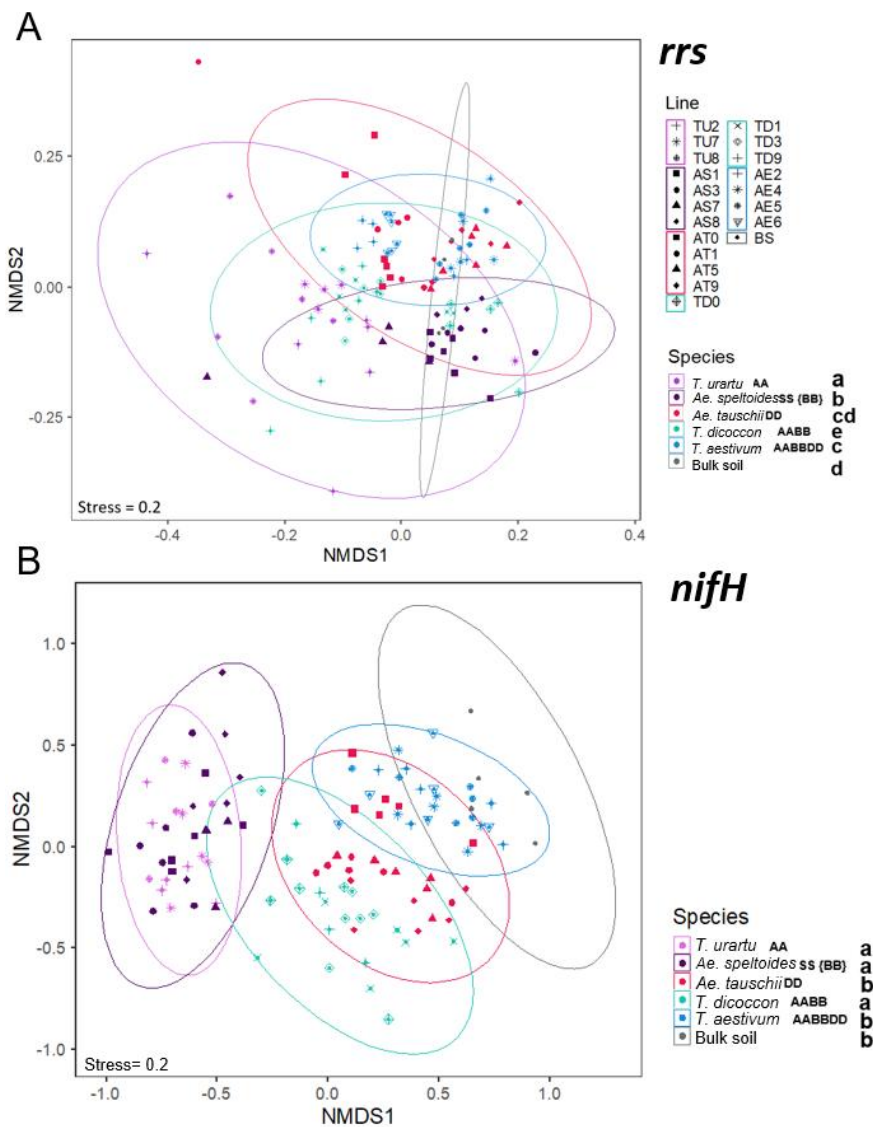
species were studied by a nested ANOVA and Tukey's HSD tests ( $P < 0.05$ ; letters a, b and c are used to show statistical differences).



**Figure 3.  $\alpha$ -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  $\alpha$ ,  $\beta$  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).



**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,  $P < 0.05$ ).