Uncovering functional deterioration in the rhizosphere microbiome associated with wheat dwarfing

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Abstract

Background:

One of the biggest developments of wheat domestication was the development of semi-dwarf cultivars that respond well to fertilisers and produce higher yields without lodging. Consequently, this change has also impacted the wheat microbiome, often resulting in reduced selection of taxa and a loss of network complexity in the rhizospheres of semi-dwarf cultivars. Given the importance of rhizosphere microbiomes for plant health and performance, it is imperative that we understand if and how these changes have affected their function. Here, we use shotgun metagenomics to classify the functional potential of prokaryote communities from the rhizospheres of tall and semi-dwarf cultivars to compare the impact of wheat dwarfing on rhizosphere microbiome functions.

Results:

We found distinct taxonomic and functional differences between tall and semi-dwarf wheat rhizosphere communities and identified that semi-dwarf wheat microbiomes were less distinct from bulk soil communities. Of the 113 functional genes that were differentially abundant between tall and semi-dwarf cultivars, 95 % were depleted in semi-dwarf cultivars and 65 % of differentially abundant reads best mapped to genes involved in staurosporine biosynthesis (antibiotic product), plant cell wall degradation (microbial mediation of plant root architecture, overwintering energy source for microbes) and sphingolipid metabolism (signal bioactive molecules).

Conclusions:

Overall, our findings indicate that green revolution breeding has developed wheat cultivars with a reduced rhizosphere effect. The consequences of this are likely detrimental to the development of microbiome-assisted agriculture which will require a strong rhizosphere

selective environment for the establishment of a beneficial plant root microbiome. We believe our results are of striking importance and highlight that implementation of microbiome facilitated agriculture as part of a sustainable crop production strategy will require an overhaul of wheat breeding programmes to consider plant-microbe interactions, especially in the root environment.

Introduction

As a result of the Green Revolution, we benefit from a huge increase in cereal grain production. This was largely due to the incorporation of reduced-height (*Rht*) mutant allele dwarfing genes into modern wheat cultivars (i.e., wheat dwarfing). Nearly all wheat grown today are semi-dwarf cultivars with increased tillering, larger seed heads producing higher yields, but on shorter stems to prevent lodging, and respond well to high fertilizer inputs. However, current high-yielding semi-dwarf crop varieties rely on unsustainable levels of agrochemical inputs, including synthetic fertilisers, which are environmentally harmful (Van Grinsven et al., 2013).

While tall heritage cultivars or landraces are mainly grown on marginal fields on organic farms (Morgounov et al., 2016; Ortman et al., 2023) they are still an important genetic resource for breeding programs (Escudero-Martinez and Bulgarelli, 2023; Lammerts van Bueren and Myers, 2012), especially since they show tolerance to extreme weather events and other stresses (Newton et al., 2011). Wheat domestication gave little to no consideration to belowground processes; as such, we are only beginning to understand how wheat dwarfing has impacted the interactions between roots and soil organisms six decades after the first semi-dwarf wheat was introduced into agriculture (Kavamura et al., 2020; Rossmann et al., 2020; Tkacz et al., 2020).

The rhizosphere, i.e., the interface between roots and soil, harbours a dynamic community of microorganisms. These communities play an important role in how plants function, ranging

67 from beneficial effects, e.g., aiding nutrient acquisition, growth promotion and plant defences
68 (Mauchline and Malone, 2017; Mendes et al., 2013; Schlaeppi and Bulgarelli, 2015), to
69 harmful effects, e.g., pathogens such as *Gaeumannomyces tritici* causative agent of take-all
70 disease in most cereals (Palma-Guerrero et al., 2021). The assembly of rhizosphere
71 communities are largely determined by the exudates and structure of plant roots (Sasse et al.,
72 2018; Vives-Peris et al., 2020) which the microbiome itself can modulate (Korenblum et al.,
73 2020; Pandey et al., 2021), therefore it is not surprising that the domestication of wheat,
74 including wheat dwarfing, has been shown to influence protist, bacterial, nematode and
75 fungal rhizosphere communities (Kavamura et al., 2020; Rossmann et al., 2020; Tkacz et al.,
76 2020). The general trend in these populations is that wheat domestication has reduced
77 selection processes in the rhizosphere. This is demonstrated by the root microbiome of
78 ancestral wheat harbouring more unique taxa and more complex microbiomes than the
79 modern cultivars. Furthermore, modern cultivars can be enriched in fungal pathogens
80 (Kinnunen-Grubb et al., 2020). Similar trends have also been found in the domestication of
81 other cereals such as barley (Bulgarelli et al., 2015), soybean (Shi et al., 2019), rice (Sun et
82 al., 2021) and durum wheat (Spor et al., 2020). While these findings have been important for
83 our understanding of microbial community dynamics there is now a requirement to
84 understand microbiome assembly and function for these resources to be harnessed in
85 sustainable agriculture programmes with less dependence on fertilizer inputs (Busby et al.,
86 2017; Gruet et al., 2022). Phylogenetic marker gene profiles can hint at the function of these
87 communities (Kavamura et al., 2020). However, to obtain a comprehensive understanding of
88 how wheat dwarfing has impacted microbiome function, there is a requirement for holistic
89 shotgun sequencing methods to be deployed.

90 Whole shotgun metagenomic sequencing provides a representation of the genomes present in
91 each sample, allowing the inference of functional potential of a microbial community. This

technique has been used to study the wheat rhizosphere microbiome and identify microbes that consume plant-derived carbon (Fan et al., 2022), to ascertain differences in microbial zinc-mobilisation genes between high and low zinc wheat cultivars (Wang et al., 2021a), and the comparison of antibiotic resistance genes in the rhizospheres of common crops (Yu et al., 2023). To date, shotgun metagenomics has not corroborated the wheat domestication-driven changes in rhizosphere communities found using phylogenetic marker gene studies. A previous attempt was made in Canadian wheat cultivars and found no effect of domestication, but this study sampled at wheat senescence (Quiza et al., 2021), a time when the structure of the root microbiome of annual plants has been found to degrade and become dominated by saprophytes (Mauchline et al., 2015). However, a similar study conducted in durum (tetraploid) wheat demonstrated that domestication led to a decline in gene diversity and a shift in microbial functional traits, particularly related to nutrient cycling (Yue et al., 2023). As yet, the impact of wheat dwarfing on the vegetative stages of hexaploid bread wheat plants has not yet been explored using shotgun metagenomics.

Given the strong evidence from previous studies that wheat dwarfing impacts the rhizosphere community structure at a taxonomic level, we predict that the same impact will be observed for a range of functional genes in these communities. We tested this hypothesis by growing two tall and two semi-dwarf wheat cultivars alongside unplanted bulk soil control pots under glasshouse conditions. At flowering stage, we sampled the rhizosphere soil and bulk soil samples and generated, analysed, and compared the shotgun metagenomic profiles, in terms of taxonomy and function, of the prokaryote communities from these sample types.

Methods

Wheat cultivars and growth conditions

115 Wheat cultivars (Table 1) were selected based on previous results by Kavamura *et al*., 2020,
116 which indicated differences in the root microbiome structure and predicted function between
117 short and tall cultivars. Soil was collected from Stackyard bare-fallow soil mine
118 (52.002997°N, 0.613058°W) in January 2019. Soil details are previously described (Reid et
119 al., 2021). Soil was sieved (2 mm mesh), mixed thoroughly, and stored at 4°C in polythene
120 bags prior to use. Seeds were surface sterilized as previously described (Robinson et al.,
121 2016) and were transferred to pre-soaked (sterile distilled water) filter paper in Petri dishes
122 and germinated for three days in the dark at room temperature. Seedlings were transplanted to
123 individual wells on a seed tray (1x seedling per well) in Stackyard bare fallow soil and grown
124 in a glasshouse at Rothamsted Research for two weeks (20 °C, 16 h/day light regime, watered
125 daily) before vernalization for twelve weeks (4 °C; 8 h light and 16 h dark). After this time, 3
126 seedlings were transplanted to 6-inch diameter pots (approximately 1 kg soil per pot), with
127 NPK granules [15% N, 9% P_2O_5, 11% K_2O, 2% MgO with micro-nutrients (B, Cu, Fe, Mn,
128 Mo, and Zn); Osmocote, United Kingdom] (~5 g per pot) added to the soil surface of each
129 pot. Five replicate pots were prepared for each wheat variety and three unplanted bulk soil
130 control pots were also set up using the same soil and fertilisation. Plants were grown in a
131 glasshouse (20°C, 16 h/day light regime) and watered daily or as required with tap water.

132 Pots were harvested at the start of flowering (Zadoks growth stage 61; approximately 10
133 weeks growth post vernalisation; Zadoks et al., 1974), resulting in twenty rhizosphere
134 samples and three bulk soil samples. Loose soil from the root system of a given pot was
135 carefully removed. A 10 g subsample of root system was transferred to a 50 ml Falcon tube
136 and 30 ml sterile water added. Next, samples were shaken vigorously for 10 min using an
137 orbital shaker to release rhizosphere soil. After this time 4 ml soil suspension was centrifuged
138 (2 min, RT, 15,000 rpm), supernatant discarded, and remaining soil was flash frozen in liquid
139 nitrogen and stored at -80 °C.

140 **Table 1. Characteristics of the cultivars used in this study.**

Cultivar	Year	Pedigree	Height
Chidham White Chaff	1790	Not recorded	Tall
Red Lammas	1850	Not recorded	Tall
Malacca	1997	Riband*(Rendezvous)*Apostle	Semi-dwarf
Gallant	2009	(Malacca*Charger)*Xi-19	Semi-dwarf

141 *Table was modified from Kavamura *et al.* (2020).

142 DNA extraction and sequencing

143 Genomic DNA was extracted from the bulk soil and rhizosphere soil sample (~0.25 g) using
144 the Dneasy PowerSoil Pro kit (Qiagen, Venlo, Netherlands) and stored at -80 °C. Extractions
145 were performed according to the manufacturer's instructions and with the use of the MP
146 Biomedicals FastPrep-24 machine twice for the bead-beating step at 30 s at 5.5 m s^{-1}. DNA
147 purity and concentrations were measured with a NanoDrop 1000 spectrophotometer (Thermo
148 Fisher Scientific, Wilmington, DE, United States), as well as a Qubit 2.0 Fluorimeter using
149 the dsDNA HS assay kit (Thermo Fisher Scientific). 0.6 µg of DNA for each sample was sent
150 to Novogene (UK) Company Limited for library preparation and sequencing using Illumina
151 NovaSeq 6000 platform (HWI-ST1276) using a 150bp paired-end sequencing strategy. An
152 average of 2.65^8 raw paired reads, ranging between 1.66^8 – 3.46^8) per sample was obtained.
153 Reads were quality checked using the FASTX-Toolkit
154 (http://hannonlab.cshl.edu/fastx_toolkit/index.html; v0.0.14) with a quality threshold of 25,
155 and Trimmomatic (http://www.usadellab.org/cms/?page=trimmomatic; v0.39; Bolger et al.,
156 2014) with a minimum length of 80 bp. Quality checked reads were assigned to taxa using
157 DIAMOND (https://github.com/bbuchfink/diamond; v2.0.13; Buchfink et al., 2015) and
158 AnnoTree (http://annotree.uwaterloo.ca/annotree/app/downloads.html; v1.2; Gautam et al.,
159 2022; Mendler et al., 2019) for prokaryote identification. KEGG Orthology (KO) molecular

160 functional identifiers were assigned by MEGAN6 Ultra (https://software-ab.cs.uni-
161 tuebingen.de/download/megan6/welcome.html; v6.24.23; Bağcı et al., 2019; Huson et al.,
162 2016) and used to extract individual gene sequences for each functional KEGG identifier.
163 Thus, processing resulted in two abundance tables for further analysis, one where reads were
164 aligned to prokaryote taxa and another to functional genes.

165 Statistical analysis

166 Taxa and functional genes were removed if their total count was < 10 reads. Only taxa
167 assigned to Phyla or lower were kept for further analysis and reads unclassified to functional
168 genes were removed. This resulted in 5,908,371,442 reads for the taxonomy table and
169 4,392,129,033 for the functional table and in both cases samples with the most reads roughly
170 double those with the lowest number of reads.

171 All statistical analysis and visualisation of results was done in R version 4.3.3 (R core Team,
172 2024). Alpha and beta diversities were calculated from rarefied data (Weiss et al., 2017)
173 while differential abundance analysis was done using DESeq2 variance stabilisation
174 technique (McMurdie and Holmes, 2014) to normalise taxonomy and function abundance
175 tables. The rarefied tables were calculated by normalizing sequence number to minimum
176 sample size (159,120,960 and 118,292,681 for taxonomy and function tables respectively) by
177 random subsampling without replacement using *rarefy_even_depth* function in the phyloseq
178 package version 1.46 (McMurdie and Holmes, 2013). Rarefaction curve analysis was used to
179 test that the subsampling of sequences still yielded sufficient resolution of prokaryote
180 communities and their functional genes (Fig. S1).

181 To test whether the sample type, including tall wheat rhizosphere, semi-dwarf wheat
182 rhizosphere and bulk soil, impacted alpha diversity, we obtained observed and Chao1
183 richness and Shannon diversity from the rarefied taxonomy and functional tables using

184 *estimate_richness* function in the phyloseq package. Normality and homogeneity of variances
185 of alpha diversity measures were tested before performing type 3 one-way ANOVA to
186 account for unbalanced design due to fewer replicates in the bulk soil samples, and Tukey's
187 honest significant differences (HSD) with sample type as a main factor.

188 *Beta diversity*

189 Sample differences of the rarefied taxonomy and functional tables were visualised with
190 principal coordinate analysis (PcoA) plots using Bray Curtis dissimilarity. To statistically test
191 for differences between sample types we used Permutational Multivariate Analysis of
192 Variance (PERMANOVA) with Bray Curtis distance matrices using *adonis2* in the vegan
193 package version 2.6-4 (Oksanen et al., 2024) with 9,999 permutations. Pairwise comparisons
194 of each group were evaluated with *pairwise.adonis* using a false discovery rate correction for
195 multiple tests. We evaluated differences among sample variability using homogeneity of
196 multivariate dispersions tests (*betadisper*), followed by ANOVAs to compare the mean
197 distance-to-centroid. Pairwise dispersion comparisons were carried out using Tukey's HSD.

198 *Differential abundance analysis*

199 Differential abundances of individual 21,805 taxa and 10,189 functional genes were
200 calculated using DESeq2 package version 1.42.1 (Love et al., 2014) which is particularly
201 powerful for small datasets (Li et al., 2022). Maximum-likelihood estimates for the log2-fold
202 change between conditions associated with each gene or taxa were calculated using a
203 negative binomial generalized linear model. Contrasts between each level of sample type
204 were made and tests of significance were conducted using Wald's test, employing $\alpha = 0.05$
205 and a Benjamin–Hochberg false discovery rate (q) of 0.05 to control type I error rate in the
206 face of multiple comparisons. Bayesian adaptive shrinkage was then applied to reduce the
207 log2-fold change towards zero for taxa or genes with low mean counts or a high dispersion in

208 their count distribution (Stephens, 2017). We then considered taxa or genes to be enriched in
209 a particular sample type only if the resulting shrunken log2-fold changes were > 1 or < -1, i.e.
210 double in abundance (Sarno et al., 2023; Semaan et al., 2023).

211 Differential abundance analysis was done on all genes but for visualisation we only showed
212 those that were considered enriched in a particular sample type and we compared the results
213 of the analysis from these against ten housekeeping genes used as a baseline. These
214 housekeeping genes were selected to cover different parts of the genome and that encode
215 proteins involved in different metabolic activity, except for the ribosomal genes, similar to
216 the gene selection in multilocus sequence typing (MLST; Takle et al., 2007; Maiden et al.,
217 2013). These included signal recognition particle protein (*ffh*), glutamine synthetase (*glnA*),
218 DNA gyrase (*gyrB*), transcription termination factor Rho (*rho*), 50S ribosomal protein L9
219 (*rplI*), 50S ribosomal subunit protein L17 (*rplQ*), RNA polymerase (*rpoZ),* DNA
220 topoisomerase I (*topA*), nucleoside-specific channel-forming protein (*tsx*), ATP synthase F1,
221 β-subunit (*atpD*).

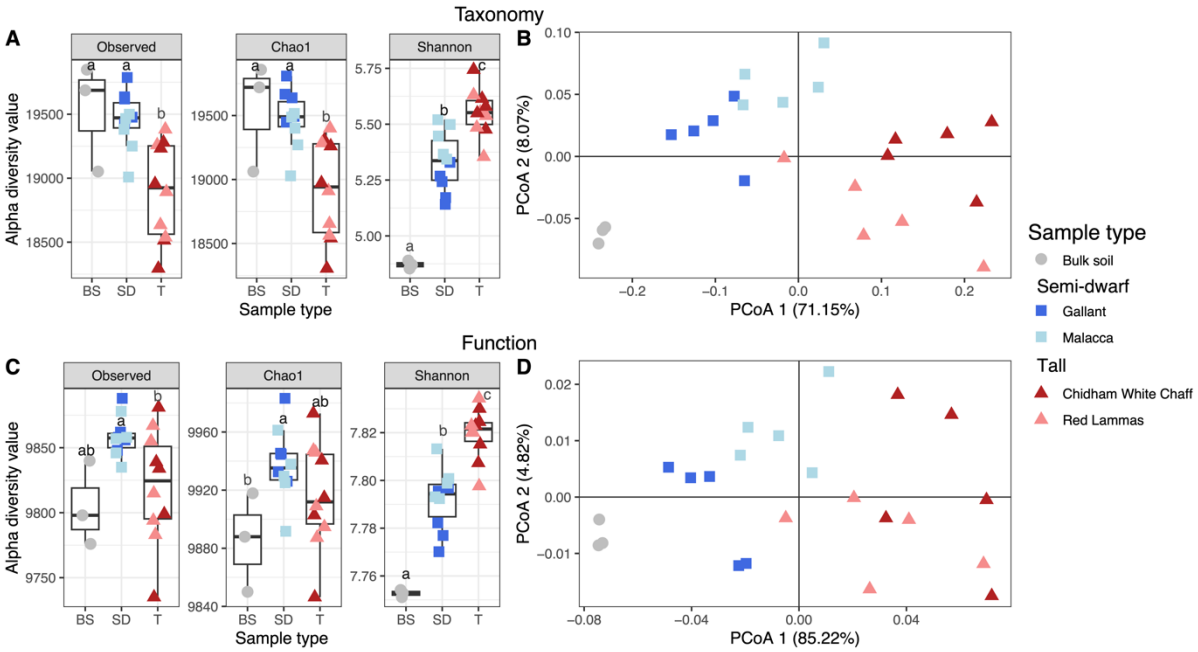
222 **Results**

223 Alpha diversity

224 Overall, alpha diversity differences were statistically significant between sample types, i.e.,
225 bulk soil and rhizosphere soil from semi-dwarf and tall cultivars, for both taxonomy and
226 function of the prokaryote communities (Fig. 1A, C). Tall cultivars harboured fewer taxa
227 (observed and chao1) in their rhizospheres than bulk soils and semi-dwarf rhizospheres;
228 however, when rarefied read abundances were considered, their communities were the most
229 diverse (Shannon's species diversity). Functional genes followed a similar pattern, though the
230 differences were less profound. Shannon's diversity was marginally greater in tall
231 rhizospheres and more genes ascribed to semi-dwarf than tall rhizospheres. Wheat

232 rhizospheres regardless of cultivar were found to have a higher functional diversity than bulk

233 soil samples.



234

235 **Fig. 1. Diversity measures of the taxonomy and function of prokaryote communities.**

236 Alpha diversity is represented in plots A and C, and Principal Coordinate Analysis (PCoA)

237 plots based on Bray-Curtis dissimilarity demonstrate beta diversity in plots B and D, for

238 taxonomy and function respectively. Significant differences are shown between sample types,

239 bulk soil samples (circles) and the rhizospheres of semi-dwarf (squares) and tall (triangles)

240 wheat. Colours differentiate between the wheat different cultivars.

241 <u>Beta diversity</u>

242 We found very similar PERMANOVA results for both taxonomy and function-assigned

243 genes, whereby a high proportion of variation between samples was explained by sample

244 type (bulk soil, semi-dwarf and tall wheat rhizospheres; Table 2). Pairwise comparisons

245 between sample types were all significantly different with the highest R^2 between tall

246 rhizosphere and bulk soils. This difference is partly explained by significantly different

247 dispersions between groups for taxonomy and function (ANOVA, F = 11.9, $p < 0.001$; F =

248 6.8, $p = 0.006$, respectively). *Posthoc* comparisons revealed that all groups differed in
249 dispersion for taxonomy, but for function, there was less dispersion in the bulk soil samples
250 compared to semi-dwarf (*padj* = 0.056) and tall (*padj* = 0.004) rhizospheres. Nevertheless,
251 the PCoA clearly supports the PERMANOVA results that sample type is an important factor
252 in determining the prokaryote taxonomy and function since they were clustered separately in
253 multivariate space (Fig. 1B, D). Most of the variation was explained by PCoA 1 for both
254 taxonomy and functional datasets which demonstrated that, from the cultivars included here,
255 semi-dwarf wheat cultivars harbour prokaryote community profiles that are more similar to
256 bulk soil than tall cultivars do. The shift in the prokaryote community was represented by an
257 increasing relative abundance of Pseudomonadota (Proteobacteria) and Bacteroidota from
258 bulk soil to rhizosphere soil of semi-dwarf and tall wheats and a higher proportion of
259 Acidobacteroidota in bulk soil samples (Fig. S2).

260 **Table 2. PERMANOVA and Tukey's pairwise results for both taxonomy and function**
261 **of Prokaryote communities.** All combinations of sample type, bulk soil (BS) and
262 rhizosphere soil of semi-dwarf (SD) and tall (T) cultivars, were present. Significant
263 differences in bold and *p* values of pairwise tests have been adjusted for multiple
264 comparisons using a false-discovery rate correction.

Variable/	Taxonomy			Function		
pairwise comparison	R^2	F	*p*	R^2	F	*p*
Sample type	0.63	21	**< 0.001**	0.72	25.6	**< 0.001**
BS vs SD	0.55	13.7	**0.004**	0.59	15.5	**0.004**
BS vs T	0.66	21.5	**0.004**	0.76	34.9	**0.004**
SD vs T	0.46	15.6	**< 0.001**	0.56	23.3	**< 0.001**

265 Differential abundance

266 Out of 21,805 taxa and 10,189 functional genes, 5,072 (23 %) and 1,719 (17 %) respectively
267 were differentially abundant for at least one contrast between the sample types (Fig. 2; Ward
268 $p < 0.05$, FDR < 0.05, shrunken log2 fold > 1 or < -1). These differentially abundant taxa and
269 functional genes make up 46 % and 5 % of total raw reads, respectively. The primary aim of
270 this work is to compare abundances of genes between wheat genotypes. However, the biggest
271 contrast was between tall rhizosphere and bulk soil prokaryote communities whereby 4,975
272 taxa and 1,712 functional genes were differentially abundant. Semi-dwarf rhizosphere
273 communities were more similar to bulk soil communities than the tall rhizosphere
274 communities with 1,114 taxa and 194 functional genes differentially abundant, 78 % and 89
275 % less than the tall vs bulk soil contrast, respectively. Despite this difference, the taxa that
276 were enriched in either the tall or semi-dwarf wheat rhizosphere samples, compared with the
277 bulk soil, belonged mostly to the same three phyla, Pseudomonadota, Bacteroidota and
278 Actinomycetota (Actinobacteria), which when combined, accounted for 95 % and 92 % of
279 the enriched taxa respectively (Fig. S3). Bulk soil samples were enriched in taxa from a wide
280 range of phyla when compared to both rhizosphere types, dominated by Bacillota
281 (Firmicutes), but the highest proportion of taxa belonging to various rare phyla (phyla that
282 contain less than 5 % of all enriched taxa). Interestingly, when assessing the abundances of
283 the differentially abundant functional genes between tall rhizospheres and bulk soil, 19 of the
284 20 most abundant genes were also more abundant in tall than semi-dwarf rhizospheres
285 (ANOVA, $p < 0.05$; Table S1). The one exception being K07305 (peptide-methionine (R)-S-
286 oxide reductase) which was equally abundant in the rhizosphere of tall and semi-dwarf but
287 has low representation in bulk soil samples. It was found that 13 genes out of these 20 most
288 abundant genes were also more abundant in semi-dwarf rhizospheres compared to bulk soil
289 samples.

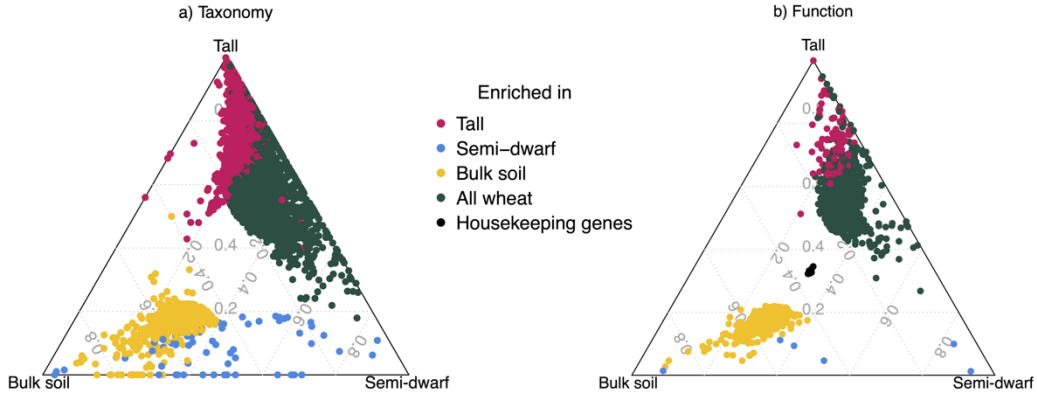


Fig. 2. Ternary plots of differentially abundant taxonomy (a) and function (b) of prokaryote communities. Each point represents a taxa (a) or functional gene (b) and their position represents the contribution of the indicated sample type, bulk soil, semi-dwarf and tall rhizospheres, to the total normalised abundance. Taxa or functional genes are significantly different in abundance for different contrasts (Ward $p < 0.05$, FDR < 0.05, shrunken log fold changes > 1) and their colours indicate the group they are enriched in (i.e., higher abundance). Taxa or functional genes enriched in 'all wheat' samples, i.e., rhizosphere samples regardless of cultivar type, were determined by contrasts with bulk soil samples and vice versa for enriched in bulk soil. Enriched in tall rhizospheres was determined compared with semi-dwarf rhizospheres, regardless of bulk soil and vice versa for semi-dwarf. Therefore, some points could be characterised by two colours but the comparison between tall and semi-dwarf rhizospheres is the one presented as this is the most relevant for our hypothesis. Prokaryote housekeeping genes were included in b as a reference for differentially abundant functional genes (see methods for a list of housekeeping genes).

There were 1,414 taxa and 113 functional genes that were differentially abundant between the semi-dwarf and tall rhizosphere communities, most of which (95 %) were enriched in the tall rhizosphere communities (Fig. 3). The taxa enriched in the tall rhizosphere communities followed the same pattern as when compared to bulk soil whereby 96 % of reads were from

309 Pseudomonadota, Bacteroidota and Actinomycetota (Fig. S3). The semi-dwarf rhizosphere
310 communities were mostly enriched in Bacillota, Pseudomonadota, Spirochaetota and the
311 highest proportion of enriched taxa belonged to rare phyla (phyla that contain less than 5 %
312 of all enriched taxa). The differentially abundant functional genes belong to 15 different
313 functional groups whereby tall rhizospheres had enriched genes belonging to each group
314 (6,574,723 raw reads) and the six functional genes enriched in the semi-dwarf rhizospheres
315 belonged to three categories (3,257 raw reads; Fig. 3b). Of the 107 genes enriched in the
316 rhizospheres of tall wheats, 40% of reads mapped to genes associated with secondary
317 metabolism, 21% with plant cell wall degradation, 10% with two-component sensor
318 regulation, 8% with membrane transport, 12% with primary metabolism (other than cell wall
319 degradation), 2% with secretion systems, 2% to transcriptional regulation and the remaining
320 5% to quorum sensing, cell cycle, biofilm formation, antibiotic resistance, motility, defence,
321 and secondary messaging functions (Table 3).

Fig. 3. Differentially abundant taxonomy (a) and function (b) of prokaryote communities between semi-dwarf and tall wheat rhizospheres. Taxa and functional genes have been assigned to Class or functional group (based on KEGG orthology) respectively and the number of differentially abundant taxa or functional genes in each group are listed to the left of each plot. Only significant (Ward $p < 0.05$, FDR < 0.05,) and large (at least double; shrunken log2 fold > 1 or < -1) differences are shown except for the 10 housekeeping genes included for reference against the differentially abundant functional genes.

The reads assigned to the differentially abundant functional genes between tall and semi-dwarf rhizospheres were re-processed though our bioinformatic pipeline and assigned to taxa. This allowed us to look for taxonomic differences and potential important taxa for these functions. Out of the 107 functional genes enriched in tall rhizospheres, 87 % had higher taxa richness in the tall rhizospheres, but only 37 % had higher diversity. This suggests that evenness in these communities may be important whereby dominant taxa could be driving these differences in function (Table 3). This was further evident by the differences in the

337 community composition, based on the relative abundance of phyla, between tall and semi-
338 dwarf rhizospheres for each differentially abundant gene (Table S2). In the tall rhizospheres
339 the dominant phyla or class often contributed to a higher proportion of reads than in the semi-
340 dwarf rhizospheres, except where a function was only carried out by taxa from a single
341 phylum.

342 **Table 3. Summaries of the taxa assigned to the 113 functional genes identified as**
343 **differentially abundant between tall and semi-dwarf wheat rhizospheres.**

344 *NOTE: Table 3 exceeds one A4 page so has been included as an additional file for reviewing*
345 *as per journal requirements.*

346 **Discussion**

347 We compared the shotgun metagenomic profiles of prokaryote communities from the
348 rhizospheres of tall and semi-dwarf wheat cultivars and found clear differences associated
349 with wheat height phenotype. We also confirmed our hypothesis that taxonomic changes
350 relate to changes in the potential function of these communities, with the main differences
351 being driven by deterioration of many taxa and functional genes in semi-dwarf rhizospheres.
352 Furthermore, the addition of unplanted bulk soil controls in our study confirm that the
353 rhizosphere microbiome of semi-dwarf cultivars differentiates less from bulk soil than
354 rhizosphere microbiomes derived from tall cultivars in terms of both taxonomy and function.
355 These observations evidence that the incorporation of mutant *Rht* gene alleles which resulted
356 in the semi-dwarf phenotype, have reduced the ability of these host plants to select a
357 rhizosphere microbiome from the bulk soil microbial reservoir.

358 1) <u>Taxonomic differences</u>

359 When using shotgun metagenomics in contrast to phylogenetic marker gene analysis, we
360 identified 10-fold more prokaryote taxa that were differentially abundant between the tall and

361 semi-dwarf cultivars than in our previous study (Kavamura et al., 2020). Both studies found
362 that most of the differentially abundant taxa belonged to Pseudomonadota, Bacteroidota and
363 Actinomycetota and were enriched in the tall cultivars, but our results were to a much larger
364 extent with 95 % enriched in tall compared with 69 % in Kavamura et al. (2020). We also
365 found that species richness was higher in the semi-dwarf rhizospheres, supporting the
366 conclusion that they are less selective than tall wheat. However, species diversity was higher
367 in tall rhizospheres in our study and not in Kavamura et al. (2020). While the overall trend
368 was the same in our previous work these minor discrepancies could also be the result of the
369 different edaphic factors and different growing conditions.

370 We demonstrated a loss of Bacteroidota through wheat dwarfing both in relative abundance
371 and in differentially abundant taxa. This has been found in wheat domestication from other
372 studies (Aleklett et al., 2015; Pérez-Jaramillo et al., 2018; Rossmann et al., 2020) and
373 Bacteroidota have been shown to be an important phylum for plant pathogen protection and
374 phosphorus uptake (Lidbury et al., 2021). Domestication has also been shown to enrich
375 Actinobacteria (Aleklett et al., 2015; Pérez-Jaramillo et al., 2018; Rossmann et al., 2020) and
376 phyla that are more commonly associated with bulk soil, such as Acidobacteriota and
377 Verrucomicrobiota (Kavamura et al., 2020). Our results showed a similar trend with
378 Acidobacteriota and Verrucomicrobiota having a reduced relative abundance in semi-dwarf
379 and tall rhizospheres compared with bulk soil, (Supplementary Figure 2) but there were taxa
380 from these phyla enriched in the rhizospheres of tall cultivars and not in semi-dwarf
381 rhizosphere samples. However, by including unplanted bulk soil controls our study confirms
382 that the rhizosphere microbiomes of semi-dwarf wheat cultivars are more similar to bulk soils
383 than the rhizosphere microbiomes of tall cultivars which would indicate less filtering of
384 Prokaryote taxa in semi-dwarf wheat varieties.

385 2) <u>Functional gene differences</u>

386 Bulk soil vs rhizosphere soils

387 As described in the results section, 19 of the 20 most abundant genes that are enriched in tall
388 rhizospheres compared to bulk soils are also more abundant in tall rhizospheres compared to
389 semi-dwarfs, but to a lesser extent (Table S1). This suggests that the selective ability of semi-
390 dwarf plants is diminished in comparison to tall wheats, and the rhizosphere microbiomes in
391 semi-dwarf are a 'middle ground' between bulk soil and tall rhizospheres (Fig. 1D). The one
392 exception being K07305 (peptide-methionine (R)-S-oxide reductase) which is equally
393 abundant in the rhizosphere of tall and short microbiomes and much higher compared to bulk
394 soil samples (approximate mean normalised read count of 500K per sample for each height
395 class and 48K for bulk soil). This enzyme plays a crucial role in repairing oxidatively
396 damaged proteins. We hypothesise that its high abundance in the rhizosphere reflects the high
397 metabolic activity of the rhizosphere with consequential generation of free radicals. It implies
398 that the high incidence of this gene and presumably expression of the enzyme it encodes is
399 crucial for rhizosphere competence regardless of wheat genotype. Future studies could also
400 determine whether expression of this gene is imperative for microbial colonisation of the
401 plant-root environment.

402 Tall vs semi-dwarf rhizospheres

403 Shotgun metagenomic analysis of samples from the rhizosphere of tall and semi-dwarf wheat
404 cultivars resulted in the detection of clear differential abundance in reads mapping to 113
405 genes. With 107 genes enriched in the rhizospheres of tall wheats, these results possibly
406 indicate that the genetic potential of the host plant to influence the root microbiome structure
407 and function has been reduced as a consequence of wheat dwarfing. We discuss the functions
408 associated with genes enriched in the rhizospheres of tall wheats (see KEGG IDs in brackets)
409 under 11 major categories below in order of highest read counts allocated per category. Not
410 all 107 genes are discussed in detail but see Table 3 and Table S2 for a summary in terms of

411 read counts and taxonomic information. The six genes enriched in the semi-dwarfs assign to
412 primary metabolism, membrane transport and motility, but in much fewer read counts (3K
413 reads) than those enriched in tall cultivars (6.5M reads).

414 *Secondary metabolism*

415 There were 13 differentially abundant genes relating to secondary metabolism with a total of
416 2.6M reads assigned over all samples. Of these reads, 98% were most similar to genes
417 associated with biosynthesis of staurosporine (K14266, K19885, K20086, K20090 and
418 K20088), a natural product antibiotic originally isolated from the bacterium *Streptomyces*
419 *staurosporeus* (Omura et al., 1977), with mode of action being through competitive protein
420 kinase inhibition, with this family of molecules exhibiting anti-cancer potential (Yadav et al.,
421 2015). In addition, reads were associated with terpenoid and polyketide antibiotic synthesis
422 (K16397, K16403, K14627, K14368, K21212, K16448 and K15968) as well as bacterial
423 toxin production (K11009). The high number of differentially abundant genes associated with
424 antibiotic production could indicate that niche occupancy competition of rhizosphere
425 microbiome community members in pre-green revolution wheat is driven by an arms race,
426 which could provide a novel underexploited resource for natural product discovery and the
427 development of the next generation of antibiotics.

428 *Cell wall degradation*

429 A total of 1.3M reads were found to map to four differentially abundant genes for plant cell
430 wall degradation (K18651, K18578, K18576 and K18786). Although this function has long
431 been associated with plant pathology (Kubicek et al., 2014; Wood, 1960), cellulolytic activity
432 has also been linked to enhanced plant root length by facilitating sloughing-off of root cap
433 cells from the root tip which assists the growing root in penetrating soil (Campillo et al.,
434 2004). It has also been found that cell wall degradation is essential for *Rhizobium* symbiotic

435 infection of legume roots (Robledo et al., 2008). It follows that this function could also be
436 important for microbial colonisation of the plant environment, microbial mediation of plant
437 root architecture and an overwintering energy source for microbes on crop residues. With
438 such high enrichment of these genes in tall wheat rhizospheres therefore demonstrates that a
439 potentially important function to facilitate plant-microbial interactions has been degraded in
440 semi-dwarf wheat.

441 *Primary metabolism*

442 In addition to cell wall degradation, a further 809K reads mapped to genes associated with
443 primary metabolism. These can primarily be subdivided into the metabolism of carbohydrates
444 (380K reads), lipids (340K reads), proteins (46K reads), co-factors/vitamins (27K reads), and
445 enzymes involved in core primary metabolism (15K reads). These observations are congruent
446 with the classes of genes most abundant in membrane transport, and it is unsurprising that
447 genes associated with the metabolism of these primary nutrient sources are abundant in these
448 data; however, it is interesting that they are highly enriched in tall rhizospheres. The most
449 abundant of the differentially abundant primary metabolism genes was associated with
450 sphingolipid metabolism through the action of galactosylceramidase (K01202, 336K reads).
451 Sphingolipids are a class of membrane bound lipid which act as signal bioactive molecules
452 (Wang et al., 2021b). It has been proposed that Bacteroidota, predominant members of the
453 mammalian gut microbiome, utilise sphingolipids as an energy source as well as to mediate
454 signal transduction and stress response pathways, facilitating their persistence in this
455 environment (An et al., 2014). Our dataset found that Actinomycetota made up the highest
456 proportion of taxa with this gene (47%) demonstrating it is not a Bacteroidota specific
457 function in the wheat root environment. It could be the case that microbe-microbe and
458 microbe-plant sphingolipid-based signalling is also crucial in commensal colonisation of the
459 plant root environment as is proposed in the human gut. There were also two genes involved

460 in propanoate metabolism (K20455, K08325) which is a building block of fatty acids which
461 are often a component of sphingolipids, the genes for their biosynthesis are highly enriched in
462 the tall rhizospheres in this dataset and have previously been found to be enriched in the
463 metabolism pathways of rhizosphere microbiota (Finkel et al., 2017; Pascale et al., 2020;
464 Zhou et al., 2020).

465 Furthermore, one of the differentially abundant carbohydrate metabolism genes was
466 associated with lipopolysaccharide (LPS) biosynthesis (K02847). LPS is an important outer
467 membrane component of Gram-negative bacteria and mutations in LPS biosynthetic genes
468 have been shown to impair rhizosphere and root colonisation of maize by *Rhizobium tropici,*
469 a root nodule bacterium (Ormeño-Orrillo et al., 2012). Finally, we also found K08961, which
470 encodes a gene for glycosaminoglycan degradation to differentially more abundant in tall
471 wheat rhizosphere microbiomes. This linear polysaccharide is not present in plant cells,
472 however it is found in animal as well as bacterial cells, so its function in the root environment
473 is likely to be implicated in nutrient cycling of microbiome community members.

474 *Sensor regulator*

475 Approximately 640K reads were found to map to differentially abundant genes associated
476 with two component sensor-regulation systems, most of which (510K reads) were assigned to
477 serine detection (K05874). In addition, histidine kinase AauS (K17060), which regulates
478 uptake and metabolism of acidic amino acids in *Pseudomonas putida* (Sonawane et al., 2006)
479 in a phosphorylation dependent manner, was also in high abundance, along with a sensor
480 receptor gene for monosaccharides (ribose and galactose; K05876). It will be interesting in
481 future work to determine the amino acid to sugar ratio and the relative contribution of amino
482 acids in the root exudates of tall compared to dwarfed wheats and determine whether
483 detection of these molecules is important for chemoattraction into the root zone as a
484 prerequisite to rhizosphere colonisation of tall wheat.

485 *Membrane transport*

486 A total of 520K reads mapped to differentially abundant genes associated with membrane
487 transport and the vast majority (435K reads) were associated with uptake systems. However,
488 export systems were largely associated with polysaccharide transport, presumably as a
489 prerequisite to biofilm formation (e.g. K16552, K16553, K16696), though the outer
490 membrane protein X (K11934), the entericidin toxin peptide and antidote system (K16347,
491 K16348) and spermidine export system (K11743; which is important at acidic pH such as
492 that of the rhizosphere (Higashi et al., 2008) are also represented). Regarding import systems,
493 these were categorised as metal (iron and nickel; K10094, K16088), purine (K10975),
494 aromatic amino acids (K11734), though the vast majority of these reads were associated with
495 ATP-binding cassette and MFS sugar uptake systems (alpha-glycosides, arabinose, lactose
496 and oligogalacturonides) with a total of 280K reads assigned (K02532, K10235, K16210,
497 K08156). The observation that bacteria in the rhizosphere have a high level of uptake
498 transport systems has previously been studied in rhizobia (Mauchline et al., 2006), though
499 this is the first time that an enhancement in these systems is associated with the root
500 microbiome of pre-green revolution wheats. It is interesting that there was no perceived
501 difference in abundance detected between tall and short wheats for genes associated with
502 organic acid uptake systems and implies that the dwarfing of wheat has perhaps not impacted
503 the root exudation profile of these molecules to the same extent of amino acids and sugars.

504 It was also found that the ratio of reads associated with amino acid:sugar detection in two
505 component systems is inversely proportional to the ratio of predicted uptake transport
506 systems for these substrates. It is possible that as sugars and organic acids are more
507 ubiquitous in root exudates, that the root microbiome of tall wheats have evolved to invest a
508 greater proportion of detection mechanisms into amino acid detection. Future work should

509 determine the differential root exudate composition between tall and semi-dwarf wheats and
510 whether these can be positively correlated with the predicted uptake systems.

511 *Biofilm formation and quorum sensing*

512 Approximately 234K reads mapped to genes associated with quorum sensing and biofilm
513 formation. Reads mapping to genes associated with mannose sensitive haemagglutinin
514 (MSHA) pilus biogenesis [*mshJ*, (K12280) *mshK* (K12281), *mshN* (K12284), *mshO*
515 (K12285) and *mshP* (K12286)] are total 83K reads. This molecule has been shown to be
516 crucial for the attachment of *Vibrio cholerae* to environmental surfaces (Marsh and Taylor,
517 1999) and more recently MSHA has also been shown to promote the attachment of
518 *Pseudoalteromonas tunicata* to cellulose surfaces (Dalisay et al., 2006). In addition,
519 approximately 28K reads are assigned to MSHA pilin protein production genes *mshC* and
520 *mshD* (K10926, K10927). These genes are important for biofilm formation, horizontal gene
521 transfer and twitching motility (Toma et al., 2002). It therefore follows that the increased
522 differential abundance of MSHA could reflect their importance for microbial persistence in
523 the tall wheat rhizosphere microbiome.

524 In addition, approximately 106K reads mapped to genes associated with quorum sensing. The
525 most abundant of these, at 56K reads, being assigned as the LuxR family transcriptional
526 regulator gene *cciR* (K19731). A study into the food-borne pathogen *Vibrio parhaemolyticus*
527 revealed that LuxR family regulators influence bacterial growth and biofilm formation and
528 highlighted that the LuxR regulator RobA regulated expression of exopolysaccharides (EPS)
529 synthesis and controlled biofilm formation (Zhong et al., 2021). Our data suggests that
530 transcriptional regulators are also important for biofilm formation in the root environment
531 and that the increased differential abundance in the rhizosphere of tall cultivars is indicative
532 of a tighter control of rhizosphere microbiome assembly in pre green revolution wheat.

533 *Transcriptional regulation*

534 Approximately 142K reads were assigned to transcriptional regulation, and almost 100K are
535 ascribed to the DeoR family transcriptional regulator (K03477). In *E. coli*, a DeoR family
536 transcriptional regulator, UlaR, was found to be responsible for suppressing transcription of
537 the divergent *ulaG* and *ulaABCDEF* operons (which catabolise L-ascorbate), under ascorbate
538 depleted conditions (Campos et al., 2004). Interestingly, ascorbate has been shown to be
539 released from plant roots under conditions of salt stress and influences root elongation (Li et
540 al., 2018; Makavitskaya et al., 2018). It could follow that exudation of ascorbate into the
541 rhizosphere profoundly affects root microbiome colonisation patterns that are employed by
542 tall wheat cultivars. In addition, 33K reads were assigned to the AraC L-arginine responsive
543 activator (K21825). It has previously been shown that arginine is an important environmental
544 cue for cyclic di-GMP signalling (c-di-GMP) signalling and biofilm formation in
545 *Pseudomonas putida* (Barrientos-Moreno et al., 2020), highlighting the potential importance
546 of detection and utilisation of this amino acid for microbiome assembly in the rhizosphere of
547 tall wheat. It will be interesting to assess whether tall cultivars produce a greater proportion
548 of arginine in their exudates than the corresponding short cultivars.

549 *Secretion systems*

550 A total of 76K reads mapped to secretion systems, and interestingly, type III (K13454,
551 K18380, K18373, K18380), IV (K03202, K12083, K120555) and type VI (K11889, K11909)
552 secretion systems are predominately over represented in tall wheat rhizosphere, all of which
553 use machinery to directly breach and deliver secreted proteins across host cell membranes as
554 opposed to other secretion system types which release toxins into the extracellular milieu
555 (Green and Mecsas, 2016). In addition, genes associated with pilus assembly proteins for
556 conjugal transfer (a total of 25K reads; K12059, K12069) were also enriched in tall

557 rhizospheres and are key to bacterial mating via synthesis of a type IV secretion system
558 (Shala-Lawrence et al., 2018).

559 *Antibiotic resistance*

560 Approximately 29K reads were assigned to differentially abundant genes involved in
561 antibiotic resistance (K19216, K18793, K19101, K22335 & K19213). These were related to
562 beta-lactamase function, which has been shown to be well represented in isolates of the soil
563 dwelling bacterium *Bacillus subtilis* (Bucher et al., 2019). Evidence suggests that its
564 production facilitates rhizosphere colonisation by the plant pathogen *Fusarium oxysporum*
565 (Chang et al., 2021). It is interesting that the number of reads in this category is far exceeded
566 by the number of reads for antibiotic production (e.g. staurosporine biosynthesis). This could
567 indicate that microbial strategy for rhizosphere colonisation of tall wheat rhizospheres has an
568 offensive emphasis, and that some of this capacity is reduced in the short wheat rhizosphere
569 microbiome.

570 *Motility*

571 There were two differentially abundant genes relating to motility, but the majority of the
572 reads (23K reads) were assigned to the flagellar chaperone biosynthesis gene *flgN* (K02399).
573 This gene has been shown to be required for flagellum-based motility in *Bacillus subtilis*
574 (Cairns et al., 2014) and is involved in the regulation and assembly of the flagellum, and its
575 enhanced differential abundance in the rhizosphere of tall wheat suggests that it is important
576 for the colonisation of this environment.

577 *Secondary messaging*

578 Approximately 10K reads were assigned to secondary messaging, especially cyclic di GMP
579 (c-di-GMP) phosphodiesterase (K20966) which has previously been shown to be important
580 for the rhizosphere colonisation of wheat by *Pseudomonas fluorescens* (Little et al., 2019).

581 The activity of c-di-GMP is important for the production of EPS and biofilm formation and
582 an increase in abundance of c-di-GMP phosphodiesterase activity which is involved in the
583 reduction of c-di-GMP level implies that biofilm formation in tall cultivars is tightly
584 regulated.

585 **Conclusions**

586 Our data showed that semi-dwarf wheat has a reduced rhizosphere effect when compared
587 with tall wheat. In addition, we observed a depletion of a wide range of functional genes in
588 semi-dwarf wheat, indicating a functional deterioration in the rhizosphere microbiome
589 associated with wheat dwarfing. Wheat dwarfing of the green revolution has profoundly
590 influenced the selection and function of the root microbiome, and this is evidenced by
591 reduced abundance in genes such as those involved in biofilm formation as well as the
592 sensing of key nutrients and their transport into microbial cells in semi-dwarf wheat.
593 However, the vast majority of reads map to genes associated with secondary metabolism as
594 well as cell wall degradation. As such it seems that the root microbiome of tall wheats have
595 adopted a two-pronged strategy of exclusion of microbial competitor reduction through
596 antibiotic production (e.g. staurosporine production) as well as the metabolism of plant
597 derived nutrients. The latter seems to be via utilisation of cell wall constituents as a nutrient
598 source to establish in this environment as well as through sphingolipid metabolism – it is
599 unclear to what extent these functions are also important for plant-microbe signalling. The
600 green revolution combined wheat dwarfing with the application of synthetic chemical
601 fertilisers. Our previous work has highlighted that application of synthetic fertiliser reduces
602 the selection of nutrient-solubilising bacteria in the rhizosphere (Reid et al., 2021). It will be
603 interesting to ascertain the impact of the combination of these factors for global microbiome
604 function and selection, and to determine the specific contribution of *Rht* mutant alleles in
605 isogenic wheats to the observed microbiome shifts caused by dwarfing which are highlighted

606 in this work. Finally, we believe our results are of striking importance and highlight that
607 implementation of microbiome facilitated agriculture as part of a sustainable crop production strategy
608 will require an overhaul of wheat breeding programmes to consider plant-microbe interactions,
609 especially in the root environment.

610 **Declarations**

611 *Ethics approval and consent to participate:* Not applicable

612 *Consent for publication:* Not applicable

613 *Availability of data and material:* The datasets generated and analysed during the current
614 study are available in the NCBI repository, BioProject PRJNA1128034. The BioProject and
615 associated SRA metadata are publicly available at
616 https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1128034. However, due to a glitch in the
617 SRA system the samples are only available via individual download until this issue is
618 resolved, see links below.

619 **BioSample**

620 SAMN42186182 DNA_from_cv.Chidham_rep1 - BioSample - NCBI

621 SAMN42186183 DNA_from_cv.Chidham_rep2 - BioSample - NCBI

622 SAMN42186184 DNA_from_cv.Chidham_rep3 - BioSample - NCBI

623 SAMN42186185 DNA_from_cv.Chidham_rep4 - BioSample - NCBI

624 SAMN42186186 DNA_from_cv.Chidham_rep5 - BioSample - NCBI

625 SAMN42186187 DNA_from_cv.Gallant_rep1 - BioSample - NCBI

626	SAMN42186188	DNA_from_cv.Gallant_rep2 - BioSample - NCBI
627	SAMN42186189	DNA_from_cv.Gallant_rep3 - BioSample - NCBI
628	SAMN42186190	DNA_from_cv.Gallant_rep4 - BioSample - NCBI
629	SAMN42186191	DNA_from_cv.Gallant_rep5 - BioSample - NCBI
630	SAMN42186192	DNA_from_cv.Malacca_rep1 - BioSample - NCBI
631	SAMN42186193	DNA_from_cv.Malacca_rep2 - BioSample - NCBI
632	SAMN42186194	DNA_from_cv.Malacca_rep3 - BioSample - NCBI
633	SAMN42186195	DNA_from_cv.Malacca_rep4 - BioSample - NCBI
634	SAMN42186196	DNA_from_cv.Malacca_rep5 - BioSample - NCBI
635	SAMN42186197	DNA_from_cv.Red_Lammas_rep1 - BioSample - NCBI
636	SAMN42186198	DNA_from_cv.Red_Lammas_rep2 - BioSample - NCBI
637	SAMN42186199	DNA_from_cv.Red_Lammas_rep3 - BioSample - NCBI
638	SAMN42186200	DNA_from_cv.Red_Lammas_rep4 - BioSample - NCBI
639	SAMN42186201	DNA_from_cv.Red_Lammas_rep4 - BioSample - NCBI
640	SAMN42186202	DNA_from_Bulk_Soil_rep1 - BioSample - NCBI
641	SAMN42186203	DNA_from_Bulk_Soil_rep2 - BioSample - NCBI
642	SAMN42186204	DNA_from_Bulk_Soil_rep3 - BioSample - NCBI

643 **SRA**

644	SRR29660204	DNA_from_cv.Chidham_rep1 - SRA - NCBI
645	SRR29660203	DNA_from_cv.Chidham_rep2 - SRA - NCBI
646	SRR29660192	DNA_from_cv.Chidham_rep3 - SRA - NCBI
647	SRR29660188	DNA_from_cv.Chidham_rep4 - SRA - NCBI
648	SRR29660187	DNA_from_cv.Chidham_rep5 - SRA - NCBI
649	SRR29660186	DNA_from_cv.Gallant_rep1 - SRA - NCBI
650	SRR29660185	DNA_from_cv.Gallant_rep2 - SRA - NCBI
651	SRR29660184	DNA_from_cv.Gallant_rep3 - SRA - NCBI
652	SRR29660183	DNA_from_cv.Gallant_rep4 - SRA - NCBI
653	SRR29660182	DNA_from_cv.Gallant_rep5 - SRA - NCBI
654	SRR29660202	DNA_from_cv.Malacca_rep1 - SRA - NCBI
655	SRR29660201	DNA_from_cv.Malacca_rep2 - SRA - NCBI
656	SRR29660200	DNA_from_cv.Malacca_rep3 - SRA - NCBI
657	SRR29660199	DNA_from_cv.Malacca_rep4 - SRA - NCBI
658	SRR29660198	DNA_from_cv.Malacca_rep5 - SRA - NCBI
659	SRR29660197	DNA_from_cv.Red_Lammas_rep1 - SRA - NCBI
660	SRR29660196	DNA_from_cv.Red_Lammas_rep2 - SRA - NCBI
661	SRR29660195	DNA_from_cv.Red_Lammas_rep3 - SRA - NCBI

SRR29660194 DNA_from_cv.Red_Lammas_rep4 - SRA - NCBI

SRR29660193 DNA_from_cv.Red_Lammas_rep5 - SRA - NCBI

SRR29660191 DNA_from_Bulk_Soil_rep1 - SRA - NCBI

SRR29660190 DNA_from_Bulk_Soil_rep2 - SRA - NCBI

SRR29660189 DNA_from_Bulk_Soil_rep3 - SRA - NCBI

Competing interests: The authors declare that they have no competing interests

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