The metatranscriptomic response of the wheat rhizosphere to drought varies with growth stages.

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Abstract

Microbes can help plant sustain abiotic stresses, such as drought. Plant-microbe interactions are, however, dynamic and the timing of the stress will affect both partners, directly and indirectly. Here, we hypothesize that the effect of drought stress on the wheat rhizosphere microbiome would change between key growth stages. We grew wheat in pots and stopped watering them for two weeks at stem elongation, booting, or heading. We then sampled the rhizosphere soil and sequenced its metatranscriptome. The timing of the drought strongly affected the transcriptional response of the microbes, but few differentially abundant transcripts were shared across all stages. Some common patterns were however observed at higher taxonomical or functional levels. Drought also affected the normal succession across wheat growth stages. Many of the differentially abundant transcripts, taxa and functions between growth stages of the control plants were not significant anymore for plants that experienced drought. Our results suggest that the timing of the drought event is paramount to the microbial rhizosphere communities, and that it could explain the heightened sensitivity of younger plants to stresses.

Keywords: metatranscriptomics, drought, rhizosphere, microbial communities, wheat.

Introduction

Drought can – depending on the growth stages – kill crops, severely reduce yields and grain quality [1], or have no effect. However, it is not clear if this is due to direct effects on the plant or indirectly through changes in beneficial plant-microbe interactions. A recent metatranscriptomic study from our group supports the indirect route. First, the microbiota – and not the plant – was the main responder to decreasing soil water content [2]. Second, this decrease resulted in higher abundance of microbial transcripts linked to osmolyte production and other functions that might be beneficial to the plant [2]. Plant tolerance to drought- or salinity-related stresses can be improved by different strains of *Actinobacteria* and *Proteobacteria* [3-6] and by fungal endophytes [7-9]. These beneficial microbial taxa and associated functions will, however, vary with plant growth stages, which could partly explain the varying sensitivity of crops to drought. This interaction between drought and growth stage is not fully understood, as most of the above-mentioned studies focused on a single sampling.

Time and water stress directly, indirectly, and interactively affects microbial communities. First, root-associated microbial communities vary with plant growth stage [10], because of changing root exudation patterns [11, 12]. Second, root exudation also changes when plants are exposed to water stress [13, 14], potentially affecting microbial communities. Finally, soil microbial communities have seasonal patterns of their own [15, 16] and respond directly to water availability [17-19]. All these factors interact to influence the microbial communities. For instance, wheat associated microbial communities were altered by historical and current water stress in interaction with plant growth stage, plant genotype, and plant compartment [17-19]. It is therefore difficult to predict the impact of the timing of drought on the microbial response and its

feedback to crops, even though this knowledge would be crucial to devise microbial approaches to help crops adapt to water stress.

Drought can reduce wheat yield by up to 50% [20, 21]. We think that if their response to drought was better understood, microorganisms could help abate these losses. As a first step, we need to understand how microbial communities will respond to water stress at different wheat growth stages. To answer this question, we grew wheat plants in pots and stopped watering them for two weeks at three distinct growth stages (stem elongation, booting, and heading). At tillering, booting, stem elongation, heading and flowering, we uprooted plants, sampled their rhizosphere soil, extracted the RNA and sequenced it. Our aim was to test the hypothesis that the effect of drought stress on the wheat rhizosphere microbiome would vary between key growth stages.

Material and methods

Experimental design and sampling

To test our hypothesis, we set-up a greenhouse pot experiment. Each 1L pot was filled with 1.5 kg (dry weight) of soil. The potting soil was collected from our experimental field at the Institut national de la recherche scientifique (Laval, QC, Canada). All the soil was air dried, sieved with a 2 mm mesh sieve and homogenized. We then sowed in each pot 8 seeds of *Triticum aestivum* cv. AC Nass, a drought-sensitive cultivar. After germination, the plants were thinned to 5 per pot. Until the stem elongation stage, all the pots were maintained at 50% SWHC (soil water holding capacity). Drought was imposed for two weeks by dropping to 8% SWHC at the beginning of stem elongation, booting and heading growth stages, resulting in 3 treatments plus a control that was kept at 50% SWHC throughout the experiment. We wanted to impose drought during the tillering stage as well, but during the pre-experiment, it was observed that the plants were unable to survive. The treatments were replicated six times in a randomized complete block design, and since our sampling was destructive, this resulted in 120 pots. After their 2-week exposure to drought, the plants were brought back to normal water content. It was observed that during drought plants were weak but continued growth, that there was no delay in the occurrence of the next growth stage and that they showed resilience after rewetting. We sampled at tillering, stem elongation, booting, heading and flowering. At stem elongation, booting and heading, we waited after the soil water content reached its target value (within a few days) before sampling all treatments and controls, to make sure we sampled during the drought event. All the plants from a pot were uprooted and firmly shaken, after which the soil remaining attached to the roots – the rhizosphere – was sampled, mixed thoroughly and immediately frozen in liquid nitrogen. This resulted in 120 rhizosphere soil samples (3 treatments + 1 control x 5 growth stages x 6

90 blocks). Rhizosphere soils were transported to the lab on dry ice and then kept at -80°C until
91 extracted.
92
93 *RNA extraction and sequencing*
94 Total RNA was extracted from 2 g of rhizosphere soil using the RNeasy PowerSoil Total RNA
95 Kit (QIAGEN, Canada). Extracted RNA was treated with DNAse (ThermoFisher, Canada) to
96 remove the DNA prior to sequencing. The absence of DNA was confirmed by the lack of PCR
97 amplification using 16S rRNA gene specific primers. Total RNA was sent for two lanes of
98 NovaSeq6000 S4 PE150 sequencing at the Centre d'Expertise et de Services Génome Québec
99 (Montréal, Québec). Libraries were created using a microbial ribosome subtraction approach.
100 The raw sequencing data produced in this study was deposited in the NCBI under Bioproject
101 accession PRJNA978601.
102
103 *Bioinformatics analyses*
104 The metatranscriptome sequencing of the 120 rhizosphere samples resulted in 12,000M reads for
105 a total of 1,800 giga bases which were processed together through our metatranscriptomics
106 bioinformatics pipeline [22], as detailed in Pande et al. [2].
107
108 *Statistical analyses*
109 All statistical analyses were performed in R version 4.2.3. [23]. Transcript differential abundance
110 analyses between the drought treatments and the growth stages were carried out using the
111 EBTest function of the EBSeq library with a false discovery rate (FDR) of 0.05. Anovas were
112 performed using the aov function of the stats package. All graphs were generated using ggplot2.

The R project folder containing the R code used for data manipulation, statistical analyses, and tables and figure generation is available on our lab GitHub repository (https://github.com/le-labo-yergeau/MT_Time_Wheat). The associated transcript abundance and annotation tables, and the metadata files used with the R code are available on Zenodo (https://doi.org/10.5281/zenodo.7995531).

118 **Results**

119 *Growth stages have a strong influence on the wheat rhizosphere metatranscriptome.*

120 We performed permanova and principal coordinate analysis (PCoA) on the entire
121 metatranscriptome (14,709,097 transcripts) to assess the effect of the four drought treatments and
122 the five growth stages. 16.1% of the variation could be explained by growth stage (P=0.001),
123 3.2% by the drought treatments (P=0.008) and 11.1% by the interaction between the two
124 variables (P=0.001). This can be visualized in the PCoA (Fig. 1b), where the clustering of the
125 samples is mostly driven by the wheat growth stage, with some, less visible in the first two
126 dimensions, effects of the drought treatments. We also looked at the treatments, but classified as
127 when the drought was applied (DR), as rewetted (RW, treatments after the drought event) or as
128 not disturbed (ND, controls and treatments before the drought event) (Fig. 1a). These results
129 show that growth stage has a strong influence on the metatranscriptome and that, depending on
130 the growth stage, the drought treatments also affected the metatranscriptome.

131 We then looked at the taxonomical affiliation of all transcripts across growth stages for
132 all treatments (Fig. 1c) and tested the difference between the control and the different treatments
133 during the drought or after rewetting using paired t-tests. Since there was quite some variability
134 between the treatments, we also kept results with 0.05<P<0.10. As compared to the controls, the
135 relative abundance of *Ascomycota* decreased 1.45 times during drought, when applied at stem
136 elongation (t=2.49, P=0.062). When this treatment was rewetted, the *Bacteroidetes* were 1.34
137 times less abundant than in the control (t=2.31, P=0.069). When drought was applied at booting,
138 none of the most abundant phyla changed their relative abundance, both during drying and
139 rewetting. When soils were dried at heading, the *Actinobacteria* were 1.34 times more abundant
140 than in the controls (t=4.95, P=0.004), whereas the *Chloroflexi* were 1.38 times more abundant

141 (t=3.94, P=0.011). For the *Chloroflexi*, this difference was sustained during rewetting, where
142 they were 1.14 times more abundant in the rewetted treatment as compared to the control
143 (t=3.27, P=0.022).

144 We also looked at the COG category of the transcripts (Fig. 2), and tested differences
145 using paired t-tests as above. As above, since there was quite some variability between the
146 treatments, we also kept results with 0.05<P<0.10.When soils were dried at the stem elongation
147 stage, transcripts related to "Carbohydrate transport and metabolism" were decreased by 1.10
148 times during drought as compared to the controls (t=2.49, P=0.055). When this treatment was
149 rewetted, no difference was found with the controls. When drought was applied at booting,
150 transcripts affiliated to "Energy production and conversion" and "Translation, ribosomal
151 structure and biogenesis" decreased 1.12 and 1.16 times, respectively (t=2.23, P=0.077 and
152 t=2.39, P=0.063, respectively), whereas transcripts classified in the "Transcription" category
153 increased 1.15 times (t=2.06, P=0.095). No significant difference remained for this treatment
154 after rewetting. When soils were dried at heading, transcripts classified in the COG categories
155 "Posttranslational modification, protein turnover, chaperones" and "Transcription" increased
156 1.24 and 1.39 times as compared to the controls, respectively (t=2.26, P=0.074 and t=3.44,
157 P=0.019, respectively). This difference was sustained at rewetting for the "Transcription"
158 category that was 1.12 times more abundant than the controls (t=2.24, P= 0.075).

159

160 *The response to drought across growth stages is idiosyncratic.*

161 To analyze the effect of the drought treatments on the metatranscriptome, we did a transcript
162 differential abundance (DA) analysis between the control and each of the three drought
163 treatments across the different growth stages. In terms of number of differentially abundant

164 transcripts, the patterns varied per treatment (Fig. 3a). When drought was applied at stem
165 elongation, there was a six-fold increase in the number of DA transcripts from stem elongation to
166 booting (from drought to rewetting), followed by a four-fold decrease at heading and another
167 four-fold decrease at flowering (Fig. 3a). When drought was applied at booting, however, there
168 was an 11-fold increase in the number of DA transcripts during drought (booting) followed by a
169 sharp decrease at rewetting (heading) (Fig. 3a). Likewise, when drought was applied at heading,
170 there was a four-fold increase in the number of DA transcripts during drought (heading) followed
171 by a two-fold reduction at rewetting (flowering) (Fig. 3a). Taken together, these results show that
172 the drought treatments resulted in large changes in the rhizosphere metatranscriptome. The
173 amount of DA transcripts varied through the plant growth stages depending on the timing of the
174 drought event.

175 We did a co-occurrence analysis of the DA transcripts found at the dry and rewetting
176 stages for the three treatments (Fig. 3c). Most of the transcripts were unique to a single treatment
177 (Fig. 3c). For the drought at stem elongation treatment, 17,166/21,399 (80.2%) and
178 121,052/126,995 (95.3%) of the DA transcripts were unique at the stem elongation and booting
179 stages, respectively. For the drought at booting treatments, 48,402/59,607 (81.2%) and
180 5,896/7,636 (77.2%) of the DA transcripts were unique at the booting and heading stages,
181 respectively. Finally, for the drought at heading treatment, 25545/32141 (79.5%) and
182 14094/15877 (88.8%) of the DA transcripts were unique at the heading and flowering stages.
183 Across the six samples (total of 247,498 DA transcripts), the largest intersection was between the
184 drought samples at booting and heading, but still only 3,512 DA transcripts were shared (Fig.
185 3c). 800 DA transcripts were shared by 3 or more treatment x growth stage combinations, 14 by
186 4 or more treatments x growth stage combinations, and none by 5 or more treatments x growth

187 stage combinations (Fig. 3c). When comparing the samples taken during the drought events, 174
188 DA transcripts were shared by the three treatments, whereas the samples taken during the
189 rewetting shared only 2 DA transcripts. Taken together, these results suggest that the response of
190 the microbial communities to the drought treatment is very different when drought is applied at
191 different plant growth stages, and that only a minority of DA transcripts are shared across the
192 different treatments.

193 We then looked at the genus and COG function annotation of DA transcripts of Fig. 3a. A
194 large portion (~75%) of the DA transcripts could not be annotated at the genus and the COG
195 function levels and were removed from the figure for clarity. As the results we are presenting are
196 the taxonomic or the functional make-up of a list of DA transcripts generated by comparing all
197 replicates, it is not possible to perform statistical comparisons as we end up with a single DA
198 transcript list per treatment. The taxonomic makeup of the DA transcripts varied across growth
199 stages across all treatments, even before the drying and rewetting events (Fig 4a). The total
200 fraction of DA transcripts that belonged to the genera listed in Fig. 4a – that made up at least
201 0.25% of the DA transcripts across all samples – was lower at the stage that showed the largest
202 increases in DA transcripts in Fig. 3a. This could be related to an increase in the unannotated DA
203 transcripts or an increase in other genera that did not cross the inclusion threshold across all
204 samples. Besides, there are no clear trends in the shifts among the genera that made up the DA
205 transcripts. For the COG functions, however, the make-up of the DA transcripts was much less
206 variable, with similar proportions of the most abundant functions across most stages for all
207 treatments (Fig. 4b). There were some shifts visible, mostly at the stage where we had seen the
208 largest number of DA transcripts (Fig. 3a), in many cases for COG functions related to amino

209 acids (Fig. 4b). In any case, the shifts in the COG functions of the DA transcripts are subtler than
210 the shifts observed at the genus level.
211
212 *Shifts in metatranscriptome across growth stages are compounded by drought.*
213 We also did differential transcript abundance analyses between each contiguous growth stage
214 (tillering vs. stem elongation, stem elongation vs. booting, booting vs. heading, heading vs.
215 flowering), for both treatments and control. We did this to disentangle the effects of growth
216 stages on the metatranscriptome vs. the temporal effects of the drought treatments. In the
217 controls, the number of DA transcripts was 2.8 to 4.6 times higher for the heading vs. flowering
218 contrast as compared to the other contrasts (Fig. 3b), suggesting large shifts in the rhizosphere
219 metatranscriptome during flowering. For the drought at stem elongation treatment, there were
220 many DA transcripts (150,083 to 287,091) for the stem elongation vs. booting, booting vs.
221 heading and heading vs. flowering contrasts, which was up to 15.5 times more than the controls
222 (for the booting vs. heading contrast) (Fig. 3b). For the drought at booting treatment, the number
223 of DA transcripts was higher than in the controls only for the stem elongation vs. booting and
224 booting vs. heading contrasts (Fig. 3b), up to a factor of 2.7. For the drought at heading
225 treatment, the number of DA transcripts was higher than the controls for the booting vs. heading
226 and the heading vs. flowering contrasts (Fig. 3b), with a 7.3 time increase for the latter case.
227 These results indicate that the microbial metatranscriptome does change with plant growth
228 stages, but that these changes are compounded by water stress.
229 Most of the DA transcripts were not shared between the treatments, for all contrasts
230 including the ones before drying (Fig. 5). This suggests that at the transcript level, there was a
231 lack of a coherent temporal response of the rhizosphere microbiome to drought and to growth

232 stage. The genus-level affiliation of the DA transcripts varied across the contrasts (Fig. 6a).
233 Although there were some common taxa represented in the DA transcripts, the treatments
234 changed the normal (control) taxonomical succession (Fig. 6a). For instance, when comparing
235 the stem elongation vs. the booting stages, the DA transcripts for the drought at stem elongation
236 and booting treatments contained less *Cylindrospermum* and *Pelomonas* genera as compared to
237 the controls (Fig. 6a). When comparing the booting and heading stages, the DA transcripts in all
238 the treatments were enriched in *Sorangium* and depleted in *Cylindrospermum* and *Nitrospira*
239 (Fig. 6a). Additionally, the drought at heading treatments transcripts were enriched in
240 *Entotheonella*, whereas it was the inverse for the two other treatments. (Fig. 6a). For the heading
241 vs. flowering contrast, the DA transcripts of the drought at booting treatments were more often
242 affiliated to *Asticcacaulis*, *Devosia*, *Cylindrospermum* and *Sorangium* than the control DA
243 transcripts (Fig. 6a). In contrast, the two other treatments showed inverse trends.
244 At the functional level – COG functions – the affiliation of the dominant DA transcripts
245 was relatively stable for the control treatment (Fig. 6b). For the drought at stem elongation
246 treatment, there were more DA transcripts identified to "Serine/threonine protein kinase" and
247 "Signal transduction histidine kinase" than in the control for both the stem elongation vs. booting
248 and booting vs. heading contrasts (Fig. 6b). For the stem elongation vs. booting contrast, there
249 were less DA transcripts related to "Chaperonin GroEL" and "DNA-directed RNA polymerase"
250 in the drought at stem elongation and at booting treatments, relative to the control (Fig. 6b). For
251 the booting vs. heading contrast, the DA transcripts identified in the three treatments were
252 depleted in "Chaperonin GroEL" and "Outer membrane membrane protein…" as compared to
253 the control (Fig. 6b). Both the drought at booting and drought at heading treatments had more
254 DA transcripts related to "ABC-type dipeptide transport system" and "ABC-type sugar transport

255 system". Finally, for the heading vs. flowering contrast, the affiliations of the DA transcripts of
256 the treatments were like the control (Fig. 6b). There was, however, more DA transcripts in the
257 "ABC-type dipeptide transport system" and "ABC-type sugar transport system" and "Outer
258 membrane protein…" for the drought at stem elongation and drought at booting treatments,
259 relative to the control (Fig. 6b). Taken together, these results suggest that at the genus and COG
260 function levels, there are some groups that are consistently showing a difference in their
261 succession when subjected to drying and rewetting.
262

263 **Discussion**

264 We had previously shown that, within the wheat holobiont, the strongest responders were
265 the microbial communities [2]. Here, we looked at how these microbial communities respond to
266 a 2-week dry spell imposed at different growth stages. For all growth stages, drying resulted in
267 many differentially abundant (DA) transcripts. However, only a small percentage of these DA
268 transcripts were shared across growth stages, suggesting idiosyncratic responses. This response
269 altered the normal microbial succession across wheat growth stages.

270 When we imposed drought at an early stage (tillering), we killed all our wheat plants.
271 Similarly, many plants resist better to disease as they age [24]. Plant aging also modulates the
272 response to drought of the associated microbial communities. For example, the root microbial
273 community composition of older sorghum plants is less impacted by drought than the one of
274 younger plants [25]. Our youngest plants that survived drought (stem elongation stage) were the
275 ones that showed the largest metatranscriptomic shifts. These shifts were larger, more intense
276 during rewetting and lasted longer, as compared to the ones observed in older plants. For these
277 older plants – at the booting and heading stages – the metatranscriptome showed smaller changes
278 that rapidly returned to unstressed control levels. It therefore appears that the rhizosphere
279 metatranscriptome of younger wheat plants reacts more to drought.

280 On top of having different magnitude, the shifts observed at the transcript level across the
281 drought treatments were idiosyncratic. Idiosyncrasy is often seen in the response of microbial
282 communities to various factors. For instance, depending on the plant compartment and the water
283 stress history of the soil, drought altered differently the wheat microbial communities [17-19].
284 The wheat microbial community is also influenced by wheat genotype, but constrained by the
285 plant growth stages, compartment and time [15]. This idiosyncrasy also depends on the

286 resolution of the analyses: at coarse taxonomic or functional levels, some patterns are conserved
287 (e.g. increased relative abundance of *Actinobacteria* following drought), but at finer levels, there
288 is no coherent response. For instance, the microbial responses to drought at two different plant
289 growth stages differed at the OTU level but resulted in the same enrichment of monoderms [25].
290 Here, most of the DA transcripts were unique for a single treatment, but when looking at
291 functional categories (i.e. COG functions), the differences were less important. This suggests that
292 shifts at fine taxonomic or at the individual transcript levels might be seemingly random but are
293 coherent at the functional category level or at coarser taxonomical levels. Alternatively, part of
294 the idiosyncratic responses observed could be due to stochastic variation, as even before the
295 drought treatments were applied, many of the DA transcripts were unique and showed distinct
296 taxonomic affiliations across treatments.

297 Some of the idiosyncrasies observed could be explained by the microbial succession
298 through plant developmental stages. As plant develops, its microbial community fluctuates, and
299 drought does not affect similarly microbial communities of different diversity and composition.
300 For instance, Gram-negative bacteria produce osmolytes in response to drought, whereas they are
301 constitutively produced in Gram-positive [26]. At the same time, Gram-positive bacteria
302 generally have a thicker cell wall that Gram-negative bacteria [27]. Accordingly, Gram-positive
303 bacteria (mostly *Actinobacteria* and *Firmicutes*) made up 90% of the wheat microbial isolates
304 that could grow under high osmotic pressure [28]. The relative activity of *Actinobacteria* also
305 increased at the flowering stage in our study. As compared to bacteria, the wheat fungal
306 community composition often doesn't respond as much to changes in soil water content [16], but
307 they strongly responded to decreasing soil water content at the metatranscriptomic level [2] and
308 many fungal isolates from wheat could grow at high osmotic pressure [28]. Hence, the

309 magnitude of the metatranscriptomic response of the microbial community to drought will likely
310 be defined by the relative dominance of fungi, Gram-positive bacteria, and Gram-negative
311 bacteria. As all these groups vary through plant development, this could partly explain the
312 contrasting responses of the microbial communities to drought imposed at different plant growth
313 stages.

314 In addition to its strong idiosyncratic effect on microbial activities, drought disrupted the
315 normal microbial transcriptomic succession. Indeed, we observed a succession in the
316 metatranscriptome of the well-watered controls. Previous studies had shown that the plant root
317 exudates varies across plant growth stages [11], shifting the microbial community and the
318 metatranscriptome of the rhizosphere [10, 12, 15, 29]. The effect of drought on microbial
319 communities was, however, shown to trump the effect of plant development [25]. Indeed, across
320 developmental stages, we found a much larger number of DA transcripts in the treated
321 rhizospheres as compared to the controls. In the controls, the largest number of DA transcripts
322 was found when comparing heading to flowering. The flowering vs. heading comparison was
323 also the one that yielded the largest number of DA transcripts in the treated samples, probably
324 because it compounded the large shifts due to the normal succession with the ones due to
325 drought. Under drought, the microbial transcriptomic succession in the rhizosphere was
326 heightened and had little to do with the normal succession, even though the development of the
327 plants was not visibly disrupted by the drought.

328 We worked with a single wheat genotype that was not bred for resistance to water stress.
329 The response of wheat to water stress does vary across genotype, with some being better at
330 tolerating water stress at particular growth stages [30]. The wheat microbiome also varies across
331 genotypes [17, 19], but this is constrained by plant compartment, growth stages and with

environmental conditions [15]. Historical exposure of the soil microbiome to water stress also modulates its interaction with wheat genotypes [17-19]. It would be important to confirm the results presented here for genotypes with a gradient of water stress resistance and across multiple soils, ideally in the field.

We showed here that drought strongly affects the wheat rhizosphere microbial transcriptome, but in a growth stage-dependent fashion. Our results imply that - as for the plant - early drought events impact more the microbial communities. These altered microbial communities might be less beneficial, which could partly explain the vulnerability of young plants to stresses. Microbial communities also responded when drought was applied at other growth stages, but the identity of the transcripts was very different. In all cases, drought altered the microbial transcriptome of the rhizosphere, which could have repercussions on wheat growth and grain quality. Understanding the interaction between crop growth stage and drought events on the rhizosphere microbiome is essential to optimize crop resistance and resilience under the current climate emergency.

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Data availability

The raw data produced in this study was deposited in the NCBI under Bioproject accession PRJNA978601. The R project folder containing the R code used for data manipulation, statistical analyses, and tables and figure generation is available on our lab GitHub repository (https://github.com/le-labo-yergeau/MT_Time_Wheat/). The associated transcript abundance and annotation tables, and the metadata files used with the R code are available in the Zenodo archive (https://doi.org/10.5281/zenodo.7995531).

Author contributions

PMP: Designed, set-up and maintained the greenhouse experiment, performed the soil sampling and molecular analyses. JT: Performed the bioinformatic analyses. MSA: Designed the experiment, supervised students and secured funding. EY: Designed the experiment, performed the statistical analyses, secured funding, supervised students and wrote the manuscript with inputs from all authors.

Competing interests

The authors declare no competing interests.

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449 **Figure legends**

450 **Figure 1. Growth stage has a strong influence on the wheat rhizosphere**
451 **metatranscriptome.** Principal coordinate analysis based on the relative abundance of all
452 transcripts (A,B) and community composition at the phylum level (C) for the rhizosphere
453 metatranscriptome of wheat plants at tillering, stem elongation, booting, heading and flowering
454 stages that were stressed at stem elongation, booting or heading, or not stressed (control).

455 **Figure 2. Growth stage also influences the functional composition of the metatranscriptome**
456 **in the wheat rhizosphere.** Community composition at the COG category level for the
457 rhizosphere metatranscriptome of wheat plants at tillering, stem elongation, booting, heading and
458 flowering stages that were stressed at stem elongation, booting or heading, or not stressed
459 (control).

460 **Figure 3. The response to drought across growth stages is idiosyncratic.** Number of DA
461 transcripts (treatment vs. control) in the rhizosphere metatranscriptome of wheat plants at
462 tillering, stem elongation, booting, heading and flowering stages that were stressed at stem
463 elongation, booting or heading (A). Number of DA transcripts between adjacent growth stages
464 for the rhizosphere metatranscriptome of wheat plants that were stressed at stem elongation,
465 booting or heading, or not stressed (B). Number of shared DA transcripts (treatment vs. control)
466 in the rhizosphere metatranscriptome of wheat plants during drought at stem elongation, booting
467 or heading, or after rewetting. Only intersection with size above 100 are shown (C). SE: stem
468 elongation, B: booting, H: heading, F: flowering.

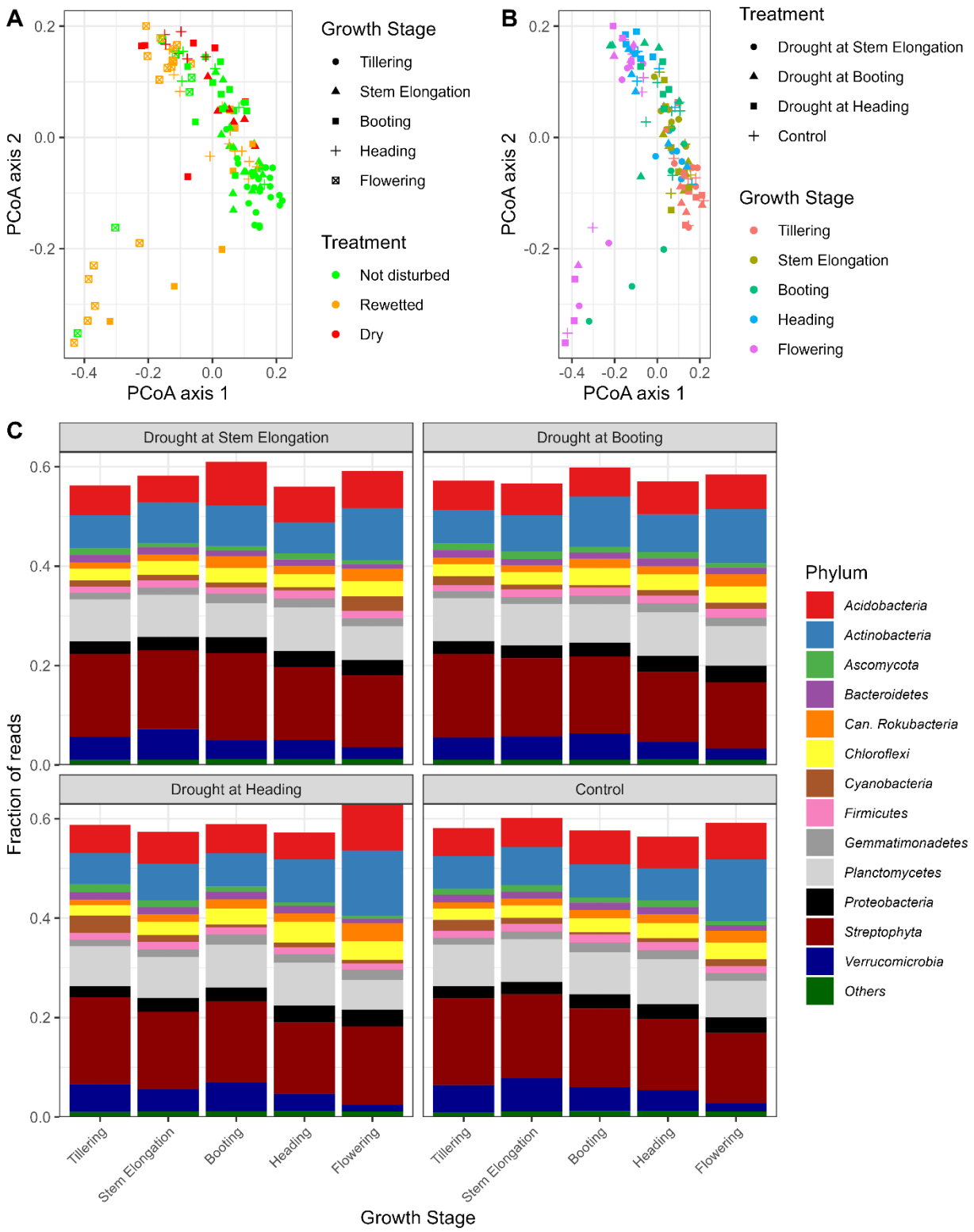
469 **Figure 4. The taxonomical and functional makeup of the DA transcripts vary without clear**
470 **patterns.** Genus (A) and COG function (B) makeup of the DA transcripts (treatment vs. control)

471 in the rhizosphere metatranscriptome of wheat plants at tillering, stem elongation, booting,
472 heading and flowering stages that were stressed at stem elongation, booting or heading.

473 **Figure 5. Drought treatments affect the normal microbial succession across growth stages.**
474 Number of shared DA transcripts between tillering and stem elongation (A), stem elongation and
475 booting (B), booting and heading (C), and heading and flowering (D) for the rhizosphere
476 metatranscriptome of wheat plants that were stressed at stem elongation, booting or heading, or
477 not stressed. T: tillering, SE: stem elongation, B: booting, H: heading, F: flowering.

478 **Figure 6. Drought treatments affect the normal microbial taxonomical and functional**
479 **succession across growth stages.** Genus (A) and COG function (B) makeup of the DA
480 transcripts between adjacent growth stages for the rhizosphere metatranscriptome of wheat plants
481 that were stressed at stem elongation, booting or heading, or not stressed**.** T: tillering, SE: stem
482 elongation, B: booting, H: heading, F: flowering.

483 **Figure 1.**



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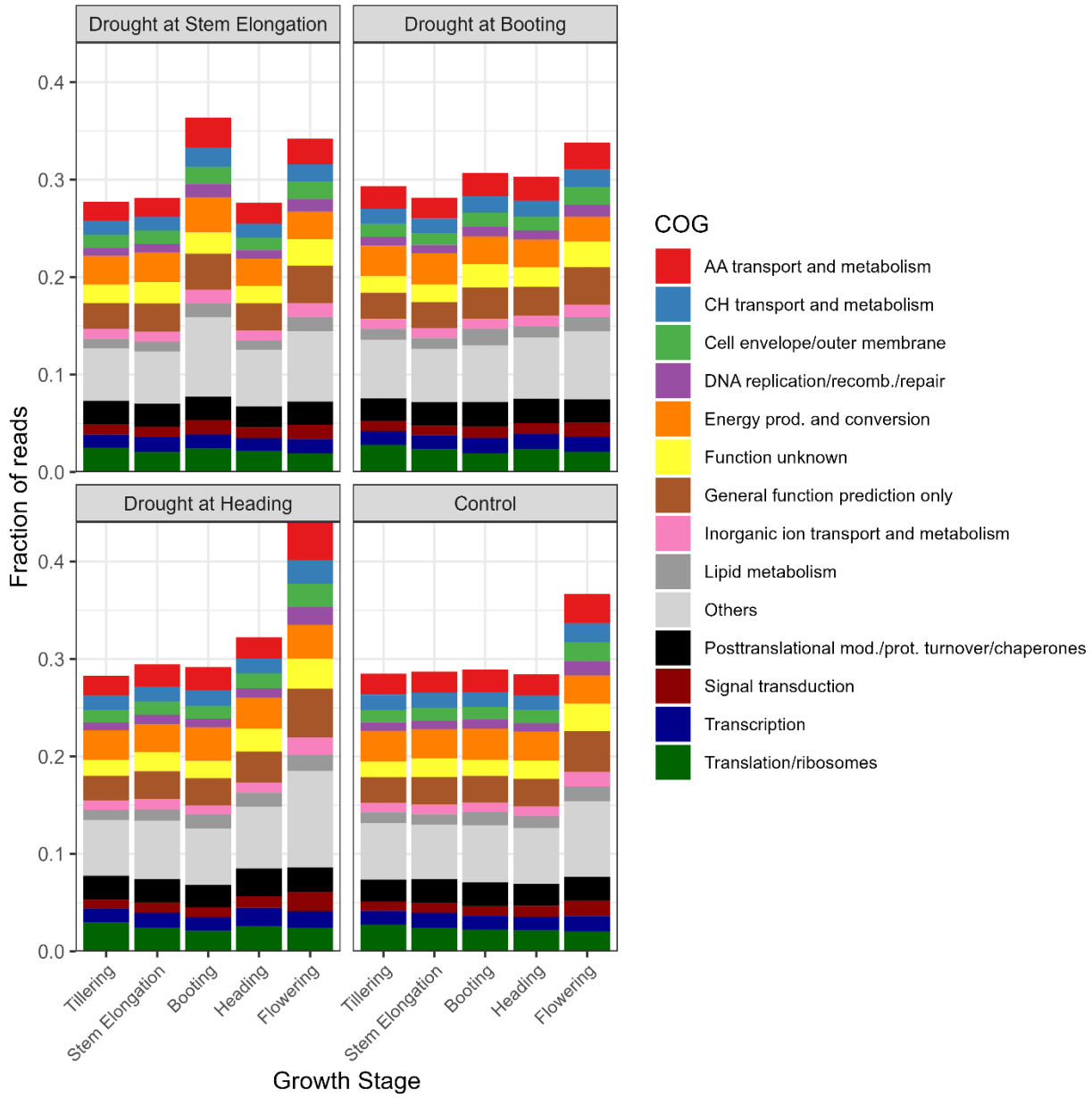
486 **Figure 2.**



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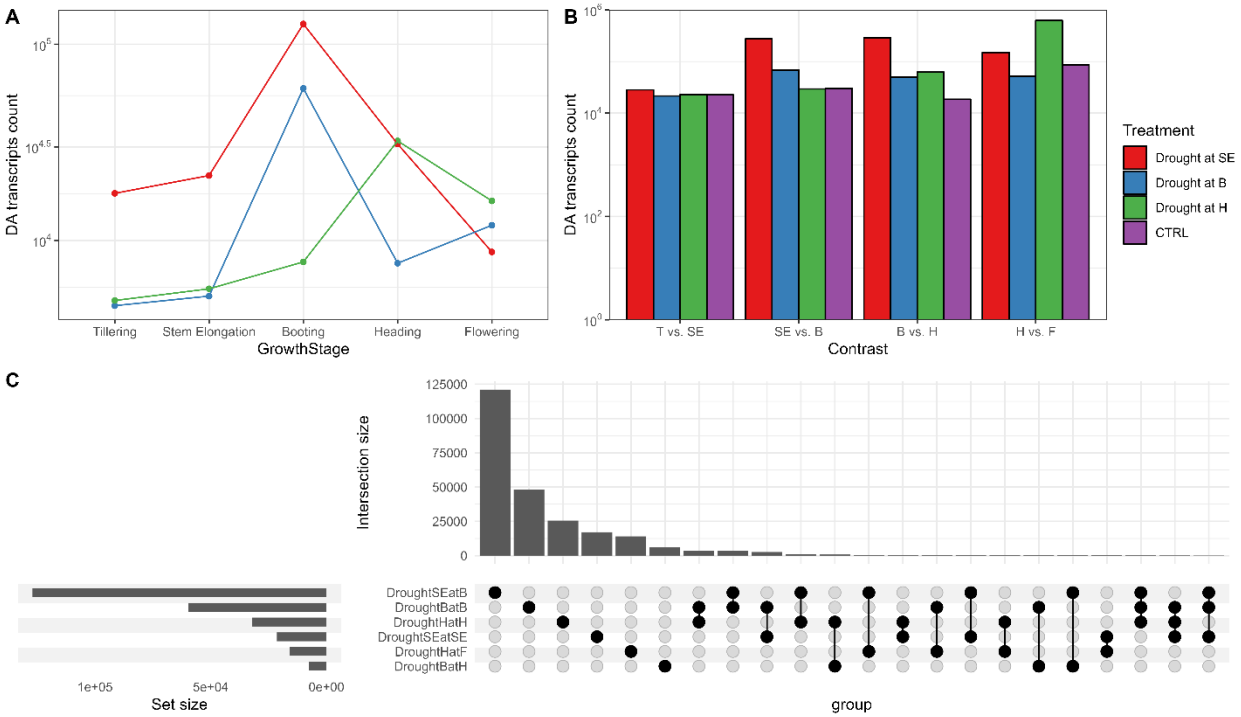

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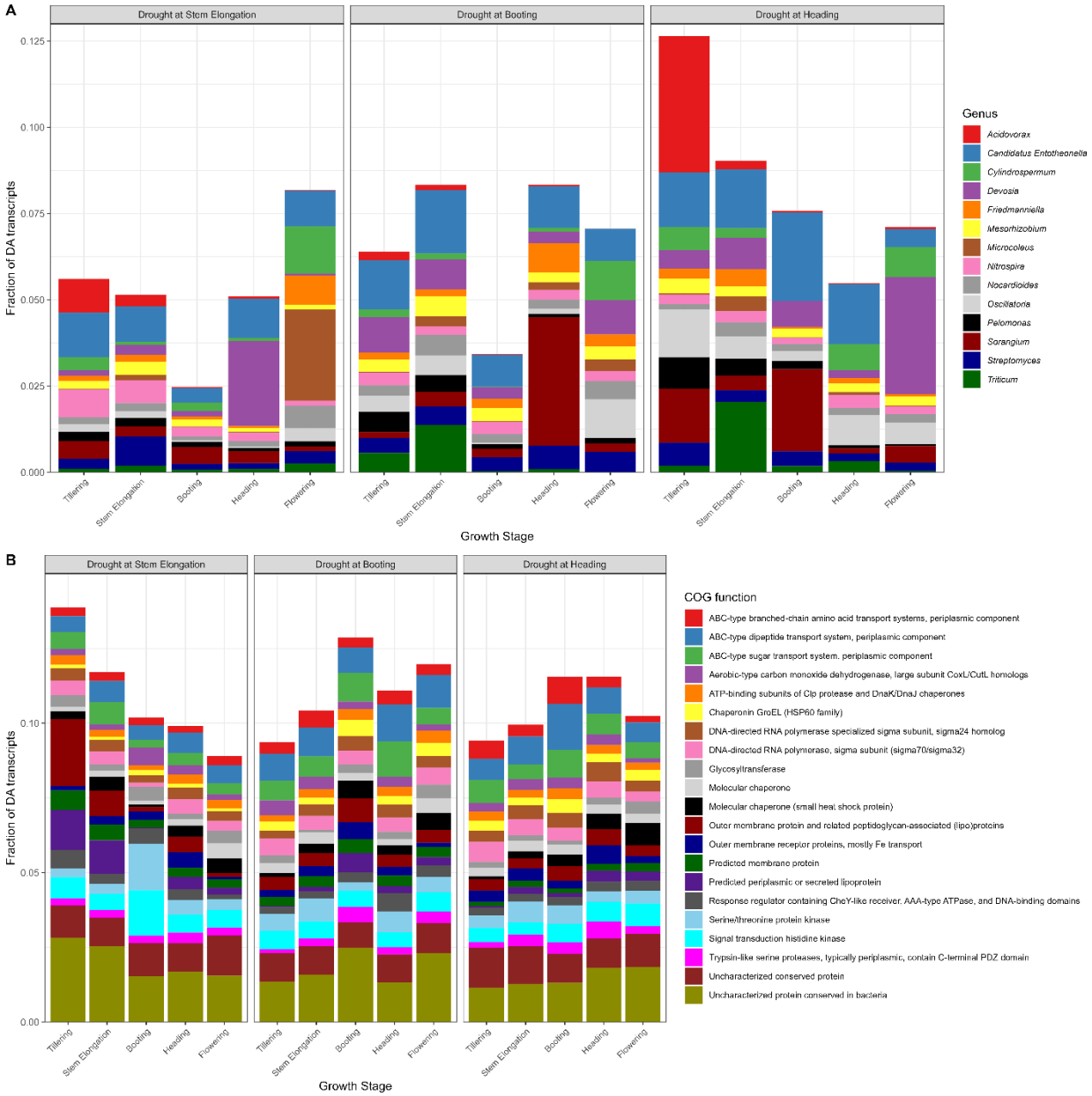

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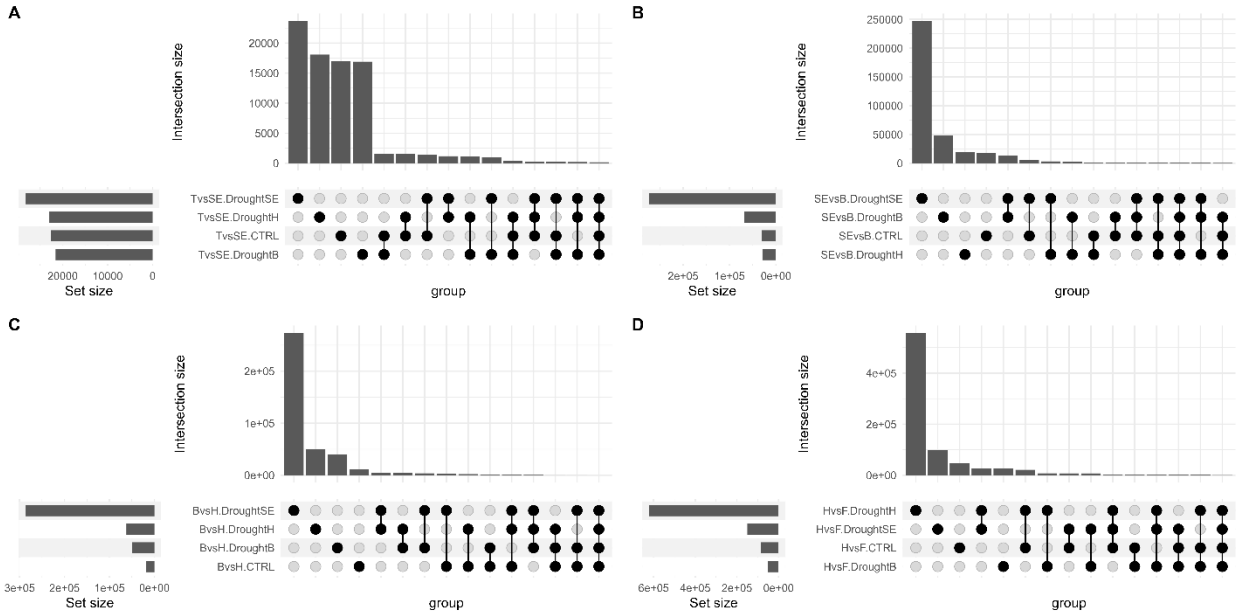

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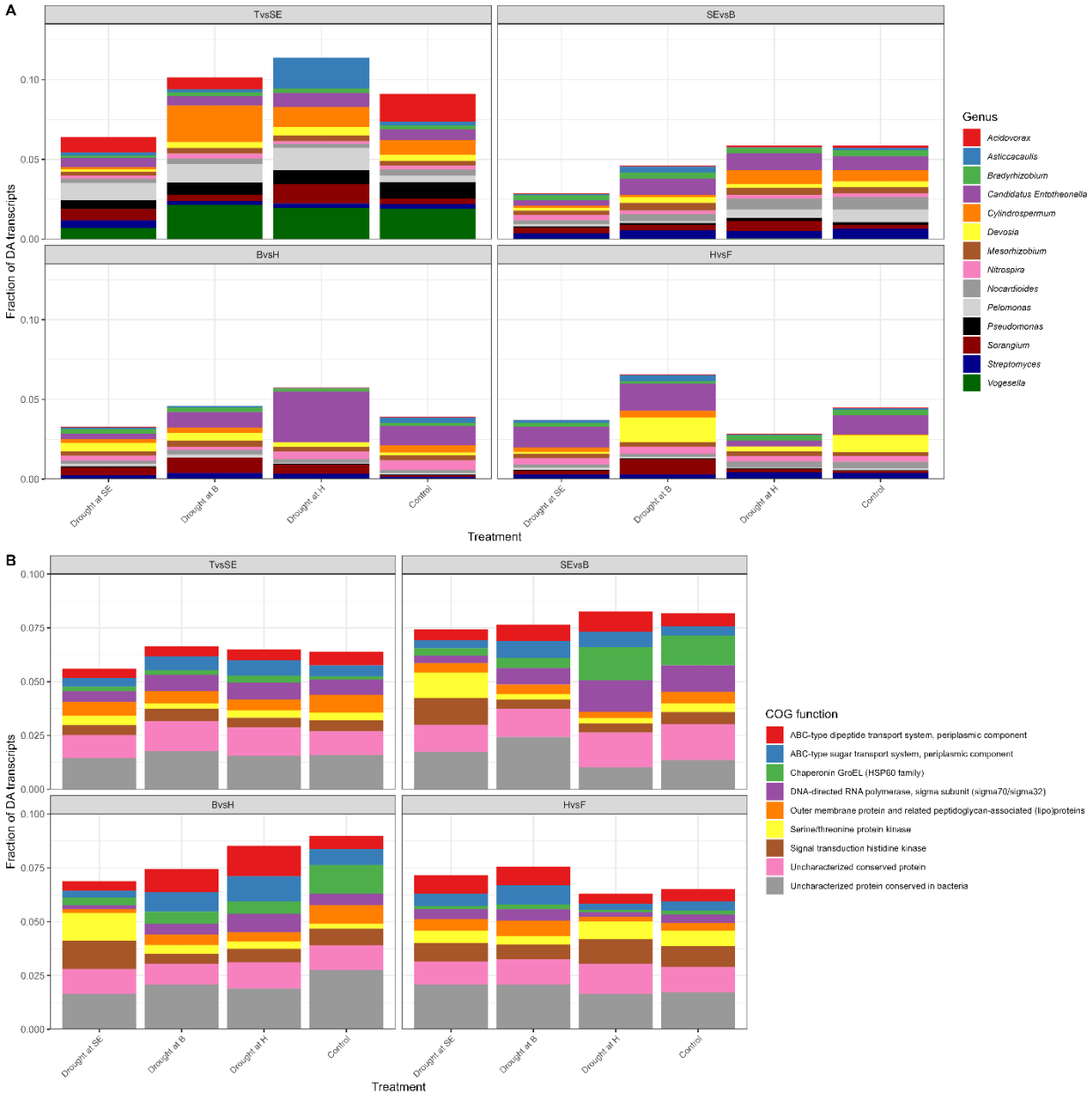
498 **Figure 6.**



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