

1 Full title: High-throughput profiling identifies resource use efficient and abiotic stress
2 tolerant sorghum varieties

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4 Short title: High-throughput phenotyping reveals abiotic stress responses in sorghum

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6 Kira M. Veley¹, Jeffrey C. Berry¹, Sarah J. Fentress¹, Daniel P. Schachtman², Ivan
7 Baxter¹, Rebecca Bart^{1*}

8

9 ¹Donald Danforth Plant Science Center, Saint Louis, MO 63132

10

11 ²Department of Agronomy and Horticulture and Center for Plant Science Innovation,
12 University of Nebraska–Lincoln, Lincoln, NE 68588

13

14 *Corresponding Author (rbart@danforthcenter.org)

15 **ABSTRACT**

16 Energy sorghum (*Sorghum bicolor* (L.) Moench) is a rapidly growing, high-biomass,
17 annual crop prized for abiotic stress tolerance. Measuring genotype-by-environment (G
18 x E) interactions remains a progress bottleneck. High throughput phenotyping within
19 controlled environments has been proposed as a potential solution. Early, measureable
20 indicators of desirable traits that translate to the field would increase the speed of crop
21 improvement efforts. Here we identify shape, color and ionomic indicators of abiotic
22 stress for genetically diverse sorghum varieties. We subjected a panel of 30 sorghum
23 genotypes to either nitrogen deprivation or drought stress and measured responses
24 within an automated phenotyping facility, followed by ionomic profiling. Images of
25 growing plants were collected every day for three weeks, and key metrics are reported.
26 Responses to stress were quantified using differences in shape (16 measureable
27 outputs), color (hue and intensity) and ionome (18 elements). We found shape
28 characteristics to be reliable indicators of performance under both stress conditions
29 tested. In contrast, color was a defining indicator of nitrogen starvation but not drought
30 stress. Through this analysis we were able to measure the speed at which specific
31 genotypes respond to stress and identify individual genotypes that perform most
32 favorably under these stress conditions. These data are made available through an
33 open access, user-friendly, web-based interface. Ionomic profiling was conducted as an
34 independent, low cost and high throughput option for characterizing G x E. The effect of
35 genotype on the ionome was consistent between the two experiments confirming the
36 robustness of the high throughput platform. In addition, multiple individual elements
37 were identified as quantitative outputs of abiotic stress. While the important challenge of
38 translation between controlled environment- and field-based experiments remains, the
39 multiple revealed quantitative outputs from different abiotic stress conditions are
40 genetically encoded. Consequently, the genetic explanations for these responses can
41 now be elucidated using classical and molecular genetics. We propose this work as a
42 time efficient method of dissecting the genetic mechanisms used by sorghum to
43 respond to abiotic stress. In summary, this work provides a mechanism to overlay high
44 throughput phenotyping with field studies to accelerate crop improvement.

45

46 **AUTHOR SUMMARY**

47 Sorghum is important for food security in developing countries and has potential as a
48 high yielding biofuel crop. In either setting, the plant is likely to experience resource
49 limited growing conditions. ‘Resource use efficiency’ and ‘abiotic stress tolerance’ are
50 distinct biologically important phenotypes. The former refers to the ability of a crop to
51 translate a provided resource, such as fertilizer or water, into yield. The latter refers to
52 the ability of a crop to survive within resource limited environments. Here we describe
53 and apply high-throughput phenotyping methods and element profiling to sorghum
54 under abiotic stress conditions. We quantify abiotic stress responses of genetically
55 diverse sorghum accessions based on color fluctuations, biomass accumulation, growth
56 rate over time and elemental profile. Through this analysis we report ‘resource use
57 efficient’ and ‘abiotic stress tolerant’ sorghum.

58 **INTRODUCTION**

59 The selection of efficient, stress-tolerant plants is essential for tackling the
60 challenges of food security and climate change, particularly in hot, semiarid regions that
61 are vulnerable to economic and environmental pressures (1–4). Many crop species,
62 having undergone both natural and human selection, harbor abundant, untapped
63 genetic diversity. This genetic diversity will be a valuable resource for selecting and
64 breeding crops to maximize yield under adverse environmental conditions (5). Sorghum
65 (*Sorghum bicolor* (L.) Moench) originated in northern Africa and was domesticated
66 8,000 – 10,000 years ago. Thousands of genotypes displaying a wide range of
67 phenotypes have been collected and described (6–8). *Sorghum bicolor*, the primary
68 species in cultivation today, has many desirable qualities including the ability to thrive in
69 arid soils with minimal inputs, and many end-uses (9,10). For example, grain varieties
70 are typically used for food and animal feed production, sweet sorghum genotypes
71 accumulate non-structural, soluble sugar for use as syrup or fuel production, and
72 bioenergy sorghum produces large quantities of structural, lignocellulosic biomass that
73 may be valuable for fuel production (11,12). Sorghum genotypes can be differentiated
74 and categorized by type according to these end-uses.

75 Rising interest in sorghum over the last forty years has led to efforts to preserve
76 and curate its diversity. To maximize utility, these germplasm collections must now be
77 characterized for performance across diverse environments (13–15). Deficits in our
78 understanding of genotype-by-environment interactions ($G \times E = P$, where $G =$
79 genotype, $E =$ environment and $P =$ phenotype) are limiting current breeding efforts
80 (16). Controlled-environment studies are quantitatively robust but are often viewed with
81 skepticism regarding their translatability to field settings. Further, they can often
82 accommodate only a limited number of genotypes at a time. In contrast, field level
83 studies allow for large numbers of genotypes to be evaluated simultaneously. However,
84 these studies provide limited resolution to resolve the effect of environment on
85 phenotype and often require multi-year replication. This conundrum has motivated
86 enthusiasm for both controlled environment and field level high throughput phenotyping
87 platforms. However, the use of large-scale phenotyping and statistical modeling to
88 predict field-based outcomes is challenging (17–19).

89 Here, we sought to define a set of measureable, environmentally-dependent
90 phenotypic outputs to aid crop improvement. We utilize automated phenotyping
91 techniques under controlled-environmental conditions to characterize $G \times E$ interactions
92 on a diverse panel of sorghum genotypes in response to abiotic stress. We describe
93 and quantify statistically robust differences among the genotypes to nutrient-poor
94 conditions and drought stress. Using image analysis to characterize leaf color and
95 biomass over time in conjunction with ionomics, we report measureable, genetically-
96 encoded, phenotypic traits that are responsive to abiotic stress. This work provides a
97 foundation for understanding the range of sorghum early-responses to abiotic stress
98 and defines biologically important characteristics that can subsequently be investigated
99 within field settings.

100

101 **RESULTS**

102 Phenotypic effects of abiotic stress on a sorghum diversity panel

103 Nitrogen and water availability are two of the most important environmental
104 factors constraining plant productivity (20,21). For this study, sorghum was chosen for
105 its genetic diversity and wide range of abilities to thrive under semi-arid, nutrient-limited

106 conditions. In order to test the role that genotype plays in response to environmental
107 stress, a panel of 30 sorghum lines was assembled (Table S1). This panel includes
108 sorghum accessions from all five cultivated races (bicolor, caudatum, durra, guinea and
109 kafir), representing a variety of geographic origins and morphologies (22,23). The
110 genotypes also display a range of photoperiod sensitivities and are categorized into
111 three general production types: grain, sweet, and bioenergy. Figure 1 illustrates the
112 overall experimental design to test the phenotypic effects of nitrogen deprivation and
113 drought stress with two independent, three-week-long experiments using high-
114 throughput phenotyping. For the nitrogen deprivation experiment, we chose to analyze
115 the effect of the source (i.e. ammonium vs. nitrate) and quantity of nitrogen on sorghum
116 development (Figure 1A, methods). Plants were watered daily with the indicated
117 solution. For the drought stress experiment, we tested the effect of mild water
118 deprivation and recovery from extreme drought (Figure 1B). Fertilizer nutrient
119 concentrations were similar to what was included in the 50%/10% ($\text{NH}_4^+/\text{NO}_3^-$) nitrogen
120 treatment, compensating for variable water volumes (see methods). Thus, the drought
121 70% and the nitrogen 50%/10% treatment groups are the most similar across the two
122 experiments.

123
124 **Figure 1. Experimental Overview.** A, B) Watering regime used for nitrogen deprivation
125 (A) and drought stress (B). The x-axis shows the age of the plants throughout the
126 experiment and the y-axis indicates the estimated volume of water plus nutrients (ml),
127 calculated based on the weight change of the pot before and after watering. Each dot
128 represents the average amount of water delivered each day with vertical lines indicating
129 error (99% confidence interval). Watering regime was increased due to plant age
130 (shades of blue). The experimental treatments are listed above the plots. A) Volume of
131 water and source of nitrogen is indicated and was scaled based on the 100% treatment
132 group. Watering was kept constant between treatment groups B) Amount of water
133 delivered was scaled based on the 100% treatment group (1 mM ammonium / 14.5 mM
134 nitrate). Arrow indicates the end of extreme drought treatment and beginning of
135 recovery. Nutrients were constant between treatment groups. C) Image analysis. From
136 left to right: Example original RGB image taken from phenotyping system, plant isolation

137 mask generating using PlantCV, two examples of attributes analyzed (area and color).
138 Scale bar = 15 cm.

139
140 All plants were photographed daily and images were processed using the open
141 source PlantCV analysis software package ((24), <http://plantcv.danforthcenter.org>).
142 Within each RGB image, the plant area was defined and isolated, allowing phenotypic
143 attributes to be analyzed (Figure 1C). In total, 18 different shape characteristics were
144 quantified (Figure S1). Principal component analysis (PCA) of all the quantified
145 attributes revealed that shape characteristics could be used to separate treatments for
146 both the nitrogen deprivation and the drought stress experiments (Figure 2A, B). Our
147 results indicated that “area” was the plant shape feature that displayed the largest
148 treatment affect (Figure 2A, B). Area measurements from plant images and biomass
149 measurements have been shown to be correlated for a number of plant species,
150 including sorghum (24,25). We also measured color attributes over time for the plants
151 within both experiments. In contrast to shape, color attributes were only strongly altered
152 by the lowest nitrogen treatment (Figure 2C). The effect of low nitrogen on plant color is
153 well established and RGB image-based methods have been described to estimate
154 chlorophyll content of leaves (26–31). Consequently, we focus on area and color to
155 investigate genotypic differences in response to nitrogen deprivation and drought stress
156 over time, though other shape attributes may prove to be useful for future analysis. To
157 view the effect that our experimental treatments had on the measured shape
158 characteristics and color for each individual genotype, an interactive version of the
159 generated plant growth curves over time is available here:
160 (http://shiny.bioinformatics.danforthcenter.org/Bart_Lab_Sorghum).

161
162 **Figure 2. Determining plant attributes affected by experimental treatments.** A) Left:
163 Principle Component Analysis (PCA) plots of shape attributes for plants subjected to
164 nitrogen deprivation (orange, green, purple) at the beginning and end of the experiment
165 (plant age 8 days and 26 days). The shape attributes included in the PCA are as
166 follows: area, hull area, solidity, perimeter, width, height, longest axis, center of mass X-
167 axis, center of mass Y-axis, hull vertices, ellipse center x-axis, ellipse center Y-axis,

168 ellipse major axis, ellipse minor axis, ellipse angle and ellipse eccentricity. Right: Bar
169 graph indicating measurability of shape attributes, showing the proportion of variance
170 explained by treatment (i. e. treatment effect, y-axis) B) Similar to A, but for plants
171 subjected to drought stress (red, blue, yellow). C) PCA plots showing analysis of color
172 values within the mask for plants subjected to nitrogen deprivation (left) and drought
173 stress (right) toward the beginning and the end of each experiment. All 360 degrees of
174 the color wheel were included, binned every 2 degrees.

175

176 Nitrogen source and availability affect growth over time in a genotype-specific manner

177 Many factors contribute to the ability of plants to utilize nutrients and presumably,
178 much of this is genetically explained. Correspondingly, genotype was a highly significant
179 variable (p -value = 0.003 when measuring area) within the nitrogen deprivation analysis.
180 To investigate how much nitrogen use efficiency is explained by major genotypic
181 groupings, we calculated the contribution of type, photoperiod, or race on treatment
182 affect. Photoperiod was the only other category that significantly contributed to biomass
183 outcomes (Figure 3A, B). To identify sorghum varieties tolerant to growth in nutrient
184 limited conditions, we considered end biomass within the most severe nitrogen
185 deprivation treatment group for all genotypes (Figure 3C). China 17 and San Chi San
186 are considered nitrogen-use-efficient genotypes, while BTx623 and CK60B are thought
187 to be less efficient (32,33). We found China 17 and San Chi San to be better than the
188 average of all other genotypes at acquiring biomass under low nitrogen conditions.
189 Similarly, CK60B was slightly smaller than average. However, BTx623 was one of the
190 higher biomass varieties in our experiment indicating limitations associated with this
191 definition of nitrogen use efficiency. Growth chamber, greenhouse and field experiments
192 are each advantageous and at the same time imperfect for distinct reasons. With this in
193 mind, next we aimed to more fully capitalize on the specific advantage of expanded
194 temporal resolution available from high throughput phenotyping platforms. For these
195 experiments we considered average growth rate across the experiment (Figure 3D).
196 Overall, end biomass measurements correlated well with overall growth rates. For
197 example, by both measures, Della displayed particularly weak growth characteristics
198 under low nitrogen conditions while BTx623 performed well. However, the correlation

199 was imperfect. San Chi San displayed the largest end biomass but was statistically
200 average in terms of growth rate across the experiment. Discrepancies between end-
201 biomass and growth rate (e.g. large plants with average or low observed growth rates)
202 may indicate differences in germination rates (e.g. being larger at the beginning of the
203 phenotyping experiment).

204

205 **Figure 3. Growth response of genotypes to nitrogen deprivation.** A) Tables
206 showing results of ANOVA indicating significance of experimental variation explained by
207 either genotype, type, photoperiod or race as found by Wald's Chi-Square tests with
208 their associated degrees of freedom (DF). Significant p -value < 0.1, bold. All three
209 nitrogen treatments are included in the calculations. B) Line graphs representing
210 changes in plant area (y-axis) with age (x-axis) for all 30 sorghum genotypes examined.
211 The experimental treatments are listed above the plots and genotypes have been
212 separated by photoperiod sensitivity (legend, right). C and D) Graphs showing average
213 end biomass (C, box plot (area), * q -values < 0.01) with outliers (colored dots), and
214 growth rate (D, average change in area per day, days 10-22) for the 10/10 treatment
215 group. The dotted lines indicate the treatment group average. Genotypes that displayed
216 greater than average (blue) or less than average (magenta) growth are indicated. Error
217 bars: 95% confidence intervals for both graphs.

218

219 To further explore nitrogen use efficiency, we factored timing of growth response
220 into our analysis. For each day, we analyzed biomass for each genotype within the
221 100% control group (100 NH_4^+ /100 NO_3^-) and compared that to the biomass within the
222 10% treatment group (10 NH_4^+ /10 NO_3^-). Comparing these two populations allowed us
223 to determine when, during the course of our experiment, those figures became
224 significantly different (Figure 4A). This analysis separated the genotypes into two broad
225 categories: "early" responding accessions and "late" responding accessions. Early- and
226 late-responding lines were not found to be significantly different in terms of size before
227 treatment administration (Figure 4B, top panel). Therefore, we hypothesized that either
228 1) lines would be late-responding because they were proficient at using any level of
229 available nitrogen or 2) because they grew slowly regardless of quantity of nitrogen

230 supplied. We found that the early-responding lines were larger, on average, than the
231 late-responding lines within the 100% treatment group (Figure 4B, bottom panel)
232 suggesting that these lines are more competent at using available nitrogen. A subset of
233 these genotypes are displayed in Figure 4C to illustrate our observations. The genotype
234 Atlas is an example of a very early responding line, and it was one of the largest plants
235 in the 100% treatment group, but also one of the worst-performing lines in the 10%
236 treatment group (Figures 3C, 3D, 4C). In contrast, China 17 performed relatively well
237 under nitrogen-limited conditions (10/10), but when nitrogen was abundant (100/100)
238 the biomass accumulation was poor (Figure 3C, 3D, 4C). Taken together, these results
239 point toward a potential inverse correlation between nitrogen use efficiency (defined as
240 the ability to translate large quantities of nitrogen into biomass) and nitrogen deprivation
241 tolerance (defined as ability to relatively thrive in low nitrogen conditions).

242

243 **Figure 4. Timing of nitrogen deprivation response: size changes in late and early**
244 **responding genotypes to nitrogen deprivation.** A) Statistical analysis of differences
245 in area over time (bottom, plant age) for the 30 sorghum genotypes analyzed. q -values
246 for the heat map are indicated in blue, with darkest coloring representing most
247 significance. The Canberra distance-based cluster dendrogram (right) was generated
248 from calculated q -values. B) Box plots showing average biomass (area) with outliers
249 (colored dots) for late- (left) and early- (right) responding lines from panel A at the
250 beginning (day 8, top) and end (day 26, bottom) of the experiment. * p -value $< 5 \times 10^{-6}$.
251 C) Scatter plots representing plant area (y-axis) by treatment (x-axis) at the beginning
252 (day 8), middle (day 19), and end (day 26) of the experiment for chosen late responding
253 (left) and early responding (right) genotypes (key, right). Each dot represents an
254 individual plant on a day and dotted lines connect genotypic averages. D and E) Color
255 analysis (late responders, left; early responders, right) at day 14. Grey areas indicate
256 standard error.

257

258 In addition to simply varying the amount of nitrogen available, we also tested
259 whether any lines harbor a preference for nitrogen source. Nitrogen is typically available
260 in two ionic forms within the soil, ammonium and nitrate, both of which are actively

261 taken up into plant roots by transporters located in the plasma membrane (34,35).
262 Expression of these gene products and others have been shown to be responsive to
263 nitrogen availability in sorghum (36). For example, San Chi San and China 17 are
264 known to have higher levels of expression of nitrate transporters when compared to
265 nitrogen-use-inefficient lines (33). Notably, San Chi San showed no change in average
266 biomass when the ammonium levels were decreased from 50% to 10% while Atlas
267 displayed the opposite response (Figure 4C). Among the 30 tested genotypes, 16
268 displayed little difference between the 50/10 and 10/10 groups in terms of biomass
269 toward the end of the experiment (Figure S2). This highlights the importance of
270 considering both quantity and source when investigating nitrogen use efficiency and
271 points to yet another complexity surrounding our understanding of nitrogen use
272 efficiency in plants.

273 In addition to affecting shape attributes, nitrogen starvation generally results in
274 reduced chlorophyll content and increased chlorophyll catabolism. Other groups have
275 used image analysis to estimate chlorophyll content and nitrogen use in rice (28). The
276 RGB images contain plant hue channel information, and this was found to be a
277 separable characteristic within our treatment groups (Figure 2C). We assessed color-
278 based responses to nitrogen deprivation in the early- and late-responding genotypes as
279 defined in Figure 4A. To facilitate this analysis we averaged the histograms of the
280 chosen individuals within the early and late categories in each treatment group. We
281 found that the histograms of the plant images contained two primary peaks: yellow and
282 green. For both early- and late-responding lines, the yellow peak was larger and the
283 green peak smaller for the plants in the 10% treatment group as compared to the 100%
284 treatment group (Figure 5). Early responding lines within the 100% treatment group
285 displayed the largest green-channel values. Late responding lines grown under
286 nitrogen-limiting conditions displayed the largest yellow channel values (Figure 5, top
287 row). In order to assign color-based treatment effects, we subtracted the 10%
288 histograms from the 100% histograms and plotted this difference (Figure 5, bottom row).
289 This revealed that although the late responding lines were more yellow, the magnitude
290 difference from the treatment was similar for early and late lines in the yellow channel
291 (Figures 5, S3). In contrast, the early-responding lines tended to have a larger green

292 channel difference between the 10% and the 100% treatment groups (Figure 5, S3),
293 with early-responding lines showing a larger difference in the green channel. This
294 analysis suggests that for nutrient-based studies known to affect chlorophyll
295 accumulation, specifically nitrogen, color-based image analysis is consistent with and
296 complimentary to the more-established biomass measures of fitness and performance.

297

298 **Figure 5. Color changes in late and early responding genotypes to nitrogen**
299 **deprivation.** Top row: Average histograms illustrating percentage of identified plant
300 image mask (y-axis) represented by a particular hue channel (x-axis). Presented is the
301 average of the chosen early and late responding lines. Second row: Difference of
302 histograms from top row. Grey areas indicate standard error.

303

304 Sorghum genotypes show a spectrum of drought tolerance over time

305 Sorghum is valued as a drought-tolerant C₄ grass (7). Just as nitrogen
306 deprivation can manifest in many different ways, so can drought stress. To define
307 relative drought tolerance among diverse sorghum varieties, we designed an
308 experiment that would simultaneously test genotypic responses to mild drought as well
309 as recovery from extreme water deprivation (Figure 1B). First we visualized the effects
310 of mild drought on growth for the diversity panel of 30 genotypes (Figure 6A). In contrast
311 to our analysis of nitrogen deprivation (Figure 3A), only genotype was a significantly
312 contributing factor for biomass accumulation by drought treatment over time. Here again
313 San Chi San accumulated the most biomass at the end of the experiment within the
314 70% treatment group (Figure 6B, C). However, growth rate measurements indicate that
315 San Chi San was average to below average in terms of accumulation per day (Figure
316 6D). Thus, the large biomass measurement is likely the product of quick germination
317 and/or growth during the first few days after germination. In contrast, accession
318 PI_297155 was one of the lowest biomass-accumulating lines but showed a strikingly
319 fast growth rate (Figure 6D). This particular genotype was very small at the beginning
320 and displayed an accelerating growth rate toward the latter-half of the experimental
321 timeframe (Figure 6B, Supplemental Shiny App

322 (shiny.bioinformatics.danforthcenter.org/Bart_Lab_Sorghum). These observations
323 highlight the importance of a multi-pronged approach to describing plant phenotypes.

324

325 **Figure 6. Growth response of genotypes to drought stress.** A) Tables showing
326 results of ANOVA indicating significance of experimental variation explained by either
327 genotype, type, photoperiod or race as found by Wald's Chi-Square tests with their
328 associated degrees of freedom (DF). Significant p -value < 0.1 , bold. Only 100 and 70
329 drought treatments are included. B) Line graphs representing changes in plant size (log
330 area, y-axis) with age (x-axis) for all 30 sorghum genotypes examined (key, right). The
331 experimental treatments are listed above the plots. C) Graphs showing average end
332 biomass (top, box plot (log area), * q -values < 0.01) with outliers (colored dots), and
333 growth rate (bottom, average change in log area per day, days 10-22) for the 70%
334 treatment group. The dotted lines indicate the treatment group average. Genotypes that
335 displayed greater than average (blue) or less than average (magenta) growth are
336 indicated. Error bars: 95% confidence intervals for both graphs.

337

338 To assess timing of response we used the biomass information for each
339 genotype per day to look for when the 100% and 70% treatment groups became
340 significantly different (Figure 7A). Using this method we found that the 30 genotypes
341 separated into two distinct groups (early-responding and late-responding), though the q -
342 value defining the difference in response timing between these groups was less
343 significant than what was observed for the nitrogen deprivation experiment. This may
344 indicate that the 70% watering regime was a relatively mild treatment as compared to
345 the 50/10 nitrogen treatment, in terms of biological response. Contrary to what was
346 seen for the nitrogen deprivation experiment, the average sizes of the early-and late-
347 responding groups at the beginning of the experiment were measurably different (Figure
348 7B, top panel, T-test p -value = 0.00167). However, bootstrapping analysis failed to
349 support a significant difference between the groups at day 8 (p -value 0.427). Therefore,
350 we conclude that this difference in mean biomass at day 8 is unlikely to be biologically
351 meaningful. At the end of the experiment, the early-responding lines were significantly
352 larger than the late-responding lines, on average, in the 100% treatment group (Figure

353 7B, bottom panel). To illustrate our observations, we chose representative early- and
354 late-responding lines to display in Figure 7C. Within the late-responding lines, Della and
355 PI_655972 performed well in the 100% treatment group. Notably, these lines were
356 unremarkable among all 30 sorghum varieties when grown in the 70% treatment group
357 (Figure 6C, D). This observation suggests a possible tradeoff between water use
358 efficiency displayed in water sufficient conditions and tolerance to water limited
359 conditions. Finally, in contrast to what was seen for nitrogen deprivation, where color
360 was an informative metric by which to analyze plant response to treatment, color was
361 not a defining factor for any of the treatment groups within the drought experiment
362 (Figures 2B, 7D).

363
364 **Figure 7. Timing of response to mild drought stress: size and color changes in**
365 **late and early responding genotypes to drought.** A) Statistical analysis of differences
366 in log area at the population level over time (bottom, plant age) for the 30 sorghum
367 genotypes analyzed. q -values for the heat map are indicated in blue, with darkest
368 coloring representing most significance. The Canberra distance-based cluster
369 dendrogram (right) was generated from calculated q -values. B) Box plots showing
370 average biomass (area) with outliers (colored dots) for late- (left) and early- (right)
371 responding lines from panel A at the beginning (day 8, top) and end (day 26, bottom) of
372 the experiment. * p -value $< 5 \times 10^{-6}$. C) Scatter plots representing plant area (y-axis) by
373 treatment (x-axis) at the beginning (day 8), middle (day 19), and end (day 26) of the
374 experiment for chosen late responding (left) and early responding (right) genotypes
375 (key, right). Each dot represents an individual plant on a day and dotted lines connect
376 genotypic averages. D) Difference of average histograms from chosen late-responding
377 and early-responding genotypes (14-day-old plants). Shown is the difference in the
378 percentage of identified plant image mask (y-axis) represented by a particular hue
379 channel (x-axis) between the 70% and 100% treatment groups. Grey areas indicate
380 standard error.

381
382 In addition to understanding sorghum response to mild drought, we aimed to
383 assess performance during, and recovery from, extreme drought. Within the ‘recovery’

384 treatment plants received decreasing amounts of water for the first half of the
385 experiment followed by a recovery period equivalent to the 70% watering regime (Figure
386 1B, 6B). With this experiment we tested the hypothesis that some genotypes may be
387 able to endure temporary, extreme drought and later compensate with increased growth
388 rates when conditions improve. During the recovery period, the majority of sorghum
389 lines did not grow at a statistically different rate than those that had been maintained in
390 the 70% water treatment group (Figure 8). However, several genotypes (highlighted in
391 blue) grew significantly faster during recovery from extreme drought than the constant
392 70% treatment. This observation indicates that some sorghum varieties have evolved
393 mechanisms to survive extreme drought conditions and are further equipped to rapidly
394 take advantage of available resources when conditions improve.

395

396 **Figure 8. Rates of recovery from extreme drought treatment.** Shown are the
397 differences in growth rates (change in log area, y-axis) for all 30 sorghum genotypes
398 analyzed (x-axis) between the 70% watering regime and the recovery samples following
399 extreme drought treatment (days 21-24, figure 1B). The dotted line indicates the
400 average growth rate difference calculated between the two treatment populations, while
401 the average growth rate difference for each genotype is plotted (dots). Genotypes that
402 displayed significantly faster (blue) or slower (magenta) growth rates following extreme
403 drought are indicated, in addition to those that were not significantly affected (black).
404 Error bars: 95% confidence intervals.

405

406

407 Ionic profiling as a heritable, independent, measurable readout of abiotic stress
408 response.

409 In addition to the image-based analysis used above to reveal measurable
410 biomass- and color-based outcomes in response to abiotic stress, we also sought
411 ionic signatures of stress response in sorghum at the end of the experiments
412 (Figures S4, S5). This analysis had two objectives: 1) to validate the robustness and
413 reproducibility of our experimental design and 2) to further define the G x E effect on
414 sorghum subjected to either nitrogen deprivation or drought conditions (Figures 9, 10).

415 We were unable to include the recovery drought samples due to the small size of many
416 of the genotypes at the end of the experiment. It has been established that both genetic
417 and environmental factors and their interactions play a significant role in determining
418 the plant ionome (37–42). Each element was modeled as a function of both genotype
419 and treatment, and genotype was a significant factor for most elements in both nitrogen
420 deprivation and drought stress experiments (Figures 9A, 10A).

421
422 **Figure 9. Ionomic profiling of genotypes at the end of nitrogen deprivation**
423 **treatments.** A) Top Panel: A total variance partition model is created for every element
424 and being shown is the percent variance explained by each partition of the model
425 (yellow: genotype, green: treatment, purple: interaction between genotype and
426 treatment, grey: unexplained variance). Bottom Panel: Significance of contribution from
427 each partition is assessed using Wald's Chi-Square statistics. Significant cells (q-value
428 < 0.05) are colored with levels of blue and non-significant cells are light grey. B) Top
429 panel: Principal components are calculated over all elements, applied to the data and
430 are color coded for treatment (left). 95% confidence ellipses are calculated for each of
431 the treatment groups and the dots indicate the center of mass (right). The percent
432 variance explained by each component is indicated in parenthesis. Bottom panel:
433 Loadings for each element from the first two principal components are shown on the y-
434 axis and are color filled based on the direction and strength of the contribution. Positive
435 direction is colored blue and negative direction is colored red. For a given element, the
436 color for PC1 and PC2 are related by the unit circle and saturation of the color is equal
437 to the length of the projection into each of the two directions. C) Heat map by genotype
438 testing the difference between the high (100/100) and low (10/10) treatment groups for
439 every element using ANOVA. Significant cells (q-value < 0.05) are colored with levels of
440 blue while non-significant cells are light grey.

441
442 **Figure 10. Ionomic profiling of genotypes after mild drought stress.** A) A total
443 variance partition model is created for every element and being shown is the percent
444 variance explained by each partition of the model (yellow: genotype, green: treatment,
445 purple: interaction between genotype and treatment, grey: unexplained variance). B)

446 Top panel: Principal components are calculated over all elements, applied to the data
447 and are color coded for treatment (left). 95% confidence ellipses are calculated for each
448 of the treatment groups and the dots indicate the center of mass (right). The percent
449 variance explained by each component is indicated in parenthesis. Bottom panel:
450 Loadings for each element from the first two principal components are shown on the y-
451 axis and are color filled based on the direction and strength of the contribution. Positive
452 direction is colored blue and negative direction is colored red. For a given element, the
453 color for PC1 and PC2 are related by the unit circle and saturation of the color is equal
454 to the length of the projection into each of the two directions. C) Scatter plot showing
455 percent contribution of genotype effect for each element (key: right) in both experiments
456 (x-axis: nitrogen y-axis: drought). Solid black line represents linear best fit. R^2 shown in
457 upper-left corner.

458

459

460 Nitrogen deprivation had a measurable effect on every element (Figures 9A, B)
461 while a treatment effect could not be resolved between 100% and 70% water availability
462 for most elements (Figure 10A). PCA of the elements revealed more separation of the
463 nitrogen deprivation treatments than the drought treatments, with the two lower nitrogen
464 treatment groups separating from the high treatment group (Figures 9B, 10B). Both
465 micro (Se, Rb, Mo, Cd) and macro (K, P) nutrients contributed strongly to the PCs
466 separating the 100/100 treatment group away from the other two treatments within the
467 PCA (Figure 9B, bottom panel). Using the change in each element between the 100/100
468 and 10/10 as the response variable we clustered the accessions (Figures 9C). These
469 groupings did not correlate with the observed biomass differences discussed above
470 (Figure 4A, 7A, S6) indicating that the ionic profile reflects different physiological
471 properties. Together, these observations provide a baseline for further investigation into
472 the role of the ionome in abiotic stress and the effect of abiotic stress on the ionome.

473

474 **DISCUSSION**

475 Crops adapted to nutrient-poor conditions will be an invaluable resource for
476 realizing the goal of dedicated bioenergy crops grown without irrigation and limited

477 fertilizer on marginal lands. Robust, quantitative phenotypes are a prerequisite for
478 genetic investigations and these can be gathered using high throughput phenotyping
479 and image analysis. In order to test for and quantify G x E interactions we designed a
480 strategy that utilized tightly controlled environmental conditions in a high-throughput
481 manner in the genetically diverse, stress-resistant crop, sorghum. Responding to stress
482 is costly to plants. As such, there may be inherent trade-offs associated with ability to
483 thrive under multiple abiotic stress conditions. Overall our results indicate that no single
484 variety is optimized for both abiotic stresses tested in terms of biomass accumulation.
485 Within this context, we went beyond characterizing biomass as an output of stress
486 tolerance and measured changes in color and elemental profile. This work highlights the
487 fact that the generally observed and measured response to a given abiotic stress may
488 often appear to be the same (i. e. stressed plants produce less biomass), but the
489 biological and chemical signaling within the plant is distinct depending on the stress.
490 These signals and responses are much more difficult to measure, but will be critical for
491 understanding and improving plant resistance to abiotic stress going forward.

492
493 Previous work has shown that plants can utilize different forms of nitrogen, yet
494 preference can be influenced greatly by genotype and the environment (35). Factors
495 such as soil pH, CO₂ levels, temperature and the availability of other nutrients have an
496 impact on nitrogen uptake (43,44). Ammonium causes acidification of the soil, which
497 affects the uptake of other nutrients and likely alters the root microbiome. Additionally,
498 root architecture is affected by nitrogen source and nutrient availability. It has been
499 shown for a number of species, including maize and barley, that ammonium causes a
500 reduction in lateral root branching that can be reversed with the addition of phosphorous
501 (45,46,42,47). In *Arabidopsis*, nitrogen-phosphorous signaling has been studied in detail
502 and the presence of nitrate has been shown to inhibit phosphorous uptake (48,49). We
503 measured the effects of nitrogen deprivation as well as the source of nitrogen
504 (ammonium vs nitrate). Some genotypes were more affected by nitrogen source in
505 terms of end biomass than others, for example the difference between San Chi San and
506 Atlas (Figure 4C). Through ionomic profiling after the nitrogen deprivation experiment,
507 we show that phosphorous was one of the elements with the largest treatment effect

508 (Figures 9). Dry weight-based concentration of phosphorous was higher in the low
509 nitrogen treatment groups, both of which received the same nitrate treatment, compared
510 to the high nitrogen treatment group (Figure S4). This highlights the value of tightly
511 controlled environments for elucidating the possible conservation and nuances of plant
512 growth responses. Taken together, these data are consistent with what other studies
513 that have shown: some genotypes have a preference for nitrogen source and other
514 environmental factors influence that preference.

515

516 Some of the most productive crops in use today are C4 grasses like corn (*Zea*
517 *mays*), sorghum (*Sorghum bicolor*), and sugarcane (primarily *Saccharum officinarum*)
518 (Reviewed in Leakey, 2009). These crops have cellular functions and chemistries that
519 result in high rates of photosynthesis in spite of drought and nutrient-poor conditions.
520 However, within each crop group, significant genetic and phenotypic variety exists. The
521 sorghum diversity panel presented here represents a wide, yet incomplete, range of
522 known sorghum genotypic and phenotypic diversity. Tens of thousands of sorghum
523 accessions are curated and maintained by a number of national and international
524 institutions (22). The largest such institution, the US National Sorghum Collection (GRIN
525 database), provides agronomic characteristic information for 40–60 % of the collection
526 (e. g. growth and morphology characteristics, insect and disease resistance, chemical
527 properties, production quality, photoperiod in temperate climates). Thus, much work is
528 yet to be done to fully characterize and maximize the potential of this hearty, productive
529 crop species.

530

531 Nitrogen and water use efficiency are traditionally defined by the difference in biomass
532 between plants grown in resource sufficient versus resource limited conditions at the
533 end of the growing season. Stated differently, this measure asks the question: How
534 efficient is a plant at translating a provided resource (nitrogen or water) into plant
535 biomass. Equally important is the ability to efficiently use a limited resource. Factors that
536 play into these distinct definitions of resource use efficiency include ability to survive
537 periods of extreme stress and rapid utilization of resources as they become available. In
538 this manuscript, we make progress toward deconstructing the building blocks that make

539 up nitrogen and water use efficiency phenotypes. These analyses reveal diverse
540 quantitative indicators of abiotic stress and genotypic differences in stress mitigation.
541 Having deconstructed these building blocks, we are now in a position to discover the
542 underlying genetic explanations for genotypic variability in resource use efficiency and
543 tolerance to resource limited growth conditions. Further, this work forms a foundation for
544 future research to overlay additional abiotic and biotic stress conditions to achieve a
545 holistic view of sorghum G x E phenotypes. The overall goal of this research is to
546 support such efforts and expedite the process of meaningful crop improvement.

547

548 **MATERIALS AND METHODS**

549 Plant growth conditions

550 Round pots (10 cm diameter) fitted with drainage trays were pre-filled with Profile® Field
551 & Fairway™ calcined clay mixture (Humert International, Earth City, Missouri) the goal
552 being to minimize soil contaminants (microbes, nutrients, etc.) and maximize drainage.
553 Before the beginning of each experiment, the thirty genotypes of *Sorghum bicolor* (L.)
554 Moench (Table S1) were planted, bottom-watered once daily using distilled water
555 (reverse osmosis), then allowed to germinate for 6 days in a Conviron growth chamber
556 (day/night temperature: 32°C/22°C, day/night humidity: 40%/50% (night), day length:
557 16hr, light source: Philips T5 High Output fluorescent bulbs (4100 K (Cool white)) and
558 halogen incandescent bulbs (2900K (Warm white)), light intensity: 400 $\mu\text{mol}/\text{m}^2/\text{s}$). On
559 day 6, plants were barcoded (including genotype identification, water treatment group,
560 and a unique pot identification number), randomized, then loaded onto the Bellwether
561 Phenotyping Platform (Conviron, day/night temperature: 32°C/22°C, day/night humidity:
562 40%/50% (night), day length: 16hr, light source: metal halide and high pressure sodium,
563 light intensity: 400 $\mu\text{mol}/\text{m}^2/\text{s}$) Plants continued to be watered using distilled water by
564 the system for another 2 days, with experimental treatments (described below) and
565 imaging beginning on day 8.

566

567 Nitrogen deprivation treatments:

568 (100% Ammonium/100% Nitrate): 6.5 mM KNO_3 , 4.0 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1.0 mM
569 $\text{NH}_4\text{H}_2\text{PO}_4$, 2.0 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, micronutrients, pH ~4.6

570
571 (50% Ammonium/10% Nitrate): 0.65 mM KNO₃, 4.95 mM KCl, 0.4 mM Ca(NO₃)₂·4H₂O,
572 3.6 mM CaCl₂·2H₂O, 0.5 mM NH₄H₂PO₄, 0.5 mM KH₂PO₄, 2.0 mM MgSO₄·7H₂O, pH
573 ~4.8

574
575 (10% Ammonium/10% Nitrate): 0.65 mM KNO₃, 4.95 mM KCl, 0.4 mM Ca(NO₃)₂·4H₂O,
576 3.6 mM CaCl₂·2H₂O, 0.1 mM NH₄H₂PO₄, 0.9 mM KH₂PO₄, 2.0 mM MgSO₄·7H₂O,
577 micronutrients, pH ~5.0

578
579 The same micronutrients were used for all above treatments: 4.6 μM H₃BO₃, 0.5 μM
580 MnCl₂·4H₂O, 0.2 μM ZnSO₄·7H₂O, 0.1 μM (NH₄)₆Mo₇O₂₄·4H₂O, 0.2 μM MnSO₄·H₂O,
581 71.4 μM Fe-EDTA

582
583 Drought stress treatments: concentrations of macro- and micronutrients adjusted for
584 volume delivered, based on formulation used in 50% Ammonium/10% Nitrate treatment
585 above.

586
587 100% (same as 50% Ammonium/10% Nitrate): 0.65 mM KNO₃, 4.95 mM KCl, 0.4 mM
588 Ca(NO₃)₂·4H₂O, 3.6 mM CaCl₂·2H₂O, 0.5 mM NH₄H₂PO₄, 0.5 mM KH₂PO₄, 2.0 mM
589 MgSO₄·7H₂O, 4.6 μM H₃BO₃, 0.5 μM MnCl₂·4H₂O, 0.2 μM ZnSO₄·7H₂O, 0.1 μM
590 (NH₄)₆Mo₇O₂₄·4H₂O, 0.2 μM MnSO₄·H₂O, 71.4 μM Fe-EDTA, pH ~4.8

591
592 70% (days 8-26) and Recovery (days 19-26): 0.93 mM KNO₃, 7.07 mM KCl, 0.57 mM
593 Ca(NO₃)₂·4H₂O, 5.14 mM CaCl₂·2H₂O, 0.71 mM NH₄H₂PO₄, 0.71 mM KH₂PO₄, 2.86
594 mM MgSO₄·7H₂O, 6.57 μM H₃BO₃, 0.71 μM MnCl₂·4H₂O, 0.29 μM ZnSO₄·7H₂O, 0.14
595 μM (NH₄)₆Mo₇O₂₄·4H₂O, 0.29 μM MnSO₄·H₂O, 102 μM Fe-EDTA, pH ~4.7

596
597 Recovery (days 8-18): 1.3 mM KNO₃, 9.9 mM KCl, 0.8 mM Ca(NO₃)₂·4H₂O, 7.2 mM
598 CaCl₂·2H₂O, 1.0 mM NH₄H₂PO₄, 1.0 mM KH₂PO₄, 4 mM MgSO₄·7H₂O, 9.2 μM H₃BO₃,
599 1.0 μM MnCl₂·4H₂O, 0.4 μM ZnSO₄·7H₂O, 0.2 μM (NH₄)₆Mo₇O₂₄·4H₂O, 0.4 μM
600 MnSO₄·H₂O, 142.8 μM Fe-EDTA, pH ~4.5

601

602 Image Processing

603 Images were analyzed by using an open-source platform named PlantCV. This package
604 primarily contains wrapper functions around the commonly used open-source image
605 analysis software called OpenCV. To get useful information from a given image, the
606 plant must be segmented out of the picture using various mask generation methods to
607 remove the background so all that remains is plant material (see figure 1). A pipeline
608 was developed to complete this task for the side-view and top-view cameras separately
609 and they were simply repeated for every respective image in a high-throughput
610 computation cluster. For this dataset of approximately 90,000 images with the
611 computation split over 40 cores, computation time was roughly four hours. Upon
612 completion, data files are created that contain parameterizations of various shape
613 features and color information from several color-spaces for every image analyzed.

614

615 Outlier Detection and Removal Criteria

616 Outliers were detected and removed by implementing Cook's distance on a linear model
617 (50) that only included the interaction effect of treatment, genotype and time. That is, for
618 each observation, an influence measure is obtained as the difference of the model with
619 and without the observation. After getting a measure for all observations in the dataset,
620 outliers were defined as having an influence greater than four times that of the mean
621 influence and were subsequently removed from the remaining analysis.

622

623 Bootstrap Methods

624 To better understand the importance of the difference between the two responder types,
625 an empirical distribution is created by randomly selecting 10 genotypes to be in one
626 group, placing the remaining 20 to be in the other, and calculating the difference in
627 mean biomass between them. This process is repeated 1000 times and the p-value is
628 calculated as the number of times the absolute value of the difference is greater than
629 the difference of the early and late responders divided by 1000.

630

631 Exploratory PCA

632 Two types of PCA's are generated: one for the shape features and the other for color
633 features. To get an idea of what shapes are important to examine further, all shape
634 parameterizations that are generated from PlantCV are included in the dimensional
635 reduction. Plotting PC1 vs PC2 shows that there is a separation of treatment groups
636 along the PC1 axis in both drought and nitrogen experiments. Examining the
637 eigenvalues and correlations for this axis shows the most important traits to be hull
638 area, area, height, and perimeter and that these four traits are all highly correlated with
639 each other. This observation is true in both experiments and the choice of using area for
640 the primary outcome is two-fold. The first is that it is the trait that explains the most
641 variance along PC1 and the second is that, relative to the other measures, area is the
642 most robust to outlier pixels from image processing.

643 Along with the shape features, color as defined by the hue channel is examined
644 with principal components. The hue channel is returned from PlantCV as the number of
645 pixels in the image mask for a particular degree range of the hue spectrum. The
646 resolution of color is within two degrees meaning that the hue circle, which is
647 understood to be continuous, is broken up in two degree increments resulting in one
648 hundred eighty bins and all of these bins are included in the dimensional reduction.
649 While color does not appear to separate the treatment groups in the drought
650 experiment, examining the eigenvalues of PC1 in the nitrogen experiment reveals that
651 the degrees that correspond to yellow-like and green-like colors are the bins that are
652 influencing the separation of the treatments.

653

654 Trends GLMM-ANOVA

655 Using area as the primary outcome, a general linear mixed model was created to
656 identify significance sources of variance adjusting for all other sources, otherwise known
657 as type III sum of squares. Designating genotype as G, treatment as E, and time as T,
658 there are six fixed effects: G, E, GxE, GxT, ExT, GxExT. The mixed effect is a random
659 slope and intercept of the repeated measures over time. To get a p-value for each
660 source of variation, Wald Chi-Square statistic was implemented and is a leave-one-out
661 model fitting procedure which allows for adjustment of all other sources.

662

663 Each Day GLM

664 Given that the ANOVA showed significance in the three-way interaction, it should be
665 true that there is variance in how long it took for a given genotype to respond. The
666 procedure was to simply loop through each genotype and each day and fit a linear
667 model that only includes the treatment effect. The p-value is obtained from a F-statistic
668 generated from the sum of squares of the treatment source of variation. After getting all
669 the raw p-values, a Benjamini-Hochberg FDR multiple comparisons correction is done
670 to aid in eliminating false positives. After the correction, there exists a small number of
671 p-values that are extremely small and a linear color gradient would be uninformative so
672 a log-transform of the q-values helps create a smoother, more informative, gradient.
673 A hierarchical-agglomerative clustering routine is done on the corrected p-values to bring
674 clusters within the data to light. Each line had an associated vector of p-values and a
675 Canberra distance is calculated for all pairwise vectors which are then grouped by
676 Ward's minimum variance method.

677

678 Color Processing

679 PlantCV returns several color-space histograms for every image that is run through the
680 pipeline (RGB, HSV, LAB, and NIR). Every channel from each color-space is a vector
681 representing values (or bins) from 0 to 255 which are black to full color respectively. All
682 image channel histograms were normalized by dividing each of the bins by the total
683 number of pixels in the image mask ultimately returning the percentage of pixels in the
684 mask that take on the value of that bin. The hue channel is a 360 degree
685 parameterization of the visible light spectrum and contains the number of pixels found at
686 each degree. The colors of most importance are between 0 and 120 degrees which
687 correspond to the gradient of reds to oranges to yellows to greens. Colors beyond this
688 range, like cyan and magenta, have values of all zeros and are not shown. Means and
689 95% confidence intervals as calculated on a per degree basis over the replicates.

690

691 Ionomics Profiling and Analysis

692 The most recent mature leaf was sampled from each plant on day 26 of each
693 experiment, placed in a coin envelope and dried in a 45°C oven for a minimum of 48

694 hours. Large samples were crushed by hand and subsampled to 75mg. Subsamples or
695 whole leaves of smaller samples were weighed into borosilicate glass test tubes and
696 digested in 2.5 mL nitric acid (AR select, Macron) containing 20ppb indium as a sample
697 preparation internal standard. Digestion was carried out by soaking overnight at room
698 temperature and then heating to 95°C for 4hrs. After cooling, samples were diluted to 10
699 mL using ultra-pure water (UPW, Millipore Milli-Q). Samples were diluted an additional
700 5x with UPW containing yttrium as an instrument internal standard using an ESI
701 prepFAST autodilution system (Elemental Scientific). A Perkin Elmer NexION 350D with
702 helium mode enabled for improved removal of spectral interferences was used to
703 measure concentrations of B, Na, Mg, Al, P, S K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se,
704 Rb, Mo, and Cd. Instrument reported concentrations are corrected for the yttrium and
705 indium internal standards and a matrix matched control (pooled leaf digestate) as
706 described (51). The control was run every 10 samples to correct for element-specific
707 instrument drift. Concentrations were converted to parts-per-million (mg analyte/kg
708 sample) by dividing instrument reported concentrations by the sample weight.

709
710 Due to low analyte concentrations, data from samples with less than 20 mg of tissue
711 were removed from further analysis. This left very few samples in the 50% field capacity
712 drought treatment, so this entire treatment was removed from further elemental
713 analysis. Outliers were identified by analyzing the variance of the replicate
714 measurements for each line in a treatment group and excluding a measurement from
715 further analysis if the median absolute deviation (MAD) was greater than 6.2 (52).

716
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723
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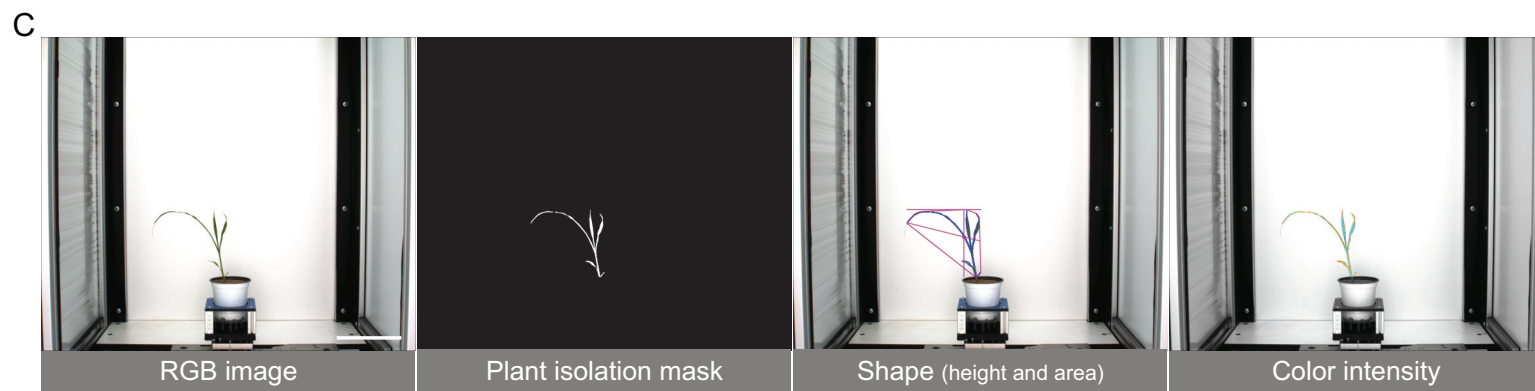
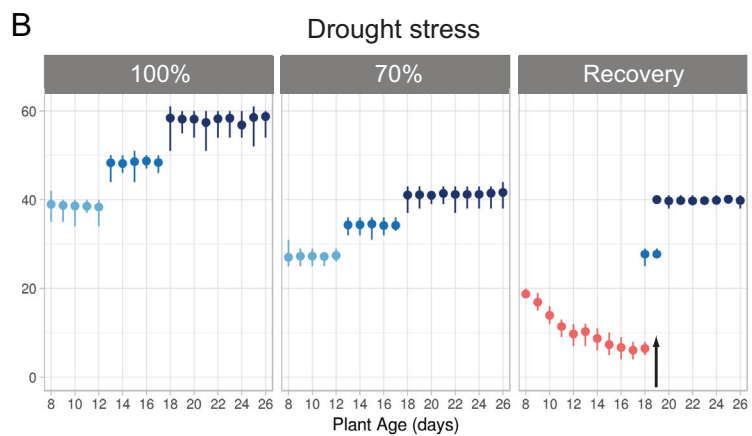
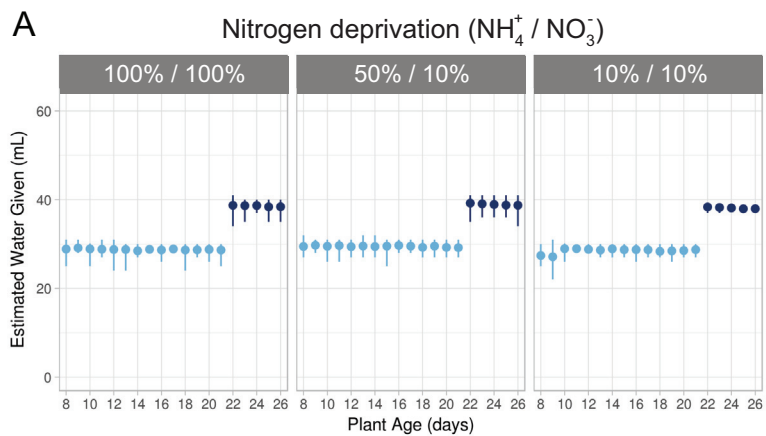
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862

863 **Supplemental raw data files:**

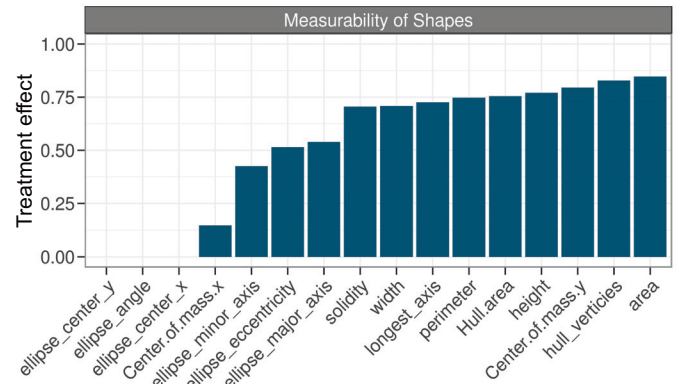
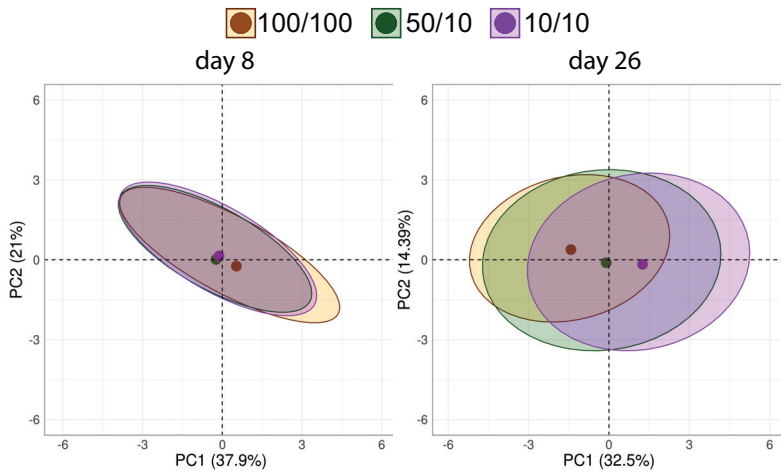
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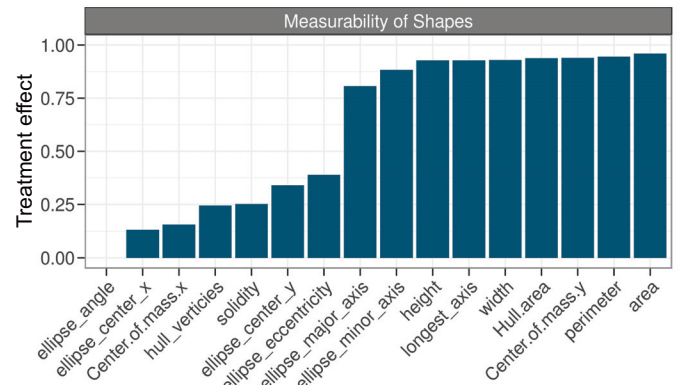
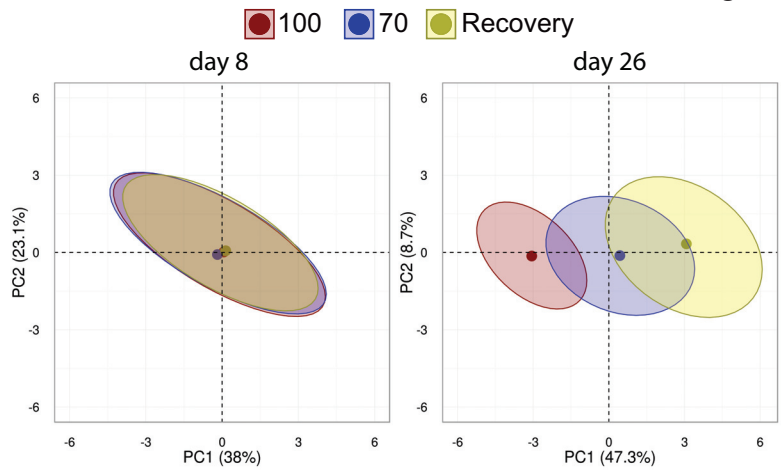
A

Nitrogen deprivation

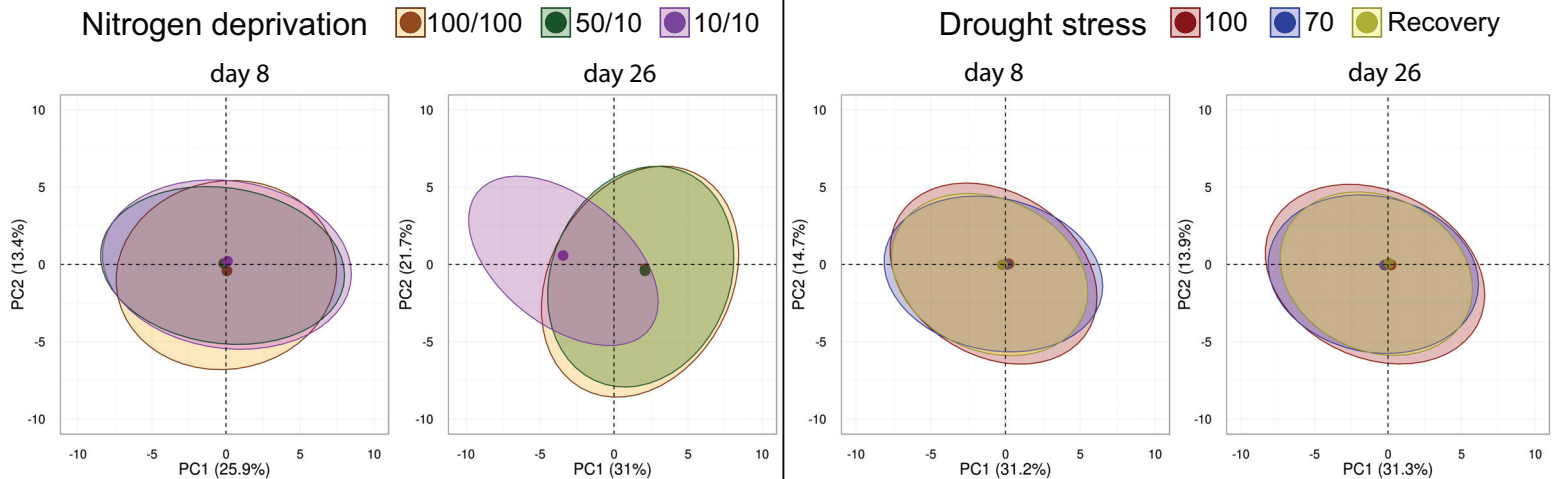


B

Drought stress



C



A

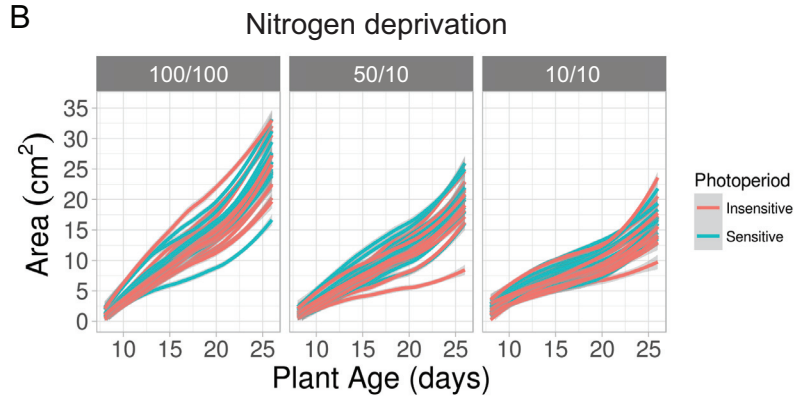
Genotype			
Source	Chisq	DF	p-value
Nitrogen	415.9	3	<0.0001
Genotype	130.1	29	<0.0001
Nitrogen x Genotype	79.27	58	0.033
Nitrogen x Time	10516	3	<0.0001
Genotype x Time	197.4	29	<0.0001
Nitrogen x Genotype x Time	91.93	58	0.003

Photoperiod			
Source	Chisq	DF	p-value
Nitrogen	3512	3	<0.0001
Photoperiod	9.017	1	0.003
Nitrogen x Photoperiod	9.147	2	0.01
Nitrogen x Time	7963	3	<0.0001
Photoperiod x Time	1.499	1	0.221
Nitrogen x Photoperiod x Time	7.585	2	0.023

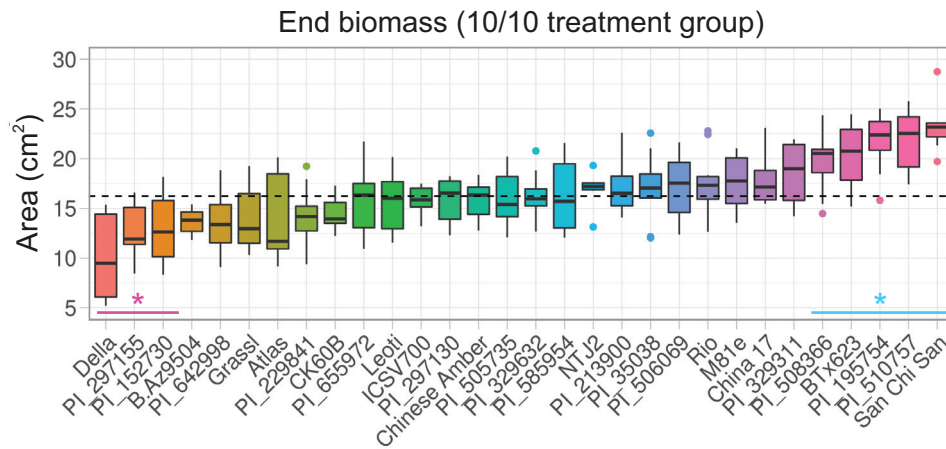
Type			
Source	Chisq	DF	p-value
Nitrogen	1875	3	<0.0001
Type	12.86	2	0.002
Nitrogen x Type	1.844	4	0.764
Nitrogen x Time	4085	3	<0.0001
Type x Time	16.51	2	0.001
Nitrogen x Type x Time	2.321	4	0.677

Race			
Source	Chisq	DF	p-value
Nitrogen	2298	3	<0.0001
Race	26.6	9	0.002
Nitrogen x Race	20.06	18	0.329
Nitrogen x Time	5338	3	<0.0001
Race x Time	39.95	9	<0.0001
Nitrogen x Race x Time	17.55	18	0.486

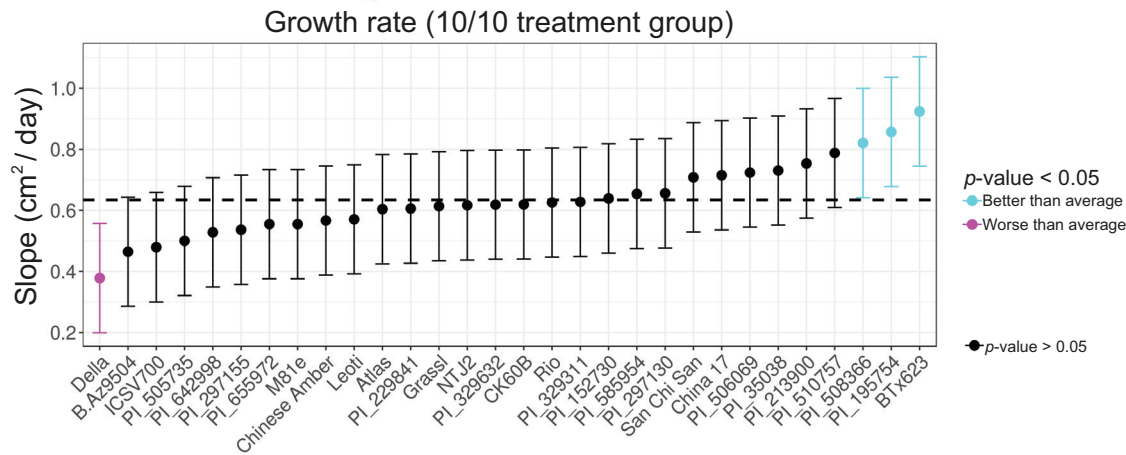
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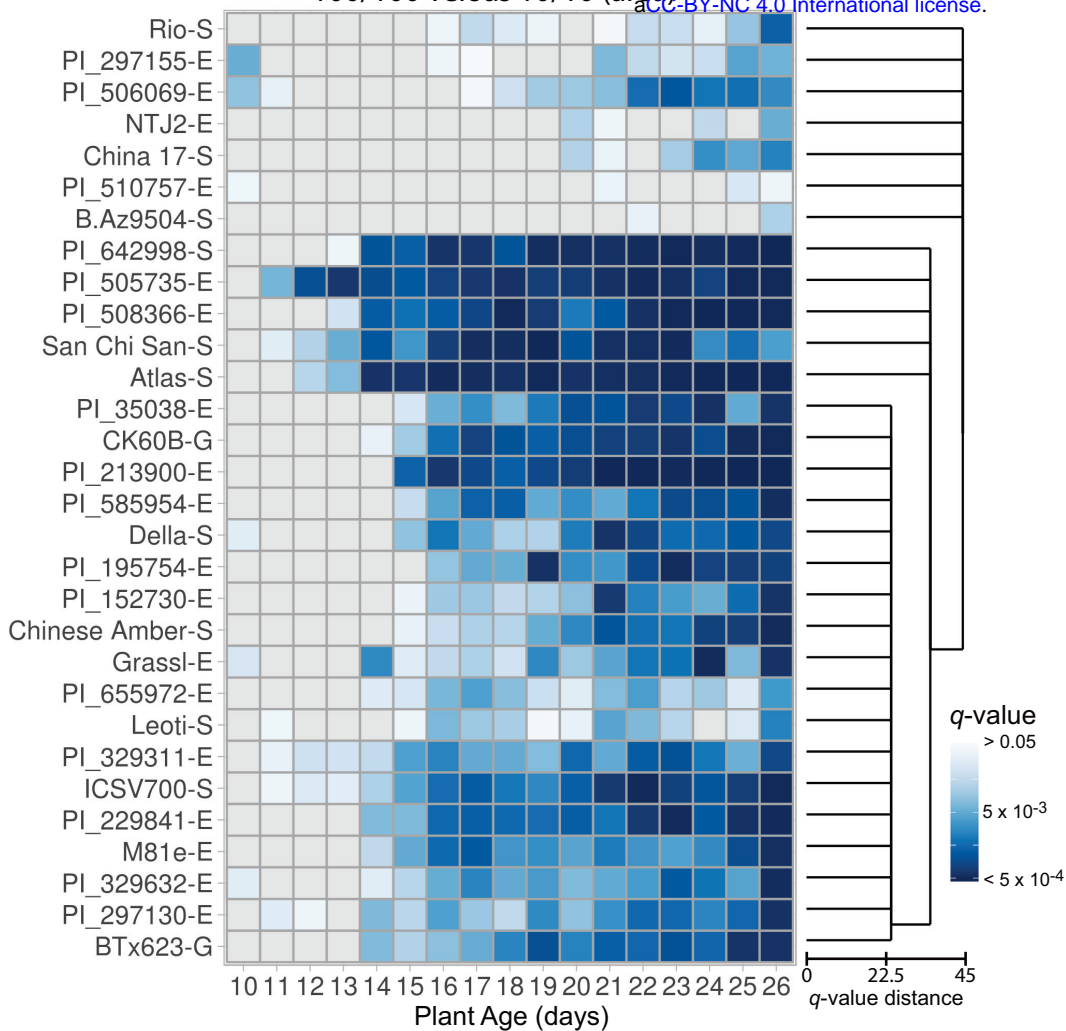
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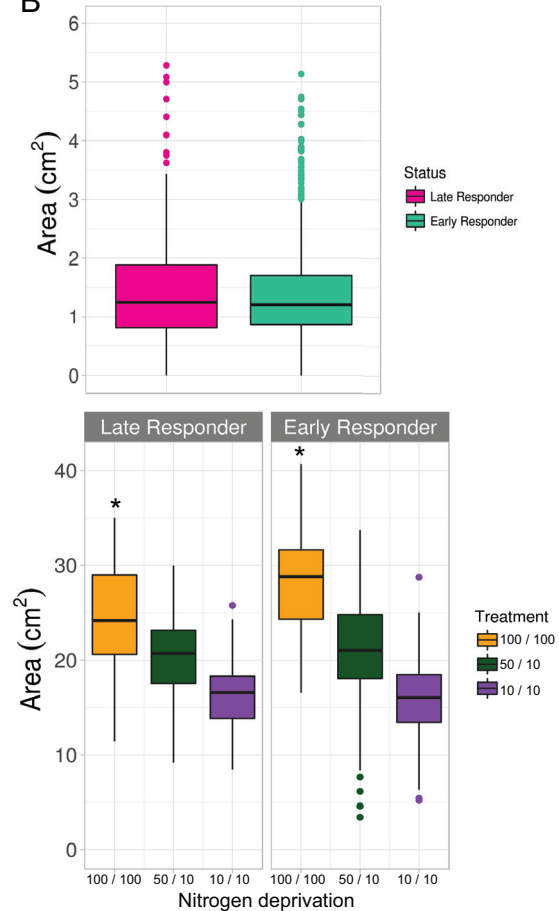
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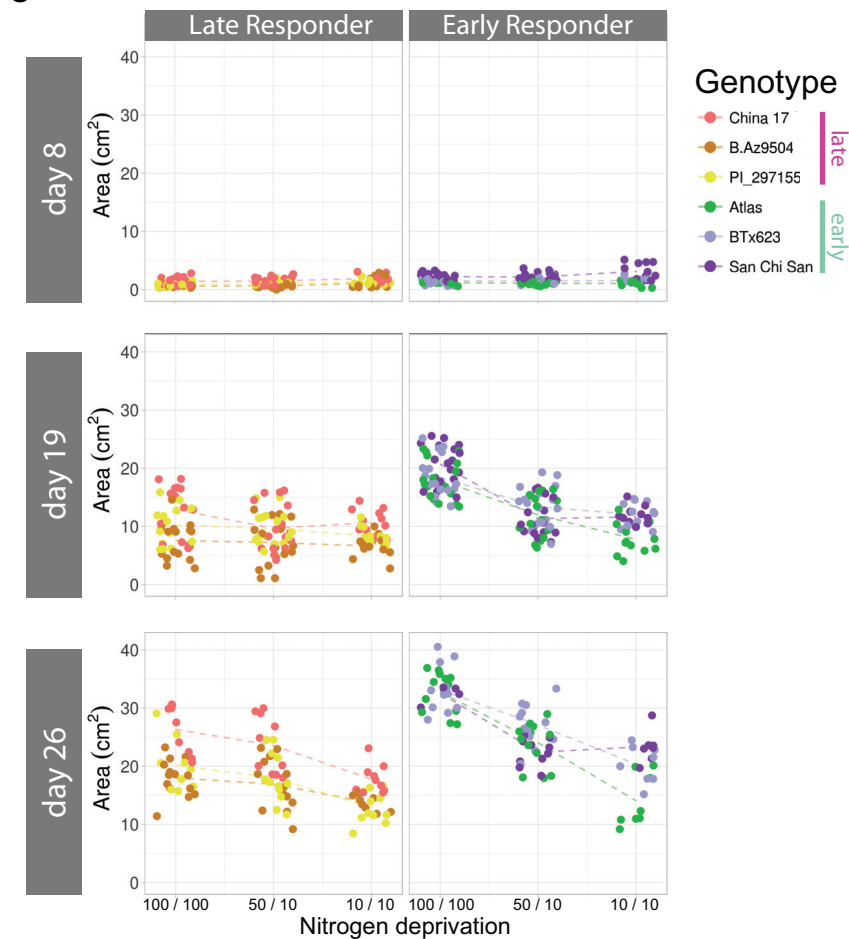
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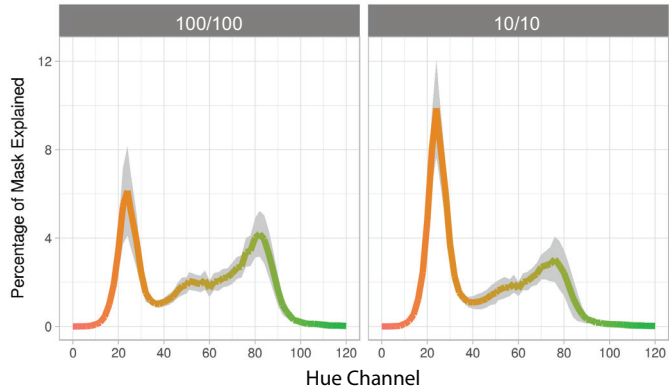
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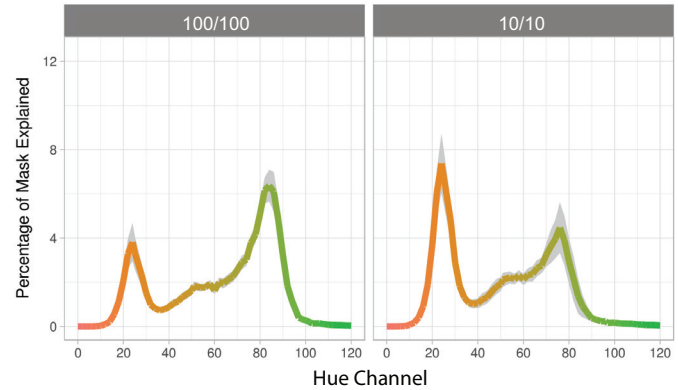
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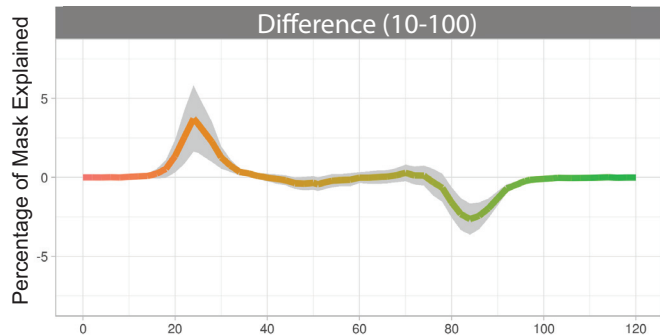
Late Responders (average)



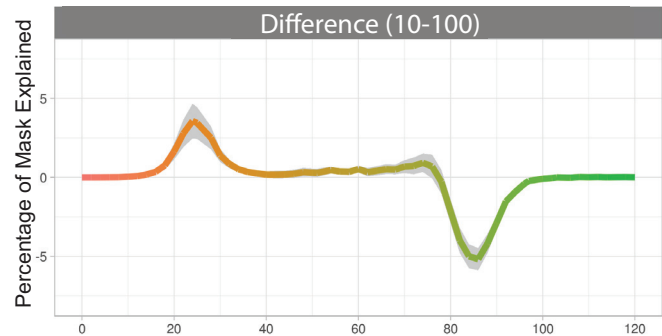
Early Responders (average)



Difference (10-100)



Difference (10-100)

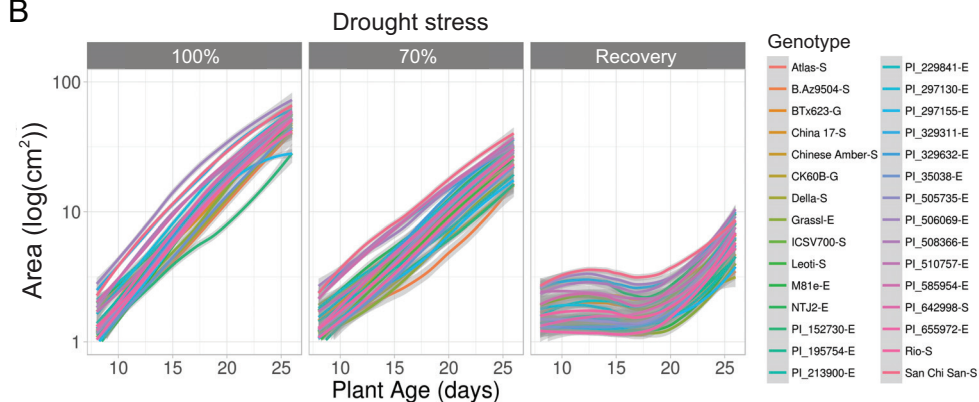


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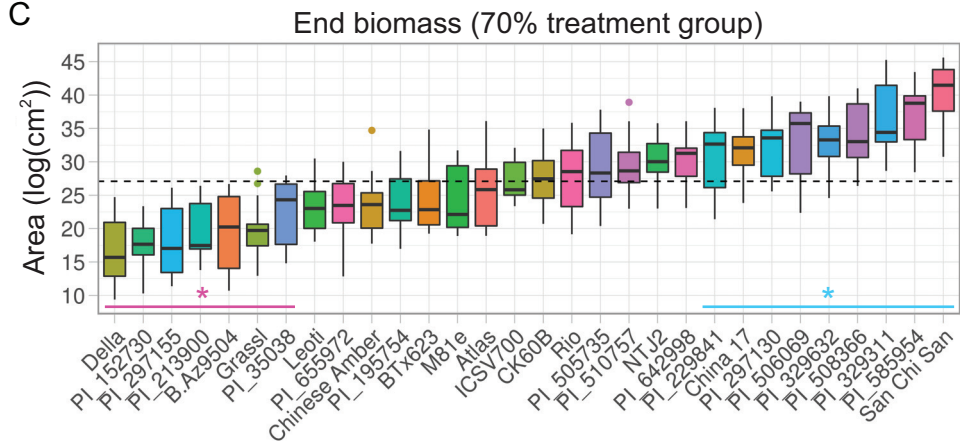
Genotype				Type			
Source	Chisq	DF	p-value	Source	Chisq	DF	p-value
Water	15042	2	<0.0001	Water	5004	2	<0.0001
Genotype	229.6	29	<0.0001	Genotype	6.502	2	0.03874
Water x Genotype	42.72	29	0.0483	Water x Type	1.608	2	0.44756
Water x Time	8860	2	<0.0001	Water x Time	3278	2	<0.0001
Genotype x Time	152.5	29	<0.0001	Type x Time	5.684	2	0.05831
Water x Genotype x Time	39.51	29	0.0923	Water x Type x Time	2.071	2	0.35502

Photoperiod				Race			
Source	Chisq	DF	p-value	Source	Chisq	DF	p-value
Water	10918	2	<0.0001	Water	7387	2	<0.0001
Photoperiod	9.966	1	0.0016	Race	87.8	9	<0.0001
Water x Photoperiod	2.191	1	0.1388	Water x Race	7.626	9	0.5722
Water x Time	7133	2	<0.0001	Water x Time	4296	2	<0.0001
Photoperiod x Time	6.879	1	0.0087	Race x Time	56.44	9	<0.0001
Water x Photoperiod x Time	0.165	1	0.6849	Water x Race x Time	9.93	9	0.3562

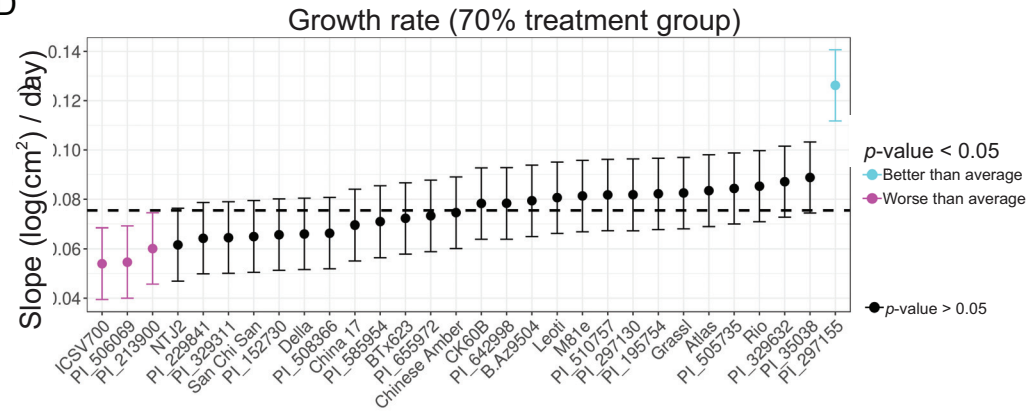
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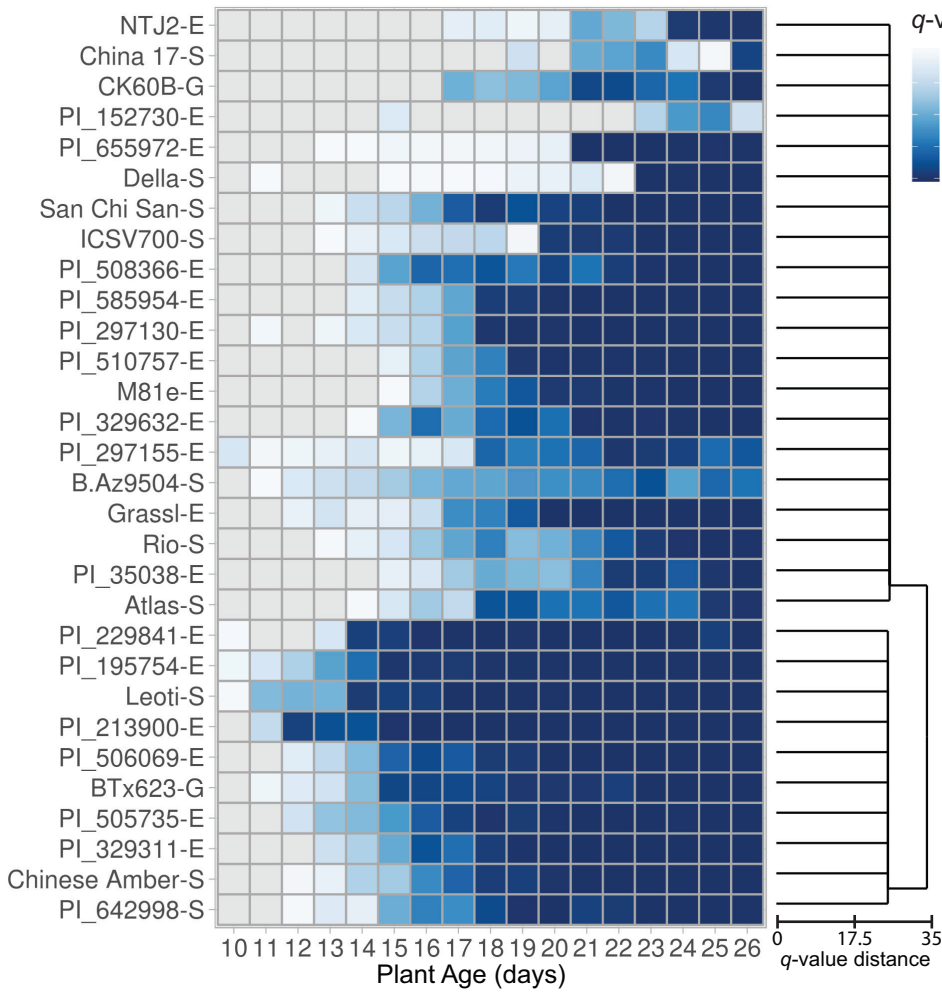


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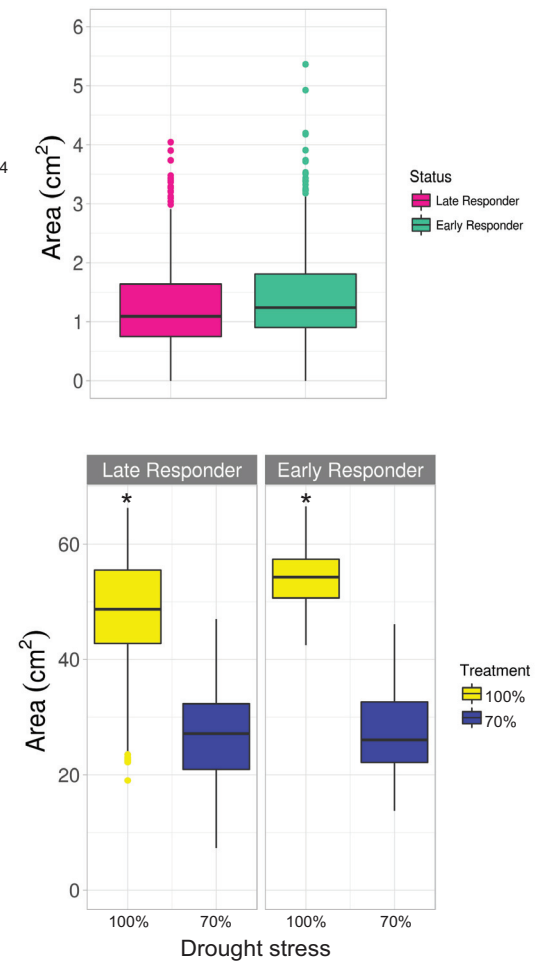


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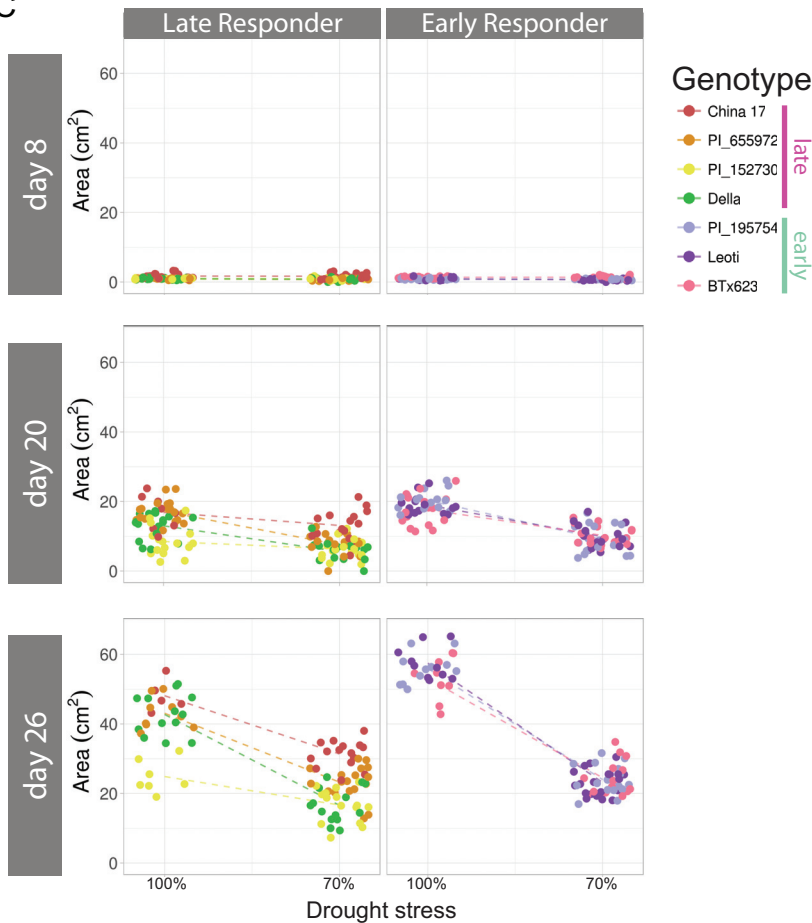
100% versus 70% (log area)



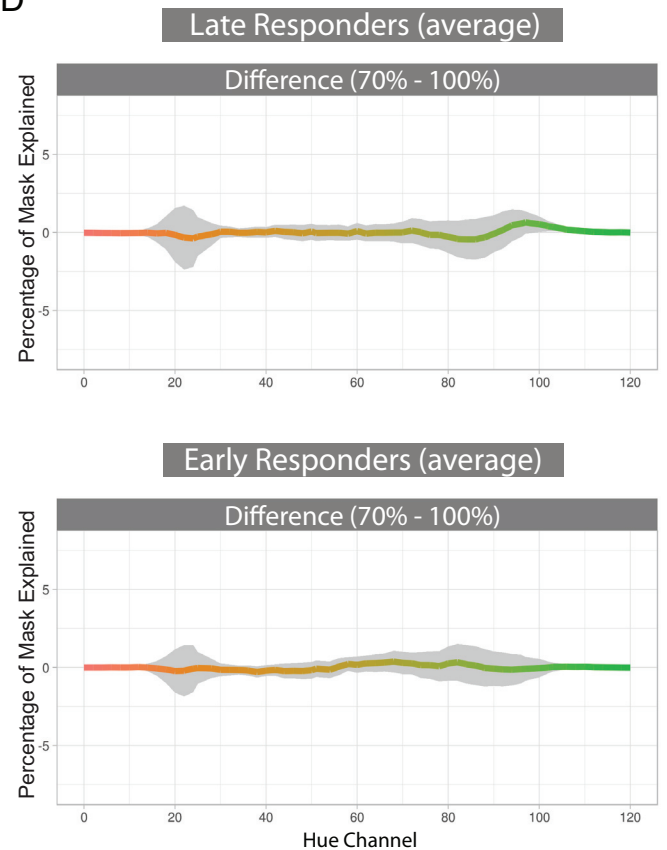
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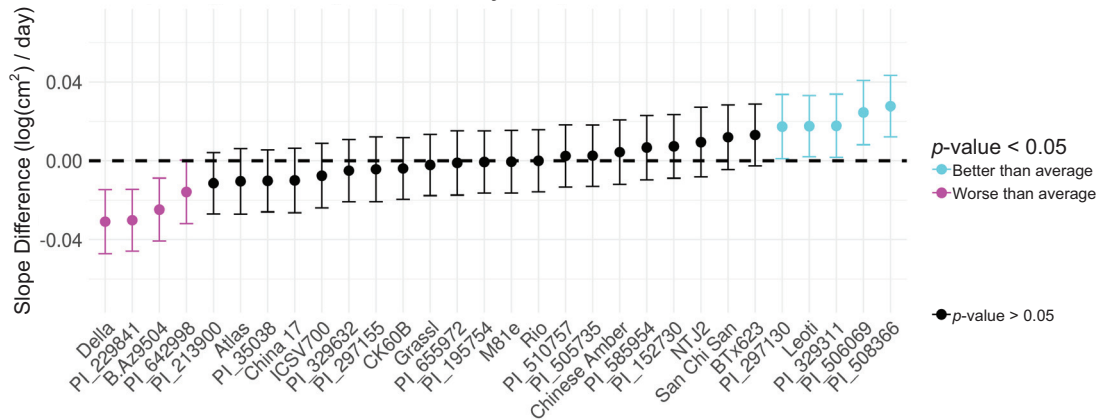
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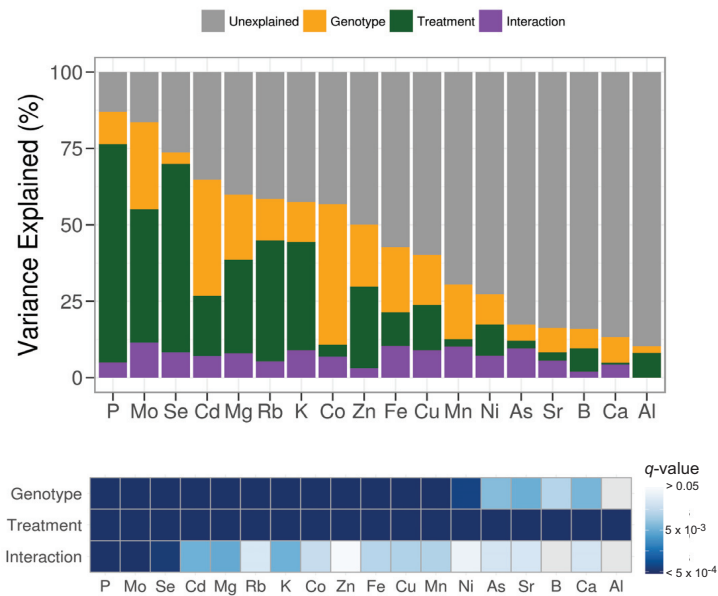
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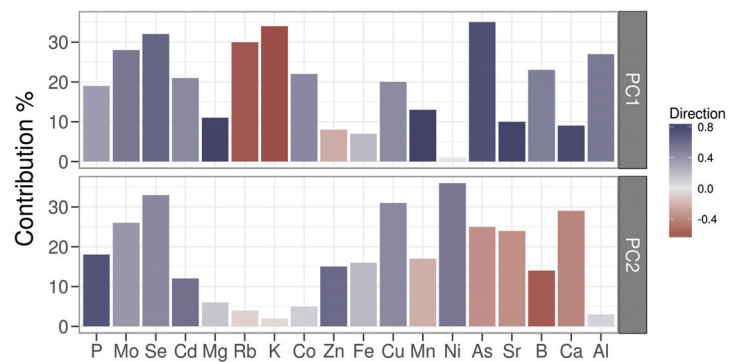
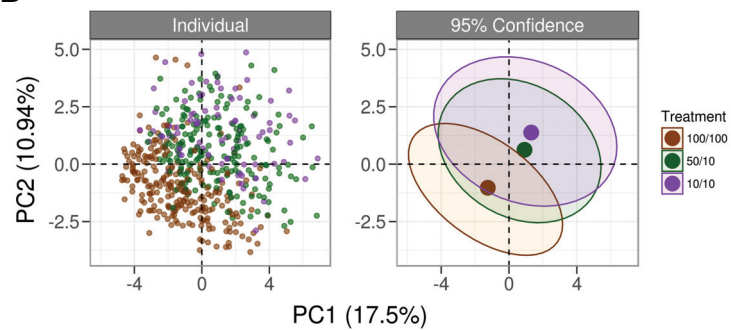
Recovery vs 70%



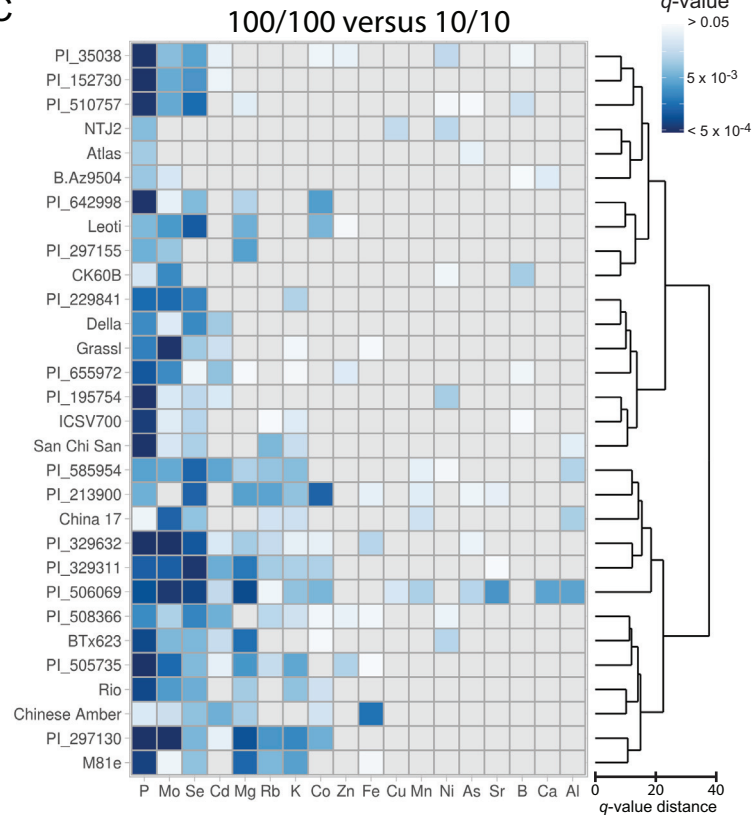
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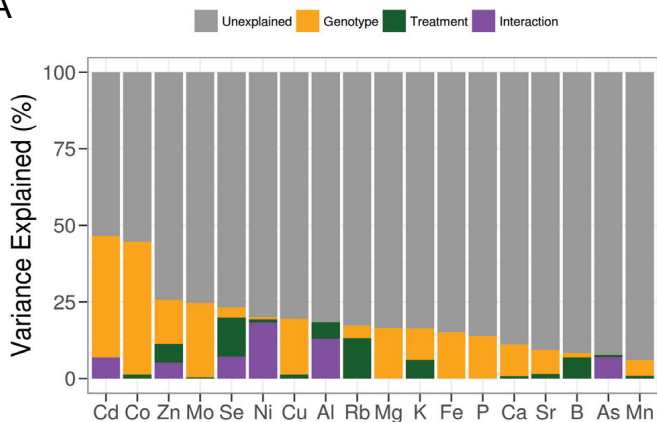
B



C



A



B

