Effects of genomic and functional diversity on stand-level productivity and performance of non-native Arabidopsis

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Abstract

Biodiversity can affect the properties of groups of organisms, such as ecosystem function and the persistence of colonizing populations. Genomic data offer a newly available window to diversity, complementary to other measures like taxonomic or phenotypic diversity. We tested whether native genetic diversity in field experimental stands of *Arabidopsis thaliana* affected their aboveground biomass and fecundity in their colonized range. We constructed some stands of genotypes that we *a priori* predicted would differ in performance or show overyielding. We found no relationship between genetic diversity and stand total biomass. However, increasing stand genetic diversity increased fecundity in high resource conditions. Polyculture (multiple genotype) stands consistently yielded less biomass than expected based on the yields of component genotypes in monoculture. This under-yielding was strongest in stands with late-flowering and high biomass genotypes, potentially due to interference competition by these genotypes. Using a new implementation of association mapping, we identified genetic loci whose diversity was associated with stand-level yield, revealing a major flowering time locus associated with under-yielding of polycultures. Our field experiment supports community ecology studies that find a range of diversity-function relationships. Nevertheless, our results suggest diversity in colonizing propagule pools can enhance population fitness. Furthermore, interference competition among genotypes differing in flowering time might limit the advantages of polyculture.

Keywords: diversity-productivity relationships, evolution, phenology, invasion

INTRODUCTION

The characteristics of groups of organisms can affect their persistence and collective functions. The emergent properties of groups of organisms are major topics across biology [1–3]. The mechanisms underlying the benefits of diversity for group-level performance can be divided into at least two categories [4]. First, greater diversity can reduce the negative effects associated with high density populations or communities. This can occur via diversity in resource use that reduces competition, diversity in susceptibility to enemies that reduces "apparent" competition, or facilitation among diverse individuals. Second, diversity can increase group-level performance via a sampling effect: greater diversity increases the chance that high-performing individuals will be present. Diversity can also reduce group-level performance. This can occur when more diverse groups tend to have lower performing individuals (e.g. when diversity is driven by immigration of maladapted individuals) [5,6] or when diversity results in interference competition among different types [7]. While diversity effects on ecosystem functions (EF) can act at both population and community levels, they have been mostly studied in communities [8,9]. However, *intraspecific* diversity can have major impacts on EF, potentially surpassing interspecific effects when a few species dominate biomass [10].

Community ecologists have often hypothesized that diversity increases ecosystem functions [11,12] while invasion biologists have hypothesized that diversity increases the growth of colonizing populations [13–15]. These fields have been largely independent and disjunct, but they share much in common (see also related topics in kin and group selection [16]). Both address hypotheses based on the same underlying biological processes: 1) sampling effects, whereby greater diversity increases the chance a group will contain high functioning/performing individuals, 2) insurance effects (a type of sampling effect), whereby greater diversity increases the chance that a group will contain an individual that is high functioning under a new environment, or 3) complementarity effects, whereby diversity relieves negative density effects. Invasion biology has long investigated how the number of individuals in a founding group affects colonization success [17,18]. Evidence suggests that increased genetic diversity, rather than increased numbers of individuals *per se*, causes improved establishment [13], growth rate [13–15], biomass production [2,19,20], and persistence of colonizing populations, and decreases vulnerability to environmental change [21]. The effects on group-level performance and EF are

70 likely to be conditional on the environment. For example, facilitation may dominate in harsh
71 environments, strengthening positive diversity effects [22,23], or novel environments might be
72 more limited by diversity due to a rarity of high-functioning individuals. Empirical studies of
73 genetic diversity in invasions have found it favorable under some conditions but not others [2,24]
74 and theoretical predictions also conflict [20,25].

75 Diversity effects on the performance of a group of organisms arise fundamentally due to
76 the effects of phenotypic diversity [26]. However, the phenotypes that affect group-level
77 performance are very often unknown. Instead, genetic, taxonomic, or phylogenetic diversity can
78 be useful proxies. Past studies of intraspecific diversity and EF have used either genotypes of
79 unknown relatedness [19,20,27], or have used a small number of genetic markers having unclear
80 connections to functional variation [2,28,29]. By contrast, large numbers of markers across the
81 genome allow precise estimation of genetic similarity between individuals, and it is now feasible
82 to measure whole genome diversity in many non-model systems. Furthermore, genetic diversity
83 effects may be most strongly associated with the specific loci that underly complex phenotypes
84 associated with resource economics. Genetic variation in complex traits corresponding to life
85 history and resource use likely involves changes in many loci [30,31], and simple genome-wide
86 additive models can often predict variation in such traits [32–34]. Additionally, genetic mapping
87 may be used to identify specific genes and mutations controlling phenotypes whose diversity
88 affects EF [23,35].

89 Here we investigate how intraspecific diversity influences productivity and fecundity for
90 the model plant *Arabidopsis thaliana* across environments in a field experiment outside its native
91 range. Arabidopsis is useful for our questions: it is a model for ecological genomics [36], diverse
92 germplasm with resquenced genomes is available [37], and it exhibits variation in resource
93 strategies and life history [38,39] suggesting that diversity may impact EF. Our study builds
94 upon previous work in Arabidopsis [19,23,35] by using ecologically realistic and relevant field
95 experimental conditions, including a low resource soil treatment, and by studying stands with up
96 to 20 genotypes. Because Arabidopsis is largely selfing and we studied a single generation, we
97 set aside the effects of diversity on population performance due to sexual reproduction, such as
98 heterosis.

99 Experimental studies of biodiversity effects typically use randomly assembled groups of
100 genotypes or species, despite existing prior knowledge of potential drivers of compositional and

101 diversity effects on EF. Here, in addition to randomly assembled groups representing a range of
102 genotypic diversity, we use knowledge of our system to develop *a priori* selected groups of
103 genotypes that we predict will exhibit particularly strong differences in EF, groups benefitting
104 from potential niche complementarity, and groups of genotypes adapted to the experimental
105 environment.
106
107
108 **METHODS**
109
110 *Plant material*
111 We studied 60 diverse Arabidopsis genotypes from across the native Eurasian range of
112 Arabidopsis, mainly from the Mediterranean, central Asia, the UK and central Europe (Figure
113 S1). All genotypes are representatives of natural inbred lines that had their genomes resequenced
114 [37]. We selected these genotypes based on diversity and seed availability from a common seed
115 production environment used in previous experiments (Methods S1).
116
117 *Field experiment*
118 To assess the impact of different levels of genetic diversity in experimentally assembled non-
119 native populations of Arabidopsis, we measured performance of Arabidopsis stands in a field
120 common garden at a site (40.8106º N, 77.8472º W) in the non-native range near other established
121 non-native populations (within ~10 km) but with none at this site (within ~1km). Thus our
122 experimental stands were likely not influenced by density-associated processes driven by wild
123 individuals nearby. To assess the environmental context dependency of diversity effects, we
124 replicated all stand genotype compositions in two treatments (described below). Each of the 60
125 genotypes were grown in monoculture in three replicate stands for each of the two treatments, for
126 a total of 360 monoculture stands (Figure S2).
127 We began by constructing polycultures using *a priori* hypotheses for factors likely to
128 affect productivity. We used five different criteria to generate these 'prediction stands': (1)
129 genomic diversity among accessions, (2) climate similarity of collection location to our field site,
130 combined with genomic diversity, (3) early flowering genotypes, (4) late flowering genotypes,
131 (5) climate similarity of collection location to our study site, combined with flowering time

132 diversity (see details in Methods S1). Two stand compositions were chosen for each of the five
133 prediction scenarios at each diversity level (2, 10, 20), for a total of ten prediction stand
134 compositions for each diversity level. Each of the prediction stands were planted into both of the
135 two treatments, for a total of 60 prediction stands.
136 After genotypes were assigned to prediction stands, we also generated random
137 polycultures. Each of these polycultures were planted into both treatments with 10 replicates of
138 diversity level each, for a total of 60 randomly assigned polyculture stands (Methods S1).
139 Seeds were initially sown into 20 positions within manure pots, with each position
140 thinned to a single plant, targeting a 20-plant stand (regardless of diversity level). High resource
141 pots were filled with potting mix and low resource pots with a high proportion of sand. Stands
142 were germinated in growth chambers and transplanted into the field site on May 24, 2018
143 (Methods S1).
144 We measured specific leaf area (SLA) on a representative, healthy leaf from a single
145 plant in each monoculture stand, and flowering date on all stands. We harvested each individual
146 plant once it reached maturity. We recorded the total silique (fruit) number and the length of one
147 representative middle silique. All remaining plants were harvested on July 18-20, as summer
148 heat stress set in and reproduction largely ceased. We measured aboveground dry biomass on all
149 plants. Canopy area was similarly measured for each stand just prior to the first harvested plant
150 using Easy Leaf Area and a red 4 cm^2 square reference [41].
151
152 *Measures of stand composition and diversity*
153 To estimate genomic diversity of stands, we used published SNPs from resequencing data [37].
154 We calculated the genetic distance matrix using the R package SNPRelate [42] and then
155 calculated the mean genetic distance among plants sown in a stand (including zero distance for
156 individuals of the same genotype). We also estimated phenotypic variation, based on our new
157 SLA data and published flowering time data [37]. We took breeding values of log(SLA)
158 measured on plants in our monocultures and calculated mean for individual plants in all stands.
159 Flowering time composition was estimated using individuals' breeding value for the average
160 flowering time across 10ºC and 16ºC in published growth chamber experiments [37].
161
162 *Statistical analyses*

To gain general insight into variation in phenotypes and performance, we estimated trait plastic responses to treatment, broad-sense heritability, and genomic signature of performance in monocultures. To test for treatment effects on trait plasticity in monocultures, we implemented linear mixed models using the R package 'VCA' [43] and included genotype and block as random effects and soil treatments and plot x-y coordinate as fixed effects. Plastic trait responses were characterized with this soil fixed effect. We estimated broad sense heritability of stand-level traits and performance for monocultures using linear models in R where genotypes were fixed effects, taking R^2 of these as broad sense heritability. We tested for a genomic signature in fecundity and aboveground biomass using genomic prediction models, where traits are a function of genotype-specific random effects that are correlated according to genome-wide similarity, using the R package 'rrBLUP' [43]. We used ten-fold cross validation to estimate the ability of genome-wide similarity to predict performance.

To address our primary research questions, we tested whether multiple measures of diversity affected stand level performance (fecundity, aboveground biomass, and leaf canopy area). In our analyses, we controlled for variation among stands in the number of plants surviving transplant or germinating late, with a minimum of 11 plants required for inclusion in analysis. We used two different counts of plants surviving transplant, on May 29 and June 12, and took the maximum, but we found results were similar when using the count on either date.

We used linear regression models to test diversity effects on stand performance while accounting for additional sources of error. The covariates were each tested in models including an interaction with the resource-limitation treatment

$$y_i = \beta_0 + \beta_1 x_i + \beta_{trt} trt_i + \beta_2 x_i trt_i + \varepsilon_i$$

where y_i is the performance measure (biomass or fecundity) of stand i and trt_i is a dummy variable for resource treatment, and x_i is a covariate of stand i's composition or diversity. Covariates x tested in separate models included diversity treatment level (1, 2, 10, or 20 genotypes), genomic similarity (mean genetic distance among plants in a stand), stand-level average of mean flowering time for each genotype, a measure of the stand-mean climate distance from the field site, or stand-mean SLA breeding value. We similarly tested for stand diversity and composition effects on fecundity [mean estimated total silique (fruit) length per fruiting plant in stand].

193 We calculated the difference between the observed polyculture biomass yields and that
194 expected based on yields of monocultures and their proportional representation in the
195 polyculture. This difference is a commonly studied metric in community diversity-ecosystem
196 function studies [4], and we refer to this quantity as the deviation from monoculture expectation
197 (DME, referred to as the "net diversity effect" by [4]). We tested how the measures of diversity
198 and composition described above were related to DME, using linear models with a parallel
199 structure to those used for aboveground biomass and fecundity (described above).
200 To determine whether our prediction stands were successful at explaining variation in
201 fecundity and biomass production, we compared stand types using ANOVA. Different prediction
202 criteria were coded as a factor level as were randomly assembled stands. Two-way ANOVA
203 showed no significant treatment-stand type interaction, thus we focused on stand type effects in a
204 two-way ANOVA without interactions. We compared each pair of stand types with post-hoc
205 Tukey's tests with FDR control. Because only two stands predicted to be later flowering
206 produced fruit, we removed this plot type from our analysis of fecundity in prediction plots.
207 To map loci across the genome where allele frequency and variance were related to stand
208 biomass and DME, we used an approach akin to genome wide association mapping. We tested
209 how both stand-level per plant biomass and DME (as response variables) were associated with
210 stand covariates of allele frequency and variance (the latter a measure of diversity for a biallelic
211 locus), for each SNP. For a biallelic SNP, variance = pq where p and q are the respective
212 frequencies of the two alleles. We used SNPs from published whole genome resequencing [37].
213 We excluded SNPs segregating at <0.05 minor allele frequency in our panel of 60 genotypes and
214 across stands in the same analysis, leading to a total of ~1.9M SNPs. For each combination of
215 resource treatment and performance measure we tested for association with SNP allele frequency
216 or variance. Because background variation across the genome can contribute to phenotypic
217 variation, we calculated genome-wide similarity among stands in either allele frequency or
218 variance at all SNPs [44]. We used a linear mixed model that included a random effect that was
219 correlated among stands according to the genome-wide similarity matrix, using the EMMA
220 software [44]. We also focused these association scans on SNPs within 3kb of 26 *a priori*
221 candidate genes for flowering time [45], based on the hypothesis that genes controlling this trait
222 might influence stand biomass and DME.
223

RESULTS

Plastic changes in traits and performance, heritability in monoculture

Comparing phenotypes for monoculture stands, SLA did not change significantly across soil treatments, nor did flowering dates (linear mixed-effects models with genotype random effects, Table S1). However, both average plant fecundity and biomass per plant in monocultures were significantly greater in the high resource treatment (Table S1, Figure 1). Date of flowering was highly heritable (~0.8 broad-sense, depending on metric and treatment), while log(SLA), fecundity, and biomass had moderate heritability (~0.4-0.5 depending on trait and treatment, Table S2).

Genomic signature of performance variation

There was a strong effect of genome-wide similarity among genotypes on biomass production in monoculture in the low resource treatment. Genomic predictions (out-of-sample) were correlated with actual breeding values for per plant biomass in monoculture in low resource ($r = 0.36$) but not high resource ($r = -0.32$) treatments. For example, in the low resource treatment a group of closely related ecotypes from the Caucasus region produced among the most biomass, while related genotypes from central Europe or Spain had low productivity. We found a similar pattern for per plant fecundity in monocultures (genomic predictions: high resource $r = -0.01$, low resource $r = 0.32$).

How did composition and diversity of stands impact stand fecundity and biomass production?

Among all stands (including monocultures and polycultures), the low resource soil treatment significantly reduced stand fecundity, canopy coverage, and biomass production, as tested in separate linear models of each response variable (Figure 2, Tables S3-5). We found that a greater number of genotypes in a stand significantly increased the per plant fecundity in the high resource treatment (Table S3) but not the low resource treatment, while other measures of composition and diversity were unrelated with fecundity. We found that higher average breeding values for flowering time were associated with significantly greater stand biomass in both treatments, while lower breeding values for SLA was associated with greater stand biomass only

255 in the high resource treatment (Table S4). None of the several diversity measures (i.e. the other
256 covariates) we tested were significantly associated with biomass production.
257 We also tested if "prediction stands" had distinct stand-level fecundity or biomass from
258 randomly assembled stands (Figure S5). We did not find a significant effect of stand type in a
259 two-way ANOVA for either plot level fecundity or biomass.
260
261 *How did polyculture productivity deviate from monoculture expectation?*
262 We found that in both treatments, the yield of polyculture stands was not closely correlated with
263 the expectation based on the yield of their component genotypes in monoculture (high resource, r
264 $= 0.051$, $p = 0.7138$; low resource, $r = 0.007$, $p = 0.9585$). Specifically, polycultures tended to
265 yield less than expected based on the yield of their component genotypes in monoculture. The
266 deviation from monoculture expectation (DME) was significantly less than zero in the high
267 resource stands (median = -61.01 mg, wilcoxon test $p = 0.0025$) and in the low resource stands
268 (median = -57.13 mg, wilcoxon test $p = 0.0158$). These results were unchanged (both $p < 0.02$)
269 when we generated randomly assembled polycultures to match the number of plants surviving
270 transplant using genotype-specific transplant survival in monoculture.
271 To evaluate potential causes of negative DME, we tested correlations between DME and
272 explanatory factors. One potential cause of negative DME was competitive inhibition of many
273 smaller plants by late flowering plants whose rosettes grew to overtop the earlier flowering
274 smaller plants. We found that in the high resource treatment, there was more negative DME in
275 stands with greater average breeding values for flowering time (mean flowering time; linear
276 model, flowering time in high resource slope = -0.0064 g/day, $p = 0.0167$, in low resource slope
277 = -0.0012 g/day, $p = 0.6532$) or stands having plants with greater flowering time (90th percentile
278 of flowering time; high resource slope = -0.0051 g/day, $p = 0.0170$, in low resource slope = -
279 0.0006 g/day, $p = 0.7883$, Table S6). We also found (perhaps unsurprisingly) that biomass in
280 monoculture of component genotypes was negatively associated with DME in polyculture:
281 stands with greater median or 90th percentile of genotypes' biomass in monoculture showed
282 significantly bigger reductions in polyculture yield in both high and low resource treatments
283 (Table S6). We tested whether DME might be due to genomic similarity, number of genotypes,
284 or climate of origin of genotypes. We found no strong effects on DME of mean kinship, total tree

285 diversity, number of genotypes, mean SLA, or mean distance to climate of origin along the first
286 five PCs (Table S6).
287 We also tested if "prediction stands" had distinct deviations from monoculture
288 expectation (DME). We found that there were significant differences in DME among prediction
289 stand criteria (two-way ANOVA with treatment and stand prediction criteria, including randomly
290 assembled stands: treatment $p = 0.8675$, prediction $p = 0.0433$, only including prediction stands:
291 treatment $p = 0.8258$, prediction $p = 0.0328$). Consistent with the regression of DME on
292 flowering time on all plots, the differences among our prediction plots were largely driven by
293 differences between the predicted early (positive diversity effect) and late flowering (negative
294 diversity effects) stands (Tukey HSD comparison of means, including randomly assembled
295 stands, only significant difference after multiple comparisons correction is: early flowering - late
296 flowering predicted stands DME = 0.27 g, FDR adjusted $p = 0.0292$).
297
298 *What genetic loci contribute to diversity effects?*
299 We tested whether per plant biomass or DME in each treatment were associated with allele
300 frequency or diversity at particular SNPs across the genome. We found that overall, there were
301 few loci with dramatically stronger (outlier) associations with biomass and DME, but rather
302 patterns suggest that the genetic basis of variation in biomass and DME were polygenic.
303 Nevertheless, we found that, in concordance with the association of late-flowering genotypes
304 with under-yielding polycultures, the top SNP (chr. 2, 9584630 bp, TAIR10, genome-wide
305 significant at FDR = 0.1, nominal $p = 8.1 \times 10^{-7}$) associated with DME in the high resource
306 treatment (both allele frequency and variance association scans) was closest to SHORT
307 VEGETATIVE PHASE (SVP, also known as AGL-22, AT2G22540). SVP is a MADS-box
308 transcription factor, one of the top loci associated with natural variation in Arabidopsis flowering
309 time in [37] (Figure 4). Indeed, the minor allele of this SNP that we identified here as having
310 more positive yields in polycultures compared to expected was also associated with earlier
311 flowering at 16ºC among the 60 genotypes we used from [37] (t-test, $t = -2.6687$, $p = 0.0279$). As
312 the frequency of this early-flowering minor allele increased and the stand variance at this locus
313 increased, DME also became more positive (Figure 4). We found the 26 flowering time
314 candidate genes had a number of SNPs with nominally significant associations with biomass and
315 DME in both treatments. Notably, SPL4 (AT1G53160), HEN2 (AT2G06990), ULP1B

316 (AT4G00690), and ATH1 (AT4G32980) were significantly associated with DME in low
317 resource stands (SNP frequency and variance associations, with candidate SNP-wide FDR <
318 0.05, Table S7).
319
320
321 **DISCUSSION**
322
323 Diversity can affect the group-level properties of organisms, including ecosystem functions like
324 carbon cycling and population trajectories. Diversity effects have been often studied via
325 observation of uncontrolled systems that vary in species or genetic diversity [29,46]. In
326 particular, species and higher levels of diversity have been demonstrated to have a range of
327 effects on ecosystem function via multiple mechanisms [8,47]. We built on this research to test
328 how intraspecific variation in genomic and trait composition and diversity affect the performance
329 of an established non-native species in the field. We found that the diversity of genotypes did not
330 increase biomass production; instead polycultures showed lower biomass production than
331 expected, suggesting interference competition. This low polyculture yield was associated with
332 stands containing late-flowering genotypes that had high productivity in monoculture. We
333 identified genetic variation in a known flowering time locus associated with these effects. We
334 also found a weak but significant increase in fecundity with the number of genotypes in a
335 treatment, for unclear reasons.
336
337 *Diversity effects on average individual fecundity*
338 Because of logistical and (in the case of invasive species) bioethical constraints, many studies of
339 the effect of diversity on population performance focus on the impact on biomass production and
340 may not assess fecundity directly (see [2,19,20]). While none of the diversity metrics we tested
341 had an effect on biomass production, we did find a small but significant effect of genotype
342 number on stand level fecundity in the high resource environment (Fig 2c). The mechanism
343 underlying this effect is unclear, given the lack of an effect on biomass. This may be due to
344 sampling effects, specifically where greater diversity increases the chance a group will contain
345 early flowering and more fecund genotypes. Alternately, interference competition may trigger

346 earlier reproduction in small (especially early flowering) genotypes to a greater degree than large
347 (especially late flowering) genotypes, leading to positive diversity-fecundity associations.
348
349 *Diversity effects on stand-level biomass*
350 We found no evidence that increased genetic diversity led to greater stand-level aboveground
351 biomass production of Arabidopsis in the field, highlighting the complexity of stand-level
352 properties in even this model plant. Our findings may be explained by a lack of such genetic
353 diversity effects in Arabidopsis populations in general, or by technical/logistical constraints that
354 made us miss such effects. First, diversity effects might not have been observed if positive
355 effects of diversity (complementarity of resource use or pathogen susceptibility) were opposed
356 by negative effects (interference competition) [7] or if diversity effects did not exist. Our finding
357 of reduced polyculture yield compared to expectation from monoculture (DME) suggests
358 interference competition as one potential explanation. Second, diversity effects might have been
359 overlooked if they occurred along dimensions that were not measured, such as seed survival or
360 nutrient use [26]. Diversity effects might also have been overlooked if they occur across
361 environmental gradients unobserved in our single year, two resource level, study [48,49].
362 By contrast, we found consistent under-yielding of polycultures of genotypes compared
363 to the expectations from their biomass in monoculture and relative representation in the
364 polycultures (deviation from monoculture expectation, DME, also known as "diversity effects")
365 [4,7]. Generally, negative DMEs are thought to arise from interference competition [7]. This
366 process could play out in our experiment as follows: when grown with genotypes having
367 different trait values, individuals exhibit reduced performance compared to in monoculture with
368 genetically identical individuals, and apparently without much benefit to the performance of the
369 higher performing genotypes. For example, [50] found that *Pseudomonas flourescens* genotypic
370 diversity lead to reduced ecosystem function (host plant protection) due to antagonistic
371 interference (mutual poisoning) among *Pseudomonas* genotypes. Similar patterns have been
372 reported in plants, where individuals have higher performance among similar genotypes and
373 reduced performance among different genotypes [24,27]. For example, [24] found reduced
374 variance in biomass among individuals and higher individual fecundity of *Plantago lanceolata*
375 when grown among related individuals, which he interpreted as being due to reduced
376 interference competition in high relatedness stands (though the term "interference competition"

377 was not used). This might occur if higher performing genotypes reduce access to resources of
378 lower performing genotypes to a greater degree than those high performing genotypes benefit by
379 being surrounded by fewer of their own type. Alternatively, higher performing genotypes in
380 monoculture might be more limited by lower performing genotypes, with little benefit to lower
381 performing genotypes. We found that polyculture plots containing later flowering and larger
382 biomass (in monoculture) genotypes were those that had the strongest negative DME, possibly
383 due to shading of different and early flowering smaller neighbors. This suggests that late-
384 flowering genotypes may have been more likely to be involved in interference competition when
385 resources were abundant, perhaps because these larger individual plants limited their competitors
386 from resource capture (e.g. by shading) more than they benefited, reducing the overall total
387 biomass [24]. Evidence suggests that size asymmetries leading to shading [51] as well as
388 allelopathy against competitors or other trophic levels [52] can also lead to such interference
389 competition. Other authors have sometimes interpreted these patterns as evidence of kin
390 selection [53,54]. The interference competition explanation for negative DME seems more likely
391 than plants' ability to first recognize kin *per se* and then reduce interference with related
392 neighbors (cf. [54]).
393 We demonstrated how genomic data on individuals in experimental stands can be used to
394 identify genetic loci potentially affecting group-level performance, although we did not identify
395 effects of whole genome diversity on stand performance. Recent studies using Arabidopsis
396 grown in pots, combined with linkage [23] or association mapping [35] have demonstrated
397 complementary genomic approaches using a smaller number of genotypes and individuals per
398 stand (two genotypes and three-four individuals/pot). Controlled experiments like these and our
399 present study, combined with group-level performance and genetic data, could be applied to
400 nearly any system. It is not clear whether such approaches could detect genetic loci operating in
401 natural uncontrolled systems, where uncontrolled environmental variation plays an important but
402 often unmeasured role in driving spatial variation in group-level performance. Here, we
403 implemented a study of association of stand level performance with both allele frequency and
404 variance in stands. However, note that to distinguish between effects of variance (diversity *per*
405 *se*) versus frequency (composition) at a biallelic SNP, a given SNP would need to span a wide
406 range of allele frequencies among stands. This condition is not likely met for most SNPs given
407 they have low minor allele frequency, thus allele frequency and variance show a large degree of

408 covariation, limiting our ability to distinguish the effects of diversity versus allelic composition
409 at most loci.
410
411 *Previous studies on Arabidopsis*
412 Previous studies in the laboratory have shown some evidence that certain combinations of
413 Arabidopsis genotypes may result in higher stand level performance. In greenhouse populations
414 of Arabidopsis, both additive and non-additive benefits of up to eight genotypes per stand (from
415 a pool of 23) were found to increase emergence, biomass, flowering duration, and fecundity [19].
416 [23] used an Arabidopsis biparental mapping population to identify a single locus causing
417 overyielding when grown in diversity in pots. Additionally, [35] used pot experiments on pairs of
418 genotypes in a greenhouse, combined with association mapping on an individual genotype's
419 association with over- versus under-yielding. Our study was conducted in the field where plants
420 were subject to a wide range of potential stressors. It is noteworthy that [55] found that natural
421 stands in Germany with a single genotype tended to have fewer individuals than stands with
422 multiple genotypes, suggesting potential positive diversity effects in nature.
423
424 A priori *prediction of stand-level performance*
425 Ecology has been criticized for not being a predictive science. Here we tested our knowledge of
426 the system with *a priori* sets of stands predicted to have divergent performance. We found that
427 our predictions were most closely associated with stand-level yield driven by flowering time
428 differences among genotypes. Going forward, if we were to construct a productive Arabidopsis
429 stand with high fecundity individuals at our site, we would choose a set of multiple distinct
430 genotypes, that are late flowering, and with low SLA (but note some late-flowering genotypes
431 did not flower here, and this strategy may only work in certain environments).
432 It is no accident that we chose flowering time as the one trait to include in our prediction
433 criteria. Flowering time has well-documented links to a major axis of life history and
434 physiological variation [39,56], and is so intensely studied for agronomic interest. The use of
435 phenotyped and genotyped individuals in such experiments are a way forward for predictive
436 approaches to group performance and diversity.
437
438 *Conclusions*

Biodiversity can affect the properties of groups of organisms and may be important to understanding the performance and persistence of both natural and introduced populations and communities. Here we did not identify strong effects of genetic diversity *per se* on the performance of stands of non-native Arabidopsis populations but we found a pattern of higher biomass yield when genetic composition was shifted to late-flowering stands. We also identified that in polyculture, the presence of later-flowering genotypes led to lower-than expected yield, possibly due to interference competition. Our research here on stand-level effects on performance in the field for the model plant Arabidopsis suggest a way forward to understanding the importance of such effects in nature.

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459 **Figure 1.** Reaction norms for aboveground biomass (AGB, left) and neighbor-joining trees,
460 where higher yielding genotypes are colored in green. ISO country codes indicate genotype
461 origins. Two genotypes with poor germination are not shown.
462

463 **Figure 2.** Stand-level biomass and fecundity versus composition and diversity metrics. Each
464 point represents a stand; red circles indicate stands in the high resource treatment, blue triangles
465 indicate the low resource treatment. Resource treatment had a significant effect on both biomass
466 and fecundity (Tables S3-4). Shaded areas represent standard error. (a) Later stand mean
467 breeding values for flowering time from published experiments [37] significantly increased stand
468 biomass at harvest. (b) log(SLA) breeding value had a significant negative effect on stand
469 biomass at harvest in the high resource treatment. (c) Experimental stand diversity level (1, 2, 10,
470 20 genotypes) significantly increased estimated fecundity (total fruit length) per flowering plant
471 in the high resource treatment.
472

473 **Figure 3.** Polyculture deviations from monoculture expectation (DME) in comparison with
474 measures of stand composition (top panels) or stand assembly criteria (bottom panels). (Top
475 panels) Stands with large biomass genotypes, or late flowering genotypes (90th percentiles) also
476 tend to show lower DME (under-yielding), even more so than when measured via mean
477 monoculture biomass or flowering time (Table S5). (Bottom panels) Stands assembled via
478 different criteria show different DME. In particular, stands with genotypes predicted to be more
479 early flowering (EF) had higher DME (over-yielding) compared to late flowering (LF) stands
480 (Tukey HSD, FDR adjusted $p = 0.0292$). Gdiv=genomic diversity, CliGdiv=similar climate to
481 study site but genomic diversity, CliFdiv=similar climate of origin to study site but flowering
482 time diversity.
483

484 **Figure 4**: Associations between DME in the high resource treatment with var(allele frequency)
485 across the genome (A) and at the top associated SNP (B). (A) Linear mixed model genome-wide
486 association with 1.9M SNPs allele frequency variance (var = pq) versus deviation from
487 monoculture expectation (DME) in high resource environments. SNPs significant with FDR <
488 0.1 are shown in red. The top SNP is highlighted with a large red dot in panel A and is featured
489 in panel B (this was also the top SNP for allele frequency associations with DME). Boxplot
490 (panel B) shows variance in allele frequency within polycultures (x-axis) at this SNP
491 (chromosome 2, 9584630 bp) associated with DME of biomass (panel B y-axis) in the high
492 resource environment.
493

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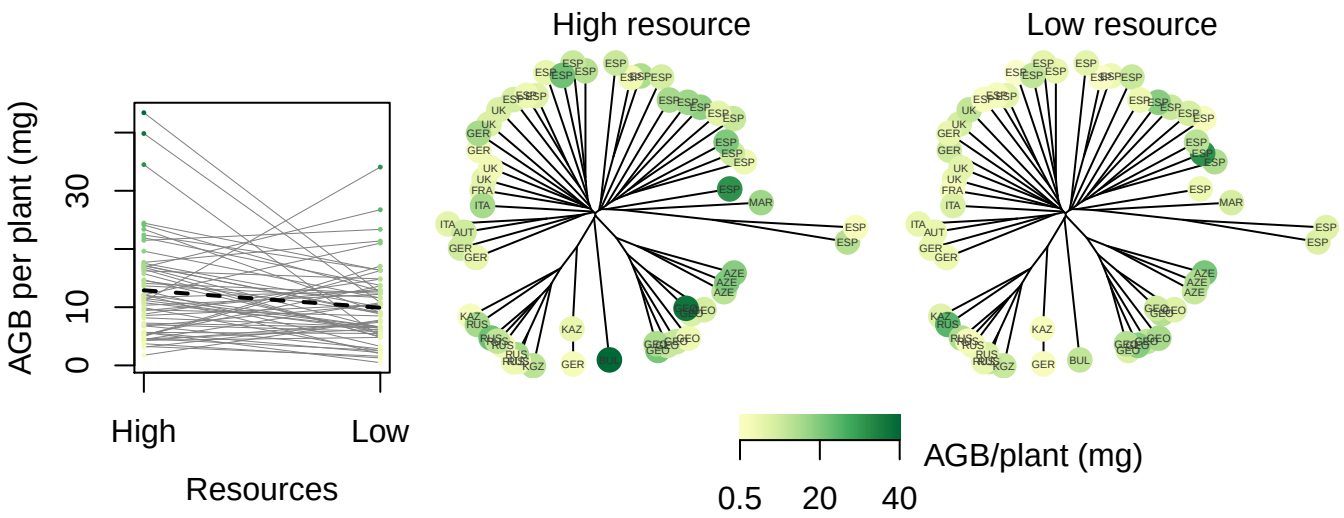
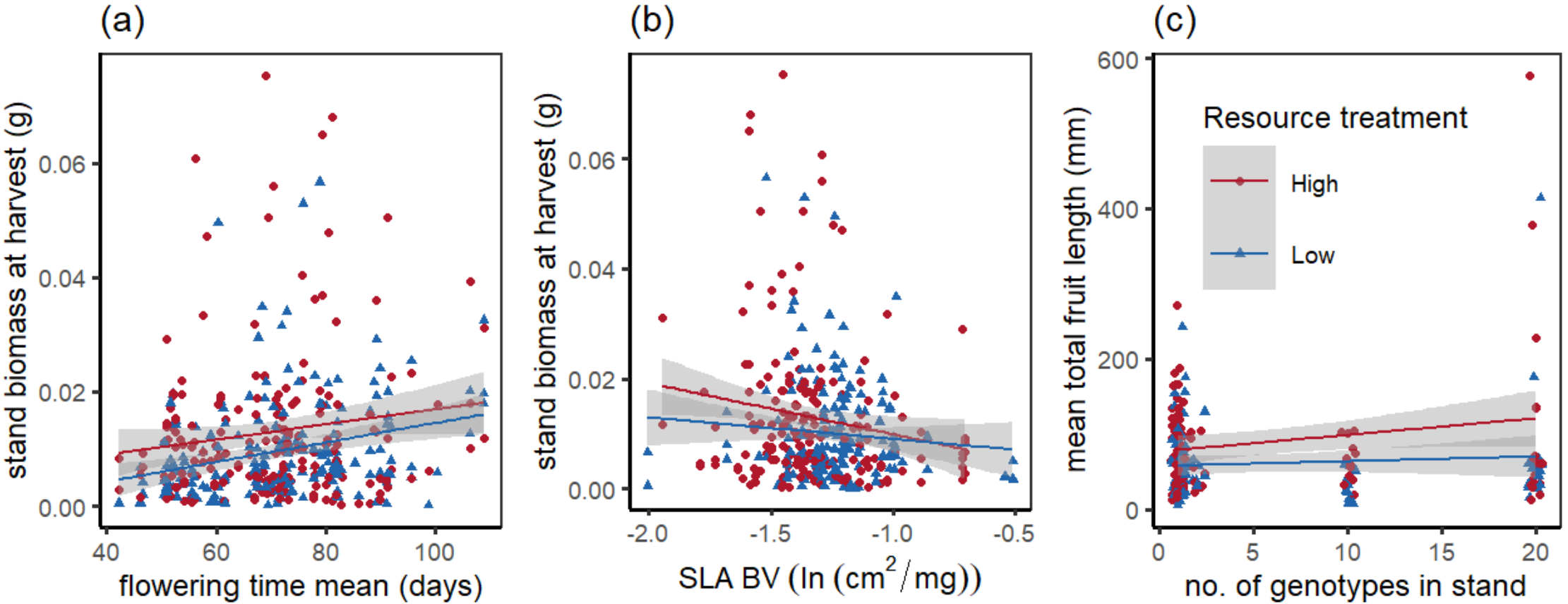
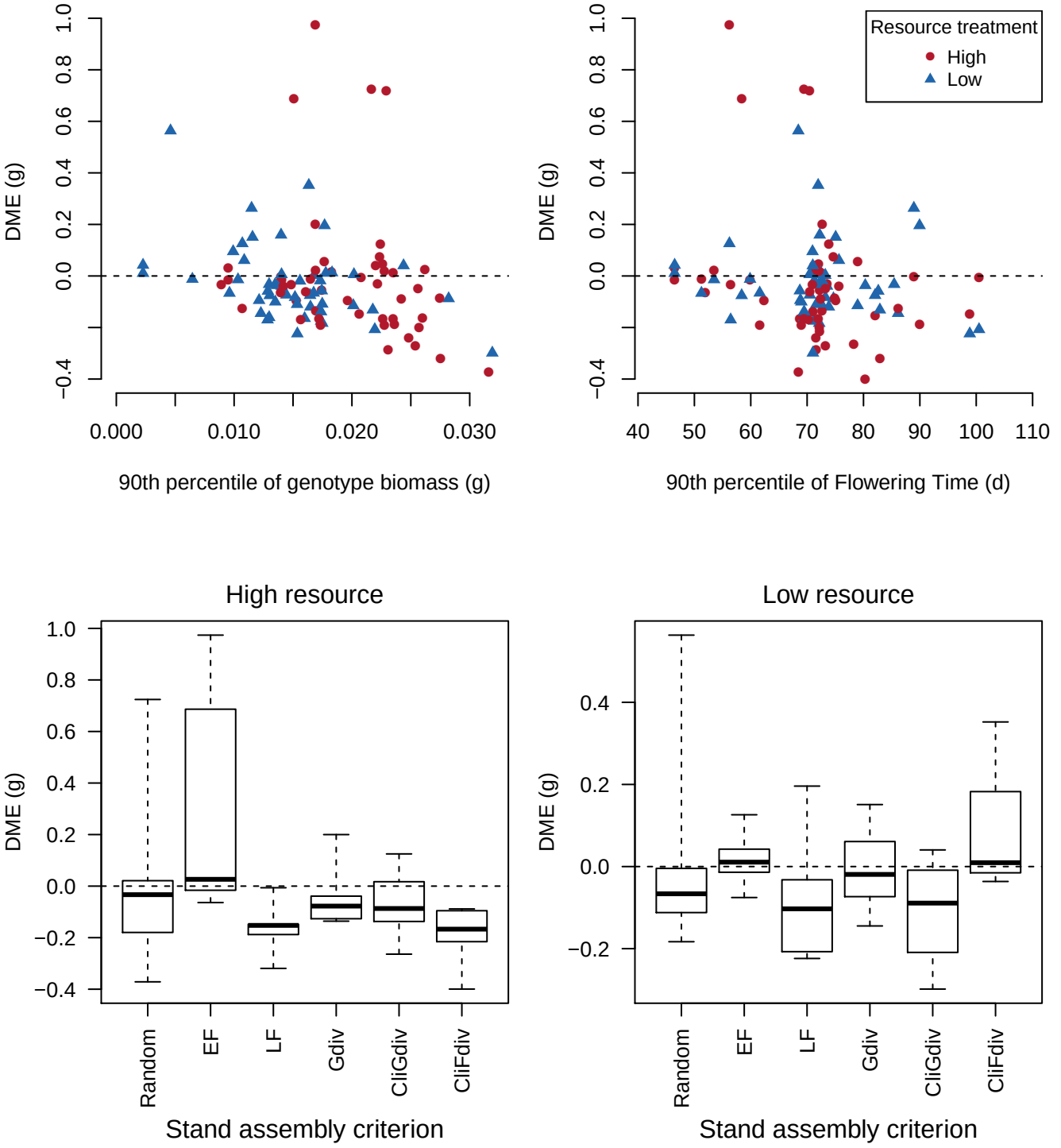
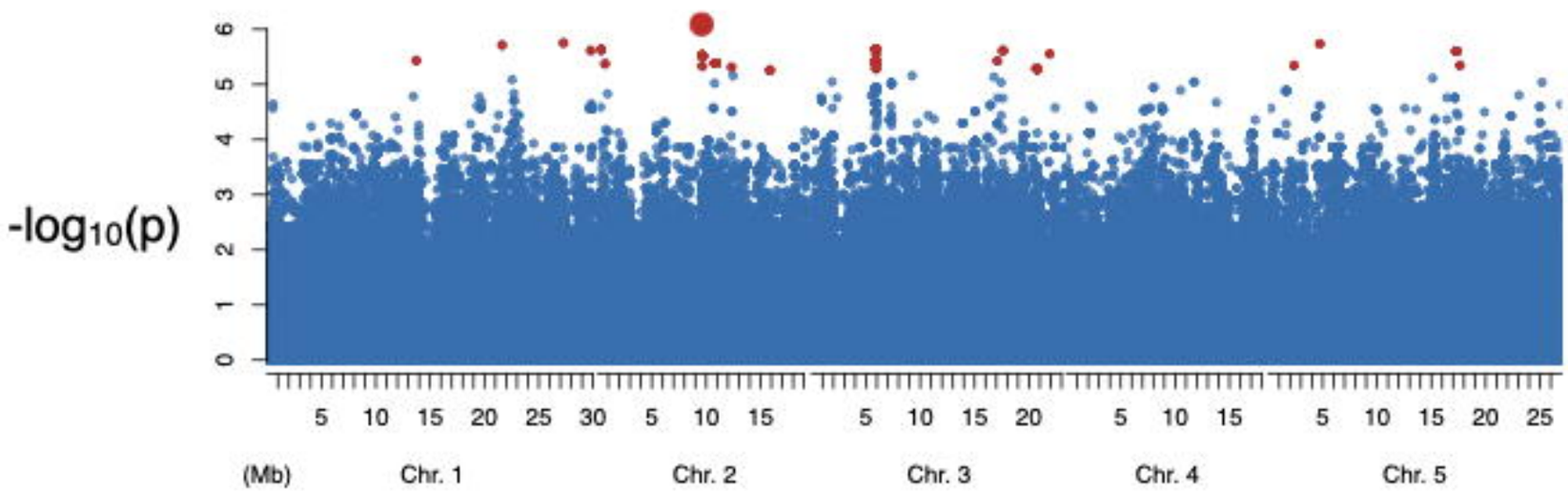
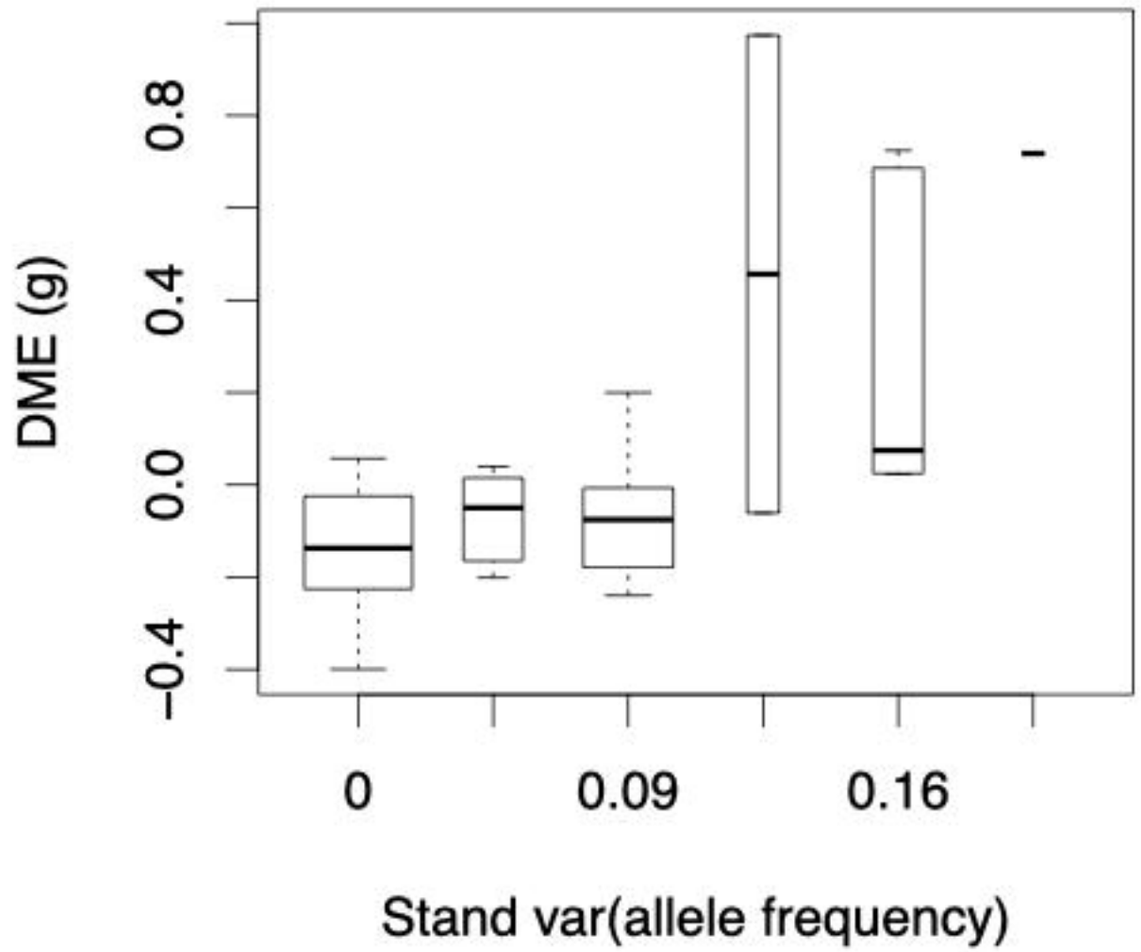

High resource
Low resource
AGB per plant (mg)
0
10
30
High
Low
Resources
AGB/plant (mg)
0.5
20
40


(a)
(b)
(c)
stand biomass at harvest (g)
0.00
0.02
0.04
0.06
40
60
80
100
flowering time mean (days)
-2.0
-1.5
-1.0
-0.5
SLA BV (ln (cm^2/mg))
mean total fruit length (mm)
0
200
400
600
0
5
10
15
20
no. of genotypes in stand
Resource treatment
High
Low


DME (g)
90th percentile of genotype biomass (g)
90th percentile of Flowering Time (d)
Resource treatment
High
Low
High resource
Low resource
Random
EF
LF
Gdiv
CliGdiv
CliFdiv
Stand assembly criterion

A. Var(allele freq.) associations with DME in high-resource



B. Top SNP for DME in high resource

Chr. 2, 9584630 bp