

# Trait plasticity and covariance along a continuous soil moisture gradient

J. Grey Monroe<sup>1,4</sup>, Haoran Cai<sup>2</sup>, & David L. Des Marais<sup>2,3</sup>

<sup>1</sup> Department of Plant Sciences, University of California at Davis, Davis, USA

<sup>2</sup> Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

<sup>3</sup> The Arnold Arboretum of Harvard University, Boston, MA, USA

<sup>4</sup> Max Planck Institute for Developmental Biology, Tübingen, DE

Water is perhaps the greatest environmental determinant of plant yield and fitness, yet our understanding of plant-water relations is limited because it is primarily informed by experiments that have treated soil moisture variability as two simple discrete levels - wet and dry - rather than a continually varying environmental gradient. Here we used experimental and statistical methods based on function-valued traits to study responses to continuously varying soil moisture gradient in a suite of physiological and morphological traits in two species and multiple genotypes of the model grass *Brachypodium*. We find that the majority of traits exhibit non-linear responses to soil moisture variability. We also observe differences in the shape of these non-linear responses between traits, species, and genotypes. Emergent phenomena arise from this variation including changes in trait correlations and evolutionary constraints as a function of soil moisture. These results point to the importance of considering non-linearity in plant-water relations to predict plastic and evolutionary responses to changing climates.

**Keywords:** drought, function-valued traits, *Brachypodium*, evolutionary constraint, phenotypic plasticity, non-linearity

## Introduction

For plants, soil moisture is one of the most important environmental factors in ecology and agriculture, acting as a major determinant of fitness and yield (Juenger 2013; Greenham *et al.* 2017). Considerable interest and effort have been placed on studying plant responses to drought, often defined conceptually and experimentally as an environmental condition of abnormally elevated aridity resulting in decreased plant performance (Passioura 1996). Most of the vast research on drought responses and tolerance strategies, including *Brachypodium*, the focal system of this work, has been limited to comparisons between discrete soil water levels - dry and water-limited (Des Marais *et al.* 2012; Edwards *et al.* 2012; El-Soda *et al.* 2014; Vasseur *et al.* 2014; Greenham *et al.* 2017). Yet soil moisture as an environmental factor is complex and multidimensional, with fluctuations varying continuously in timing, duration, and degree. Here we investigate trait responses to one important dimension of soil moisture variability - degree - with experimental and statistical approaches treating soil moisture content as a continuous variable rather than a set of fixed levels.

The limited extent to which continuous environmental vari-

ables have been studied is not unique to investigations of plant responses to soil moisture. Recent decades have seen increased appreciation for modeling traits as mathematical functions of continuous variables across diverse organisms (Pettay *et al.* 2008; Rocha and Klaczko 2012; Mason *et al.* 2020), a wide range of traits (Robinson *et al.* 2009; McGuigan *et al.* 2010; Stinchcombe *et al.* 2010), and numerous components of the environment (Brommer *et al.* 2008; McGuigan 2009; Pearse *et al.* 2019). This burgeoning interest in the importance of "function-valued traits" has evolved from a call-to-arms to a field experiencing rapid theoretical and empirical advances (Kirkpatrick and Heckman 1989; Kingsolver 2001; Griswold *et al.* 2008; Stinchcombe *et al.* 2012; Wilby 2015; Gomulkiewicz *et al.* 2018). For plant biologists this presents a compelling framework for gaining a deeper understanding of plant adaptations to the environment.

A deep body of literature aims to understand the traits and strategies used by plants to acclimate to variable soil water content. Quantitative genetic variation in myriad drought resistance traits have been observed in natural populations and laboratory model systems. In particular, natural populations of Brassicaceae species (including *Arabidopsis thaliana*) and *Brachypodium* harbor variation both constitutive and inducible traits mediating plant-water relations, including water use efficiency (Des Marais *et al.* 2012, 2017; Edwards *et al.* 2012; Greenham *et al.* 2017), leaf chemistry (Kesari *et al.* 2012; Des Marais *et al.* 2017), leaf anatomy (Skirycz *et al.* 2011; Verelst *et al.* 2013; Dittberner *et al.* 2018), root-shoot biomass partitioning (Des Marais *et al.* 2012, 2017),

Correspondence concerning this article should be addressed to J. Grey Monroe, Current address: 1 Max Planck Ring, 72076 Tübingen, DE. E-mail: greymonroe@gmail.com

and many others (Verslues and Juenger 2011; Edwards *et al.* 2012; Juenger 2013; Luo *et al.* 2016; Yarkhunova *et al.* 2016; Lenk *et al.* 2019). Among the numerous plant traits that can and have been measured, few have been studied more extensively than specific leaf area (SLA - often reported as it's inverse, leaf mass per area or LMA). SLA provides a description of leaf architecture, which is part of the leaf economics spectrum, a theory that links variation in leaf traits to more productive contexts of drought stress. In the context of drought stress, SLA is often considered adaptive as lower leaf surface area is expected to reduce water loss through transpiration. In several cases, reductions in SLA have been reported under drought conditions (Casper *et al.* 2001). For all of these traits, the shape of plastic responses to variation in water availability remain largely unmeasured.

Despite abundant evidence of natural selection in organism-environment interaction, little is known about how trait covariance changes across environmental gradients (but see Robinson *et al.* 2009). Trait variance and covariance structure is important as trait variances and covariances result in differential evolutionary constraints in different environments. For example, if total genetic variance in trait space changes depending on the environment, then the capacity to respond to selection will vary accordingly, with reduced responses to selection under conditions where trait variation is lower and vice versa. Similarly, if trait covariances depend on environment, conditions which increase trait covariances may limit evolutionary potential across a range of environments by reducing the effective axes of variation (Levins 1968; Via and Lande 1985; Kingsolver and Gomulkiewicz 2003; Gomulkiewicz *et al.* 2018). These phenomena are made more complex by the possibility that the relationship between trait variances and covariances with the environment may be non-linear. Investigating complexities of genetic architecture is therefore needed for developing more complete quantitative predictions of evolvability in rapidly changing environments. Fortunately, advances have been made in statistical methods to reduce the dimensionality of genetic variance covariance matrices and produce meaningful summaries describing evolutionary constraints (Houle 1992; Blows 2007; Kirkpatrick 2009; Kingsolver *et al.* 2015).

In this study we combined these approaches with function-valued trait analyses to investigate trait plasticity, covariance and evolutionary constraints across soil moisture gradients. We model trait responses to a continuous gradient of soil moisture for a range of genotypes of two species of the model grass genus *Brachypodium*. Because the shapes of trait responses cannot necessarily be known *a priori*, we use model selection among linear and non-linear environmental predictors to estimate the response function for each trait. We then estimate genotype means at different levels of soil moisture

and compute the variance-covariance parameters for all traits. Finally, we ask whether patterns of variance and covariance in drying-responsive traits in *Brachypodium* species may lead to variation in evolutionary constraint as a function of soil moisture.

## Materials and Methods

**Species and species.** *Brachypodium* is a model genus for studies of genetics and genomics of C3 grasses (Brkljacic *et al.* 2013). In this work we studied natural variation between accessions and among two species of *Brachypodium*: the annual *B. distachyon* and the perennial *B. sylvaticum*. Both species are endemic to Eurasia, with *B. distachyon* more prevalent in seasonally dry habitats in Southern Europe, North Africa, and the Middle East, and *B. sylvaticum* more widely distributed throughout Eurasia (Catalan *et al.* 2016) (Figure S1).

Five genotypes of each species were studied to characterize patterns of variation in plant traits across an environmental gradient: *Brachypodium distachyon* inbred lines ABR2, Adi-10, Bd21, Bd3-1, and Koz-1 and *Brachypodium sylvaticum* inbred lines Ain-1, Ast-1, Kry-1, Osl-1, Vel-1. For each species, these genotypes represent a range of geographical origins and phenotypic diversity (Steinwand *et al.* 2013; Des Marais *et al.* 2017). Both species are self-compatible and each of the lines used here have been maintained as inbred lines for greater than six generations (Vogel *et al.* 2009; Steinwand *et al.* 2013); as such, experimental replicates may be considered nearly homozygous.

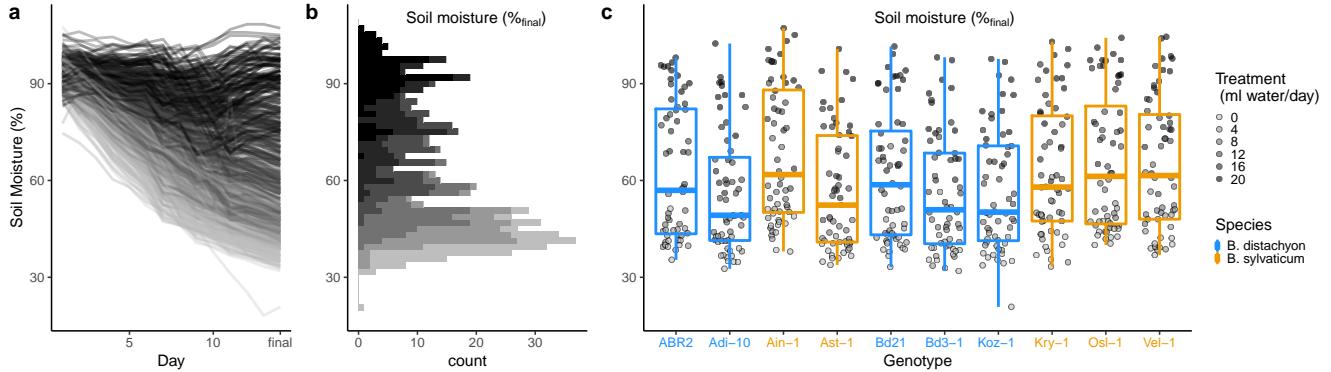
**Plant growth and dry down experiment.** Plant growth and experimental soil dry down were performed in the greenhouses of the Arnold Arboretum of Harvard University. To synchronize germination across genotypes within each species, seeds were placed on damp filter paper in the dark at 4°C for 14 days prior to planting. To synchronize the developmental stage at the timing of the drought treatments between the two species, *B. sylvaticum* seeds were planted thirteen days before *B. distachyon* (Oct 7 and 20, 2015, respectively). For each genotype, 1200 seeds were planted two to a pot and were subsequently thinned to one plant, for a total of 600 experimental plants in a randomized block design. All plants germinated within four days of sowing. Individual seeds of plants were sown in Greens Grade Profile porous ceramic rooting media (Profile Products, Buffalo Grove, IL, USA) in Deepot D40H Conetainers (650mL; Stuewe & Sons, Tangent, OR, USA) and grown at 25°C/20°C days/nights. Ambient sunlight was supplemented to 1000 umol/m<sup>2</sup>/s for 12hr/day.

Dry down treatments began 29 and 42 days after sowing (DAS) for *B. distachyon* and *B. sylvaticum*, respectively. Because the harvesting was divided over five consecutive days (see section below), plants were split into five equal harvest

Add something here  
about the onset of stress and  
why drought is a great  
candidate for FVT

More refs, here

Constraints is a  
bit loaded — maybe  
relate selection,  
here



**Figure 1. Effect of the experimental dry down on soil water content.** (A) Time series of gravimetric soil moisture for all pots during the 14-day dry down period. (B) Distribution of final (day 14) soil moisture content across all pots. The data are distinguished by color according to the watering treatment. (C) Final soil moisture content by genotype.

cohorts, with each cohort containing equal numbers of each watering treatment to avoid confounding harvest day with soil moisture content. Thus, though each consecutive cohort differed in age by a single day, each experienced the dry down treatment for the same amount of time. Nevertheless, we expected the difference in age between harvest cohorts to potentially impact trait expression and we therefore included harvest day (cohort) as a covariate in subsequent models. To generate a continuous gradient of final soil moisture by the end of the dry down period, plants were split into five watering treatments, receiving approximately 0, 4, 8, 12, 16, or 20 ml of water per day for 14 days. Prior to initiating the experiment pots were weighed with dry soil ( $mass_{dry}$ ) and field capacity ( $mass_{max}$ ) at the beginning of the experiment. These measurements provide the basis for calculating the gravimetric soil moisture content on each day and at the conclusion of the experiment. During the course of the dry down experiment, soil moisture content was calculated during the morning of day  $d$  for each pot as  $mass_d/(mass_{max} - mass_{dry})$ .

**Plant harvesting and phenotyping.** To characterize phenotypic responses to our experimental soil moisture gradient, we measured a suite of developmental and physiological traits. Plants were harvested in five cohorts over five days. Each day, half of the sampled plants were harvested for above and below ground biomass, total above ground green area,  $\delta C_{13}$ , N content, C content. The other half were harvested and assessed for specific leaf area (SLA) and relative water content (RWC). Above ground leaf area was estimated by laying freshly harvested plants flat between plates of clear plexiglass and imaging with a Nikon 5300 digital camera at a fixed distance with a 35mm Nikkor lens. Total green pixels were counted for each image with Easy Leaf Area (<https://github.com/heaslon/Easy-Leaf-Area>) with settings shown in Figure S3. Above ground biomass was measured after drying leaf material overnight at 60°C and then for several weeks at room temperature. Below ground

biomass was measured after washing the soil matrix from roots and drying them overnight at 60°C and then for several weeks at room temperature. Above and belowground biomass was measured after leaves and roots were dried. Leaf tissues for  $\delta C_{13}$ ,  $\delta N_{15}$ , nitrogen (hereafter “N”) content, and carbon (hereafter “C”) content were ground to a fine powder and processed by the UC Davis Stable Isotope Facility. SLA was calculated by scanning the two youngest fully emerged leaves with a 1 cm<sup>2</sup> red square. Leaf area in mm<sup>2</sup> was calculated from these same images using Easy Leaf Area. SLA was calculated as  $leaf\ area/biomass_{dry}$ . These leaves were also used to calculate RWC. Prior to drying, fresh leaves were weighed ( $biomass_{fresh}$ ) and then submerged under water in 15mL falcon tubes for several hours. They were then weighed ( $biomass_{turgid}$ ), oven-dried overnight, and weighed again ( $biomass_{dry}$ ). RWC was calculated as  $(biomass_{fresh} - biomass_{dry})/(biomass_{turgid} - biomass_{dry})$ .

## Analyses

We used R for all statistical analyses. Code and data to generate this manuscript can be found at [https://github.com/greymonroe/brachypodium\\_fvt](https://github.com/greymonroe/brachypodium_fvt).

**Function-valued traits.** For the purposes of modeling phenotypic responses to variation in soil moisture content, we considered soil moisture content as the final soil moisture on day 14 of the dry down period for each plant, referred to in figures as *Soil moisture(%final)*. A major challenge in studying function-valued traits is model selection. That is, identifying the functions that best describe the curvature (or lack thereof) in the shape of phenotypic responses to environmental gradients. Quadratic and natural splines have been suggested as potential functions to model non-linearities (Meyer 2005), but assuming the appropriate function is problematic. Akaike information criterion (AIC) selection based on contrasting multiple complex models offers an effective

Table 1

*Model selection.* \*\*\* = predictor variable  $p < 1e-5$ , \*\* = predictor variable  $p < 1e-3$ , \* = predictor variable  $p < 0.5$ , - = predictor variable included in selected model but  $p > 0.05$ . H = harvest day, S = species, G = genotype, E = final soil moisture,  $E^2$  = quadratic parameter,  $ns(E)$  = 2nd degree natural spline parameter

	G	E	$E^2$	$ns(E)$	H	$G*E$	$G*E^2$	$G*ns(E)$
RWC - B. distachyon				***	***	*		
RWC - B. sylvaticum	*				***	*		
SLA - B. distachyon	-		***	***	***			*
SLA - B. sylvaticum	***				-			
Green Area - B. distachyon	**				***	***		
Green Area - B. sylvaticum	***		*		**			-
Shoot Mass - B. distachyon	***		***			***		
Shoot Mass - B. sylvaticum	***		-			*		
Root Mass - B. distachyon	***		***		-	*		
Root Mass - B. sylvaticum	***							
Root:Shoot - B. distachyon	***			*		***		
Root:Shoot - B. sylvaticum	***		*			***		*
Biomass - B. distachyon	***			***	***			
Biomass - B. sylvaticum	***					*		
C content - B. distachyon				*				
C content - B. sylvaticum	*				-			
d13c - B. distachyon	***		***			***		
d13c - B. sylvaticum	***			**	***			
N content - B. distachyon	***		-			*		
N content - B. sylvaticum	***			***			**	
d15n - B. distachyon	**			**	***			
d15n - B. sylvaticum			***	-	**			
C:N ratio - B. distachyon	***		**			*		
C:N ratio - B. sylvaticum	***		***				*	

means to balance predictability with over-fitting (Griswold *et al.* 2008; Gomulkiewicz *et al.* 2018). Thus, we began with the complex model for traits as described below.

$$Trait = H + G + E + E^2 + ns(E)_{df=2} + G*E + G*E^2 + G*ns(E)_{df=2}$$

where  $H$  = harvest day,  $G$  = genotype,  $E$  = soil moisture,  $E^2$  = quadratic parameter,  $ns(E)_{df=2}$  = second degree natural spline parameter

We then selected a model for each trait using stepwise AIC model selection with the *stepAIC* function from the package *MASS* (Venables and Ripley 2002) in R with the “direction” parameter set to “both.” The two species were analyzed separately to avoid biases introduced by enforcing the same model on species with different sizes, developmental trajectories and evolutionary histories.

**Genetic correlations as a function of soil moisture content.** We calculated trait correlations at different levels of soil moisture to characterize how genetic correlations between

traits vary as a function of soil moisture content. Predicted genotypic means for each trait were calculated at 20 levels of soil moisture content (from 0.3 to 1.0 gravimetric water content) based on the model chosen by AIC (see above). Next at each level of soil moisture, pairwise Pearson correlation coefficients between genotype means were calculated within each species.

**Plasticity through multidimensional trait space.** We quantified total plasticity through multidimensional trait space as a function of soil moisture by scaling each trait to a mean of 0 and calculating distance matrix between genotype means at all soil moisture levels. We looked specifically at total plasticity between consecutive soil moisture levels for each genotype. At each level of soil moisture, we then compared the two species by T-tests. To visualize plasticity of each genotype through multivariate trait space further, we performed a principal component analysis from the matrix of scaled genotype trait means using the *prcomp* function in R.

**Analysis of evolutionary constraints among traits.** We calculated several statistics summarizing evolutionary constraint as described in Kirkpatrick (2009). First, for each species, we estimated the  $G$  matrix of genetic covariances between mean scaled genotype trait means at different levels of soil moisture. We then calculated, using the *prcomp* function in R, the eigenvalues of each mean standardized (trait values divided by mean)  $G$  matrix,  $\lambda_i$ . From these we then calculated the *number of effective dimensions*,  $n_D$ , equal to the sum of the eigenvalues divided by the largest eigenvalue:

$$n_D = \sum_{n=1}^N \lambda_i / \lambda_1$$

We also calculated the *maximum evolvability*,  $e_{max}$ , equal to the square root of the largest eigenvalue,  $\lambda_1$  (Houle 1992; Kirkpatrick 2009):

$$e_{max} = \sqrt{\lambda_1}$$

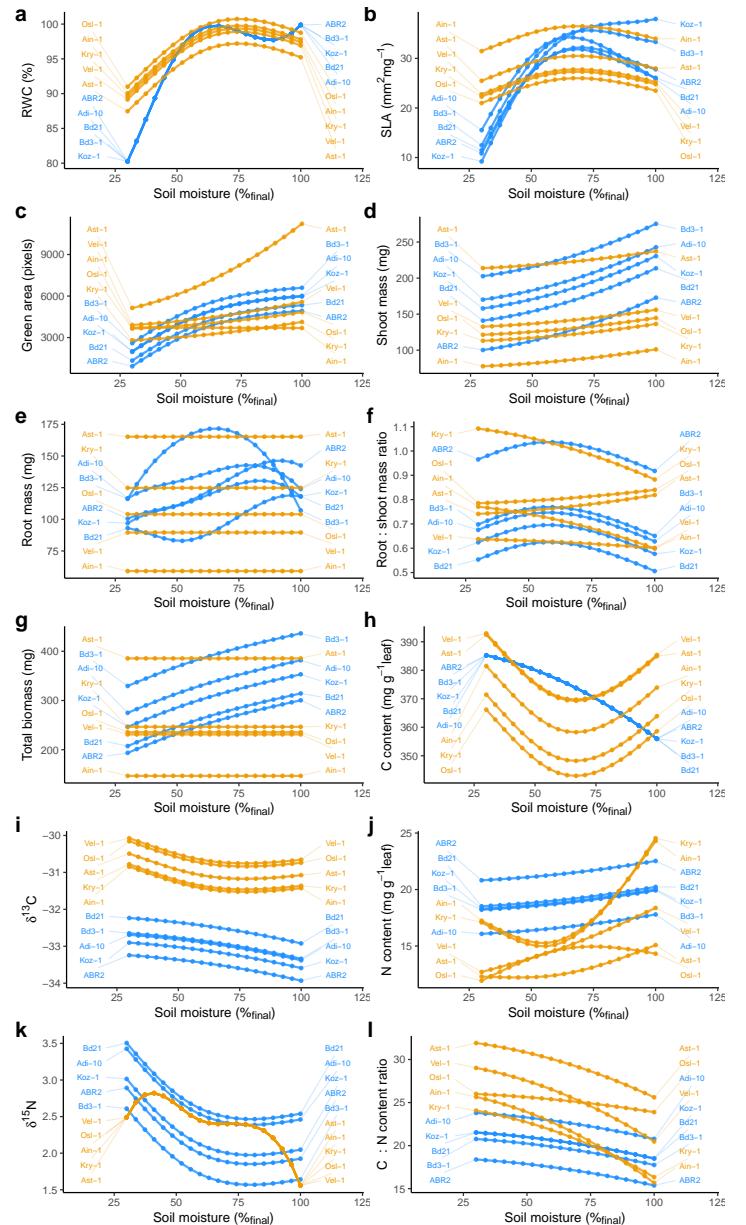
Finally, we calculated the *total genetic variance* (Kirkpatrick 2009), equal to the sum of the eigenvalues of  $G$ :

$$v_T = \sum_{n=1}^N \lambda_i$$

## Results

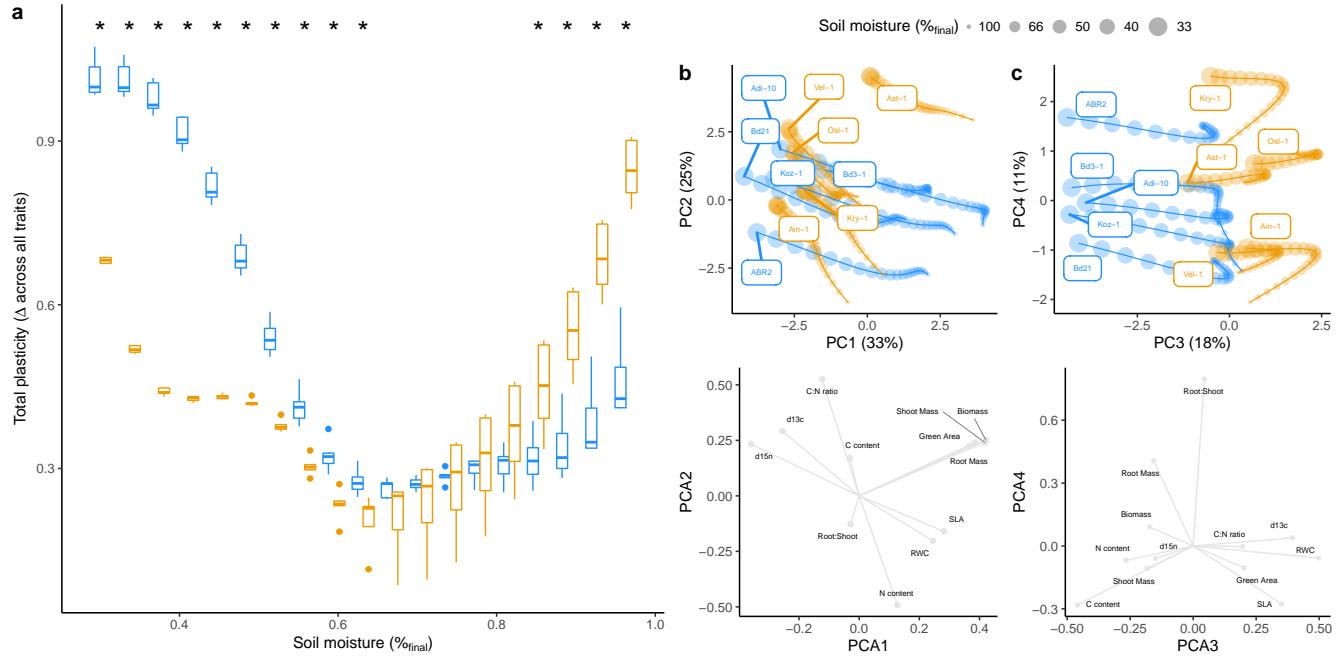
**The dry down experiment resulted in a continuous soil moisture gradient.** Across the six watering treatments, combined with random variation in water capacity of pots (Figure S4), the dry down period resulted in a continuous environmental gradient of final soil moisture, but with a higher frequency of plants near the driest extreme of soil moisture variation (Figure 1). This gradient provides the basis for analyzing phenotypes in relation to soil moisture treated as a continuous gradient rather than limited set of discrete factors.

The observed reduction in leaf relative water content under the driest conditions in both *B. distachyon* and *B. sylvaticum* indicates that at this extreme, plants were physiologically stressed (Figure 2A). Mean leaf RWC for plants in the 10% tail of soil water content was 85.21% which is drier than that observed in the dry treatment of Des Marais *et al.* (Des Marais *et al.* 2017). Additional observations made during the experiment such as leaf rolling, another symptom of dehydration stress, was evident in plants at the lowest water treatment by the end of the dry down period.



**Figure 2. Variation in phenotypic responses to soil moisture gradient as function-valued traits.** *B. sylvaticum* genotypes are colored orange and *B. distachyon* blue. (a) RWC (b) SLA (c) Green Area (d) Shoot Mass (e) Root Mass (f) Root:Shoot (g) Biomass (h) C content (i)  $\delta^{13}\text{C}$  (j) N content (k)  $\delta^{15}\text{N}$  (l) C:N ratio

**Non-linearity in trait responses to soil moisture is pervasive.** We evaluated the degree to which traits show linear or non-linear shapes using an AIC model selection approach from a full model which included quadratic and natural spline parameters relating soil moisture content to plant phenotypes. We observed significant ( $\alpha < 0.05$ ) non-linear components (quadratic, spline, or both) in the final models for all of the



**Figure 3. Plasticity through multivariate trait space.** (a) Plasticity across all traits was calculated as distance between scaled phenotype for each genotype between different levels of soil moisture. Box plots indicate species median and 25th and 75th percentiles with whiskers extending to 1.5 times the interquartile range. "\*" indicates significant differences between species (t-test,  $\alpha < 0.05$ ). Principal component analysis of scaled phenotypic responses to soil moisture gradient among genotypes of both species. Upper panels show genotype means across soil moisture content. Percent values in axis titles indicate percent variance explained by that principal component. Lower panels show eigenvectors of each trait. (b) PC1 and PC2. (c) PC3 and PC4.

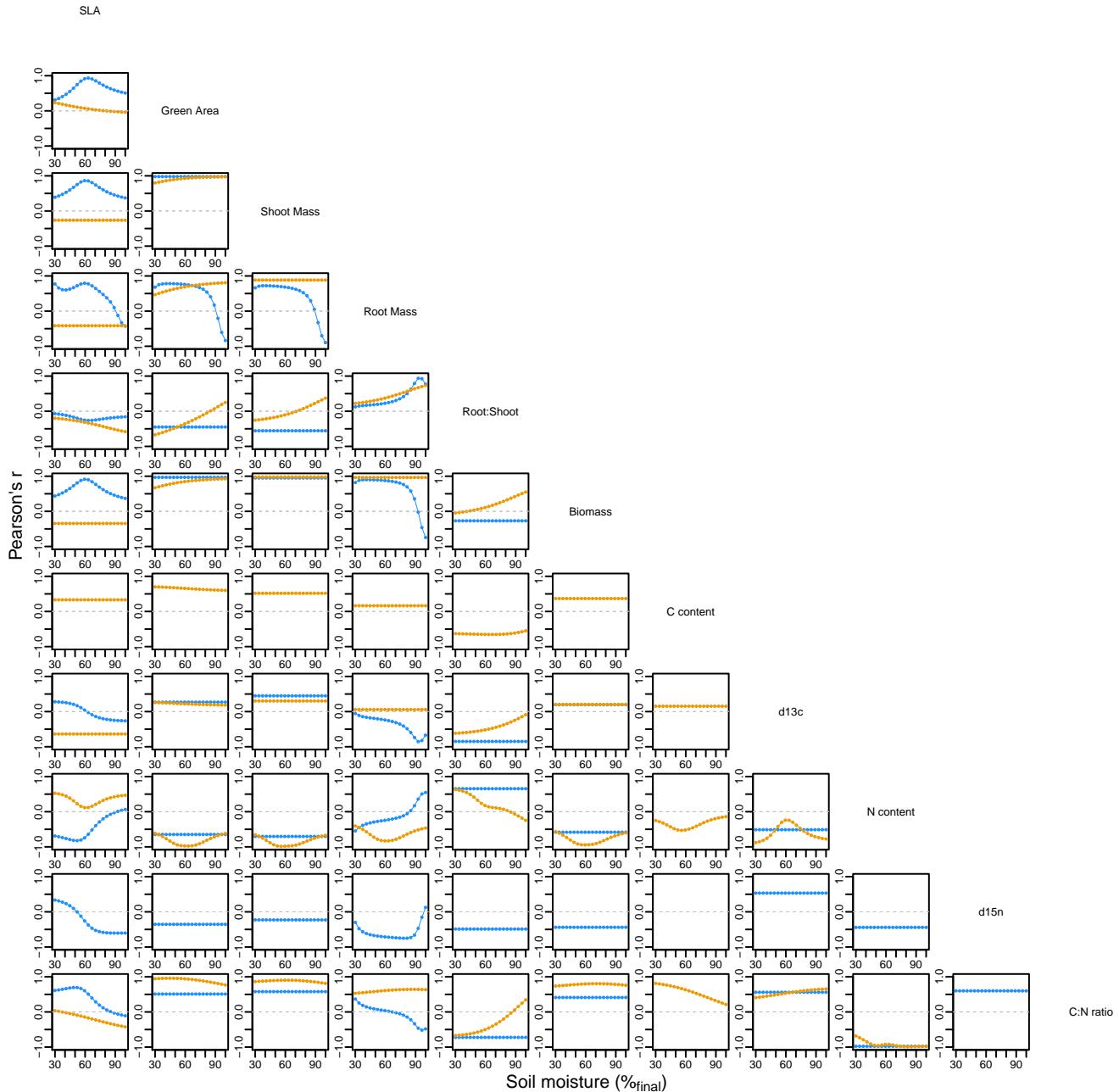
traits which included an environmental (water content) predictor (Table 1, Figure 2) except N content in *B. distachyon*, C content in *B. sylvaticum*, and shoot mass in *B. sylvaticum* where non-linear environmental predictors were included in the final models chosen by AIC but did not significantly explain trait variance ( $p > 0.05$ ). In *B. distachyon*, all of the traits included at least one non-linear environmental predictor. In contrast, SLA, total biomass and shoot mass did not include environmental predictor in selected models in *B. sylvaticum*. Interestingly, of all the traits which were predicted by environment in both *B. distachyon* and *B. sylvaticum*, the shape – whether a quadratic versus a spline function was included in the model – differed between the two species with the exception of carbon to nitrogen ratio.

When considering plasticity across multidimensional trait space (Figure 3), it appears that most of the variation is attributable to responses to low soil moisture *B. distachyon* which was significantly more responsive to low soil moisture values (Figure 3A). In contrast, *B. sylvaticum* was more responsive to extreme wet conditions than *B. distachyon*. Across PC2 and PC3, we observed, particularly in *B. sylvaticum*, that phenotypes were similar between extreme dry and extreme wet soil moisture contents. This similarity may be explained

by the quadratic parameters of trait functions where the curvature of trait responses leads to similar phenotypes at both environmental extremes.

**Nearly all traits show significant genetic variation.** We also tested whether there was significant natural variation for the traits measured between genotypes in each species by looking at the parameters in the final model for each trait. Interestingly, in across species, most traits (20/24) included a significant genotype term in the final model, indicating significant differences between genotypes in magnitude of traits across all levels of soil moisture (Table 1, Figure 2). Though not formally tested, for other traits there are clear distinctions between the two species. For example,  $\delta C_{13}$  was considerably higher in *B. sylvaticum* (Figure 2). For SLA, while *B. distachyon* showed a strong response to soil moisture, especially under the driest conditions, SLA in *B. sylvaticum* was not responsive to soil moisture. In contrast, *B. sylvaticum* appears to show a more dramatic response in leaf composition estimated by N content and C:N ratio.

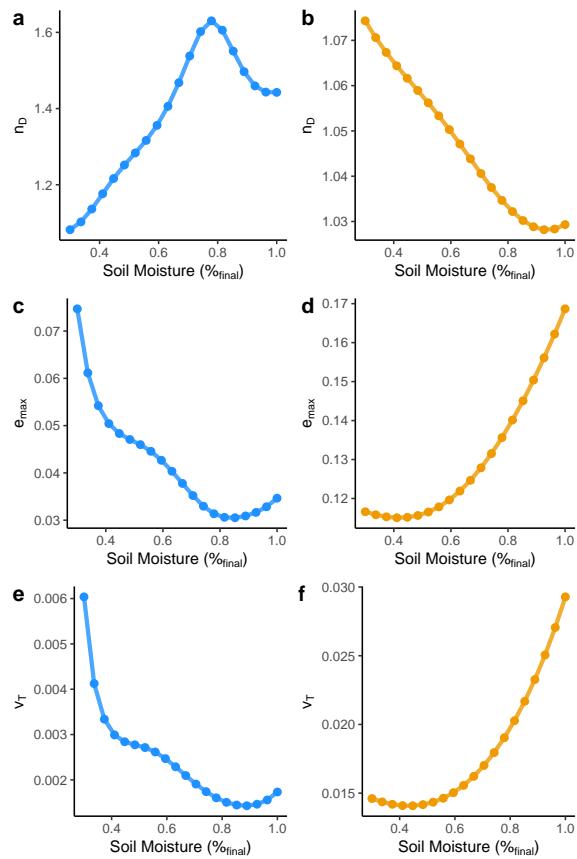
**Several traits show interactions between genotype and non-linear responses to the environment.** Significant interactions between genotype and environmental parameters in a final model indicate the presence of genetic variation



**Figure 4. Trait correlations as a function of soil moisture content.** Correlations were calculated among genotypes by species (blue = *B. distachyon*, orange = *B. sylvaticum*). Note that correlations are not shown for traits in species where genotype was not included in final model (Table 1).

for plasticity (GxE) (Via and Lande 1985). For those GxE interactions where the environmental parameter is non-linear, significant GxE indicates genetic variation for the shape of reaction norms. SLA showed a significant interaction between genotype and soil moisture in *B. distachyon*. In *B. sylvaticum*, nitrogen content, carbon to nitrogen ratio, and root to shoot ratio all showed significant interactions between genotype and

soil moisture (Table 1, Figure 2). In each of these cases, the interaction between genotype and environment involved a non-linear environmental predictor, indicating not only variation for the magnitude of plasticity (i.e. slope) but also variation in the shape of responses.



**Figure 5. Contrasting patterns of evolutionary constraint between *B. distachyon* and *B. sylvaticum*.** Summary statistics of evolutionary constraint as a function of soil moisture in *B. distachyon* (blue) and *B. sylvaticum* (orange). (a and b) The number of effective dimensions,  $n_D$ , estimates number of unconstrained axes of variation (c and d) The maximum evolvability,  $e_{max}$ , corresponds to the square root of the largest eigenvalue of the genetic covariance matrix. (e and f) The total genetic variance,  $V_T$ , is equal to the sum of the eigenvalues of the genetic covariance matrix.

**Correlations between traits change as a function of soil moisture, often in a non-linear fashion.** We examined correlations between genotype trait means across soil moisture for traits where genotypic differences were observed (i.e. genotype predictor in trait models). Certain traits were strongly correlated regardless of environment. For example, correlations near 1 were observed between biomass and green area in both species across soil moisture. More complex relationships between trait correlations and soil moisture are observed in other trait combinations. For traits with genotype by non-linear environment interactions (Table 1), trait correlations showed non-linear relationships with soil moisture as well. Because more of these interactions were found in *B. sylvaticum* the number of trait combinations showing non-linear

relations between correlation and soil moisture appears to be higher than in *B. distachyon*. In some cases, the relationship between soil moisture and correlation was dramatic. The correlation coefficient and root:shoot ratio in *B. sylvaticum*, for example, were approximately 0.3 under the wettest environment and approximately -0.7 under the driest environment.

Say why this matters  
in order to set up next section

**Evolutionary constraints differ as a function of soil moisture and show contrasting patterns between *Brachypodium* species.** To assess evidence of evolutionary constraint on the sampled traits, we estimated and analyzed parameters of the genetic covariance matrix,  $G$ , in each species across the soil moisture gradient. These analyses revealed contrasting patterns of evolutionary constraint both in relation to soil moisture and between *B. distachyon* and *B. sylvaticum*. In *B. distachyon*, the number of effective dimensions ( $n_D$ , which estimates number of axes of variation unconstrained by covariance) was lower when soils were drier. In contrast,  $n_D$  was lower in *B. sylvaticum* when soils were wetter. The maximum evolvability ( $e_{max}$ , variance through largest eigenvector of multidimensional trait space) also showed opposite trends between the two species. Whereas in *B. distachyon*  $e_{max}$  was highest under the driest conditions, in *B. sylvaticum*  $e_{max}$  was highest under the wettest conditions. The same trend was seen in total genetic variance ( $V_T$ , which summarizes all genetic variance through multidimensional trait space). These results indicate that *B. distachyon* has increased genetic variance under dry conditions as compared to wet conditions, but that natural selection may be more constrained to act on this variation due to covariance between traits. In contrast, our results suggest that *B. sylvaticum* has decreased genetic variance under dry conditions on which selection might act but that this variation is less constrained by covariance between traits.

## Discussion

Environmental conditions vary along continuous gradients in space, time, and degree. Natural populations of plants may therefore be exposed to a range of values for any particular dimension of the environment. The efficacy of natural selection to shape evolutionary response of populations depends on the magnitude of genetic variation in response to these environmental gradients and on the genetic correlation between traits as a function of the environment. Here, we explicitly model trait variation in two plant species as a continuous function of soil water availability and consider how genetic variance and co-variance in these functional responses may affect the evolution of plant-environment interaction.

**Non-linearity in soil moisture response is pervasive in *Brachypodium*.** We found significant non-linearity in response to a soil moisture gradient for all measured traits in at least one of the two species sampled. The best-fit function

for some traits was quadratic, while other traits showed more complex responses to the environment which were best fit by a spline function. These results offer new insights with respect to the study of plant response to soil drying. By focusing on the curvature of phenotypic response as the explicitly modeled trait, we avoid contrasts of trait values expressed at arbitrary levels of soil water content which may obscure different thresholds of response among the diverse genotypes under study. SLA in *B. distachyon* exhibits this pattern, as two accessions show a threshold-like response in decreasing SLA as soils become drier, and three accessions express their highest SLA at intermediate SWC. Leaf N content (on a leaf-mass basis) in *B. sylvaticum* likewise shows considerable diversity of response with two accessions expressing their lowest values at intermediate SWC, one accession expressing its highest Leaf N at intermediate SWC, and one accession showing a nearly linear response along the SWC gradient. Such nonlinearities in trait responses to soil moisture reinforce the need to consider the severity of extreme weather events when predicting plastic responses, especially when scaling up to investigating the ecosystem consequences of plant responses to environmental stress (Felton and Smith 2017).

**Implications for evolution of *Brachypodium*.** Leaf N and SLA are two axes of the classic Leaf Economic Spectrum (Wright *et al.* 2004) and so the contrasting responses of these traits between the annual *B. distachyon* and perennial *B. sylvaticum* may reflect broader differences in their life history strategies. We recently reviewed evidence for physiological, anatomical and developmental differences between herbaceous annual and perennial species, finding support for generally higher SLA in annuals, befitting a generally resource-acquisitive strategy (Lundgren and Des Marais). Garnier (Garnier 1992) argued that small changes in leaf anatomy (e.g. SLA) will likely have large effects on plant growth rate and resource use and could therefore tip the balance between perennial and annual strategies. We also found that signatures of evolutionary constraint differ along our imposed soil water content gradient. Specifically, we find evidence of highest evolvability in multitrait space (measured by  $e_{max}$  and  $v_T$ ) in *B. distachyon* under the driest soils. In contrast, *B. sylvaticum* exhibited evidence of greater evolvability by the same measures under the highest soil water content studied here. We speculate that the pattern observed could be a reflection of the different life history strategies of these two species. Annuality is considered a drought adaptive strategy characterized by escaping drought through phenology, by flowering before and remaining dormant as seeds during the most drought prone seasons (Friedman and Rubin 2015; Monroe *et al.* 2019). Thus, because of their life history, populations of annuals may actually experience fewer episodes of strong selection from extreme drought, which could explain why we find elevated genetic variance under these environments. In contrast, perennials such as *B. sylvaticum* are subjected to all seasons and

might therefore, experience more frequent episodes of selection caused by dehydration stress, despite paradoxically being found in environments where droughts are less frequent on an annual basis. This pattern is consistent with the predictions of cryptic genetic variation revealed under environments where selection is less frequent or severe (Schlichting 2008).

**Implications for breeding drought adaptation.** We found that genetic correlations between traits can vary dramatically even over relatively small changes in soil moisture (Fig. 4). Responses to selection for drought tolerance may therefore depend on drought severity because of these patterns in genetic correlations. In the context of breeding, exploratory studies such as this may be valuable for identifying conditions for which genetic correlations are aligned with breeding objectives. Similarly, we found that signatures of evolutionary constraint varied across the environmental gradient, suggesting that responses to selection may be improved or restricted in accordance with patterns of constraint in relation to environment. Interestingly, we also found that some species may be more responsive to selection in a given environment based on patterns of constraint.

From a practical perspective this work highlights the value of function-valued trait approaches that may be extended to studying plant-water relations in agricultural settings. In this experiment, we investigated variation in plant responses to a gradient of soil moisture using six watering levels, which in combination with random variation in water capacity of experimental pots, produced a continuous gradient of soil moisture ranging from field capacity of the soil to strongly water-limited. In the field, multiple watering regimes in combination with random variability between plots may produce similar gradients of soil moisture. Here, water content was measured gravimetrically. New sensing technologies may be useful for quantifying soil moisture in an analogous fashion, to define soil moisture quantitatively and then apply function-valued statistical approaches to contrast trait expression among genotypes. Finally, while in this experiment we used destructive phenotyping methods to measure traits, non-destructive (and high throughput) phenotyping will enable measurement of yield or fitness data as well, to examine explicit connections between trait variation and adaptation to different degrees of soil moisture.

## Acknowledgements

We thank Chase Mason and Eric Goolsby for insightful conversations about function-valued trait approaches. This work was supported by an Eco-Evo-Devo Network training grant, USDA-NIFA Award 2014-38420-21801, and Max Planck Society support for JGM.

### Contributions

JGM and DD funded, planned, and conducted the experiment.  
JGM, HC, and DD contributed to analyses and writing.

- Blows M. W., 2007 A tale of two matrices: Multivariate approaches in evolutionary biology. *Journal of evolutionary biology* 20: 1–8.
- Brkljacic J., E. Grotewold, R. Scholl, T. Mockler, and D. F. Garvin *et al.*, 2011 Brachypodium as a model for the grasses: Today and the future. *Plant Physiology* 157: 3–13.
- Brommer J. E., K. Rattiste, and A. J. Wilson, 2008 Exploring plasticity in the wild: Laying date–temperature reaction norms in the common gull larus canus. *Proceedings of the Royal Society B: Biological Sciences* 275: 687–693.
- Casper B., I. Forseth, H. Kempenich, S. Seltzer, and K. Xavier, 2001 Drought prolongs leaf life span in the herbaceous desert perennial cryptantha flava. *Functional Ecology* 15: 740–747.
- Catalan P., D. Lopez-Alvarez, A. Diaz-Perez, R. Sancho, and M. L. Lopez-Herranz, 2016 Phylogeny and evolution of the genus brachypodium, in *Genetics and genomics of brachypodium*, Plant genetics and genomics: Crop models. edited by Vogel J. Springer International.
- Des Marais D. L., J. K. McKay, J. H. Richards, S. Sen, and T. Wayne *et al.*, 2012 Physiological genomics of response to soil drying in diverse arabidopsis accessions. *The Plant Cell* 24: 893–914.
- Des Marais D. L., J. R. Lasky, P. E. Verslues, T. Z. Chang, and T. E. Juenger, 2017 Interactive effects of water limitation and elevated temperature on the physiology, development and fitness of diverse accessions of brachypodium distachyon. *New Phytologist* 214: 132–144.
- Dittberner H., A. Korte, T. Mettler-Altmann, A. P. Weber, and G. Monroe *et al.*, 2018 Natural variation in stomata size contributes to the local adaptation of water-use efficiency in arabidopsis thaliana. *Molecular ecology* 27: 4052–4065.
- Edwards C. E., B. E. Ewers, C. R. McClung, P. Lou, and C. Weinig, 2012 Quantitative variation in water-use efficiency across water regimes and its relationship with circadian, vegetative, reproductive, and leaf gas-exchange traits. *Molecular Plant* 5: 653–668.
- El-Soda M., M. P. Boer, H. Bagheri, C. J. Hanhart, and M. Koornneef *et al.*, 2014 Genotype–environment interactions affecting preflowering physiological and morphological traits of brassica rapa grown in two watering regimes. *Journal of experimental botany* 65: 697–708.

- Felton A. J., and M. D. Smith, 2017 Integrating plant ecological responses to climate extremes from individual to ecosystem levels. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372: 20160142.
- Friedman J., and M. J. Rubin, 2015 All in good time: Understanding annual and perennial strategies in plants. *American journal of botany* 102: 497–499.
- Garnier E., 1992 Growth analysis of congeneric annual and perennial grass species. *Journal of Ecology* 80: 665–675.
- Gomulkiewicz R., J. G. Kingsolver, P. A. Carter, and N. Heckman, 2018 Variation and evolution of function-valued traits. *Annual Review of Ecology, Evolution, and Systematics* 49: 139–164.
- Goolsby E. W., 2015 Phylogenetic comparative methods for evaluating the evolutionary history of function-valued traits. *Systematic biology* 64: 568–578.
- Greenham K., C. R. Guadagno, M. A. Gehan, T. C. Mockler, and C. Weinig *et al.*, 2017 Temporal network analysis identifies early physiological and transcriptomic indicators of mild drought in brassica rapa. *Elife* 6: e29655.
- Griswold C. K., R. Gomulkiewicz, and N. Heckman, 2008 Hypothesis testing in comparative and experimental studies of function-valued traits. *Evolution* 62: 1229–1242.
- Houle D., 1992 Comparing evolvability and variability of quantitative traits. *Genetics* 130: 195–204.
- Juenger T. E., 2013 Natural variation and genetic constraints on drought tolerance. *Current opinion in plant biology* 16: 274–281.
- Kesari R., J. R. Lasky, J. G. Villamor, Des MaraisD. L., and Y.-J. C. Chen *et al.*, 2012 Intron-mediated alternative splicing of arabidopsis p5cs1 and its association with natural variation in proline and climate adaptation. *Proceedings of the National Academy of Sciences* 109: 9197–9202.
- Kingsolver J. G., R. Gomulkiewicz, and P. A. Carter, 2001 Variation, selection and evolution of function-valued traits, pp. 87–104 in *Microevolution rate, pattern, process*, Springer.
- Kingsolver J. G., and R. Gomulkiewicz, 2003 Environmental variation and selection on performance curves. *Integrative and Comparative Biology* 43: 470–477.
- Kingsolver J. G., N. Heckman, J. Zhang, P. A. Carter, and J. L. Knies *et al.*, 2015 Genetic variation, simplicity, and evolutionary constraints for function-valued traits. *The American Naturalist* 185: E166–E181.
- Kirkpatrick M., and N. Heckman, 1989 A quantitative genetic model for growth, shape, reaction norms, and other infinite-dimensional characters. *Journal of mathematical biology* 27: 429–450.
- Kirkpatrick M., 2009 Patterns of quantitative genetic variation in multiple dimensions. *Genetica* 136: 271–284.
- Lenk I., L. H. C. Fisher, M. Vickers, A. Akinyemi, and T. Didion *et al.*, 2019 Transcriptional and metabolomic analyses indicate that cell wall properties are associated with drought tolerance in brachypodium distachyon. *Int J Mol Sci* 20. <https://doi.org/10.3390/ijms20071758>
- Levins R., 1968 *Evolution in changing environments: Some theoretical explorations*. Princeton University Press.
- Lundgren M. R., and Des MaraisD. L., Life history variation as a model for understanding trade-offs in plant-environment interactions. *Current Biology*.
- Luo N., X. Yu, G. Nie, J. Liu, and Y. Jiang, 2016 Specific peroxidases differentiate brachypodium distachyon accessions and are associated with drought tolerance traits. *Ann Bot* 118: 259–70. <https://doi.org/10.1093/aob/mcw104>
- Mason C. M., M. C. LaScaleia, De La PascuaD. R., J. G. Monroe, and E. W. Goolsby, 2020 Learning from dynamic traits: Seasonal shifts yield insights into ecophysiological trade-offs across scales from macroevolutionary to intraindividual. *International Journal of Plant Sciences* 181: 88–102.
- McGuigan K., 2009 Condition dependence varies with mating success in male drosophila bunnanda. *Journal of evolutionary biology* 22: 1813–1825.
- McGuigan K., N. Nishimura, M. Currey, D. Hurwit, and W. A. Cresko, 2010 Quantitative genetic variation in static allometry in the threespine stickleback. *Integrative and comparative biology* 50: 1067–1080.
- Meyer K., 2005 Random regression analyses using b-splines to model growth of australian angus cattle. *Genet. Sel. Evol.* 37: 473–500.
- Monroe J. G., B. Gill, K. G. Turner, and J. K. McKay, 2019 Drought regimens predict life history strategies in heliophila. *New Phytologist* 223: 2054–2062.
- Passioura J., 1996 Drought and drought tolerance. *Plant growth regulation* 20: 79–83.
- Pearse I. S., J. M. Aguilar, and S. Y. Strauss, 2019 Life history plasticity and water use trade-offs associated with

- drought resistance in a clade of California jewelflowers
- Pettay J. E., A. Charmantier, A. J. Wilson, and V. Lummaa, 2008 Age-specific genetic and maternal effects in fecundity of preindustrial Finnish women. *Evolution: International Journal of Organic Evolution* 62: 2297–2304.
- Robinson M. R., A. J. Wilson, J. G. Pilkington, T. H. Clutton-Brock, and J. M. Pemberton *et al.*, 2009 The impact of environmental heterogeneity on genetic architecture in a wild population of soay sheep. *Genetics* 181: 1639–1648.
- Rocha F. B., and L. B. Klaczko, 2012 Connecting the dots of nonlinear reaction norms unravels the threads of genotype–environment interaction in *Drosophila*. *Evolution: International Journal of Organic Evolution* 66: 3404–3416.
- Schlüchting C. D., 2008 Hidden reaction norms, cryptic genetic variation, and evolvability. *Annals of the New York Academy of Sciences* 1133: 187–203.
- Skirycz A., H. Claeys, De Bodt S., A. Oikawa, and S. Shinoda *et al.*, 2011 Pause-and-stop: The effects of osmotic stress on cell proliferation during early leaf development in *Arabidopsis* and a role for ethylene signaling in cell cycle arrest. *Plant Cell* 23: 1876–88. <https://doi.org/10.1105/tpc.111.084160>
- Steinwand M. A., H. A. Young, J. N. Bragg, C. M. Tobias, and J. P. Vogel, 2013 *Brachypodium sylvaticum*, a model for perennial grasses: Transformation and inbred line development. *PLoS One* 8: e75180. <https://doi.org/10.1371/journal.pone.0075180>
- Stinchcombe J. R., R. Izem, M. S. Heschel, B. V. McGoey, and J. Schmitt, 2010 Across-environment genetic correlations and the frequency of selective environments shape the evolutionary dynamics of growth rate in *Impatiens capensis*. *Evolution: International Journal of Organic Evolution* 64: 2887–2903.
- Stinchcombe J. R., M. Kirkpatrick, F.-v. T. W. Group, and others, 2012 Genetics and evolution of function-valued traits: Understanding environmentally responsive phenotypes. *Trends in Ecology & Evolution* 27: 637–647.
- Vasseur F., T. Bontpart, M. Dauzat, C. Granier, and D. Vile, 2014 Multivariate genetic analysis of plant responses to water deficit and high temperature revealed contrasting adaptive strategies. *Journal of Experimental Botany* 65: 6457–6469.
- Venables W. N., and B. D. Ripley, 2002 *Modern applied statistics with s*. Springer, New York.
- Verelst W., E. Bertolini, De Bodt S., K. Vandepoele, and M. Demeulenaere *et al.*, 2013 Molecular and physiological analysis of growth-limiting drought stress in *Brachypodium distachyon* leaves. *Mol Plant* 6: 311–22. <https://doi.org/10.1093/mp/sss098>
- Verslues P. E., and T. E. Juenger, 2011 Drought, metabolites, and *Arabidopsis* natural variation: A promising combination for understanding adaptation to water-limited environments. *Current Opinion in Plant Biology* 14: 240–245.
- Via S., and R. Lande, 1985 Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39: 505–522.
- Vogel J. P., M. Tuna, H. Budak, N. Huo, and Y. Q. Gu *et al.*, 2009 Development of SSR markers and analysis of diversity in Turkish populations of *Brachypodium distachyon*. *BMC Plant Biology* 9: 88.
- Wright I. J., P. B. Reich, M. Westoby, D. D. Ackerly, and Z. Baruch *et al.*, 2004 The worldwide leaf economics spectrum. *Nature* 428: 821.
- Yarkhunova Y., C. E. Edwards, B. E. Ewers, R. L. Baker, and T. L. Aston *et al.*, 2016 Selection during crop diversification involves correlated evolution of the circadian clock and ecophysiological traits in *Brassica rapa*. *New Phytologist* 210: 133–144.

**Supplement**

**Table S1**  
*final models*

	Df	Sum.Sq	Mean.Sq	Fvalue	Pr.F.	trait
I(Day_14^2)	1.00000	1,749.17374	1,749.17374	113.13775	0.00000	Relative_WC_b_dist
ns(Day_14,df=2)	2.00000	1,875.17736	937.88868	60.64387	0.00000	Relative_WC_b_dist
Harv	4.00000	205.30059	51.32515	3.31975	0.01248	Relative_WC_b_dist
Residuals	137.00000	2,118.09770	15.46057	NA	NA	Relative_WC_b_dist
Geno	4.00000	323.29367	80.82342	3.39597	0.01138	Relative_WC_b_sylv
ns(Day_14,df=2)1	2.00000	671.68506	335.84253	14.11116	0.00000	Relative_WC_b_sylv
Harv1	4.00000	268.15302	67.03826	2.81676	0.02817	Relative_WC_b_sylv
Residuals1	121.00000	2,879.77449	23.79979	NA	NA	Relative_WC_b_sylv
Geno1	4.00000	140.92870	35.23217	1.75884	0.14122	SLA_b_dist
I(Day_14^2)1	1.00000	2,414.64758	2,414.64758	120.54272	0.00000	SLA_b_dist
ns(Day_14,df=2)2	2.00000	2,327.88690	1,163.94345	58.10575	0.00000	SLA_b_dist
Harv2	4.00000	651.79297	162.94824	8.13461	0.00001	SLA_b_dist
Geno(Day_14^2)	4.00000	337.42496	84.35624	4.21119	0.00310	SLA_b_dist
Residuals2	127.00000	2,543.99629	20.03147	NA	NA	SLA_b_dist
Geno2	4.00000	1,572.00031	393.00008	12.05355	0.00000	SLA_b_sylv
ns(Day_14,df=2)3	2.00000	162.19048	81.09524	2.48724	0.08735	SLA_b_sylv
Residuals3	122.00000	3,977.75094	32.60452	NA	NA	SLA_b_sylv
Geno3	4.00000	32,376.338.95160	8,094.084.73790	5.44806	0.00043	aboveground_greenarea_b_dist
ns(Day_14,df=2)4	2.00000	192,517,942.01652	96,258,971.00826	64.79115	0.00000	aboveground_greenarea_b_dist
Harv3	4.00000	59,381,207.50609	14,845.301.87652	9.99225	0.00000	aboveground_greenarea_b_dist
Residuals4	134.00000	199,081,240.28441	1,485,680.89764	NA	NA	aboveground_greenarea_b_dist
Geno4	4.00000	219,846,397.86896	54,961,599.46724	15.60576	0.00000	aboveground_greenarea_b_dist
I(Day_14^2)2	1.00000	24,060,407.68683	24,060,407.68683	6,831.70	0.01005	aboveground_greenarea_b_sylv
Harv4	4.00000	77,050,113,87350	19,262,528.46838	5.46939	0.00043	aboveground_greenarea_b_sylv
Geno(Day_14^2)1	4.00000	33,700,623,60514	8,245,155,90129	2.39223	0.05412	aboveground_greenarea_b_sylv
Residuals5	126.00000	443,756,646,65128	3,521,878,14803	NA	NA	aboveground_greenarea_b_sylv
Geno5	4.00000	151,815,09343	37,953,77336	17.16469	0.00000	Shoot_Mass_b_dist
I(Day_14^2)3	1.00000	66,562,14458	66,562,14458	30.10291	0.00000	Shoot_Mass_b_dist
Harv5	4.00000	102,805,02689	25,701,25672	11.62346	0.00000	Shoot_Mass_b_dist
Residuals6	136.00000	300,716,87291	2,211,15348	NA	NA	Shoot_Mass_b_dist
Geno6	4.00000	282,513,50822	70,628,37705	24.41054	0.00000	Shoot_Mass_b_sylv
I(Day_14^2)4	1.00000	6,122,54739	6,122,54739	2.11607	0.14821	Shoot_Mass_b_sylv
Harv6	4.00000	50,339,74373	12,584,93593	4.34960	0.00248	Shoot_Mass_b_sylv
Residuals7	128.00000	370,349,46472	2,893,35519	NA	NA	Shoot_Mass_b_sylv
Geno7	4.00000	52,681,25005	13,170,31251	23.75364	0.00000	Root_Mass_b_dist
I(Day_14^2)5	1.00000	13,857,56690	13,857,56690	24,99316	0.00000	Root_Mass_b_dist
ns(Day_14,df=2)5	2.00000	2,853,05835	1,426,52918	2.57285	0.08032	Root_Mass_b_dist
Harv7	4.00000	6,739,85641	1,684,96410	3.03896	0.01976	Root_Mass_b_dist
Genons(Day_14,df=2)	8.00000	8,723,71652	1,090,46457	1.96673	0.05586	Root_Mass_b_dist
Residuals8	126.00000	69,861,25916	554,45444	NA	NA	Root_Mass_b_dist
Geno8	4.00000	176,944,42509	44,236,10627	24.22780	0.00000	Root_Mass_b_sylv
Residuals9	133.00000	242,836,80767	1,825,84066	NA	NA	Root_Mass_b_sylv
Geno9	4.00000	2,81680	0.70420	36.70369	0.00000	Shoot_Root_Ratio_b_dist
ns(Day_14,df=2)6	2.00000	0.15610	0.07805	4.06809	0.01925	Shoot_Root_Ratio_b_dist
Harv8	4.00000	1,14490	0.28623	14,91840	0.00000	Shoot_Root_Ratio_b_dist
Residuals10	135.00000	2,59012	0.01919	NA	NA	Shoot_Root_Ratio_b_dist
Geno10	4.00000	2,15066	0.53767	58.14315	0.00000	Shoot_Root_Ratio_b_sylv
I(Day_14^2)6	1.00000	0.04624	0.04624	5.00027	0.02713	Shoot_Root_Ratio_b_sylv
Harv9	4.00000	0.38527	0.09632	10,41571	0.00000	Shoot_Root_Ratio_b_sylv
Geno(Day_14^2)2	4.00000	0.13787	0.03447	3.72728	0.00671	Shoot_Root_Ratio_b_sylv
Residuals11	124.00000	1,14666	0.00925	NA	NA	Shoot_Root_Ratio_b_sylv
Geno11	4.00000	312,787,50625	78,196,87656	18,62845	0.00000	biomass_b_dist
ns(Day_14,df=2)7	2.00000	147,377,96199	73,688,98099	17,55455	0.00000	biomass_b_dist
Harv10	4.00000	145,877,82793	36,469,45698	8,68793	0.00000	biomass_b_dist
Residuals12	135.00000	566,691,30055	4,197,71334	NA	NA	biomass_b_dist
Geno12	4.00000	862,484,81413	215,621,20353	24,16680	0.00000	biomass_b_sylv
Harv11	4.00000	98,371,42927	24,592,85732	2,75636	0.03065	biomass_b_sylv
Residuals13	129.00000	1,150,964,71805	8,922,20712	NA	NA	biomass_b_sylv
I(Day_14^2)7	1.00000	9,393,36268	9,393,36268	4,44262	0.03680	c_content_b_dist
Residuals14	143.00000	302,355,81423	2,114,37632	NA	NA	c_content_b_dist
Geno13	4.00000	16,887,94249	4,221,98562	4.20111	0.00314	c_content_b_sylv
ns(Day_14,df=2)8	2.00000	3,997,44433	1,998,72217	1.98884	0.14106	c_content_b_sylv
Residuals15	128.00000	128,635,92473	1,004,96816	NA	NA	c_content_b_sylv
Geno14	4.00000	14,72235	3,68059	24,76573	0.00000	d13c_b_dist
I(Day_14^2)8	1.00000	4,81710	4,81710	32,41303	0.00000	d13c_b_dist
Harv12	4.00000	7,67007	1,90175	12,79639	0.00000	d13c_b_dist
Residuals16	135.00000	20,06317	0.14862	NA	NA	d13c_b_dist
Geno15	4.00000	12,87800	3,21950	14,09960	0.00000	d13c_b_sylv
ns(Day_14,df=2)9	2.00000	3,81605	1,90802	8,35607	0.00039	d13c_b_sylv
Harv13	4.00000	25,16097	6,29024	27,54772	0.00000	d13c_b_sylv
Residuals17	124.00000	28,31414	0.22834	NA	NA	d13c_b_sylv
Geno16	4.00000	332,26225	83,06556	8,93282	0.00000	n_content_b_dist
I(Day_14^2)9	1.00000	15,55323	15,55323	1,67259	0.19812	n_content_b_dist
Harv14	4.00000	139,69914	34,92478	3,75579	0.00626	n_content_b_dist
Residuals18	135.00000	1,255,35355	9,29892	NA	NA	n_content_b_dist
Geno17	4.00000	456,11421	114,02855	25,06977	0.00000	n_content_b_sylv
ns(Day_14,df=2)10	2.00000	325,38436	162,69218	35,76872	0.00000	n_content_b_sylv
Genons(Day_14,df=2)	8.00000	145,98218	18,24777	4,01187	0.00030	n_content_b_sylv
Residuals19	120.00000	545,81385	5,45845	NA	NA	n_content_b_sylv
Geno18	4.00000	14,25665	3,56416	5,34810	0.00050	d15n_b_dist
ns(Day_14,df=2)11	2.00000	14,26839	7,13419	10,70500	0.00005	d15n_b_dist
Harv15	4.00000	21,75070	5,43768	8,15934	0.00001	d15n_b_dist
Residuals20	134.00000	89,30239	0.66644	NA	NA	d15n_b_dist
I(Day_14^2)10	1.00000	17,12283	17,12283	32,60495	0.00000	d15n_b_sylv
ns(Day_14,df=2)12	2.00000	2,16282	1,08141	2,05920	0.13179	d15n_b_sylv
Harv16	4.00000	13,73616	3,43404	6,53903	0.00008	d15n_b_sylv
Residuals21	127.00000	66,69536	0.52516	NA	NA	d15n_b_sylv
Geno19	4.00000	434,41850	108,60463	18,40460	0.00000	c_n_b_dist
I(Day_14^2)11	1.00000	79,78162	79,78162	13,53538	0.00034	c_n_b_dist
Harv17	4.00000	75,29789	18,82447	3,19007	0.01537	c_n_b_dist
Residuals22	135.00000	796,62842	5,90095	NA	NA	c_n_b_dist
Geno20	4.00000	1,484,86836	371,21709	40,40652	0.00000	c_n_b_sylv
I(Day_14^2)12	1.00000	488,17121	488,17121	53,13683	0.00000	c_n_b_sylv
Geno(Day_14^2)3	4.00000	90,41398	22,60349	2,46036	0.04877	c_n_b_sylv
Residuals23	125.00000	1,148,38252	9,18706	NA	NA	c_n_b_sylv

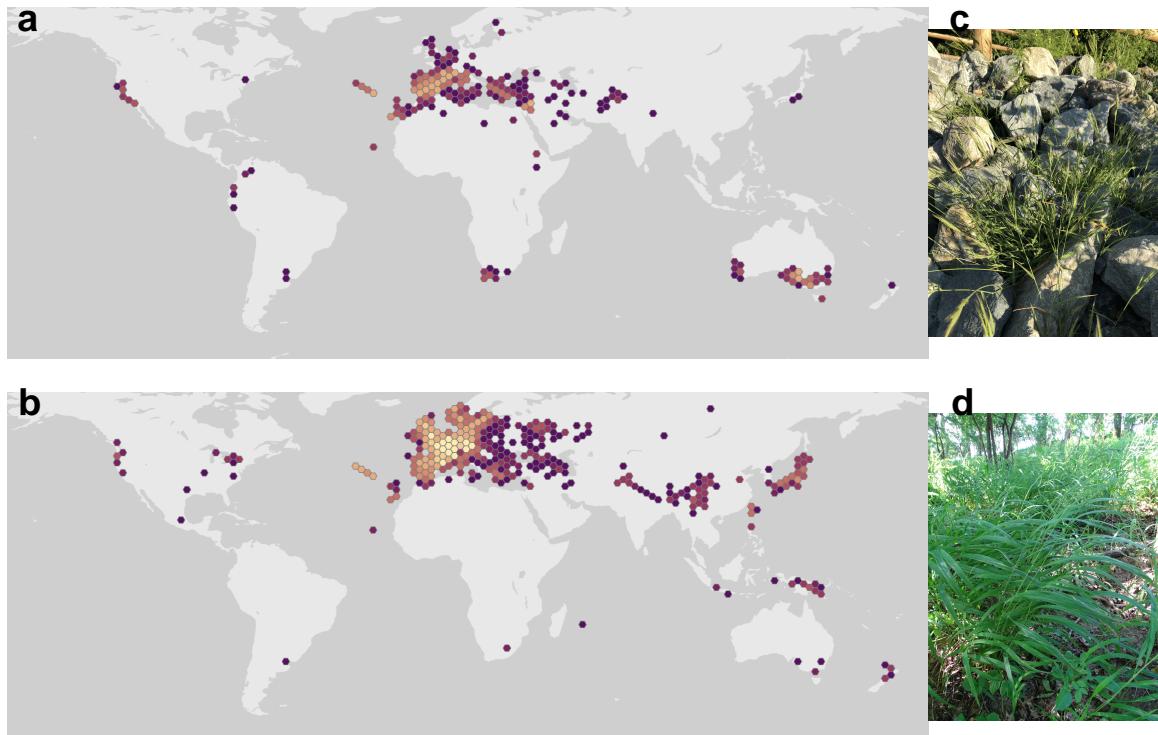


Figure S1. Distributions of (a) *B. distachyon* and (b) *B. sylvaticum* reported on GBIF as of 2019.18.08. Examples of (c) *B. distachyon* Carly Slawson (CC BY 4.0, <https://www.inaturalist.org/photos/42532397>) and (d) *B. sylvaticum* Grzegorz Grzejszczak (CC BY-NC 4.0, <https://www.inaturalist.org/photos/36088991>) (GBIF.org (26 February 2020) GBIF Occurrence Download <https://doi.org/10.15468/dl.rau5v9>).

1	12	2	18	11
15	5	13	19	16
3	4	17	14	10
6	7	9	8	20
24	33	26	32	38
25	29	39	30	37
28	23	40	31	21
35	22	36	27	34
53	44	50	58	60
52	49	45	46	59
55	42	48	43	41
56	54	51	47	57
78	77	71	75	68
62	79	63	61	76
72	64	67	73	69
66	80	70	74	65
96	90	97	84	83
91	89	82	95	93
81	88	85	98	99
92	87	94	86	100
113	107	118	110	102
105	114	116	108	120
106	117	112	119	109
111	103	104	101	115
135	130	126	137	128
133	127	139	125	138
132	122	129	121	123
136	124	131	140	134
144	153	146	152	158
145	149	159	150	157
148	143	160	151	141
155	142	156	147	154
176	170	177	164	163
171	169	162	175	173
161	168	165	178	179
172	167	174	166	180
194	198	196	197	183
181	192	190	199	195
184	186	187	188	191
200	182	193	189	185
210	208	220	215	214
202	201	211	217	213
209	219	204	206	218
216	203	207	212	205
236	240	233	224	222
231	238	221	227	225
234	226	237	228	230
239	232	229	235	223
241	252	242	258	251
255	245	253	259	256
243	244	257	254	250
246	247	249	248	260
274	278	276	277	263
261	272	270	279	275
264	266	267	268	271
280	262	273	269	265
290	288	300	295	294
282	281	291	297	293
289	299	284	286	298
296	283	287	292	285
313	304	310	318	320
312	309	305	306	319
315	302	308	303	301
316	314	311	307	317
338	337	331	335	328
322	339	323	321	336
332	324	327	333	329
326	340	330	334	325
353	347	358	350	342
345	354	356	348	360
346	357	352	359	349
351	343	344	341	355
366	368	364	375	370
361	362	363	369	365
372	367	378	380	377
373	376	371	379	374
382	393	395	392	389
391	383	399	394	397
388	396	385	400	398
381	390	387	386	384
413	418	417	414	416
411	408	407	403	412
401	409	419	410	405
404	415	406	402	420
440	428	433	432	436
425	434	424	431	430
438	423	437	429	435
427	421	426	439	422
456	460	453	444	442
451	458	441	447	445
454	446	457	448	450
459	452	449	455	443
471	463	465	464	466
470	478	473	461	476
467	474	468	462	480
475	472	479	477	469
502	513	515	512	509
511	503	519	514	517
508	516	505	520	518
501	510	507	506	504
535	530	526	537	528
533	527	539	525	538
532	522	529	521	523
536	524	531	540	534
553	558	557	554	556
551	548	547	543	552
541	549	559	550	545
544	555	546	542	560
580	568	573	572	576
565	574	564	571	570
578	563	577	569	575
567	561	566	579	562
591	583	585	584	586
590	598	593	581	596
587	594	588	582	600
595	592	599	597	589

Figure S2. Planting scheme.

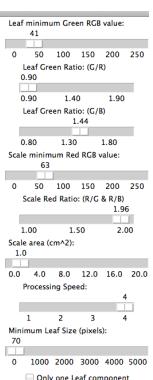


Figure S3. Settings used in Easy Leaf Area.

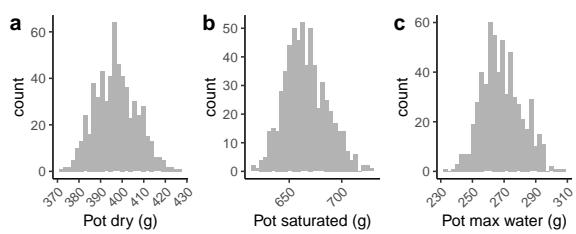


Figure S4. Variation in pot field capacity.