New Phytologist

Research

Changes in leaf economic trait relationships across a precipitation gradient are related to differential gene expression in a C_4 perennial grass

Robert W. Heckman[1], Michael J. Aspinwall[1,2], Samuel H. Taylor[1,3], David B. Lowry[1,4], Albina Khasanova[1], Jason E. Bonnette[1], Samsad Razzaque[1,5], Philip A. Fay[6] and Thomas E. Juenger[1]

[1]Department of Integrative Biology, University of Texas at Austin, Austin, TX 78712, USA; [2]Formation Environmental LLC, Sacramento, CA 95816, USA; [3]Lancaster Environmental Centre, Lancaster University, Lancaster, LA1 4YQ, UK; [4]Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA; [5]Plant Molecular and Cellular Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA; [6]U.S. Department of Agriculture Agricultural Research Service, Grassland Soil and Water Research Lab, Temple, TX 76502, USA

Authors for correspondence:
Robert W. Heckman
Email: robert.heckman@usda.gov

Thomas E. Juenger
Email: tjuenger@austin.utexas.edu

Received: *5 April 2024*
Accepted: *23 February 2025*

New Phytologist (2025) **246:** 1583–1596
doi: 10.1111/nph.70089

Key words: extreme precipitation, functional ecology, gene expression, leaf economics strategy, leaf mass per area, local adaptation, switchgrass, transcriptomics.

Summary

- The leaf economics spectrum (LES) describes a suite of functional traits that consistently covary at large spatial and taxonomic scales. Despite its importance at these larger scales, few studies have examined the major drivers of intraspecific variation in the LES – phenotypic plasticity and standing genetic variation.
- Using experimental precipitation manipulations, we examined whether covariation among leaf economics traits and selection on leaf economics traits and trait combinations change as diverse genotypes of the widespread perennial grass *Panicum virgatum* are exposed to differences in precipitation. We also used RNA-Seq to examine whether groups of co-expressed genes that align with leaf economics traits function in processes hypothesized to underlie the LES.
- Water availability impacted leaf economics trait covariation in important ways – covariation between leaf economics traits and selection on covariation between traits (i.e. correlational selection) tended to be strongest when water availability was high. Additionally, many genes associated with leaf economics traits functioned in processes that may explain how the LES originates, such as chloroplasts, cell walls, and nitrogen metabolism.
- Water availability is likely an important modulator of selection and evolution of the LES in *P. virgatum* that can be better understood by examining gene expression.

Introduction

The leaf economics spectrum (LES) is an example of phenotypic integration, where several functionally related traits covary. It is highly consistent at large taxonomic and spatial scales (Wright *et al.*, 2004; Anderegg *et al.*, 2018), but often breaks down at lower taxonomic levels, such as within genera or species (Edwards *et al.*, 2014; Martin *et al.*, 2017). Leaf economic trait covariation within species can be driven by the environment and genetic architecture and variation (i.e. pleiotropy, linkage, and epistasis) (Westerband *et al.*, 2021). When leaf economics traits are largely genetically determined, the LES should be more consistent across environments, especially in the absence of genotype-by-environment interactions. When the environment influences leaf economics traits (i.e. environment or genotype-by-environment effects), intraspecific trait covariation occurring in one environment may be unpredictable or entirely absent in another environment (Schlichting, 1986). Leaf economics trait covariation may increase if selection favors particular trait combinations (i.e. correlational selection occurs) or if leaf economics traits are controlled by the same set of genes and respond similarly to stress (e.g. pleiotropy or shared genetic networks). Phenotypic integration can decline if extreme stress reduces developmental stability (Wang & Zhou, 2022) or if traits differ in their environmental responsiveness (Gianoli & Palacio-López, 2009; Matesanz *et al.*, 2021). This may occur when the genetic or molecular bases of these traits only partially overlap or when selection for traits independently becomes stronger than selection for traits in combination. An important question, then, is whether covariation among leaf economics traits changes across environments (Matesanz *et al.*, 2021), and if so, what causes this change.

Two potentially important processes that could explain changes in leaf economics traits and their covariation are phenotypic plasticity and intraspecific genetic variation (Anderegg *et al.*, 2018;

Present address: Robert W. Heckman, U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station, Cedar City, UT 84721, USA.

Westerband *et al.*, 2021). Phenotypic plasticity, which is the ability of a single genotype to produce different phenotypes in response to changing environmental conditions (Schlichting, 1986), can be active – driven by metabolic or physiological responses to the environment – or passive – resulting from stress responses or genetic correlations with other biological processes – and can increase or decrease fitness (i.e. adaptive vs maladaptive plasticity; Ghalambor *et al.*, 2007). The strength and speed of plastic responses can vary among traits (Famiglietti *et al.*, 2024) and along resource gradients (e.g. nutrients, light, water; Mooney, 1972, Wright *et al.*, 2004, Keenan & Niinemets, 2016), which may cause correlations between traits to change or break down (Pigliucci & Preston, 2004). For instance, photosynthesis often declines precipitously and reversibly under acute drought, while foliar nutrient concentration and leaf mass per area (LMA) may change more gradually and only under extended drought (Aspinwall *et al.*, 2023; Melton *et al.*, 2023; Famiglietti *et al.*, 2024). Intraspecific genetic variation is an important driver of leaf economics strategies that is difficult to isolate from environmental effects in the natural field environments where the LES is typically studied (e.g. Wright *et al.*, 2004; Pérez-Harguindeguy *et al.*, 2013). To partition variation in traits between genetic and environmental sources, then, diverse genotypes can be grown across experimental gradients in an environmental variable (e.g. Aspinwall *et al.*, 2017; Exposito-Alonso *et al.*, 2019).

While both phenotypic plasticity and genetic variation are potentially important general determinants of leaf economics strategies, neither phenomenon alone can explain which lower-level processes underlie leaf economics trait variation, the subject of several studies (e.g. Shipley *et al.*, 2006; Blonder *et al.*, 2015; Mason *et al.*, 2016; Onoda *et al.*, 2017). An early attempt to identify the lower-level physiological and molecular processes that cause the LES used structural equation modeling to propose that one or more latent variables (i.e. a theoretical construct that cannot be directly measured) were necessary to explain the relationships between leaf economics traits (Shipley *et al.*, 2006). The authors posited that these latent variables were likely associated with a trade-off between structural tissue and carbon fixation or a trade-off between leaf longevity, photosynthetic rate, and construction cost (Shipley *et al.*, 2006). Onoda *et al.* (2017) built upon this model using detailed, low-level anatomical and physiological traits to assess two potential trade-offs: a trade-off in nitrogen (N) allocation between chloroplasts and nonphotosynthetic components, particularly cell walls, and a CO_2 diffusion trade-off associated with allocation to mesophyll cell walls vs mesophyll surface area. Another approach with many advantages to identifying the physiological and molecular functions (i.e. the latent variable) underlying the LES is to examine the links between transcriptomic responses and the morphological and physiological traits that comprise the LES. Transcriptomics requires no prior knowledge of the traits that could underlie leaf economics trade-offs and is high throughput and relatively cheap compared with the laborious methods used in past studies (Blonder *et al.*, 2015; John *et al.*, 2017; Onoda *et al.*, 2017). These attributes make transcriptomics a promising approach for assessing the importance of the lower-level functions previously proposed by Shipley *et al.* (2006) and Onoda *et al.* (2017) to leaf economics strategies and for identifying other potentially important functions that can be tested in future studies. This can be done by identifying groups of genes with similar expression patterns across diverse genotypes, different environments, or both, and comparing these groups of co-expressed genes to leaf economics traits (Horvath, 2011). When a strong relationship between a gene co-expression network and leaf economics traits exists, analyses can assess whether these co-expressed genes function in particular processes more frequently than would be expected by chance across the entire transcriptome (Alexa & Rahnenfuhrer, 2024).

Although transcriptomic responses are more dynamic than the morphological and physiological traits that comprise the LES (Volaire, 2018), they can still reveal much about how plasticity and genetic variation influence leaf morphology and physiology. First, plants with different leaf economics strategies often construct leaves that differ in tissue composition (Poorter *et al.*, 2009; Onoda *et al.*, 2017). Because gene expression can vary among the diverse cell types that comprise the leaf (e.g. Marand *et al.*, 2021; Xia *et al.*, 2022), leaves that are constructed in different ways may possess different transcriptomic profiles. Second, examining transcriptomic profiles across a range of genotypes and environments may help to distinguish genes that are strongly associated with plant traits that vary little with the environment from genes specifically associated with traits in a particular environment (Cruz *et al.*, 2020; Darwiche & Struhl, 2020) as well as whether the relationship between gene expression and leaf economics traits strengthens as leaf economics traits become more integrated.

In this study, we examined phenotypic plasticity and genetic variation in leaf economics traits in multiple genotypes of the widespread, phenotypically diverse grass *Panicum virgatum* L. (Poaceae). Ecotypes of *P. virgatum* experience substantially different growing season lengths across their native range in the eastern and central United States, which may explain differences in leaf economics strategies among its ecotypes (Casler, 2012; Lovell *et al.*, 2021; Heckman *et al.*, 2024): The upland ecotype, which is more common in the northern portion of the range of *P. virgatum*, often exhibits acquisitive phenotypes, while the lowland ecotype, which is more common in the southern portion of the range, typically exhibits conservative phenotypes (Aspinwall *et al.*, 2013; Heckman *et al.*, 2020). Within these plants, we measured three leaf economics traits – leaf nitrogen concentration (N_{MASS}), LMA, and photosynthetic rate (A_{MASS}) – and gene expression across an experimental gradient of precipitation to address the following five questions. (1) How do changes in precipitation impact variation in, and covariation among, leaf economics traits? (2) Does selection for leaf economics traits and trait combinations change along a precipitation gradient? (3) Do patterns of gene expression align with the acquisitive–conservative leaf economics trade-off? (4) Does the relationship between gene expression and leaf economics traits strengthen in environments with stronger leaf economics trait covariation? (5) Does the alignment between gene expression and the leaf economics trade-off give insight into physiological functions underlying the LES?

Materials and Methods

Plant system

Panicum virgatum L. is an ecologically and economically important perennial C_4 grass species possessing high phenotypic variation in leaf economics traits. *Panicum virgatum* is common throughout eastern and central North American grasslands, occupying habitats that vary considerably in season length and mean annual temperature and precipitation (Lowry *et al.*, 2014). To occupy these diverse habitats, *P. virgatum* has diverged into three ecotypes – upland, coastal, and lowland – with the coastal ecotype exhibiting characteristics intermediate between the other two ecotypes (Lovell *et al.*, 2021). The upland ecotype typically occurs in northern regions with short growing seasons and possesses an acquisitive strategy; the lowland ecotype occurs in southern regions with long growing seasons and possesses a conservative strategy (Aspinwall *et al.*, 2013; Heckman *et al.*, 2020; Chen *et al.*, 2021).

Experimental design

We performed this study at the USDA Grassland Soil and Water Research Lab near Temple, TX, USA. At this site, soils are moderately deep (50–100 cm) and fine-textured (Austin silty clay, fine silty, carbonatic, and Udorthentic Haplustol); mean annual precipitation is 910 mm, mean annual minimum air temperature is 13.0°C, and mean annual maximum air temperature is 25.6°C (data from NOAA weather station located at 31.078078, −97.318268; see Aspinwall *et al.*, 2013 for details).

Our study utilized a subset of genotypes and precipitation treatments within a larger precipitation manipulation experiment described by Aspinwall *et al.* (2017). Specifically, we focus on six *P. virgatum* genotypes, which originated between 27°N and 40°N, including the genome reference genotype and several genotypes that represent important cultivars (Supporting Information Table S1). *Panicum virgatum* cultivars do not differ genetically in appreciable ways from natural populations, primarily because they have not undergone substantial selection (Casler *et al.*, 2015; Lovell *et al.*, 2021). We focused on the tetraploid (4×) subset of genotypes planted in this experiment rather than pooling tetraploid and octoploid (8×) genotypes to facilitate gene expression analysis, which resulted in an unbalanced distribution of genotypes across ecotypes – four of the six genotypes were southern lowlands and only one was a northern upland (SUM). We sampled single genotypes from diverse populations to assess how these genotypes respond to precipitation variation within the limited space available in our experimental facility (Aspinwall *et al.*, 2017). We acknowledge, however, that this choice limits our ability to predict responses to precipitation extremes across the phenotypic and genetic diversity that exists within this widespread species.

Before planting in our experimental facility, each genotype was clonally propagated by first dividing, then multiplying, rhizomes from a single genet collected at each location (i.e. plants from each genotype are genetically identical). Individual plants were initially grown outdoors in separate 3.79-l pots and then transplanted directly into the soil in plots in March 2011. Plants were arranged in plots on 1-m centers in duplicate, such that individuals of the same genotype never abutted one another. During establishment in 2011, plants were well-watered.

This experimental facility included 24 5-m × 5-m plots arranged in four spatial blocks, of which 12 plots were used in the current study. Within each block, plots were 0.25 m apart; blocks were 2.76 m apart. We surrounded each plot with pond liner (1.84 mm thick; Firestone Specialty Products, Indianapolis, IN, USA) that extended 10 cm above the soil surface to limit overland flow of water into and out of plots and 120 cm below the soil surface to limit the movement of subsurface water and roots between plots. The experiment was covered by open-sided 18.3-m × 73.0-m rainout shelters with 2.1-m-high walls and 4.2-m-high leaves on each end (Windjammer Cold Frame; International Greenhouse Co., Danville, IL, USA), which maximized air movement and heat dissipation (Aspinwall *et al.*, 2013). The shelters excluded precipitation year-round with a clear 150-µm polyethylene greenhouse film, which transmits *c.* 90% of photosynthetically active radiation (PAR; Aspinwall *et al.*, 2013). We applied precipitation treatments using programmable 90° sprinklers placed in each corner of the plot (LEIT XRC Series Ambient Powered Irrigation Controller; DIG Corp., Vista, CA, USA; Hunter HP2000; Hunter Industries Inc., San Marcos, CA, USA).

We assigned each of our 12 plots to one of three precipitation treatments – low (severe drought, 530 mm annual precipitation), mean (average precipitation, 978 mm annual precipitation), and high (extremely wet conditions, 1541 mm annual precipitation), which began in March 2012 and continued through the end of 2012. These treatments were applied to approximate the seasonality, size distribution, and spacing of rainfall events typical of the desired conditions at this site using a stochastic weather generator that was calibrated to the 102-yr site-level precipitation record (data spanning 1900–2002 downloaded from a NOAA weather station located at 31.078078, −97.318268; LARS-WG 5.5; Semenov *et al.*, 1998, Aspinwall *et al.*, 2017). Varying the seasonality, size distribution, and spacing of rainfall events more accurately mimics the historical differences between years typical of each precipitation treatment at this site than would simply altering the total amount of experimental rainfall applied to each treatment. In general, the high treatment received larger and more frequent precipitation applications than the low and mean treatments; all treatments were relatively dry during summer.

In total, this study began with 144 plants (3 precipitation treatments × 4 spatial blocks × 6 genotypes × 2 replicates), but mortality reduced the final sample size to 137 plants.

Phenotypic responses

To examine variation in leaf economics strategies, we measured three commonly studied leaf economics traits – mass-based carbon assimilation (A_{MASS}), mass-based leaf nitrogen concentration (N_{MASS}), and LMA. We sampled these 1-yr-old plants over 2 d in mid-June 2012 when plants were actively growing. Two blocks

were measured each day, and the sequence of measurements on each day was randomized across treatments, genotypes, and replicates. To quantify leaf net CO_2 assimilation (*ACO2*, μmol m^{-2} s^{-1}) on each plant, we measured leaf-level gas exchange on two fully expanded, mature upper-canopy leaves from separate tillers using a portable photosynthesis system (LI-6400; Li-Cor, Lincoln, NE, USA). All measurements occurred between 11:00 h and 14:00 h. For all measurements, ambient midday photosynthetic photon flux density was *c.* 1500 μmol m^{-2} s^{-1} and PAR within the cuvette was maintained at 1500 μmol m^{-2} s^{-1}. Chamber supply [CO_2] was controlled at 400 μmol mol^{-1}, resulting in cuvette [CO_2] of 389.0 ± 7.0 (mean ± SD). The cuvette block temperature was set at the forecasted ambient air temperature, T_a (32°C). Water vapor content of incoming air was controlled (29 ± 3 mmol H_2O mol^{-1}), such that reference relative humidity was consistent across measurements. After gas exchange measurements, single-sided leaf area (cm^2) of one sampled leaf was measured using a portable leaf area meter (LI-3000A; Li-Cor). The leaf was then dried to a constant mass at 65°C, and LMA (g m^{-2}) was calculated as dry leaf mass divided by leaf area. The dried leaves were ground into a fine powder using a ball mill (SPEX 8000D Mixer/Mill; SPEX SamplePrep, Metuchen, NJ, USA), and N_{MASS} (% N) was measured using a combustion elemental analyzer (Flash 2000; Thermo Scientific, Waltham, MA, USA). A_{MASS} was calculated as LMA/*ACO2*. While no single leaf can adequately characterize the traits of an entire plant, we focused on sampling a small number of leaves at a similar developmental stage and canopy position to ensure that comparisons are standardized and valid, as is common in leaf economics trait sampling (e.g. Wright *et al.*, 2004; Pérez-Harguindeguy *et al.*, 2013). At the end of the growing season (November 2012), we measured aboveground plant biomass by clipping at 10 cm above the soil surface and then weighed the biomass after drying at 65°C for at least 48 h in forage drying ovens (kg per plant).

Tissue collection and library preparation

To examine the relationship between leaf economics traits and gene expression, we collected tissue for RNA sequencing (RNA-Seq) from two representative, fully expanded upper-canopy leaves at the same time as photosynthesis measurements (see Lovell *et al.*, 2016 for details). Then, we cut 2-cm sections from the proximal portion of both leaves, removed their midribs, placed the leaf sections into a 2-ml Eppendorf tube, and flash-froze them in liquid nitrogen. Samples were transported to the laboratory on dry ice. The samples were then homogenized (SPEX Geno/Grinder 2000; SPEX SamplePrep) after adding three stainless steel beads to the tubes. RNA was extracted with the standard TRIzol protocol and treated with DNase I to remove contaminating genomic DNA (Meyer *et al.*, 2011). Finally, we prepared RNA-Seq library samples using a modified version of the 3′ Tag-Seq protocol (Meyer *et al.*, 2011). To do this, we amplified purified RNA and individually barcoded samples before sequencing on the Illumina HiSeq platform (Genomic Sequencing and Analysis Facility, University of Texas at Austin, Austin, TX, USA) with a goal of obtaining 5 million reads per sample (Lovell *et al.*, 2016). The 3′ Tag-Seq protocol selects molecules using a poly-A capture and employs a known inline adapter on the 5′ end of the molecule (Meyer *et al.*, 2011; Lohman *et al.*, 2016).

Bioinformatic analysis of RNA-Seq data

We received sequencing samples demultiplexed on a per-run basis and processed each fastq file individually. To generate the count matrix from the raw sequence data, we first checked the quality of raw sequence reads (FastQC; Andrews, 2010) and then filtered raw sequences by removing a poly-A tail of 15-mer length and known adapter sequence (cutadapt; Martin, 2011). Next, we removed trimmed sequences shorter than 70 base pairs and mapped the remaining sequences against the *P. virgatum* v.5.0 reference (Lovell *et al.*, 2021) using BWA-mem with default parameters (Li, 2013). Only uniquely mapped reads (--primary) based on the *P. virgatum* v.5.1 annotation file (available at https://phytozome-next.jgi.doe.gov/info/Pvirgatum_v5_1) were used to quantify the RNA-Seq counts (featureCounts; Liao *et al.*, 2013).

Data analysis

To answer the first question – how do changes in precipitation impact variation in, and covariation among, leaf economics traits – we took three approaches. First, we quantified genotype-specific variation in leaf economics traits in response to precipitation by fitting linear mixed models with one of the three leaf economics traits (i.e. LMA, N_{MASS}, or A_{MASS}) as the response and genotype, precipitation, and genotype-by-precipitation as fixed effect predictors and whole plot nested within spatial block as a random effect; this model also estimated variances for each combination of genotype and precipitation treatment separately (lme() function in package nlme; Pinheiro *et al.*, 2023). Second, we evaluated the degree of covariation in leaf economics traits and whether covariation differed among genotype or precipitation treatment by fitting linear mixed models in which one of the three leaf economics traits was the response and another was a fixed effect predictor that was allowed to interact with genotype and precipitation treatment (i.e. $trait_1 \sim trait_2 \times genotype \times precipitation$); this model also included a nested random effect of whole plot within spatial block (lme() function in package nlme). We then performed *post hoc* tests to estimate the slope of the $trait_1 \sim trait_2$ relationship at different levels of precipitation and genotype and to determine whether these slopes differed between levels of precipitation and/or genotype (emtrends() and test() functions in package emmeans; Lenth, 2018). Third, we evaluated the degree of integration among these three leaf economics traits using the phenotypic integration index (PINT), which quantifies phenotypic integration as the variance of the eigenvalues of the correlation matrix among traits (phenix package; Wagner, 1984, Torices & Muñoz-Pajares, 2015).

To assess the second question – does selection for leaf economics traits change along a precipitation gradient – we performed phenotypic selection analyses (Lande & Arnold, 1983;

Stinchcombe *et al.*, 2008). We used aboveground biomass as a fitness/performance proxy, which we first mean-standardized separately by precipitation treatment by dividing each plant's aboveground biomass by treatment-level mean aboveground biomass. As is standard in phenotypic selection analyses, this resulted in a relativized fitness proxy for each treatment where average fitness = 1; above-average fitness > 1; and below-average fitness < 1. To assess the linear and nonlinear effects of leaf economics traits on aboveground biomass, we also mean-centered and variance-standardized (mean = 0, SD = 1) leaf economics traits separately by precipitation treatment. We fit two sets of models – selection differentials, where each leaf economics trait was modeled separately to detect both direct and indirect selection on each trait, and selection gradients, where all three leaf economics traits were modeled together to detect only direct selection on each trait – to examine how these standardized traits impacted mean-standardized biomass (terHorst *et al.*, 2015). As is customary, we fit both selection differential and selection gradient models in two parts (lmer() function in package LME4). We first assessed linear directional selection by fitting a model that only included the main effects of leaf economics traits and their interactions with precipitation. Then, we assessed nonlinear stabilizing or disruptive selection (both selection differentials and selection gradients) and nonlinear correlational selection (selection gradients only) by fitting a second set of models that included main effects, quadratic effects, all two-way interactions between N_{MASS}, LMA, and A_{MASS}, and interactions between all leaf economics predictors and precipitation treatment. Each model also included a nested random effect of whole plot within spatial block and a random slopes and intercepts effect of precipitation treatment and genotype. To evaluate selection separately in each precipitation treatment, we performed *post hoc* tests on these phenotypic selection models as well as whether selection differed among precipitation treatments (using the emtrends(), test(), and joint_tests() functions in EMMEANS). We then calculated bootstrapped confidence intervals (CI) on selection differentials and selection gradients (bootstraps() function with 1000 iterations in package RSAMPLE; Frick *et al.*, 2024). When there was a significant quadratic effect in selection differential models, we determined whether the quadratic effect represented a hump (i.e. stabilizing selection) or a nonlinear increase or decrease (i.e. directional selection; Mitchell-Olds & Shaw, 1987) by testing the null hypothesis that the hump of the quadratic relationship is at the minimum or maximum value (MOStest() function in package VEGAN; Oksanen *et al.*, 2022).

To examine the third, fourth, and fifth questions – which molecular functions underlie correlations between gene expression and leaf economics traits across precipitation treatments – we used gene co-expression network analysis. Before analyzing transcriptomic data, we removed all transcripts with fewer than five counts per individual plant, which left 22 329 of *c.* 65 000 *P. virgatum* v.5.0 gene models. To account for variation in sequencing depth, we normalized all gene expression counts (DESeq2 package; Love *et al.*, 2014). Specifically, we created a design matrix with interactive effects of precipitation treatment and genotype (DESeqDataSetFromMatrix() function), then normalized the counts (estimateSizeFactors() function with the ratio method, followed by the counts() function with option 'normalize = T') and $\log_2$-transformed them.

Next, we evaluated the relationship between the LES and the transcriptome using weighted gene co-expression network analysis (WGCNA), an approach that identifies clusters of genes with highly correlated expression profiles, which can then be associated with phenotypic traits of interest (WGCNA package; Langfelder & Horvath, 2008, 2012). We set minModuleSize to 100 and merge cutHeight to 0. The soft thresholding power, which was determined by scale-free topology model fit (R^2 cutoff = 0.8), differed between analyses (16 for high + low precipitation; 12 for high precipitation only; and 17 for low precipitation only). The WGCNA identified 23 distinct gene expression modules across high + low precipitation, 23 for high precipitation only, and 14 for low precipitation only. We calculated Pearson correlations between gene expression modules and leaf economics traits, leaf economics trait combinations (the product of the standardized values for each pair of traits), and LES factor 1 – the scores generated from exploratory factor analysis on these three traits (fa() function in package PSYCH; Revelle, 2024). When Pearson correlations were significant, we examined whether expression modules were enriched in particular functions using Gene Ontology (GO) enrichment analysis (TOPGO package; Alexa & Rahnenfuhrer, 2024). The GO enrichment analysis assesses whether genes with particular functions (GO terms) are present within an expression module more often than expected based on the frequency of these GO terms throughout the analyzed transcriptome (i.e. all transcripts that met filtering criteria and had an assigned GO term). Specifically, we performed Fisher's exact test on GO terms containing at least 50 annotated genes using topGO's 'weight01' algorithm (Alexa & Rahnenfuhrer, 2024).

Results

How do changes in precipitation impact variation in, and covariation among, leaf economics traits?

The main sources of variation differed among leaf economics traits (Table S2; Fig. 1). Genotypes had different LMA responses to precipitation – three genotypes (AP13, KAN, and K×S) exhibited large reductions in LMA as precipitation increased, while the other three genotypes exhibited relative stability in LMA ($P < 0.001$; Table S2; Fig. 1a). Genotypes differed significantly from one another in N_{MASS} ($P < 0.001$), but there was no precipitation ($P = 0.236$) or genotype-by-precipitation effect ($P = 0.892$; Table S2; Fig. 1a). A_{MASS} was highly responsive to precipitation ($P = 0.006$) – increasing approximately threefold, on average, between the low and high precipitation treatments – but exhibited no genotype ($P = 0.186$) or genotype-by-precipitation effects ($P = 0.340$; Table S2; Fig. 1a).

Across all genotypes and treatments, these three leaf economics traits generally covaried as predicted by previous leaf economics studies (Table S3). However, the degree of covariation

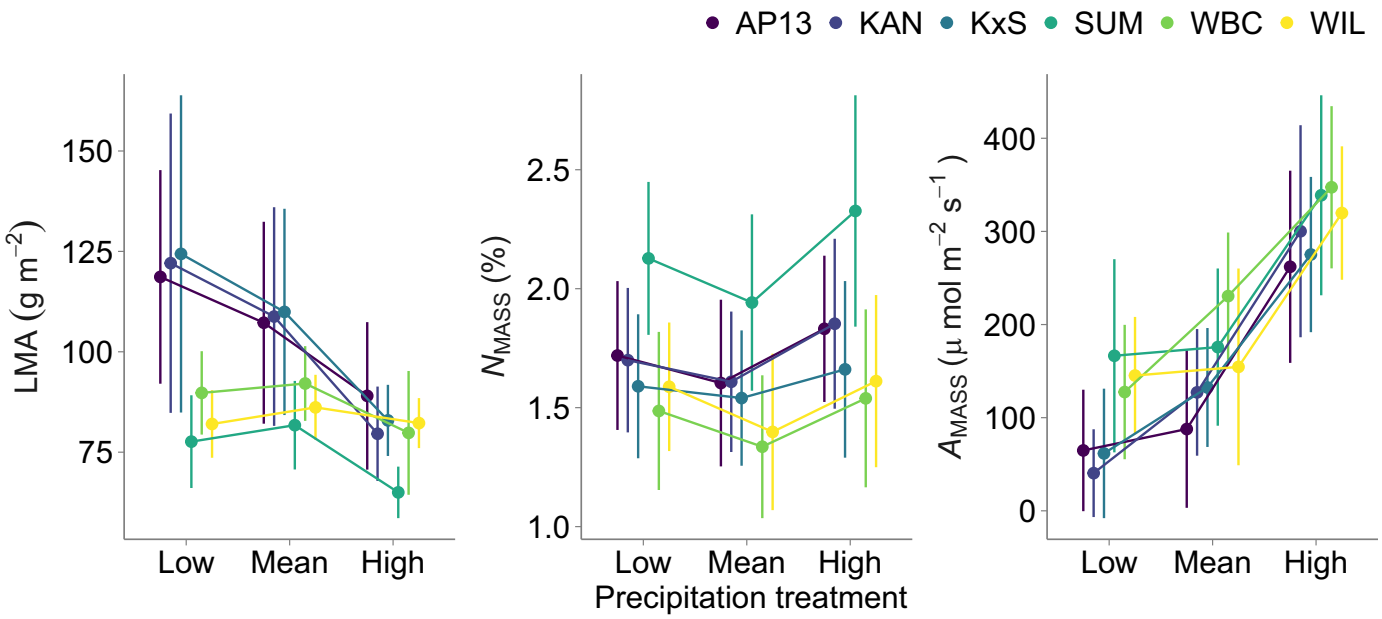

Fig. 1 Effects of genotype, precipitation, and genotype-by-precipitation on leaf economics traits in *Panicum virgatum*. Points are model-derived; error bars represent 95% confidence intervals. A_{MASS}, mass-based photosynthetic rate; AP13, Alamo; KAN, Kanlow; K×S, Kanlow × Summer; LMA, leaf mass per area; N_{MASS}, leaf % N concentration; SUM, Summer; WBC, West Bee Cave; WIL, Wilson.

between traits was often weak at low and mean precipitation relative to high precipitation. Specifically, A_{MASS} and N_{MASS} covaried positively within the mean and high precipitation treatments ($P = 0.007$ and $P = 0.021$, respectively), but did not covary in the low precipitation treatment ($P = 0.861$; Table S4; Fig. 2a). A_{MASS} and LMA only negatively covaried at high precipitation ($P = 0.044$), but not at low or mean precipitation ($P = 0.328$ and $P = 0.142$, respectively; Table S4; Fig. 2a). N_{MASS} and LMA positively covaried in all three precipitation treatments (low, $P = 0.066$; mean, $P = 0.005$; high, $P = 0.019$; Table S4; Fig. 2a).

Like these bivariate relationships, overall phenotypic integration was highest at high precipitation (PINT = 0.408, 95% CI = 0.165, 0.712; Fig. 2b). However, phenotypic integration was intermediate at low precipitation (PINT = 0.289, 95% CI = 0.142, 0.568; Fig. 2b) and lowest at mean precipitation (PINT = 0.178, 95% CI = 0.066, 0.414; Fig. 2b). Together, these results suggest that leaf economics covariation breaks down largely, but not solely, due to the sensitivity of A_{MASS} to water availability.

Does selection for leaf economics traits or trait combinations change along a precipitation gradient?

We found support for the prediction that selection for leaf economics traits changes along a precipitation gradient (Tables S5–S7; selection differential coefficient estimates and bootstrapped CIs in Table S7). When fitting univariate selection differential models to estimate total selection on individual traits, we found directional selection on N_{MASS} at both low and high precipitation ($P = 0.001$ and $P = 0.019$, respectively; Table S7) and marginal directional selection on A_{MASS} at low precipitation ($P = 0.087$). There were also significant quadratic effects of LMA on biomass at low precipitation ($P = 0.038$; Table S7) and A_{MASS} on biomass at high precipitation ($P = 0.076$; Table S7), indicating that these relationships between LES traits and biomass were nonlinear. However, we found no evidence that the humps of these relationships occurred at an intermediate value ($P = 0.17$ and $P = 0.49$, respectively), supporting directional selection on both traits.

We fit multivariate selection gradient models to test for direct selection on each leaf economics trait. Here, we found that directional selection on individual leaf economics traits occurred at low precipitation, while correlational selection for combinations of leaf economics traits occurred at high precipitation (Tables S8–S10; selection gradient coefficient estimates and bootstrapped CIs in Table S10). At low precipitation, significant linear selection gradients were common (Fig. S1). Specifically, there was a significant interaction between precipitation treatment and A_{MASS} ($P = 0.023$; Table S9): biomass and A_{MASS} were positively associated at low precipitation, but not at high precipitation ($P = 0.005$ and $P = 0.858$, respectively; Table S10). Similarly, there was a marginally significant interaction between precipitation and N_{MASS} ($P = 0.076$; Table S9): biomass and N_{MASS} were negatively associated at low precipitation but not at high precipitation ($P < 0.001$ and $P = 0.124$, respectively; Table S10). Finally, there was a marginally significant positive relationship between LMA and biomass across both treatments ($P = 0.079$; Table S9), but not within either the low or high precipitation treatments ($P = 0.122$ and $P = 0.224$, respectively; Table S10).

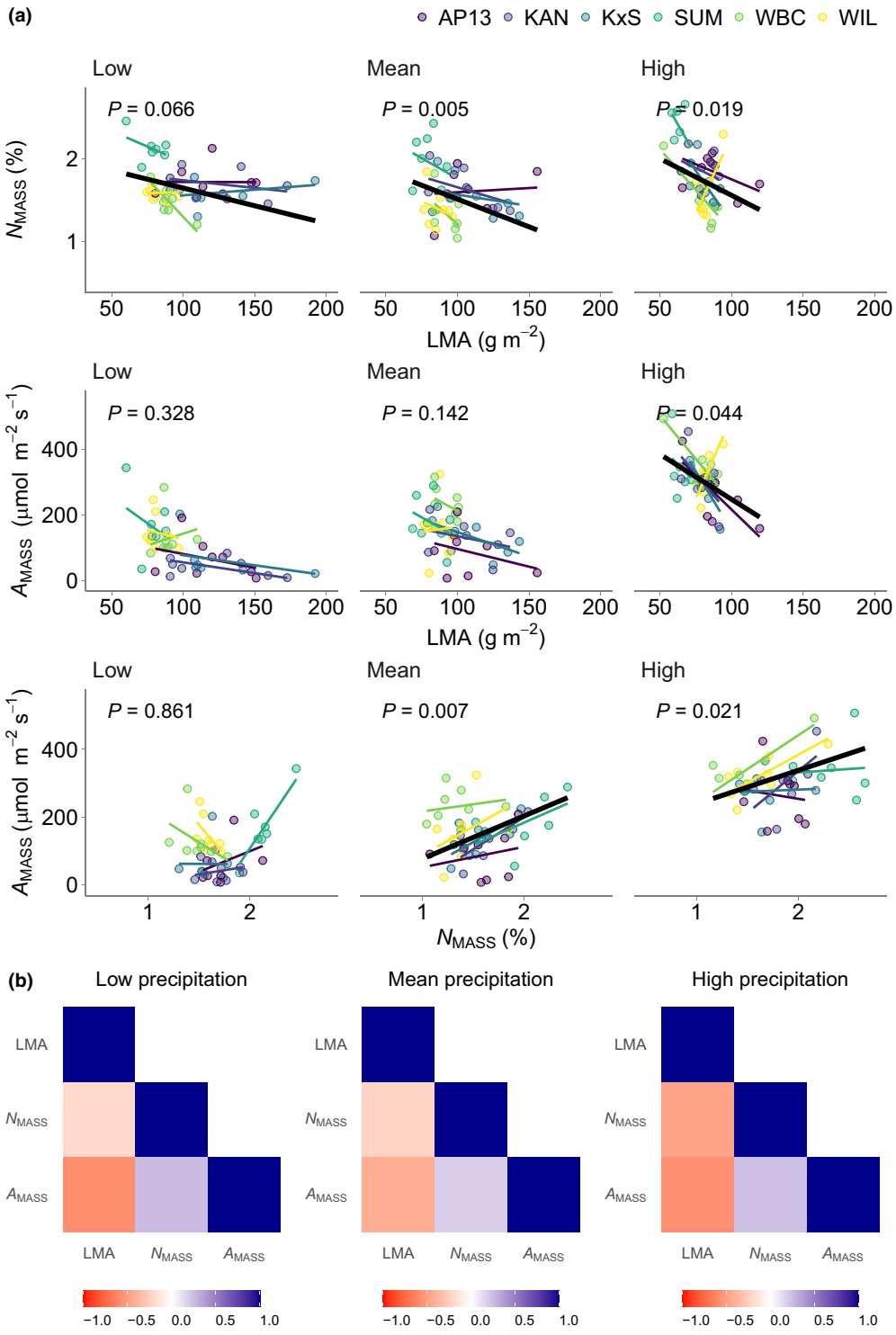

Fig. 2 Bivariate relationships between leaf economics traits in *Panicum virgatum* across a gradient in precipitation. (a) The bold black trend line is the overall relationship with trait pairs derived from a linear mixed model, while the colored lines are genotype-specific relationships. Points represent individual plants. *P*-values from a *post hoc* test evaluate whether the covariation between traits at each precipitation level differs from zero. (b) Heatmaps showing pairwise correlations among leaf economics traits. A_{MASS}, mass-based photosynthetic rate; AP13, Alamo; KAN, Kanlow; K×S, Kanlow × Summer; LMA, leaf mass per area; N_{MASS}, leaf % N concentration; SUM, Summer; WBC, West Bee Cave; WIL, Wilson.

In addition to directional selection, there were also significant nonlinear selection gradients. Most notably, the interactive effect of N_{MASS} and LMA on biomass changed marginally with precipitation treatment ($P = 0.079$; Table S9). At high precipitation, but not at low precipitation, LMA and N_{MASS} interacted to influence biomass ($P = 0.024$ and $P = 0.620$, respectively; Table S10): Plants with high LMA and low N_{MASS} or low LMA and high N_{MASS} performed better than plants with high LMA and high N_{MASS} or low LMA and low N_{MASS} (Fig. 3). While the sample size for these analyses was lower than

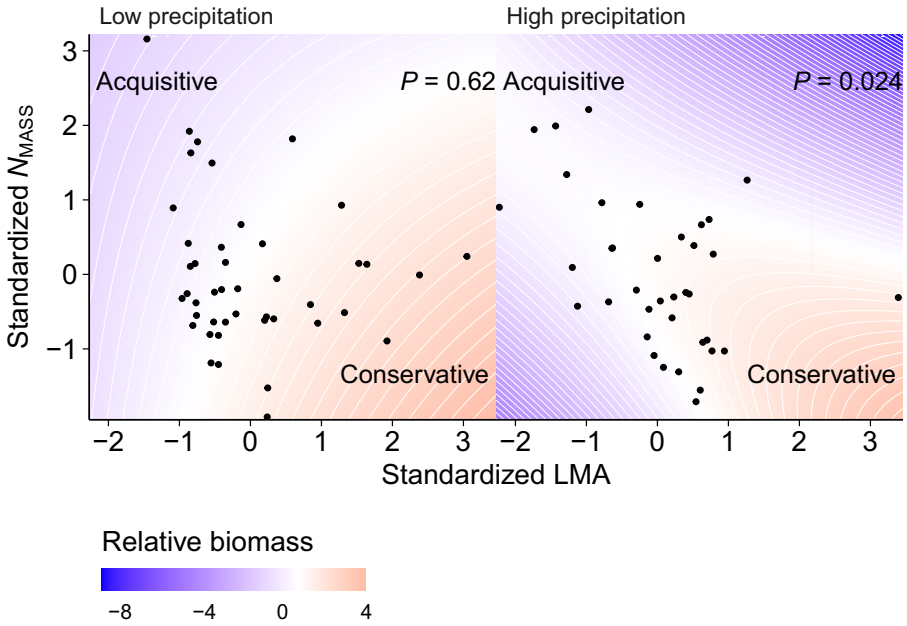

Fig. 3 Effects of standardized LMA and N_{MASS} (transformed such that across all plants within each treatment, mean = 0 and SD = 1) on relative biomass in *Panicum virgatum* while controlling for A_{MASS}. Points represent individual plants. Model-derived quadratic parameter estimates are doubled. *P*-values from a *post hoc* test represent the interactive LMA × N_{MASS} effect for each treatment. A_{MASS}, mass-based photosynthetic rate; LMA, leaf mass per area; N_{MASS}, leaf % N concentration.

recommended for detecting selection gradients (Kingsolver *et al.*, 2012), bootstrapped CIs on selection gradient estimates support these significant results (Table S10).

Do patterns of gene expression align with the acquisitive – conservative leaf economics trade-off? Does the relationship between gene expression and leaf economics traits strengthen in environments with stronger leaf economics trait covariation?

Correlations between gene expression modules and leaf economics traits varied considerably among precipitation treatments. When the high and low precipitation treatments were combined, LMA × N_{MASS} was significantly correlated with three gene expression modules and marginally correlated with two additional modules; LMA and N_{MASS} were each individually correlated with five modules (Fig. S2). This is consistent with evidence that the bivariate relationship between LMA and N_{MASS} was strong across precipitation treatments. Moreover, A_{MASS} was correlated with three modules and LMA × A_{MASS} was correlated with two modules (Fig. 4). Under high precipitation, LMA was significantly correlated with 4/23 modules (*c.* 17%), while N_{MASS}, LMA × N_{MASS}, and LES factor 1 were each significantly correlated with two modules, and A_{MASS} and LMA × A_{MASS} were each correlated with one module (Fig. S3). At low precipitation, 29% of expression modules (4/14) exhibited significant correlations with N_{MASS}, two modules were significantly correlated with A_{MASS} and LMA × N_{MASS}, and only one module was significantly correlated with LES factor 1. None of the other traits or trait combinations were significantly correlated with > 1 module (LMA and LMA × A_{MASS} = 1/14 modules; Fig. S4). Thus, while covariation between leaf economics traits was highest at high precipitation, links between leaf economics traits and gene expression were similar between low and high precipitation.

Does the alignment between gene expression and the leaf economics trade-off give insight into the physiological functions underlying the LES?

In aggregate, LES factor 1 – which explained 46% of the variation in these three leaf economics traits in exploratory factor analysis across the high and low precipitation treatments – was correlated with four gene expression modules (Fig. 4). In total, these four modules were enriched in over 100 GO terms. Here, we highlight GO terms that describe functions hypothesized by Shipley *et al.* (2006) to underlie the LES (Table S11). Specifically, photosynthetic functions were represented by at least 18 GO terms in three modules, including chloroplast organization and photosynthetic electron transport chain in the darkgreen module (GO:0009658; $P = 0.015$; GO:0009767; $P = 0.010$, respectively); Chl biosynthetic processes and chloroplast inner membrane in the paleturquoise module (GO:0015995, $P = 0.043$; GO:0009706, $P = 0.043$, respectively); and cellular response to light stimulus and response to blue light in the royalblue module (GO:0071482, $P = 0.044$; GO:0009637, $P < 0.001$, respectively). Additionally, cell wall functions were represented by at least seven GO terms in three modules, including plant-type cell wall and regulation of cellular macromolecule biosynthetic processes in the paleturquoise module (GO:0009505, $P = 0.023$; GO:2000112, $P = 0.037$, respectively); cell wall and hydrolase activity, hydrolyzing O-glycosyl compounds in the royalblue module (GO:0005618, $P = 0.012$; GO:0004553, $P = 0.003$, respectively); and glycosyltransferase activity in the saddlebrown module (GO:0016757, $P = 0.031$). Finally, nitrogen metabolism was represented by at least four GO terms in two modules, including response to organonitrogen compound, amine metabolic process, and proteolysis involved in the protein catabolic process in the saddlebrown module (GO:0010243, $P = 0.020$; GO:0009308, $P = 0.021$; GO:0051603, $P = 0.024$) and response to nitrogen

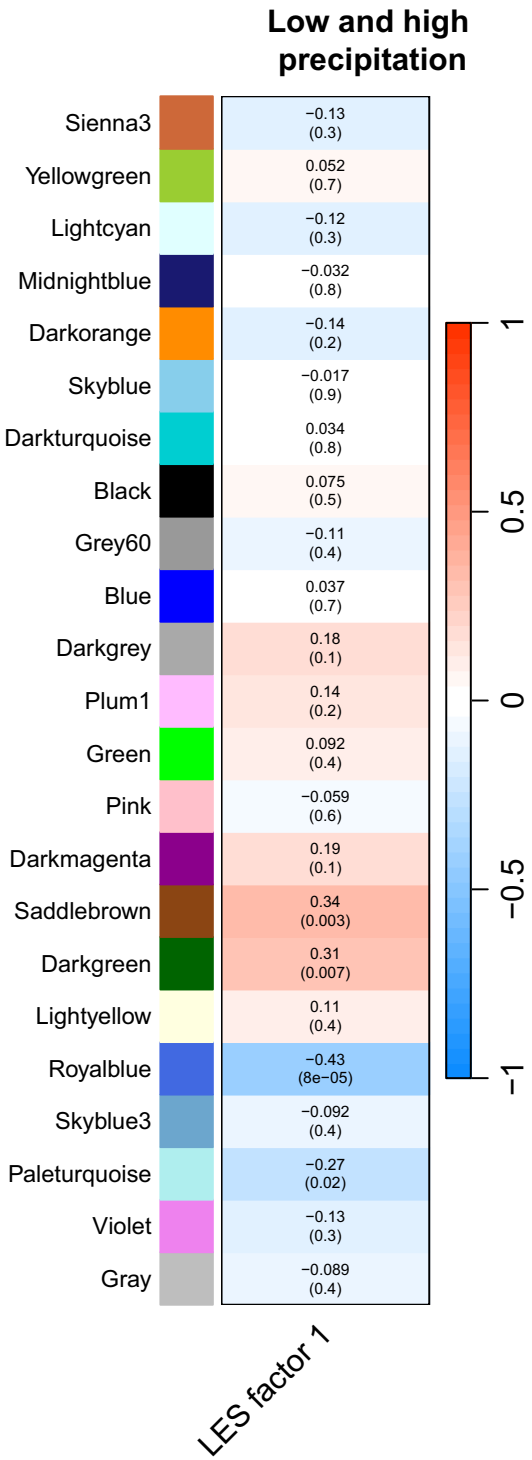

Fig. 4 Relationship between gene expression modules and leaf economics factor score (LES factor 1) in *Panicum virgatum*. Gene expression modules were calculated using weighted gene co-expression network analysis, and LES factor 1 was calculated through exploratory factor analysis on three leaf economics traits – N_{MASS}, LMA, and A_{MASS} – across high + low precipitation. The top number in each box represents the Pearson correlation coefficient, and the number in parentheses is the *P*-value of the correlation. Gene expression modules were clustered so that each module included at least 100 genes. A_{MASS}, mass-based photosynthetic rate; LMA, leaf mass per area; N_{MASS}, leaf % N concentration.

compound in the darkgreen module (GO:1901698, $P < 0.001$). Aside from these key leaf economics functions, another large class of GO terms significantly associated with LES factor 1 was related to biotic and abiotic stress, including response to virus and jasmonic acid-mediated signaling pathway in the royalblue module (GO:0009615, $P = 0.014$; GO:0009867, $P = 0.049$) and abscisic acid-activated signaling pathway and positive regulation of defense response in the saddlebrown module (GO:0009738, $P = 0.030$; GO:0031349, $P = 0.015$).

Discussion

The LES is observed across communities and biogeographic locations at a variety of scales, but often breaks down within species and across resource gradients (Wright *et al.*, 2004; Edwards *et al.*, 2014; Martin *et al.*, 2017; Anderegg *et al.*, 2018). We found that precipitation treatment (i.e. water-availability-related phenotypic plasticity), genotype (i.e. standing genetic variation), and precipitation-by-genotype were significant predictors of different leaf economics traits, highlighting the importance of these sources of variation in understanding leaf economics strategies in *P. virgatum*. At high precipitation, bivariate relationships between LMA, N_{MASS}, and A_{MASS} were most consistent with LES predictions, and integration among these three traits was highest. At lower precipitation, these bivariate relationships and multivariate integration weakened primarily, although not exclusively, because A_{MASS} was more sensitive to changes in precipitation than LMA and N_{MASS}. Similarly, correlational selection for leaf economics covariation was strongest at high precipitation when LMA–N_{MASS} combinations were significantly associated with plant performance. Together, these results are consistent with the idea that phenotypic integration declines with decreasing water availability (Gianoli & Palacio-López, 2009; Matesanz *et al.*, 2021). Finally, transcriptomic results provide support for the functional identity of a previously hypothesized latent variable underlying the LES. Specifically, genes associated with leaf economics strategies functioned in chloroplasts and photosynthesis, cell walls, and nitrogen metabolism.

Changes in precipitation impact leaf economics traits differently

Despite the importance of intraspecific trait variation, its two major sources – phenotypic plasticity and genetic variation – have been challenging to disentangle (Westerband *et al.*, 2021). Here, both phenotypic plasticity (precipitation treatment effect) and standing genetic variation (genotype effect and genotype-by-precipitation effects) were important contributors to leaf economics trait expression. Moreover, the contributions of plasticity and genetic variation differed among traits – the highly dynamic trait, A_{MASS}, was more plastic than LMA and N_{MASS}, which typically reflect conditions over several weeks to months. This difference in plasticity was particularly apparent in bivariate analyses, where the LMA–N_{MASS} relationship changed less across the precipitation gradient than the relationships between A_{MASS} and

these other two traits. One possible explanation for the changes in leaf economics relationships is a change in resource limitation: at higher precipitation, N_{MASS} and A_{MASS} become more coupled, which is consistent with a shift from water- to nitrogen-limited growth (Hooper & Johnson, 1999).

Correlational selection supports the evolution of the LES at high precipitation

Because plants are often functionally integrated, selection on combinations of traits – correlational selection – is likely to be an important evolutionary process, but correlational selection has been studied considerably less than other forms of selection (Svensson, 2023). In fact, the most extensive survey of studies of selection on the LES found little evidence for correlational selection (Donovan *et al.*, 2011). Here, we found evidence for correlational selection on the LES: concordant LMA – N_{MASS} combinations – a conservative strategy of high LMA and low N_{MASS} or an acquisitive strategy of low LMA and high N_{MASS} – were more advantageous at high precipitation than discordant LMA – N_{MASS} combinations that do not correspond to either LES strategy. This may occur because the advantage of low N_{MASS} – low resource requirements to maintain metabolic function – is most beneficial when leaves are sufficiently strong (i.e. high LMA) to live long enough to recoup the carbon costs of their construction (Wright *et al.*, 2004). Thus, our results are consistent with the idea that phenotypic integration is strongest in high-resource environments because selection favors particular trait combinations. In the low precipitation treatment, phenotypic integration appears to have broken down – directional selection favored high A_{MASS} and low N_{MASS} rather than combinations of traits. This decline in phenotypic integration at low precipitation may have occurred because the stress imposed by this treatment was beyond the adaptive range of many genotypes (Riley *et al.*, 2023), resulting in high relative performance among the plants that were best able to continue assimilating carbon when water availability was low. Another possibility that we cannot rule out is that our sample size was too low to detect weaker, but still biologically meaningful, correlational selection on LES trait combinations at low precipitation (Kingsolver *et al.*, 2012). Bootstrapped CIs on selection gradient estimates, however, support our conclusion that selection favors more integrated phenotypes at high precipitation than at low precipitation.

Gene expression identifies functions underlying the LES

Identifying the genes and gene pathways that impact leaf economics traits can contribute substantially to understanding the evolution of leaf economics strategies. Here, we found that many genes involved in functions hypothesized to drive leaf economics strategies were associated with our leaf economics traits, including many genes associated with chloroplasts and photosynthesis, cell walls, and nitrogen metabolism (Shipley *et al.*, 2006; Onoda *et al.*, 2017). A previous study also found that acquisitive liana species exhibited gene expression profiles consistent with rapid growth, high photosynthetic rates, and limited stress responses (Sezen *et al.*, 2022). This suggests that transcriptomics can contribute substantially to identifying the latent variable or variables underlying the LES. While not hypothesized by Shipley *et al.* (2006) or Onoda *et al.* (2017), we also detected notable enrichment in genes associated with biotic stress. This could occur because the acquisitive–conservative trade-off described by the LES extends to defense against herbivores and pathogens (Mason & Donovan, 2015; Welsh *et al.*, 2016; Heckman *et al.*, 2019). Some evidence supports this in *P. virgatum*: high-LMA, southern genotypes tend to have stronger leaves that are more resistant to insect herbivory and fungal disease than low-LMA, northern genotypes (VanWallendael *et al.*, 2020; Durant *et al.*, 2024; Headrick *et al.*, 2024).

Comparing leaf economics traits to transcriptional profiles across different conditions may help to identify the genes that consistently underlie the LES (Cruz *et al.*, 2020; Darwiche & Struhl, 2020). Supporting this, our co-expression network analysis found stronger relationships between gene modules and leaf economics traits or trait combinations when high and low precipitation treatments were combined than when either treatment was examined in isolation. These core genes that exhibit strong relationships with leaf economics traits or trait combinations across treatments, then, are compelling targets for further study of leaf economics strategies. Future studies could improve our understanding of the molecular mechanisms driving the LES by examining whether these core genes are associated with leaf economics traits using genetic mapping approaches, like linkage or association mapping (Napier *et al.*, 2023). Future studies could also functionally validate these results through genome editing or manipulative experiments using mutants for a particular gene or molecular mechanism. While this is not yet possible in most wild species, some model organisms like *Arabidopsis thaliana* possess mutants for many genes and CRISPR/Cas9 now has many experimental applications (Scholl *et al.*, 2000; Moradpour & Abdulah, 2020). Similarly, the genes associated with leaf economics traits in a single environment may also be valuable for future leaf economics research. Future studies could examine whether these genes have important environment-specific effects on leaf economics traits or whether we lacked the statistical power to detect their effect in the other environment.

The links between gene expression and leaf economics traits that we observed may arise from differences in leaf functional anatomy between plants with different leaf economics strategies, such as differences in cell size and tissue composition. For instance, high photosynthetic rates may be promoted by large bundle sheath cells, and long leaf lifespans may arise from proportionally large amounts of cell wall (Sage, 2004; Onoda & Wright, 2018; Watcharamongkol *et al.*, 2018). If these compositional differences are associated with different transcriptional profiles across cell types, aggregating these differences across entire leaf sections could elicit differential gene expression (Marand *et al.*, 2021; Xia *et al.*, 2022). Thus, even though gene expression at a single time point may represent different time scales than some of the leaf economics traits that we measured – A_{MASS} is far more dynamic than LMA or N_{MASS}, for instance – all three traits may be linked to gene expression through leaf morphology or

composition. Future studies could distinguish between variation in gene expression that is driven by leaf composition and variation driven by gene expression differences within a given tissue type (i.e. do plants with an acquisitive strategy have inherently acquisitive cells?) by using single cell RNA sequencing (scRNA-Seq) (Wang *et al.*, 2021).

Conclusions

Intraspecific variation in leaf economics traits may be an important determinant of species responses to environmental variation and their impacts on ecosystem functioning (Anderegg *et al.*, 2018; Westerband *et al.*, 2021). Intraspecific variation is also critical to the evolution of the LES (Donovan *et al.*, 2011; Anderegg *et al.*, 2018), however, relatively little is known about its origins. Here, we demonstrate that leaf economics traits and trait combinations exhibit considerable variability in response to changes in precipitation. Specifically, the selective pressure on different leaf economics traits changed with precipitation – from directional selection on individual traits at low precipitation to correlational selection on combinations of LMA and N_{MASS} at high precipitation. This is particularly important because the LES may evolve differently than predicted when different precipitation regimes select for different combinations of leaf economics traits and plasticity alters the expression of these traits. Finally, our gene expression results support the long-standing hypothesis that a latent variable underlying the LES includes major functions related to chloroplasts and photosynthesis, cell walls, and nitrogen metabolism, which can improve our knowledge of LES evolution across broader scales.

Acknowledgements

We thank K. Dodge, A. Gibson, Q. Hiers, T. Logan, A. Naranjo, K. Tiner, and B. Whitaker for technical support, members of the Juenger laboratory for discussion and helpful suggestions on earlier versions of this manuscript, J. Edwards for providing genome annotations, and L. Bartley for interpreting Gene Ontology results. This research was supported by the Office of Science (BER), the US Department of Energy (grant no. DE-SC0014156), the National Science Foundation (awards PGRP IOS-0922457 and IOS1444533), the US Department of Agriculture National Institute of Food and Agriculture (award no. 2019-67013-29161), and in part by the US Department of Agriculture Forest Service, Rocky Mountain Research Station. The findings and conclusions of this publication are those of the authors and should not be construed to represent any official USDA or US Government determination or policy.

Competing interests

None declared.

Author contributions

RWH analyzed data and led writing. SR analyzed data. MJA, PAF and TEJ conceived and implemented the experiment. MJA, SHT, DBL, AK and JEB collected data. All authors contributed to writing.

ORCID

Michael J. Aspinwall https://orcid.org/0000-0003-0199-2972
Jason E. Bonnette https://orcid.org/0000-0003-1835-8409
Philip A. Fay https://orcid.org/0000-0002-8291-6316
Robert W. Heckman https://orcid.org/0000-0002-2281-3091
Thomas E. Juenger https://orcid.org/0000-0001-9550-9288
Albina Khasanova https://orcid.org/0000-0001-6652-012X
David B. Lowry https://orcid.org/0000-0002-8182-1059
Samsad Razzaque https://orcid.org/0000-0003-0836-2076
Samuel H. Taylor https://orcid.org/0000-0001-9714-0656

Data availability statement

The Tag-Seq data have been deposited in the National Center for Biotechnology Information (NCBI) short read archive (SRA) under BioProject ID PRJNA1108763 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1108763). Phenotypic data associated with this study are available at the USDA Forest Service Research Data Archive (doi: 10.2737/RDS-2024-0043).

References

Alexa A, Rahnenfuhrer J. 2024. *topGO: enrichment analysis for gene ontology.* R package v.2.56.0. [WWW document] URL https://bioconductor.org/packages/topGO.

Anderegg LDL, Berner LT, Badgley G, Sethi ML, Law BE, HilleRisLambers J, Penuelas J. 2018. Within-species patterns challenge our understanding of the leaf economics spectrum. *Ecology Letters* **21**: 734–744.

Andrews S. 2010. *FastQC: a quality control tool for high throughput sequence data.* [WWW document] URL https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Aspinwall MJ, Blackman CJ, Maier C, Tjoelker MG, Rymer PD, Creek D, Chieppa J, Griffin-Nolan RJ, Tissue DT. 2023. Aridity drives clinal patterns in leaf traits and responsiveness to precipitation in a broadly distributed Australian tree species. *Plant-Environment Interactions* **4**: 70–85.

Aspinwall MJ, Fay PA, Hawkes CV, Lowry DB, Khasanova A, Bonnette J, Whitaker BK, Johnson N, Juenger TE. 2017. Intraspecific variation in precipitation responses of a widespread C_4 grass depends on site water limitation. *Journal of Plant Ecology* **10**: 310–321.

Aspinwall MJ, Lowry DB, Taylor SH, Juenger TE, Hawkes CV, Johnson M-VV, Johnson M-VV, Kiniry JR, Fay PA. 2013. Genotypic variation in traits linked to climate and aboveground productivity in a widespread C_4 grass: evidence for a functional trait syndrome. *New Phytologist* **199**: 966–980.

Blonder B, Vasseur F, Violle C, Shipley B, Enquist BJ, Vile D. 2015. Testing models for the leaf economics spectrum with leaf and whole-plant traits in *Arabidopsis thaliana*. *AoB Plants* **7**: plv049.

Casler MD. 2012. Switchgrass breeding, genetics, and genomics. In: Monti A, ed. *Switchgrass: a valuable biomass crop for energy.* London, UK: Springer, 29–53.

Casler MD, Vogel KP, Harrison M. 2015. Switchgrass germplasm resources. *Crop Science* **55**: 2463–2478.

Chen W, Wu Y, Fritschi FB, Juenger TE. 2021. The genetic basis of the root economics spectrum in a perennial grass. *Proceedings of the National Academy of Sciences, USA* **118**: e2107541118.

Cruz DF, De Meyer S, Ampe J, Sprenger H, Herman D, Van Hautegem T, De Block J, Inzé D, Nelissen H, Maere S. 2020. Using single-plant-omics in the

field to link maize genes to functions and phenotypes. *Molecular Systems Biology* **16**: e9667.

Darwiche R, Struhl K. 2020. Pheno-RNA, a method to associate genes with a specific phenotype, identifies genes linked to cellular transformation. *Proceedings of the National Academy of Sciences, USA* **117**: 28925–28929.

Donovan LA, Maherali H, Caruso CM, Huber H, de Kroon H. 2011. The evolution of the worldwide leaf economics spectrum. *Trends in Ecology & Evolution* **26**: 88–95.

Durant PC, Bhasin A, Juenger TE, Heckman RW. 2024. Genetically correlated leaf tensile and morphological traits are driven by growing season length in a widespread perennial grass. *American Journal of Botany* **111**: e16349.

Edwards EJ, Chatelet DS, Sack L, Donoghue MJ. 2014. Leaf life span and the leaf economic spectrum in the context of whole plant architecture. *Journal of Ecology* **102**: 328–336.

Exposito-Alonso M, Gómez Rodríguez R, Barragán C, Capovilla G, Chae E, Devos J, Burbano HA, Bossdorf O, Nielsen R, Weigel D *et al.* 2019. Natural selection on the *Arabidopsis thaliana* genome in present and future climates. *Nature* **573**: 126–129.

Famiglietti CA, Worden M, Anderegg LDL, Konings AG. 2024. Impacts of climate timescale on the stability of trait–environment relationships. *New Phytologist* **241**: 2423–2434.

Frick H, Chow F, Kuhn M, Mahoney M, Silge J, Wickham H. 2024. *RSAMPLE: general resampling infrastructure.* [WWW document] URL https://CRAN.R-project.org/package=rsample.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**: 394–407.

Gianoli E, Palacio-López K. 2009. Phenotypic integration may constrain phenotypic plasticity in plants. *Oikos* **118**: 1924–1928.

Headrick KC, Juenger TE, Heckman RW. 2024. Plant physical defenses contribute to a latitudinal gradient in resistance to insect herbivory within a widespread perennial grass. *American Journal of Botany* **111**: e16260.

Heckman RW, Halliday FW, Mitchell CE. 2019. A growth–defense trade-off is general across native and exotic grasses. *Oecologia* **191**: 609–620.

Heckman RW, Khasanova AR, Johnson NS, Weber S, Bonnette JE, Aspinwall MJ, Reichmann LG, Juenger TE, Fay PA, Hawkes CV. 2020. Plant biomass, not plant economics traits, determines responses of soil CO_2 efflux to precipitation in the C_4 grass *Panicum virgatum*. *Journal of Ecology* **108**: 2095–2106.

Heckman RW, Pereira CG, Aspinwall MJ, Juenger TE. 2024. Physiological responses of C_4 perennial bioenergy grasses to climate change: causes, consequences, and constraints. *Annual Review of Plant Biology* **75**: 737–769.

Hooper DU, Johnson L. 1999. Nitrogen limitation in dryland ecosystems: responses to geographical and temporal variation in precipitation. *Biogeochemistry* **46**: 247–293.

Horvath S. 2011. Weighted network analysis. In: *Applications in genomics and systems biology*. New York, NY, USA: Springer.

John GP, Scoffoni C, Buckley TN, Villar R, Poorter H, Sack L. 2017. The anatomical and compositional basis of leaf mass per area. *Ecology Letters* **20**: 412–425.

Keenan TF, Niinemets U. 2016. Global leaf trait estimates biased due to plasticity in the shade. *Nature Plants* **3**: 16201.

Kingsolver JG, Diamond SE, Siepielski AM, Carlson SM. 2012. Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. *Evolutionary Ecology* **26**: 1101–1118.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.

Langfelder P, Horvath S. 2008. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* **9**: 559.

Langfelder P, Horvath S. 2012. Fast R functions for robust correlations and hierarchical clustering. *Journal of Statistical Software* **46**: i11.

Lenth R. 2018. *EMMEANS: estimated marginal means, aka least-squares means.* [WWW document] URL https://CRAN.R-project.org/package=emmeans.

Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv*. doi: 10.48550/arXiv.1303.3997.

Liao Y, Smyth GK, Shi W. 2013. FEATURECOUNTS: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**: 923–930.

Lohman BK, Weber JN, Bolnick DI. 2016. Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. *Molecular Ecology Resources* **16**: 1315–1321.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550.

Lovell JT, MacQueen AH, Mamidi S, Bonnette J, Jenkins J, Napier JD, Sreedasyam A, Healey A, Session A, Shu S *et al.* 2021. Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. *Nature* **590**: 438–444.

Lovell JT, Shakirov EV, Schwartz S, Lowry DB, Aspinwall MJ, Taylor SH, Bonnette J, Palacio-Mejia JD, Hawkes CV, Fay PA *et al.* 2016. Promises and challenges of eco-physiological genomics in the field: tests of drought responses in switchgrass. *Plant Physiology* **172**: 734–748.

Lowry DB, Behrman KD, Grabowski P, Morris GP, Kiniry JR, Juenger TE. 2014. Adaptations between ecotypes and along environmental gradients in *Panicum virgatum*. *American Naturalist* **183**: 682–692.

Marand AP, Chen Z, Gallavotti A, Schmitz RJ. 2021. A *cis*-regulatory atlas in maize at single-cell resolution. *Cell* **184**: 3041–3055.

Martin AR, Rapidel B, Roupsard O, Van den Meersche K, de Melo Virginio Filho E, Barrios M, Isaac ME. 2017. Intraspecific trait variation across multiple scales: the leaf economics spectrum in coffee. *Functional Ecology* **31**: 604–612.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal* **17**: 2156.

Mason C, Donovan L. 2015. Does investment in leaf defenses drive changes in leaf economic strategy? A focus on whole-plant ontogeny. *Oecologia* **177**: 1053–1066.

Mason CM, Goolsby EW, Humphreys DP, Donovan LA. 2016. Phylogenetic structural equation modelling reveals no need for an 'origin' of the leaf economics spectrum. *Ecology Letters* **19**: 54–61.

Matesanz S, Blanco-Sánchez M, Ramos-Muñoz M, de la Cruz M, Benavides R, Escudero A. 2021. Phenotypic integration does not constrain phenotypic plasticity: differential plasticity of traits is associated to their integration across environments. *New Phytologist* **231**: 2359–2370.

Melton AE, Moran K, Martinez P, Ellestad P, Milliken E, Morales W, Child AW, Richardson BA, Serpe M, Novak SJ *et al.* 2023. A genotype × environment experiment reveals contrasting response strategies to drought between populations of a keystone species (*Artemisia tridentata*; Asteraceae). *Plant-Environment Interactions* **4**: 201–214.

Meyer E, Aglyamova GV, Matz MV. 2011. Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. *Molecular Ecology* **20**: 3599–3616.

Mitchell-Olds T, Shaw RG. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**: 1149–1161.

Mooney HA. 1972. The carbon balance of plants. *Annual Review of Ecology and Systematics* **3**: 315–346.

Moradpour M, Abdulah SNA. 2020. CRISPR/dCas9 platforms in plants: strategies and applications beyond genome editing. *Plant Biotechnology Journal* **18**: 32–44.

Napier JD, Heckman RW, Juenger TE. 2023. Gene-by-environment interactions in plants: molecular mechanisms, environmental drivers, and adaptive plasticity. *Plant Cell* **35**: 109–124.

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, Weedon J. 2022. *VEGAN: community ecology package.* R package v.2.6-4. [WWW document] URL https://CRAN.R-project.org/package=vegan

Onoda Y, Wright IJ. 2018. The leaf economics spectrum and its underlying physiological and anatomical principles. In: Adams WW III, Terashima I, eds. *The leaf: a platform for performing photosynthesis*. Cham, Switzerland: Springer International Publishing, 451–471.

Onoda Y, Wright IJ, Evans JR, Hikosaka K, Kitajima K, Niinemets Ü, Poorter H, Tosens T, Westoby M. 2017. Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytologist* **214**: 1447–1463.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE *et al.* 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**: 167–234.

Pigliucci M, Preston K, eds. 2004. *Phenotypic integration: studying the ecology and evolution of complex phenotypes.* Oxford, UK: Oxford University Press.

Pinheiro J, Bates D, R Core Team. 2023. *NLME: linear and nonlinear mixed effects models.* R package v.3.1-164. [WWW document] URL https://CRAN.R-project.org/package=nlme.

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**: 565–588.

Revelle W. 2024. *PSYCH: procedures for psychological, psychometric, and personality research.* R package v.2.4.6.

Riley CL, Oostra V, Plaistow SJ. 2023. Does the definition of a novel environment affect the ability to detect cryptic genetic variation? *Journal of Evolutionary Biology* **36**: 1618–1629.

Sage RF. 2004. The evolution of C_4 photosynthesis. *New Phytologist* **161**: 341–370.

Schlichting CD. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics* **17**: 667–693.

Scholl RL, May ST, Ware DH. 2000. Seed and molecular resources for Arabidopsis. *Plant Physiology* **124**: 1477–1480.

Semenov MA, Brooks RJ, Barrow EM, Richardson CW. 1998. Comparison of the WGEN and LARS-WG stochastic weather generators for diverse climates. *Climate Research* **10**: 95–107.

Sezen UU, Worthy SJ, Umaña MN, Davies SJ, McMahon SM, Swenson NG. 2022. Comparative transcriptomics of tropical woody plants supports fast and furious strategy along the leaf economics spectrum in lianas. *Biology Open* **11**: 489.

Shipley B, Lechowicz MJ, Wright I, Reich PB. 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology* **87**: 535–541.

Stinchcombe JR, Agrawal AF, Hohenlohe PA, Arnold SJ, Blows MW. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution* **62**: 2435–2440.

Svensson EI. 2023. Phenotypic selection in natural populations: what have we learned in 40 years? *Evolution* **77**: 1493–1504.

terHorst CP, Lau JA, Cooper IA, Keller KR, Rosa RJL, Royer AM, Schultheis EH, Suwa T, Conner JK. 2015. Quantifying nonadditive selection caused by indirect ecological effects. *Ecology* **96**: 2360–2369.

Torices R, Muñoz-Pajares AJ. 2015. PHENIX: an R package to estimate a size-controlled phenotypic integration index. *Applications in Plant Sciences* **3**: 1400104.

VanWallendael A, Bonnette J, Juenger TE, Fritschi FB, Fay PA, Mitchell RB, Lloyd-Reilley J, Rouquette FM Jr, Bergstrom GC, Lowry DB. 2020. Geographic variation in the genetic basis of resistance to leaf rust between locally adapted ecotypes of the biofuel crop switchgrass (*Panicum virgatum*). *New Phytologist* **227**: 1696–1708.

Volaire F. 2018. A unified framework of plant adaptive strategies to drought: crossing scales and disciplines. *Global Change Biology* **24**: 2929–2938.

Wagner GP. 1984. On the eigenvalue distribution of genetic and phenotypic dispersion matrices: evidence for a nonrandom organization of quantitative character variation. *Journal of Mathematical Biology* **21**: 77–95.

Wang S, Zhou D-W. 2022. Associations between leaf developmental stability, variability, canalization, and phenotypic plasticity in *Abutilon theophrasti*. *Ecology and Evolution* **12**: e8845.

Wang Y, Huan Q, Li K, Qian W. 2021. Single-cell transcriptome atlas of the leaf and root of rice seedlings. *Journal of Genetics and Genomics* **48**: 881–898.

Watcharamongkol T, Christin P-A, Osborne CP. 2018. C_4 photosynthesis evolved in warm climates but promoted migration to cooler ones. *Ecology Letters* **21**: 376–383.

Welsh ME, Cronin JP, Mitchell CE. 2016. The role of habitat filtering in the leaf economics spectrum and plant susceptibility to pathogen infection. *Journal of Ecology* **104**: 1768–1777.

Westerband AC, Funk JL, Barton KE. 2021. Intraspecific trait variation in plants: a renewed focus on its role in ecological processes. *Annals of Botany* **127**: 397–410.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M *et al.* 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

Xia K, Sun H-X, Li J, Li J, Zhao Y, Chen L, Liu G, Yin R, Mu B, Wang X *et al.* 2022. The single-cell stereo-seq reveals region-specific cell subtypes and transcriptome profiling in *Arabidopsis* leaves. *Developmental Cell* **57**: 1299–1310.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Univariate effects of leaf economics traits on plant relative biomass separately by precipitation treatment, selection gradients.

Fig. S2 Relationship between gene expression modules and leaf economics traits at high + low precipitation.

Fig. S3 Relationship between gene expression modules and leaf economics traits at high precipitation.

Fig. S4 Relationship between gene expression modules and leaf economics traits at low precipitation.

Table S1 Summary of genotypes studied.

Table S2 ANOVAs showing effects of genotype, precipitation, and genotype-by-precipitation on leaf economics traits.

Table S3 ANOVAs of bivariate leaf economics relationships.

Table S4 *Post hoc* test results examining bivariate leaf economics relationships separately by precipitation treatment.

Table S5 Standardized effects of leaf economics traits and precipitation on plant relative biomass, selection differentials.

Table S6 ANOVA showing effects of leaf economics traits and precipitation on plant relative biomass, selection differentials.

Table S7 Effects of leaf economics traits on plant relative biomass separately by precipitation treatment, selection differentials.

Table S8 Standardized effects of leaf economics traits and precipitation on plant relative biomass, selection gradients.

Table S9 ANOVA showing effects of leaf economics traits and precipitation on plant relative biomass, selection gradients.

Table S10 Effects of leaf economics traits on plant relative biomass separately by precipitation treatment, selection gradients.

Table S11 Significant Gene Ontology enrichment terms for gene expression modules from WGCNA performed on the low and high treatments combined.