Codominant grasses differ in gene expression under experimental climate extremes in native tallgrass prairie (#22086)

First submission

Editor guidance

Please submit by **21 Dec 2017** for the benefit of the authors (and your $200 publishing discount).

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Raw data check
Review the raw data. Download from the location described by the author.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files

Download and review all files from the materials page.

3 Figure file(s)
2 Table file(s)
1 Other file(s)

Custom checks

Field study

Have you checked the authors field study permits?

Are the field study permits appropriate?

Structure and Criteria

Structure your review

The review form is divided into 5 sections.
Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- ✓ Clear, unambiguous, professional English language used throughout.
- ✓ Intro & background to show context. Literature well referenced & relevant.
- ✓ Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- ✓ Figures are relevant, high quality, well labelled & described.
- ✓ Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- ✓ Original primary research within Scope of the journal.
- ✓ Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- ✓ Rigorous investigation performed to a high technical & ethical standard.
- ✓ Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- ℹ Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- ✓ Data is robust, statistically sound, & controlled.
- ✓ Conclusions are well stated, linked to original research question & limited to supporting results.
- ✓ Speculation is welcome, but should be identified as such.

Standout reviewing tips

The best reviewers use these techniques

Tip	***Example***
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Codominant grasses differ in gene expression under experimental climate extremes in native tallgrass prairie

Ava M Hoffman Corresp., [1, 2] , **Meghan L Avolio** [3] , **Alan K Knapp** [1, 2] , **Melinda D Smith** [1, 2]

[1] Department of Biology, Colorado State University, Fort Collins, Colorado, United States
[2] Graduate Degree Program in Ecology, Colorado State University, Fort Collins, Colorado, United States
[3] Department of Earth & Planetary Sciences, The Johns Hopkins University, Baltimore, Maryland, United States

Corresponding Author: Ava M Hoffman
Email address: ava.hoffman@colostate.edu

Extremes in climate, such as heat waves and drought, are expected to become more frequent and intense with forecasted climate change. Plant species will almost certainly differ in their responses to these stressors. We experimentally imposed a heat wave and drought in the tallgrass prairie ecosystem near Manhattan, Kansas, USA to assess transcriptional responses of two ecologically important C_4 grass species, *Andropogon gerardii* and *Sorghastrum nutans*. Based on previous research, we expected that *S. nutans* would regulate more genes, particularly those related to stress response, under high heat and drought. Across all treatments, *S. nutans* showed greater expression of negative regulatory and catabolism genes while *A. gerardii* upregulated cellular and protein metabolism. As predicted, *S. nutans* showed greater sensitivity to water stress, particularly with downregulation of non-coding RNAs and upregulation of water stress and catabolism genes. *A. gerardii* was less sensitive to drought, although *A. gerardii* tended to respond with upregulation in response to drought versus *S. nutans* which downregulated more genes under drier conditions. Surprisingly, *A. gerardii* only showed minimal gene expression response to increased temperature, while *S. nutans* showed no response. Gene functional annotation suggested that these two species may respond to stress via different mechanisms. Specifically, *A. gerardii* tends to maintain molecular function while *S. nutans* prioritizes avoidance. *Sorghastrum nutans* may strategize abscisic acid response and catabolism to respond rapidly to stress. These results have important implications for success of these two important grass species under a more variable and extreme climate forecast for the future.

Codominant grasses differ in gene expression under experimental climate extremes in native tallgrass prairie

Ava M. Hoffman[1,2], Meghan L. Avolio[3], Alan K. Knapp[1,2], Melinda D. Smith[1,2]
[1] Department of Biology, Colorado State University, Fort Collins, CO, USA.
[2] Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA.
[2] Department of, The Johns Hopkins University, Baltimore, MD, USA.

Corresponding Author:
Ava Hoffman[1,2]

Email address: ava.hoffman@colostate.edu or avamariehoffman@gmail.com

Abstract

Extremes in climate, such as heat waves and drought, are expected to become more frequent and intense with forecasted climate change. Plant species will almost certainly differ in their responses to these stressors. We experimentally imposed a heat wave and drought in the tallgrass prairie ecosystem near Manhattan, Kansas, USA to assess transcriptional responses of two ecologically important C_4 grass species, *Andropogon gerardii* and *Sorghastrum nutans*. Based on previous research, we expected that *S. nutans* would regulate more genes, particularly those related to stress response, under high heat and drought. Across all treatments, *S. nutans* showed greater expression of negative regulatory and catabolism genes while *A. gerardii* upregulated cellular and protein metabolism. As predicted, *S. nutans* showed greater sensitivity to water stress, particularly with downregulation of non-coding RNAs and upregulation of water stress and catabolism genes. *A. gerardii* was less sensitive to drought, although *A. gerardii* tended to respond with upregulation in response to drought versus *S. nutans* which downregulated more genes under drier conditions. Surprisingly, *A. gerardii* only showed minimal gene expression response to increased temperature, while *S. nutans* showed no response. Gene functional annotation suggested that these two species may respond to stress via different mechanisms. Specifically, *A. gerardii* tends to maintain molecular function while *S. nutans* prioritizes avoidance. *Sorghastrum nutans* may strategize abscisic acid response and catabolism to respond rapidly to stress. These results have important implications for success of these two important grass species under a more variable and extreme climate forecast for the future.

Introduction

Climatic extremes, such as drought and heat waves, are predicted to increase in frequency and magnitude with forecasted climate change (IPCC 2013). These extreme events may significantly impact ecosystem structure and function depending on their severity (Ciais et al. 2005; García-Herrera et al. 2010; Smith 2011; Knapp et al. 2015). Climate extremes may affect plants in species-specific ways, such as through timing of bud development (Bokhorst et al. 2008), variation in tissue die-back (Kreyling et al. 2008), and differences in physiological performance (Hoover et al. 2014a) or chemical composition (AbdElgawad et al. 2014). Several recent studies have found species to respond differentially to extreme events like drought and heat waves (Beierkuhnlein et al. 2011; Hoover et al. 2014a; Nardini et al. 2016). However, the mechanisms that lead to differences in plant performance are not always clear (McDowell et al. 2008). Understanding gene regulation may help explain the mechanisms of plant response to novel stressful environments (Leakey et al. 2009; Swarbreck et al. 2011). Gene regulation may also be more sensitive to periods of extreme climate compared with physiological performance and growth traits that may have delayed response. Likewise, gene regulation may reveal variation which can affect fitness, selection, and adaptation to new environmental conditions (Ouborg and Vriezen 2007; Gibson 2008; Avolio and Smith 2013; Vázquez et al. 2015). However, most molecular studies of plant responses to drought and heat stress are focused on model organisms with limited ecological relevance (Leakey et al. 2009), although awareness and sequencing costs are improving our understanding (Voesenek et al. 2014; Ellegren 2014)

Here, we present a comparison of gene regulation responses of two C_4 grass species, *Andropogon gerardii* and *Sorghastrum nutans*, to an experimentally induced heat wave and drought in the field. These two dominant grasses are native to the tallgrass prairie ecosystem of the Central U.S. and play an important role in determining community and ecosystem structure and function (Smith and Knapp 2003; Whitham et al. 2006; Whitham et al. 2008; Koerner et al. 2014). They are often assumed to be functionally similar (i.e., both closely related phylogenetically, rhizomatous, C_4 warm-season tallgrasses, Weaver and Fitzpatrick 1934; Benson and Hartnett 2006; Estep et al. 2014), and both are relatively resistant to stress (Knapp 1985; Swemmer et al. 2006; Tucker et al. 2011). However, *A. gerardii* and *S. nutans* differ in physiological response and abundance under different temperature and water availability (Silletti and Knapp 2002; Silletti et al. 2004; Swemmer et al. 2006; Nippert et al. 2009; Hoover et al. 2014b; Hoover et al. 2014a). At the level of gene regulation, *A. gerardii* has been shown to be more sensitive to thermal stress (Travers et al. 2007; Travers et al. 2010; but see Smith et al. 2016) while *S. nutans* is more sensitive to moderate water stress (Smith et al. 2016; Hoffman and Smith 2017). Specifically, *S. nutans* was more responsive to both a year-round 2°C increase in temperature and more variable precipitation patterns (and lower average soil water availability) than *A. gerardii* (Smith et al. 2016). *Sorghastrum nutans* also showed greater plasticity for dealing with water stress at the gene level (Hoffman and Smith 2017). To date, much of the research assessing sensitivity of these grasses to heat and water stress has focused on chronic, subtle changes in temperature (2°C increase in temperature) and water availability (on average 14% reduction in soil moisture; Fay et al. 2011). It remains unknown whether these two species would regulate genes differently under more extreme conditions, such as heat waves and droughts, which are predicted increase in frequency and severity in the Central US with climate change (Cook et al. 2015).

To increase our mechanistic understanding of the response of *A. gerardii* and *S. nutans* to climate extremes typical of the region, we analyzed the transcriptional profiles of both grass species during an 18-day controlled heat wave under both watered and drought conditions within natural field plots. As in past research (Travers et al. 2007; Travers et al. 2010; Smith et al. 2016), we measured gene expression using heterologous hybridization with cDNA microarrays designed for a closely related model species, *Zea mays.* We coupled the microarray data with filtering through each species' RNA-seq transcriptome (Hoffman and Smith 2017). We hypothesized that gene regulation (number of genes, functional groups) would differ between *A. gerardii* and *S. nutans* in response to the heat wave under both watered and drought conditions, with these grasses employing different strategies for coping with extreme heat and water stress.

Methods

Site description and experimental treatments

The study was carried out within the context of an existing long-term climate change experiment, the Rainfall Manipulation Plots (RaMPs), located at the Konza Prairie Biological Station in north-eastern Kansas (39º05` N, 96º35` W). The RaMPs is located in a native, annually burned site and consists of twelve 14 x 9 m greenhouse shelters (without walls) equipped with a clear (UV transparent) polyethylene roof to exclude natural rainfall inputs (Fay et al. 2011). Our experimental plots were located in two RaMPs (RaMP 12 and 13) in areas outside the 6 x 6 m experimental plots, but still located underneath the shelter infrastructure. Each of these areas is approximately 3 x 8 m in size, within which we located a 3 x 6 m experimental sampling plot. The RaMP 12 sampling plot was watered from late-May to mid-Aug to create a watered condition, whereas all ambient rainfall was excluded from the RaMP 13 sampling plot to create a drought. For both the watered and drought plots, a controlled high heat treatment was achieved by installing pairs of rectangular infrared heating lamps (Kalglo 2000 W, Kalglo Electronic Co Inc., Bethlehem, PA, USA) (Online Resource Fig. 1). This resulted in a high heat treatment zone with a daytime target maximum of +8°C above ambient midday temperature (Online Resource Fig. 2), alongside ambient temperature treatment zones. The four treatments allowed us to examine the effects of drought and heat individually along with their interaction. The high heat treatment was imposed for an 18-day period (July 17 to August 4), when heat waves have generally occurred in the past (Hoover et al. 2014b).

Prior to initiation of the experiment, canopy temperature in the watered sampling plot was measured using an infrared thermometer mounted on a movable platform (approx. 0.5 m above the canopy). Soil moisture was monitored at a depth of 0-15 cm with 30-cm time-domain reflectometery probes (Model CS616, Campbell Scientific, Logan, Utah, USA) inserted at a 45° angle (see supplementary information).

Plant sampling and measurements

The focal species, *A. gerardii* and *S. nutans,* are both are rhizomatous C$_4$ grasses that reproduce primarily vegetatively via belowground buds on rhizomes (Brejda et al. 1989; Carter and VanderWeide 2014), which form dense intermixed stands, making it virtually impossible to differentiate between clones in the field (Avolio et al. 2011). We sampled individuals of *A. gerardii* and *S. nutans* from native populations growing within the experimental treatment plots during two sampling campaigns conducted at Day 4 and Day 18 of the heat wave. Each sampling campaign was conducted between 11:00 and 15:00 CDT to allow for collection of leaf temperature and water status (see below).

During each sampling campaign, we sampled two, morphological similar individuals (tiller or ramets, with 3-5 fully expanded leaves) of each species within the high heat zone and ambient temperature zone in both the watered and drought sampling plots (n = 2 samples per species, four treatments, and two campaign dates, or n = 16 per species, N = 32 total samples). While a sample size of two per species and treatment combination is relatively small, we believe this sample size was appropriate given that our focus was on broadly detecting interspecific differences under the high heat and drought conditions. Although we did not control for plant genotype, we collected our samples within a limited sampling area (10 x 10 cm) to minimize genotypic differences among samples. Leaf tissue was collected from individuals located within each treatment within a five-minute window. For each individual, the first or second fully expanded leaf was randomly selected for genomic analysis to ensure similar leaf age. The entire leaf was clipped and immediately flash-frozen and stored in liquid nitrogen until brought to the laboratory. Immediately after, we measured leaf temperature (T_{leaf}) and midday leaf water potential (Ψ_{mid}) on the remaining fully expanded leaf. T_{leaf} was measured using a LI-6400 system (LiCOR, Inc., Lincoln, NE, USA).The whole leaf was then collected for determination of mid-day leaf water potential (LWP) using an Scholander-type pressure chamber (PMS Instruments, Inc., Corvallis, OR, USA).

RNA preparation and microarray hybridization

Leaf tissue samples were stored in an -80 freezer prior to RNA extraction. Total RNA was extracted from the 32 leaf samples for both species using TRIzol reagent (Invitrogen, Carlsbad, CA) (McCarty 1986), and further purified with the RNeasy kit (Invitrogen, Carlsbad, CA). RNA quantity was measured by a NanoDrop spectrophotometer (Nanodrop products, Thermo Scientific, Wilmington, DE). The verification of RNA quality, preparation of cDNA, and the subsequent steps leading to hybridization and array scanning were performed by Biotechnology Resources of Keck facility at Yale University (http://keck.med.yale.edu/). We used maize spotted cDNA arrays (SAM 1.2, GEO platform GPL4521) produced by the Center for Plant Genomics at Iowa State University for hybridization. The arrays included 15,680 maize cDNA probes (14,118 informative) isolated from maize ear tissue.

Quality control of heterologous hybridizations

In total, there were eight hybridizations for each species per sampling campaign (Online Resource Table 1). Array image data were collected using GenePix software (Version 6, Axon, Downingtown, PA). Prior to normalization across arrays, features with obvious abnormality and saturated signal were flagged and excluded from statistical analysis. Two steps were taken to minimize the probability of mishybridization and sequence divergence between the focal species and the model species (Leakey et al. 2009). First, we used stringent criteria by excluding spots with signal to noise ratios less than 3 or larger than 10 to decrease the inclusion of cross-hybridization artefacts (Verdnik et al. 2002). Second, the cDNA sequences of the maize microarray SAM1.2 (18,862 sequences) were aligned against the de novo RNA-seq transcriptome data sets of *A. gerardii* and *S. nutans* (Hoffman and Smith 2017), previously generated using Trinity (version 2.1.1, Haas et al. 2013). We only included BLASTN (Altschul et al. 1990; Altschul et al. 1997) hits with an e-value cutoff of $1e^{-10}$ and alignment length larger than 150 base pairs from the *A. gerardii* and *S. nutans* transcript data sets. After these two steps, 7,964 and 6,035 probe sequences were included in the analysis, accounting for 61.4% and 56.6% of the maize SAM 1.2 array probes for *A. gerardii* and *S. nutans* respectively. 5,109 features were common to both species. Because features were screened by both the intensity of hybridization signal and sequence similarity, the intensity values of the included features were reliable for further expression analysis. These same techniques have also been validated previously using quantitative real-time PCR (qPCR) (Smith et al. 2016).

Array data normalization and statistical analysis

An important source of systematic errors in two-color microarray experiments is the different properties of the dyes used to label the two samples (Tseng et al. 2001; Yang et al. 2001; Yang et al. 2002) and the hybridization variability from array to array. We used dye-swap design for the same pair of samples in the hybridizations (Online Resource Table 1) to account for the dye effect (Dabney and Storey 2007). Background signals were removed from median signal intensity and modelled similarly to Travers *et al*. (2010) to remove the array and dye effect:

$$y_{ijk} = A_i + D_j + A_iD_j + \varepsilon_{ijk},$$

where y is the median intensity for the kth gene on each array (i) with each dye (j), A is the array effect for each array (i), D is the dye effect for each dye (j), AD is the array × dye interaction, and ε_{ijk} is the stochastic error. Residuals from this model were adjusted by the minimum value to produce all positive residuals. To examine overall statistical effects, we used the residuals in the following model:

$$r_{klmno} = S_l + W_m + T_n + C_o + S_lW_m + S_lT_n + W_mT_n + \varepsilon_{klmno},$$

where r is the residual for each gene (k) with each species (l), water treatment (m), temperature (n), and sampling date (o), S is the species effect, W is the water treatment effect (plot), T is the temperature effect, and C is the sampling date effect. Residuals were used to generate $\log_2$ expression ratios for the four variables: species (*A. gerardii* / *S. nutans*), water treatment (watered / droughted), temperature (ambient / heated), date (day 4 / day 18). Any genes with missing signals were removed. We plotted the $\log_2$ expression ratio against the $\log_{10}$ intensity for each gene and performed a loess correction to normalize each set of $\log_2$ values (Online Resource Fig. 3). Then for each gene without missing values, a linear model was performed to test each main effect (species, water treatment, temperature, and date) as well as selected interactions (species × water treatment, species × temperature, and water treatment × temperature). Because of the variation in genes present across arrays, each model was constructed only if appropriate data was present. In other words, to test species effect, both species had to express the given gene. P-values were adjusted using a Bonferroni correction to account false discovery across multiple tests. All analyses were performed using R (version 3.3.2).

Functional annotation, enrichment, and clustering

The functional annotation of transcripts was based on the Trinotate pipeline (version 3.0.1). We matched microarray probe sequences to known sequences using BLAST against the SwissProt annotated database (Apweiler et al. 2004), identified protein sequence homology using HMMER and Pfam (Finn et al. 2011; Finn et al. 2015), and searched for known annotations within eggNOG and GO databases (The Gene Ontology Consortium 2015; Huerta-Cepas et al. 2016). Ontology enrichment was determined using GOSeq (version 3.4, Young et al. 2010), a statistical package for R which accounts for multiple testing as well as differing probe lengths. Finally, clustering of gene modules was performed using the WGCNA package for R (version 1.51, Langfelder and Horvath 2008) with a minimum module size of five genes.

Results

Efficacy of the heat wave and drought treatments and impacts on T_{leaf} and Ψ_{mid}

On average, the heated (heat wave) treatment resulted in an 8°C increase in canopy temperature (Online Resource Fig. 2a). The drought treatment decreased volumetric soil water content by ~4% midway through the heat wave (day 9) when compared to the watered treatment, and the high heat treatment further decreased soil water content by 2% for the watered and 5% for the drought treatments (Online Resource Fig. 2b). The increase in canopy temperature with the high heat treatment was reflected in greater leaf temperature (T_{leaf}) for both species; *A. gerardii* and *S. nutans* had significantly higher T_{leaf} at both day 4 and 18 of the heat wave (Online Resource Fig. 4). Similarly, the decrease in soil water content with the drought and high heat treatment were reflected in greater water stress in both species (i.e., more negative Ψ_{mid}, Online Resource Fig. 4). For *A. gerardii*, the high heat treatment caused a large decrease in Ψ_{mid}, with this decline greatest at day 4 of the heat wave combined with drought (-0.9 MPa, Online Resource Fig. 4). The decrease in Ψ_{mid} with the high heat treatment was most pronounced in *S. nutans* after 18 days of heat wave under drought (-1.7 MPa, Online Resource Fig. 4).

Environment affects gene regulation in *A. gerardii* and S. *nutans*

Overall, 1131 genes were shared across both species, 1515 were shared across water treatment, 1653 were shared across temperature treatment, and 1390 were shared across date. Species ($p<0.001$), water treatment ($p<0.001$), and their interaction ($p<0.001$) most significantly impacted gene expression. In other words, species gene expression response strongly depended on the drought environment. Temperature was only a weakly significant predictor of gene expression ($p=0.048$) with no significant species by temperature interaction. Gene expression did not vary across sample date/duration of the heat wave.

Overall differences between *A. gerardii* and *S. nutans*

Of 1131 genes found in both species, 160 differed significantly in their regulation between species. Genes with greater expression in *A. gerardii* were enriched in cellular metabolic process, biological regulation, and protein metabolic process, while genes with greater expression in *S. nutans* were enriched in negative regulation of metabolism, biological, and cellular processes, macromolecule catabolic process, and protein kinase activity (Fig. 1). Within cellular metabolic process, the most extreme differences were found in a methyltransferase and other transferases, GTP binding protein, Dihydrouridine synthase (Dus), as well as several transcription factors (Table 1). Among biological regulation genes, several transcription factors were strongly upregulated in *A. gerardii.* Protein metabolic processes included several ribosomal-related genes as well as fibrillarin upregulated in *A. gerardii*. Within genes significantly upregulated in *S. nutans*, the negative regulation (inhibition) category consisted of a finger protein as well as several membrane proteins like CMP-sialic acid transporter homolog (Table 1). Macromolecule catabolism genes included several proteasomes, 1,2-alpha-mannosidase, and a ubiquitin-conjugating enzyme. Among genes annotating to the term “stress”, 18 were upregulated in *S. nutans* versus 31 upregulated in *A. gerardii.* Genes annotating broadly to “regulation” showed 91 upregulated in *A. gerardii* versus 74 in *S. nutans.*

Gene clustering was performed for day 18 samples to detect species differences for both plots at the end of the heat wave. Similarly regulated modules or groups of genes may lead to a greater understanding of gene networks contributing to different species responses. One gene module significantly explained species differences in the watered treatment ($p<0.001$, Fig. 2a) with genes generally expressed more highly in *S. nutans.* Two gene modules significantly explained species differences in the drought treatment (p=0.01, Fig. 2b and p=0.02, Fig. 2c respectively). Under drought, genes generally had lower expression in *S. nutans*.

Genes regulated in *A. gerardii*

In *A. gerardii*, 61 genes were significantly regulated in response to drought (5% of 1148 total genes), with 24 genes upregulated under watered conditions and 37 upregulated under drought conditions. Few GO categories had strong enrichment (i.e., few genes per category). The drought treatment showed enrichment in response to osmotic stress, chromatin silencing, and lysosome. The watered treatment suggested greater abundance of xylose metabolism, sucrose metabolism, and ion transport (although each group contained only one gene) (Fig. 3a). Osmotic stress genes included an RNA-binding protein, ribosomal protein S3, and aconitate hydratase (Table 1). Within chromatin silencing genes, both histone acetyltransferases were upregulated in under drought. Among all genes, 24 genes annotating to "stress" were upregulated in the watered treatment, versus 29 under drought. Only two genes (both within *A. gerardii*) responded significantly to temperature. One gene was upregulated in response to higher temperatures (Hsp70 protein); another was downregulated under higher temperatures (high mobility group-box domain).

Genes regulated in *S. nutans*

Sorghastrum nutans regulated more genes in response to drought than *A. gerardii* (23% of 762 genes total). Of these, 92 showed greater expression in the watered treatment while 82 showed greater expression under drought. Genes upregulated in the watered treatment showed GO enrichment in non-coding RNA (ncRNA) and RNA metabolism and nitrogen response. Genes upregulated under drought showed enrichment in response to water stress, external encapsulating structure, organophosphate metabolism, and cellular catabolism (Fig. 3b). Within the watered treatment ncRNA metabolism genes including ERBB-3 binding ribonuleoprotein, serrate RNA effector molecule, and pseudouridine synthase were upregulated (Table 1). *Sorghastrum nutans* in the watered treatment also showed greater expression of aquaporin NIP3-1, NEP1-interacting protein, and a transcriptional corepressor.

In contrast, *S. nutans* under drought showed greater expression of osmotic stress genes E3 ubiquitin ligase SUD1, 9 aldo-keto reductase, and hydrophobic protein LTI6A (Table 1). Among encapsulating structures, CMP-sialic acid transporter homolog, phosphatidylinositol kinase, pectin acetylesterase 8, and two glucuronosyltransferases (ranged from fold change of -1.47 to -1.71) were upregulated under drought. Catabolism genes within the drought treatment included 26S protease, DNA-directed RNA polymerase II Rpb7p, and phosphatidylinositol kinase. Lastly, the drought treatment showed increased expression of organophosphate metabolism genes including GDP-mannose 4,6 dehydratase, triosephosphate isomerase and phosphatidylinositol-4-phosphate 5-kinase. Among all genes, 12 (1.5%) genes annotating to "stress" were upregulated in the watered treatment, versus 20 (2.6%) under drought.

Discussion

Increasingly, ecological studies are using molecular techniques to study gene-level responses to global change in non-model organisms (Travers et al. 2007; Leakey et al. 2009; Alvarez et al. 2015; Smith et al. 2016). Genomic tools like microarrays have revealed mechanisms behind plant environmental responses in natural plant populations (Jackson et al. 2002; Travers et al. 2007; Ungerer et al. 2008; Leakey et al. 2009; Travers et al. 2010; Smith et al. 2016). Heterologous hybridization has proven useful for studying non-model organisms when the proper precautions are taken and stringent criteria are utilized to control for mishybridizations (Leakey et al. 2009; Travers et al. 2010; Alvarez et al. 2015). Both environmental (Gong et al. 2005; Hammond et al. 2006; Sharma et al. 2006; Travers et al. 2010; Alvarez et al. 2015) and biotic (Horvath et al. 2007; Broz et al. 2008) stress responses have been explored. Our study used heterologous hybridization to compare transcriptional responses of two non-model grasses under field conditions. We used stringent criteria to control for mishybridizations, multiple steps to normalize the array data, and sequence alignment with RNA-seq transcriptomes. One significant caveat of the microarray technique is the inability of microarray technology to distinguish between two scenarios: no microarray signal due to true low expression versus no microarray signal due to probe-to-gene mismatch. In other words, this study is limited to low versus high expression contrasts while excluding presence/absence analysis, and may fail to detect larger, significant shifts in gene expression. Moreover, these two species have almost certainly evolved unique genes to adapt to harsh conditions sometimes experienced in the tallgrass prairie. These species-specific genes may be the most insightful but are undetectable using these methods.

We expected that *A. gerardii* and *S. nutans*, two closely related and functionally similar species, would differ in their gene responses to heat-wave and drought. Specifically, *S. nutans* would regulate a greater number of genes from different groups compared with *A. gerardii*. This expectation was based on past evidence for greater sensitivity of *S. nutans* to more moderate water stress (Nippert et al. 2009; Hoover et al. 2014a; Smith et al. 2016). Overall, our hypothesis was supported; *S. nutans* had greater sensitivity to the imposed drought compared to *A. gerardii* in the percentage of regulated transcripts. Despite similar T_{leaf} and Ψ_{mid} measurements, *A. gerardii* appeared less responsive with a smaller proportion of genes (5%) exhibiting a significant change under drought. A similar pattern of gene regulation was observed when *A. gerardii* and *S. nutans* were exposed to more moderate changes in water availability in the field (Smith et al. 2016). Thus, in line with past research, our results suggest that *A. gerardii* is more resistant to and/or better able to cope with water stress than *S. nutans*. According to gene modules detected using statistical clustering, *S. nutans* genes tended toward downregulation on day 18 of the drought compared to *A. gerardii* (Fig. 2), which could represent a surpassed stress response threshold. *Sorghastrum nutans* has also shown loss of function under stress with respect to net photosynthetic rate and biomass production (Hoover et al. 2014a).

Despite strong support for the non-additive effects of water and temperature stress in some systems (Atkinson and Urwin 2012; Johnson et al. 2014; Suzuki et al. 2014), the two did not show a significant interaction. However, previous work comparing these two species also found no environmental interaction (Hoover et al. 2014a). In our study, only two genes within *A. gerardii* responded to the high heat treatment. Previous ecophysiological research has shown greater relative temperature sensitivity in *A. gerardii* (Nippert et al. 2009). Gene expression did not vary across sampling date, despite evidence for plasticity in other species (Hayano-Kanashiro et al. 2009; Meyer et al. 2014). However, it is important to acknowledge that fewer genes overlapped across sample date, and only these genes were contrasted. Many genes may have been expressed during the first sampling date but not during the second date and vice versa.

Over all treatments, *A. gerardii* tended to have greater expression of metabolic and regulatory genes compared to *S. nutans*, suggesting it maintains high levels of metabolic function in many environmental conditions and may strategize plasticity at the regulatory level (i.e., utilizes more transcription factors, tRNA enzymes, and ribosomal enzymes). In other words, gene expression remains fairly constant but may be modified downstream. Expression of transcription factors has been widely implicated in drought adaptation and response (Yamaguchi-Shinozaki and Shinozaki 2006; Yoshida et al. 2015; Kudo et al. 2016; Gahlaut et al. 2016). On the other hand, greater transcription of negative regulators and catabolism genes in *S. nutans* may reflect an ability to respond more rapidly to drought stress. Over-expression of negatively regulating PHD finger proteins in *Arabidopsis* inhibits pathways and leads to enhanced stress tolerance (W[illegible] et al. 2015) and the 26S proteastome helps modulate ABA response as well as degrade proteins not needed under non-stressed conditions (Stone 2014). Both species appear equipped to handle stressful conditions, though *S. nutans* seems to focus on rapid response via molecular breakdown and pathway inhibition whereas *A. gerardii* maintains higher levels of metabolic process and regulates transcription via transcription factors. Due to multiple statistical tests performed, only the most significant genes responding to drought were examined. Only two of these overlapped in *A. gerardii* and *S. nutans*, further highlighting their different drought response strategies.

Andropogon gerardii has previously shown greater ecophysiological response to temperature (Nippert et al. 2009), but may actually be less sensitive at the gene expression level to mild temperature stress (Smith et al. 2016). A consensus regarding temperature response may remain elusive considering only two genes significantly responded to temperature in *A. gerardii*. Hsp70 is well known to be upregulated under stress to assist protein folding (Hayano-Kanashiro et al. 2009; Wang et al. 2015), while high mobility group (HMG) genes are known to be negatively correlated with stress response (Kim et al. 2010). The general lack of response may be due to our stringent gene filtering criteria, but may also reflect presence of unique genes in these species. Non-targeted methods (such as RNA-seq, Hoffman and Smith 2017) have been successful in these species and would likely reveal more comprehensive differences under temperature extremes.

Of osmotic stress-related genes upregulated in *A. gerardii* in response to drought, Glycine-rich RNA-binding protein 2 is known to have RNA chaperone activity during abiotic stress (Kim et al. 2007), 40S ribosomal protein may be upregulated to compensate for mild osmotic stress (Ma et al. 2016), and aconitate hydratase has been shown to increase under water and heat stress (Johnson et al. 2014) in a compensatory manner due to its sensitivity to oxidative damage (Budak et al. 2013). Osmotic stress-related genes were also upregulated in *S. nutans* under drought, however their function was quite different. E3 ubiquitin ligase is understood to play a role in regulating response to ABA (Doblas et al. 2013; Zhao et al. 2014), aldo-keto reductase 4C9 is involved in scavenging toxins produced under stress (Simpson et al. 2009), and hydrophobic LTI6A is a transmembrane protein which responds to low temperature stress, drought, and ABA (Wang et al. 2016). These focal genes tied to osmotic stress response suggest that while both species are responding to drought, their strategies differ. In this case, *S. nutans* not only regulates a greater percentage of genes but also focuses on ABA response, whereas *A. gerardii* appears to upregulate genes to compensate for lost function. Among its many roles, ABA may help with stomatal closure and drought avoidance (Jones and Mansfield 1970).

Within *S. nutans*, ncRNAs (transcriptional regulators) declined under drought, which have been shown to downregulate in response to drought (Hackenberg et al. 2015). In this study, many of these genes mapped to transcription factors or RNA binding, which are typically upregulated under drought (Yamaguchi-Shinozaki and Shinozaki 2006; Yoshida et al. 2015; Kudo et al. 2016; Gahlaut et al. 2016; but see Baldoni et al. 2015). This could indicate that *S. nutans* experienced mechanistic loss of function under drought conditions. Catabolism related genes upregulated under drought may indicate salvaging of important functions. For example, phosphatidylinositol-4-phosphate 5-kinase is known to modulate ABA response as well as prevent breakdown of proline, an important ROS scavenger (Leprince et al. 2014). The 26S protease regulatory subunit lends additional breakdown of molecules potentially involved in signaling (Stone 2014). Similarly, RNA polymerase subunit Rpb7p is thought to help degrade mRNAs as a counteractive measure (Shalem et al. 2011). Of the genes not involved in cellular catabolism, some were tied to cell wall integrity (e.g., pectin acetylesterase) and may serve as a last resort for survival under extreme stress (Houston et al. 2016). Meanwhile, few genes suggested loss of function or disassembly role in *A. gerardii*, which further emphasizes *S. nutans*' greater sensitivity to drought stress. Of note is *A. gerardii*'s more consistent regulation of stress transcripts: this species shifted from 2.1% to 2.5% "stress" annotations following drought, while *S. nutans* shifted from 1.6% to 2.6% "stress" annotations. This could mean that *A. gerardii* tolerates stress and avoids sensitivity by constitutively expressing some stress responses. This makes sense considering the broad array of stressors *A. gerardii* is likely to experience (Hulbert 1988; Turner and Knapp 1996; Silletti et al. 2004; Swemmer et al. 2006; Koerner et al. 2014). Overall, these results suggest that *S. nutans*' ecophysiological sensitivity may be mechanistically tied to downregulation of genes under stress coupled with rapid avoidance strategies, such as the regulation of ABA. *Andropogon gerardii*'s apparent lack of sensitivity may result from upregulation of stress sensitive transcripts coupled with maintenance of cellular processes despite extreme stress.

Conclusions

Our results suggest that *A. gerardii* is more resistant to extremes in water stress and does not downregulate as many processes as *S. nutans*. Surprisingly, response to the heat wave was minimal. While *A. gerardii* contributes proportionally more aboveground biomass (Smith and Knapp 2003) and is an important mediator of species diversity in the tallgrass prairie ecosystem (Collins 2000; Smith et al. 2004), *S. nutans* is able to attain greater photosynthetic rates that could be linked to carbon storage (Hoover et al. 2014a). Differences in sensitivity and stress response mechanisms could ultimately alter community structure and ecosystem function in the tallgrass prairie ecosystem.

Acknowledgements

We thank S. Yuan for preparing the samples for this study. J.M. Blair in part conceived the rainfall manipulation plots experiment. We thank J.C. Cahill for comments on a previous version of the manuscript.

References

AbdElgawad H, Peshev D, Zinta G, Van Den Ende W, Janssens IA, Asard H (2014) Climate extreme effects on the chemical composition of temperate grassland species under ambient and elevated CO2: A comparison of fructan and non-fructan accumulators. PLoS One 9: . doi: 10.1371/journal.pone.0092044

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410 . doi: 10.1016/S0022-2836(05)80360-2

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402 . doi: 9254694

Alvarez M, Schrey AW, Richards CL (2015) Ten years of transcriptomics in wild populations: what have we learned about their ecology and evolution? Mol Ecol 24:710–725 . doi: 10.1111/mec.13055

Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M, Martin MJ, Natale DA, O'Donovan C, Redaschi N, Yeh L-SL (2004) UniProt: the Universal Protein knowledgebase. Nucleic Acids Res 32:D115–D119 . doi: 10.1093/nar/gkh131

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: From genes to the field. J. Exp. Bot. 63:3523–3544

Avolio ML, Chang CC, Smith MD (2011) Assessing Fine-Scale Genotypic Structure of a Dominant Species in Native Grasslands. Am Midl Nat 165:211–224 . doi: 10.1674/0003-0031-165.2.211

Avolio ML, Smith MD (2013) Mechanisms of selection: Phenotypic differences among genotypes explain patterns of selection in a dominant species. Ecology 94:953–965 . doi: 10.1890/12-1119.1

Baldoni E, Genga A, Cominelli E (2015) Plant MYB transcription factors: Their role in drought response mechanisms. Int. J. Mol. Sci. 16:15811–15851

Beierkuhnlein C, Thiel D, Jentsch A, Willner E, Kreyling J (2011) Ecotypes of European grass species respond differently to warming and extreme drought. J Ecol 99:703–713 . doi: 10.1111/j.1365-2745.2011.01809.x

Benson EJ, Hartnett DC (2006) The Role of Seed and Vegetative Reproduction in Plant Recruitment and Demography in Tallgrass Prairie. Plant Ecol 187:163–178 . doi: 10.1007/s11258-005-0975-y

Bokhorst S, Bjerke JW, Bowles FW, Melillo J, Callaghan T V., Phoenix GK (2008) Impacts of extreme winter warming in the sub-Arctic: Growing season responses of dwarf shrub heathland. Glob Chang Biol 14:2603–2612 . doi: 10.1111/j.1365-2486.2008.01689.x

Brejda JJ, Moser LE, Waller SS (1989) Rhizome and Tiller Development of Three Nebraska Sandhills Warm-Season Grasses. Proc Elev North Am Prairie Conf 211–215

Broz AK, Manter DK, Callaway RM, Paschke MW, Vivanco JM (2008) A molecular approach to understanding plant–plant interactions in the context of invasion biology. Funct Plant Biol 35:1123–1134 . doi: 10.1071/FP08155

Budak H, Akpinar BA, Unver T, Turktas M (2013) Proteome changes in wild and modern wheat leaves upon drought stress by two-dimensional electrophoresis and nanoLC-ESI-MS/MS. Plant Mol Biol 83:89–103 . doi: 10.1007/s11103-013-0024-5

Carter DL, VanderWeide BL (2014) Belowground bud production is linked to population establishment in Sorghastrum nutans (Poaceae). Plant Ecol 215:977–986 . doi:

10.1007/s11258-014-0353-8

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529–533 . doi: 10.1038/nature03972

Collins SL (2000) Disturbance Frequency and Community Stability in Native Tallgrass Prairie. Am Nat 155:311–325 . doi: 10.1086/303326

Cook BI, Ault TR, Smerdon JE (2015) Unprecedented 21st century drought risk in the American Southwest and Central Plains. Sci Adv 1: . doi: 10.1126/sciadv.1400082

Dabney AR, Storey JD (2007) A new approach to intensity-dependent normalization of two-channel microarrays. Biostatistics 8:128–139 . doi: 10.1093/biostatistics/kxj038

Doblas VG, Amorim-Silva V, Posé D, Rosado A, Esteban A, Arró M, Azevedo H, Bombarely A, Borsani O, Valpuesta V, Ferrer A, Tavares RM, Botella MA (2013) The SUD1 gene encodes a putative E3 ubiquitin ligase and is a positive regulator of 3-hydroxy-3-methylglutaryl coenzyme a reductase activity in Arabidopsis. Plant Cell 25:728–43 . doi: 10.1105/tpc.112.108696

Ellegren H (2014) Genome sequencing and population genomics in non-model organisms. Trends Ecol Evol 29:51–63 . doi: 10.1016/j.tree.2013.09.008

Estep MC, McKain MR, Vela Diaz D, Zhong J, Hodge JG, Hodkinson TR, Layton DJ, Malcomber ST, Pasquet R, Kellogg E a (2014) Allopolyploidy, diversification, and the Miocene grassland expansion. Proc Natl Acad Sci 111:15149–15154 . doi: 10.1073/pnas.1404177111

Fay PA, Blair JM, Smith MD, Nippert JB, Carlisle JD, Knapp AK (2011) Relative effects of precipitation variability and warming on tallgrass prairie ecosystem function. Biogeosciences 8:3053–3068 . doi: 10.5194/bg-8-3053-2011

Finn RD, Clements J, Eddy SR (2011) HMMER web server: Interactive sequence similarity searching. Nucleic Acids Res 39: . doi: 10.1093/nar/gkr367

Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, Salazar GA, Tate J, Bateman A (2015) The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res 279–285 . doi: 10.1093/nar/gkv1344

Gahlaut V, Jaiswal V, Kumar A, Gupta PK (2016) Transcription factors involved in drought tolerance and their possible role in developing drought tolerant cultivars with emphasis on wheat (Triticum aestivum L.). Theor Appl Genet 129:2019–2042 . doi: 10.1007/s00122-016-2794-z

García-Herrera R, Díaz J, Trigo RM, Luterbacher J, Fischer EM (2010) A Review of the European Summer Heat Wave of 2003. Crit. Rev. Environ. Sci. Technol. 40:267–306

Gibson G (2008) The environmental contribution to gene expression profiles. Nat Rev Genet 9:575–581 . doi: 10.1038/nrg2383

Gong Q, Li P, Ma S, Indu Rupassara S, Bohnert HJ (2005) Salinity stress adaptation competence in the extremophile Thellungiella halophila in comparison with its relative Arabidopsis thaliana. Plant J 44:826–839 . doi: 10.1111/j.1365-313X.2005.02587.x

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD, Ott M, Orvis J, Pochet N, Strozzi F, Weeks N, Westerman R, William T, Dewey CN, Henschel R, LeDuc RD, Friedman N, Regev A (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc 8:1494–1512 . doi: 10.1038/nprot.2013.084

Hackenberg M, Gustafson P, Langridge P, Shi BJ (2015) Differential expression of microRNAs and other small RNAs in barley between water and drought conditions. Plant Biotechnol J 13:2–13 . doi: 10.1111/pbi.12220

Hammond JP, Bowen HC, White PJ, Mills V, Pyke KA, Baker AJM, Whiting SN, May ST, Broadley MR (2006) A comparison of the Thlaspi caerulescens and Thlaspi arvense shoot transcriptomes. New Phytol 170:239–260 . doi: 10.1111/j.1469-8137.2006.01662.x

Hayano-Kanashiro C, Calderón-Vásquez C, Ibarra-Laclette E, Herrera-Estrella L, Simpson J (2009) Analysis of gene expression and physiological responses in three Mexican maize landraces under drought stress and recovery irrigation. PLoS One 4: . doi: 10.1371/journal.pone.0007531

Hoffman AM, Smith MD (2017) Gene expression differs in co-dominant prairie grasses under drought. Mol Ecol Resour. doi: 10.1111/1755-0998.12733

Hoover DL, Knapp AK, Smith MD (2014a) Contrasting sensitivities of two dominant C4 grasses to heat waves and drought. Plant Ecol 215:721–731 . doi: 10.1007/s11258-014-0345-8

Hoover DL, Knapp AK, Smith MD (2014b) Resistance and resilience of a grassland ecosystem to climate extremes. Ecology 95:2646–2656 . doi: 10.1890/13-2186.1

Horvath DP, Llewellyn D, Clay SA (2007) Heterologous Hybridization of Cotton Microarrays with Velvetleaf (Abutilon Theophrasti) Reveals Physiological Responses Due to Corn Competition. Weed Sci 55:546–557 . doi: 10.1614/WS-07-008.1

Houston K, Tucker MR, Chowdhury J, Shirley N, Little A (2016) The Plant Cell Wall: A Complex and Dynamic Structure As Revealed by the Responses of Genes under Stress Conditions. Front Plant Sci 7:1–18 . doi: 10.3389/fpls.2016.00984

Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, Jensen LJ, von Mering C, Bork P (2016) eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res 44:286–293 . doi: 10.1093/nar/gkv1248

Hulbert LC (1988) Causes of Fire Effects in Tallgrass Prairie. Ecology 69:46–58 . doi: 10.2307/1943159

Jackson RB, Linder CR, Lynch M, Purugganan M, Somerville S, Thayer SS (2002) Linking molecular insight and ecological research. Trends Ecol Evol 17:409–414 . doi: 10.1016/S0169-5347(02)02571-5

Johnson SM, Lim F-L, Finkler A, Fromm H, Slabas AR, Knight MR (2014) Transcriptomic analysis of Sorghum bicolor responding to combined heat and drought stress. BMC Genomics 15: . doi: 10.1186/1471-2164-15-456

Jones RJ, Mansfield TA (1970) Suppression of Stomatal Opening in Leaves Treated with Abscisic Acid. J Exp Bot 21:714–719 . doi: 10.1093/jxb/21.3.714

Kim JM, To TK, Nishioka T, Seki M (2010) Chromatin regulation functions in plant abiotic stress responses. Plant, Cell Environ 33:604–611 . doi: 10.1111/j.1365-3040.2009.02076.x

Kim JY, Park SJ, Jang B, Jung C-H, Ahn SJ, Goh C-H, Cho K, Han O, Kang H (2007) Functional characterization of a glycine-rich RNA-binding protein 2 in Arabidopsis thaliana under abiotic stress conditions. Plant J 50:439–451 . doi: 10.1111/j.1365-313X.2007.03057.x

Knapp AK (1985) Effect of Fire and Drought on the Ecophysiology of Andropogon gerardii and Panicum virgatum in a Tallgrass Prairie. Ecology 66:1309–1320 . doi: 10.2307/1939184

Knapp AK, Hoover DL, Wilcox KR, Avolio ML, Koerner SE, La Pierre KJ, Loik ME, Luo Y, Sala OE, Smith MD (2015) Characterizing differences in precipitation regimes of extreme wet and dry years: implications for climate change experiments. Glob Chang Biol 21:2624–2633 . doi: 10.1111/gcb.12888

Koerner SE, Burkepile DE, Fynn RWS, Burns CE, Eby S, Govender N, Hagenah N, Matchett KJ, Thompson DI, Wilcox KR, Collins SL, Kirkman KP, Knapp AK, Smith MD (2014) Plant community response to loss of large herbivores differs between North American and South African savanna grasslands. Ecology 95:808–816 . doi: 10.1890/13-1828.1

Kreyling J, Wenigmann M, Beierkuhnlein C, Jentsch A (2008) Effects of extreme weather events on plant productivity and tissue die-back are modified by community composition. Ecosystems 11:752–763 . doi: 10.1007/s10021-008-9157-9

Kudo M, Kidokoro S, Yoshida T, Mizoi J, Todaka D, Fernie AR, Shinozaki K, Yamaguchi-Shinozaki K (2016) Double overexpression of DREB and PIF transcription factors improves drought stress tolerance and cell elongation in transgenic plants. Plant Biotechnol J. doi: 10.1111/pbi.12644

Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9:559 . doi: 10.1186/1471-2105-9-559

Leakey ADB, Ainsworth EA, Bernard SM, Markelz RJC, Ort DR, Placella SA, Rogers A, Smith MD, Sudderth EA, Weston DJ, Wullschleger SD, Yuan S (2009) Gene expression profiling: Opening the black box of plant ecosystem responses to global change. Glob Chang Biol 15:1201–1213 . doi: 10.1111/j.1365-2486.2008.01818.x

Leprince A-S, Magalhaes N, De Vos D, Bordenave M, Crilat E, Clément G, Meyer C, Munnik T, Savouré A (2014) Involvement of Phosphatidylinositol 3-kinase in the regulation of proline catabolism in Arabidopsis thaliana. Front Plant Sci 5:772 . doi: 10.3389/fpls.2014.00772

Ma J, Dong W, Zhang D, Gao X, Jiang L, Shao Y, Tong D, Li C (2016) Proteomic profiling analysis reveals that glutathione system plays important roles responding to osmotic stress in wheat (*Triticum aestivum* L.) roots. PeerJ 4:e2334 . doi: 10.7717/peerj.2334

McCarty DR (1986) A simple method for extraction of RNA from maize tissue. Maize Genet Coop Newsl 60:

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez E a (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739 . doi: 10.1111/j.1469-8137.2008.02436.x

Meyer E, Aspinwall MJ, Lowry DB, Palacio-Mejía JD, Logan TL, Fay P a, Juenger TE (2014) Integrating transcriptional, metabolomic, and physiological responses to drought stress and recovery in switchgrass (Panicum virgatum L.). BMC Genomics 15: . doi: 10.1186/1471-2164-15-527

Nardini A, Casolo V, Dal Borgo A, Savi T, Stenni B, Bertoncin P, Zini L, Mcdowell NG (2016) Rooting depth, water relations and non-structural carbohydrate dynamics in three woody angiosperms differentially affected by an extreme summer drought. Plant, Cell Environ 39:618–627 . doi: 10.1111/pce.12646

Nippert JB, Fay PA, Carlisle JD, Knapp AK, Smith MD (2009) Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. Acta Oecologica 35:400–408 . doi: 10.1016/j.actao.2009.01.010

Ouborg NJ, Vriezen WH (2007) An ecologist’s guide to ecogenomics. J Ecol 95:8–16 . doi: 10.1111/j.1365-2745.2006.01197.x

Shalem O, Groisman B, Choder M, Dahan O, Pilpel Y (2011) Transcriptome kinetics is governed by a genome-wide coupling of mRNA production and degradation: A role for RNA pol II. PLoS Genet 7: . doi: 10.1371/journal.pgen.1002273

Sharma N, Cram D, Huebert T, Zhou N, Parkin IAP (2006) Exploiting the wild crucifer Thlaspi arvense to identify conserved and novel genes expressed during a plant’s response to cold stress. Plant Mol Biol 63:171–184 . doi: 10.1007/s11103-006-9080-4

Silletti A, Knapp A (2002) Long-term responses of the grassland co-dominants Andropogon gerardii and Sorghastrum nutans to changes in climate and management. Plant Ecol 163:15–22 . doi: 10.1023/A:1020320214750

Silletti AM, Knapp AK, Blair JM (2004) Competition and coexistence in grassland codominants: responses to neighbour removal and resource availability. Can J Bot 82:450–460 . doi: 10.1139/b04-016

Simpson PJ, Tantitadapitak C, Reed AM, Mather OC, Bunce CM, White SA, Ride JP (2009) Characterization of Two Novel Aldo-Keto Reductases from Arabidopsis: Expression Patterns, Broad Substrate Specificity, and an Open Active-Site Structure Suggest a Role in Toxicant Metabolism Following Stress. J Mol Biol 392:465–480 . doi: 10.1016/j.jmb.2009.07.023

Smith MD (2011) An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. J Ecol 99:656–663 . doi: 10.1111/j.1365-2745.2011.01798.x

Smith MD, Hoffman AM, Avolio ML (2016) Gene expression patterns of two dominant tallgrass prairie species differ in response to warming and altered precipitation. Sci Rep 1–14 . doi: 10.1038/srep25522

Smith MD, Knapp AK (2003) Dominant species maintain ecosystem function with non-random species loss. Ecol Lett 6:509–517 . doi: 10.1046/j.1461-0248.2003.00454.x

Smith MD, Wilcox J, Kelly T, Knapp AK (2004) Dominance not richness determines invasibility of tallgrass prairie. Oikos 2:253–262

Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (2013) IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA

Stone SL (2014) The role of ubiquitin and the 26S proteasome in plant abiotic stress signaling. Front Plant Sci 5:135 . doi: 10.3389/fpls.2014.00135

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic and biotic stress combinations. New Phytol 203:32–43 . doi: 10.1111/nph.12797

Swarbreck SM, Sudderth E a., St.Clair SB, Salve R, Castanha C, Torn MS, Ackerly DD, Andersen GL (2011) Linking leaf transcript levels to whole plant analyses provides mechanistic insights to the impact of warming and altered water availability in an annual grass. Glob Chang Biol 17:1577–1594 . doi: 10.1111/j.1365-2486.2010.02359.x

Swemmer AM, Knapp AK, Smith MD (2006) Growth responses of two dominant C4 grass species to altered water availability. Int J Plant Sci 167:1001–1010 . doi: 10.1086/505611

The Gene Ontology Consortium (2015) Gene Ontology Consortium: going forward. Nucleic Acids Res 43:1049–1056 . doi: 10.1093/nar/gku1179

Travers SE, Smith MD, Bai J, Hulbert SH, Leach JE, Schnable PS, Knapp AK, Milliken GA, Fay PA, Saleh A, Garrett KA (2007) Ecological genomics: making the leap from model systems in the lab to native populations in the field. Front Ecol Environ 5:19–24 . doi: 10.1890/1540-9295(2007)5[19:EGMTLF]2.0.CO;2

Travers SE, Tang Z, Caragea D, Garrett KA, Hulbert SH, Leach JE, Bai J, Saleh A, Knapp AK, Fay PA, Nippert J, Schnable PS, Smith MD (2010) Variation in gene expression of Andropogon gerardii in response to altered environmental conditions associated with climate change. J Ecol 98:374–383 . doi: 10.1111/j.1365-2745.2009.01618.x

Tseng GC, Oh MK, Rohlin L, Liao JC, Wong WH (2001) Issues in cDNA microarray analysis: quality filtering, channel normalization, models of variations and assessment of gene effects. Nucleic Acids Res 29:2549–2557 . doi: 10.1093/nar/29.12.2549

Tucker SS, Craine JM, Nippert JB (2011) Physiological drought tolerance and the structuring of tallgrass prairie assemblages. Ecosphere 2: . doi: 10.1890/ES11-00023.1

Turner CL, Knapp AK (1996) Responses of a C 4 Grass and Three C 3 Forbs to Variation in Nitrogen and Light in Tallgrass Prairie. Ecology 77:1738–1749 . doi: 10.2307/2265779

Ungerer MC, Johnson LC, Herman MA (2008) Ecological genomics: understanding gene and genome function in the natural environment. Heredity (Edinb) 100:178–183 . doi: 10.1038/sj.hdy.6800992

Vázquez DP, Gianoli E, Morris WF, Bozinovic F (2015) Ecological and evolutionary impacts of changing climatic variability. Biol Rev 7: . doi: 10.1111/brv.12216

Verdnik D, Handran S, Pickett S (2002) Key considerations for accurate microarray scanning and image analysis. In: Kamberova G (ed) DNA arrray image analysis: nuts and bolts. DNA Press, Salem, MA, pp 83–98

Voesenek LACJ, Van Veen H, Sasidharan R (2014) Learning from nature: The use of non-model species to identify novel acclimations to flooding stress. AoB Plants 6

Wang W, Qin Q, Sun F, Wang Y, Xu D, Li Z, Fu B (2016) Genome-Wide Differences in DNA Methylation Changes in Two Contrasting Rice Genotypes in Response to Drought Conditions. Front Plant Sci 7:1–13 . doi: 10.3389/fpls.2016.01675

Wang X, Dinler BS, Vignjevic M, Jacobsen S, Wollenweber B (2015) Physiological and proteome studies of responses to heat stress during grain filling in contrasting wheat cultivars. Plant Sci 230:33–50 . doi: 10.1016/j.plantsci.2014.10.009

Weaver JE, Fitzpatrick TJ (1934) The Prairie. Ecol. Monogr. 4

Wei W, Zhang Y-Q, Tao J-J, Chen H-W, Li Q-T, Zhang W-K, Ma B, Lin Q, Zhang J-S, Chen S-Y (2015) The Alfin-like homeodomain finger protein AL5 suppresses multiple negative factors to confer abiotic stress tolerance in Arabidopsis. Plant J 81:871–883 . doi: 10.1111/tpj.12773

Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf E V, Allan GJ, DiFazio SP, Potts BM, Fischer DG, Gehring C a, Lindroth RL, Marks JC, Hart SC, Wimp GM, Wooley SC (2006) A framework for community and ecosystem genetics: from genes to ecosystems. Nat Rev Genet 7:510–523 . doi: 10.1038/nrg1877

Whitham TG, DiFazio SP, Schweitzer JA, Shuster SM, Allan GJ, Bailey JK, Woolbright SA (2008) Extending Genomics to Natural Communities and Ecosystems. Science 320:492–495 . doi: 10.1126/science.1153918

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57:781–803 . doi: 10.1146/annurev.arplant.57.032905.105444

Yang YH, Dudoit S, Luu P, Lin DM, Peng V, Ngai J, Speed TP (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res 30: . doi: 10.1093/nar/30.4.e15

Yang YH, Dudoit S, Luu P, Speed TP (2001) Normalization for cDNA microarray data. In: Microarrays Optical Technologies and Informatics. pp 141–152

Yoshida T, Fujita Y, Maruyama K, Mogami J, Todaka D, Shinozaki K, Yamaguchi-Shinozaki K (2015) Four A rabidopsis AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signalling in response to osmotic stress. Plant Cell Environ 38:35–49 . doi: 10.1111/pce.12351

Young MD, Wakefield MJ, Smyth GK, Oshlack A (2010) Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol 11: . doi: 10.1186/gb-2010-11-2-r14

Zhao H, Zhang H, Cui P, Ding F, Wang G, Li R, Jenks MA, Lu S, Xiong L (2014) The Putative E3 Ubiquitin Ligase ECERIFERUM9 Regulates Abscisic Acid Biosynthesis and Response during Seed Germination and Postgermination Growth in Arabidopsis1[W][OPEN]. Plant Physiol 165:1255–1268 . doi: 10.1104/pp.114.239699

Table 1(on next page)

Selected differentially expressed genes.

Maize gene	Description	Log$_2$ fold-change	Upregulated in:	GO category
Regulation between *A. gerardii* and *S. nutans*				
CB331760	methyltransferase	**3.56**	***A. gerardii***	Cellular metabolic process
DV621283	GTP binding protein	**3.28**	***A. gerardii***	Cellular metabolic process
DV490673	Dihydrouridine synthase (Dus)	**2.99**	***A. gerardii***	Cellular metabolic process
DV491165	transcription factor	**2.60**	***A. gerardii***	Biological regulation
BM331929	transcription factor	**2.56**	***A. gerardii***	Biological regulation
CD510408	fibrillarin	**2.56**	***A. gerardii***	Protein metabolic processes
DV491840	finger protein	**-2.66**	***S. nutans***	Negative regulation
DV491692	CMP-sialic acid transporter homolog	**-2.64**	***S. nutans***	Negative regulation
DV942581	Proteasome	**-2.37**	***S. nutans***	Macromolecule catabolism
DV490558	1,2-alpha-mannosidase	**-2.13**	***S. nutans***	Macromolecule catabolism
DV493085	ubiquitin-conjugating enzyme	**-1.51**	***S. nutans***	Macromolecule catabolism
Regulation within *A. gerardii*				
CB331250	RNA-binding protein	**-1.01**	***Drought***	Osmotic stress
CA989232	ribosomal protein S3	**-1.51**	***Drought***	Osmotic stress
BM347878	aconitate hydratase	**-1.17**	***Drought***	Osmotic stress
CD651535	histone acetyltransferase	**-1.33**	***Drought***	Chromatin silencing
CB815849	histone acetyltransferase	**-1.86**	***Drought***	Chromatin silencing
DY576254	Hsp70 protein	**-1.06**	***Heat wave***	Protein folding
CD662140	high mobility group-box domain	**1.08**	***Ambient temp.***	DNA binding
Regulation within *S. nutans*				
DV489871	ERBB-3 binding ribonuleoprotein	**1.33**	***Watered***	ncRNA metabolism
DV489639	serrate RNA effector molecule	**1.24**	***Watered***	ncRNA metabolism
DV943322	pseudouridine synthase	**1.16**	***Watered***	ncRNA metabolism
DV942798	ribosome production factor 2	**1.04**	***Watered***	ncRNA metabolism
BM073337	polyribonucleotide nucleotidyltransferase	**1.02**	***Watered***	ncRNA metabolism
CD651136	Cysteinyl-tRNA synthetase	**2.14**	***Watered***	ncRNA metabolism

BM078961	methionine-tRNA ligase	**1.26**	***Watered***	ncRNA metabolism
CD651793	valine-tRNA ligase with editing activity	**1.24**	***Watered***	ncRNA metabolism
DV492155	aquaporin NIP3-1	**1.22**	***Watered***	Transmembrane activity
BM340348	NEP1-interacting protein	**1.19**	***Watered***	Methyltransferase activity
DV492743	transcriptional corepressor	**1.17**	***Watered***	Negative regulation of transcription
CD527890	E3 ubiquitin ligase SUD1	**-2.24**	***Drought***	Osmotic stress
DV489949	aldo-keto reductase	**-2.16**	***Drought***	Osmotic stress
BM348293	hydrophobic protein LTI6A	**-1.01**	***Drought***	Osmotic stress
DV491692	CMP-sialic acid transporter homolog	**-2.35**	***Drought***	Encapsulating structures
DV492287	phosphatidylinositol kinase	**-2.12**	***Drought***	Encapsulating structures
BM333861	pectin acetylesterase 8	**-1.65**	***Drought***	Encapsulating structures
DV491662	26S protease	**-2.40**	***Drought***	Catabolism
DV492129	DNA-directed RNA polymerase II Rpb7p	**-2.35**	***Drought***	Catabolism
DV492287	phosphatidylinositol kinase	**-2.12**	***Drought***	Catabolism
DV942393	GDP-mannose 4,6 dehydratase	**-4.99**	***Drought***	Organophosphate metab.
DV493244	triosephosphate isomerase	**-2.47**	***Drought***	Organophosphate metab.
DV491451	phosphatidylinositol-4-phosphate 5-kinase	**-2.10**	***Drought***	Organophosphate metab.

Figure 1 (on next page)

Differentially expressed genes in *A. gerardii* and *S. nutans.*

Differentially expressed genes have a $\log_2$ fold change greater than one, shown as colored points where $p<0.05$. Positive values indicate greater expression in *A. gerardii* while negative values indicate greater expression in *S. nutans*. Selected Gene Ontology groups are represented by filled circles. Open circles: red = differentially expressed, gray = not significantly different

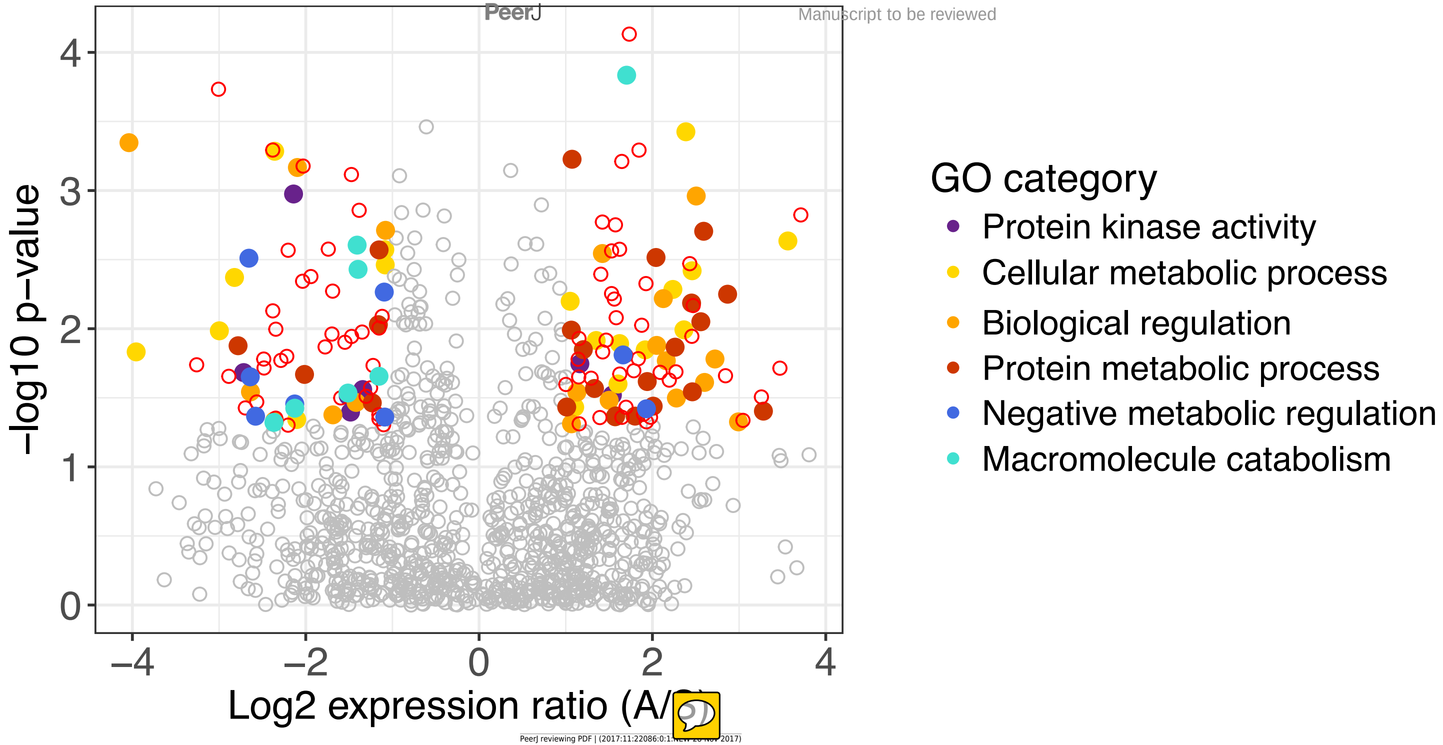

4
3
2
1
0
−log10 p−value
−4
−2
0
2
4
Log2 expression ratio (A/S)
GO category
● Protein kinase activity
● Cellular metabolic process
● Biological regulation
● Protein metabolic process
● Negative metabolic regulation
● Macromolecule catabolism

Figure 2 (on next page)

Gene modules explaining species differences under different water availability.

Gene modules detected explaining species differences in under watered (A) and drought (B, C) conditions. Sample names are presented on the x-axis, where each label applies to two columns of the same description (e.g. Ag.W.H applies to the first two columns, but both are replicates of *A . gerardii* in Watered plot with Heated treatment). Ag = *A. gerardii*, Sn = *S. nutans*, W = watered, D = drought, H = heated, A = ambient temperature. No annotation found = N.A.

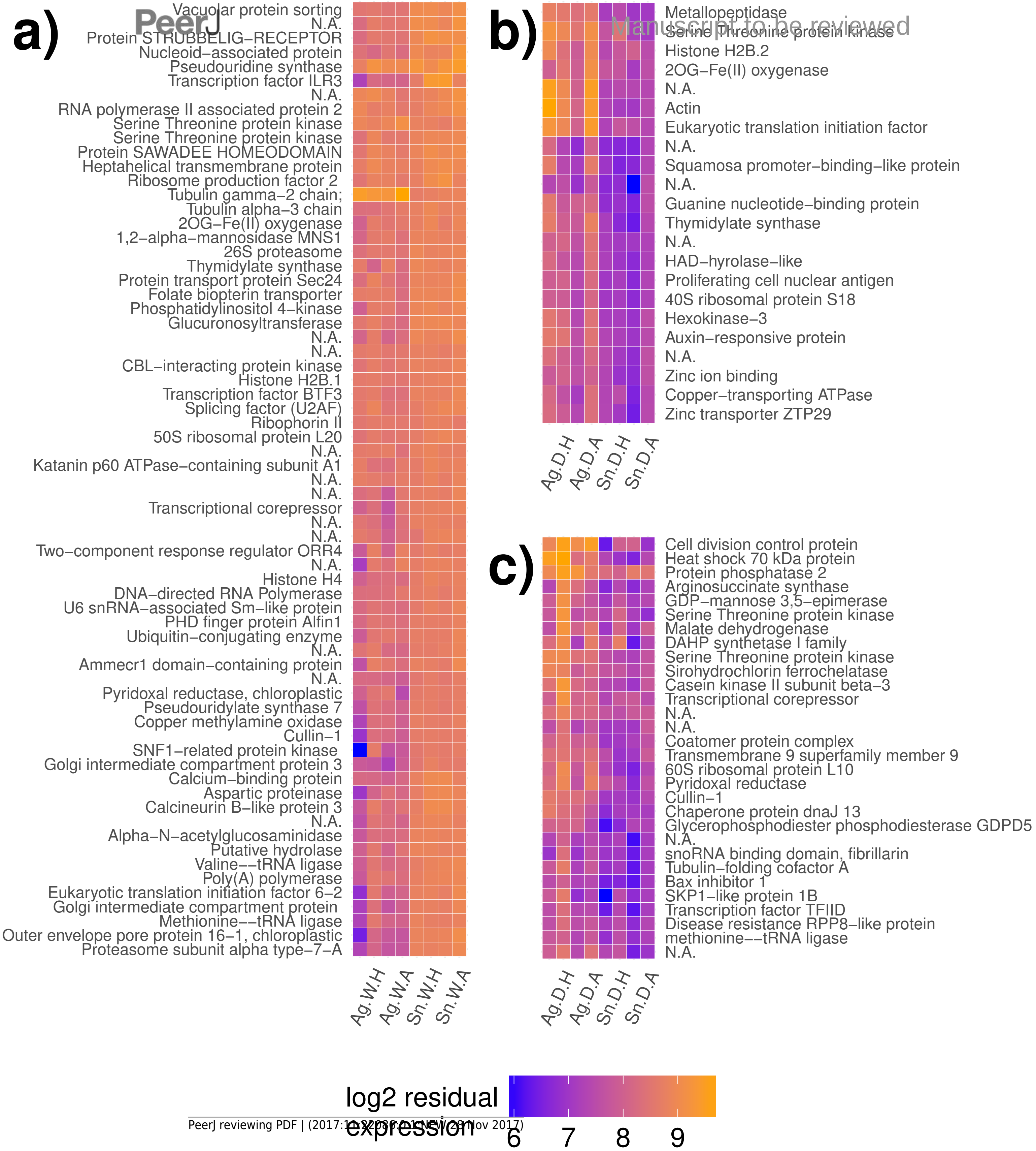
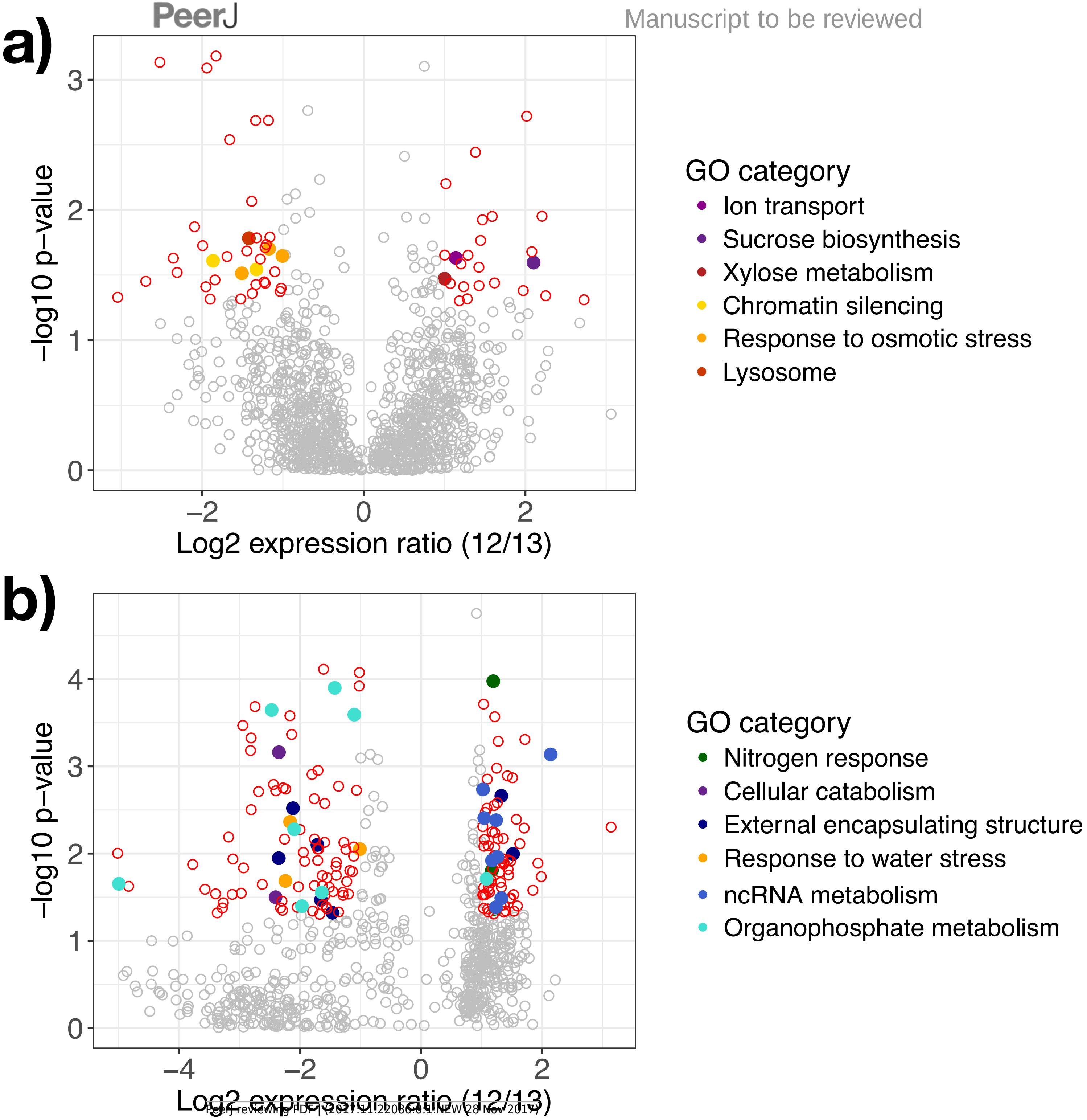

a)
Vacuolar protein sorting
N.A.
Protein STRUBBELIG−RECEPTOR
Nucleoid−associated protein
Pseudouridine synthase
Transcription factor ILR3
N.A.
RNA polymerase II associated protein 2
Serine Threonine protein kinase
Serine Threonine protein kinase
Protein SAWADEE HOMEODOMAIN
Heptahelical transmembrane protein
Ribosome production factor 2
Tubulin gamma−2 chain;
Tubulin alpha−3 chain
2OG−Fe(II) oxygenase
1,2−alpha−mannosidase MNS1
26S proteasome
Thymidylate synthase
Protein transport protein Sec24
Folate biopterin transporter
Phosphatidylinositol 4−kinase
Glucuronosyltransferase
N.A.
N.A.
CBL−interacting protein kinase
Histone H2B.1
Transcription factor BTF3
Splicing factor (U2AF)
Ribophorin II
50S ribosomal protein L20
N.A.
Katanin p60 ATPase−containing subunit A1
N.A.
N.A.
Transcriptional corepressor
N.A.
N.A.
Two−component response regulator ORR4
N.A.
Histone H4
DNA−directed RNA Polymerase
U6 snRNA−associated Sm−like protein
PHD finger protein Alfin1
Ubiquitin−conjugating enzyme
N.A.
Ammecr1 domain−containing protein
N.A.
Pyridoxal reductase, chloroplastic
Pseudouridylate synthase 7
Copper methylamine oxidase
Cullin−1
SNF1−related protein kinase
Golgi intermediate compartment protein 3
Calcium−binding protein
Aspartic proteinase
Calcineurin B−like protein 3
N.A.
Alpha−N−acetylglucosaminidase
Putative hydrolase
Valine−−tRNA ligase
Poly(A) polymerase
Eukaryotic translation initiation factor 6−2
Golgi intermediate compartment protein
Methionine−−tRNA ligase
Outer envelope pore protein 16−1, chloroplastic
Proteasome subunit alpha type−7−A
Ag.W.H
Ag.W.A
Sn.W.H
Sn.W.A
b)
Metallopeptidase
Serine Threonine protein kinase
Histone H2B.2
2OG−Fe(II) oxygenase
N.A.
Actin
Eukaryotic translation initiation factor
N.A.
Squamosa promoter−binding−like protein
N.A.
Guanine nucleotide−binding protein
Thymidylate synthase
N.A.
HAD−hyrolase−like
Proliferating cell nuclear antigen
40S ribosomal protein S18
Hexokinase−3
Auxin−responsive protein
N.A.
Zinc ion binding
Copper−transporting ATPase
Zinc transporter ZTP29
Ag.D.H
Ag.D.A
Sn.D.H
Sn.D.A
c)
Cell division control protein
Heat shock 70 kDa protein
Protein phosphatase 2
Arginosuccinate synthase
GDP−mannose 3,5−epimerase
Serine Threonine protein kinase
Malate dehydrogenase
DAHP synthetase I family
Serine Threonine protein kinase
Sirohydrochlorin ferrochelatase
Casein kinase II subunit beta−3
Transcriptional corepressor
N.A.
N.A.
Coatomer protein complex
Transmembrane 9 superfamily member 9
60S ribosomal protein L10
Pyridoxal reductase
Cullin−1
Chaperone protein dnaJ 13
Glycerophosphodiester phosphodiesterase GDPD5
N.A.
snoRNA binding domain, fibrillarin
Tubulin−folding cofactor A
Bax inhibitor 1
SKP1−like protein 1B
Transcription factor TFIID
Disease resistance RPP8−like protein
methionine−−tRNA ligase
N.A.
Ag.D.H
Ag.D.A
Sn.D.H
Sn.D.A
log2 residual expression
6
7
8
9

Figure 3(on next page)

Differentially expressed genes in response to water availability.

Differentially expressed genes in a) *A. gerardii* and b) *S. nutans* only compared between watered and drought plots (12 and 13). Significantly different genes with $\log_2$ fold change greater than one are represented by colored points where $p<0.05$. Positive values indicate greater expression in the watered plot while negative values indicate greater expression in the drought plot. Selected Gene Ontology groups are represented by filled circles. Open circles: red = differentially expressed, gray = not significantly different.


a)
3
2
1
0
−log10 p−value
−2
0
2
Log2 expression ratio (12/13)
GO category
● Ion transport
● Sucrose biosynthesis
● Xylose metabolism
● Chromatin silencing
● Response to osmotic stress
● Lysosome
b)
4
3
2
1
0
−log10 p−value
−4
−2
0
2
Log2 expression ratio (12/13)
GO category
● Nitrogen response
● Cellular catabolism
● External encapsulating structure
● Response to water stress
● ncRNA metabolism
● Organophosphate metabolism