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COMPARATIVE AND INTERACTIVE EFFECTS OF REDUCED PRECIPITATION FREQUENCY AND VOLUME ON THE GROWTH AND FUNCTION OF TWO PERENNIAL GRASSLAND SPECIES

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Premise of research. Water deficits are a common limiting factor of plant growth. Many studies have looked at the effects of drought, but few have compared the independent and interactive effects of multiple dimensions of changing precipitation patterns (e.g., reduced rainfall frequency and reduced rainfall volume) on overall plant growth of individuals with snapshots of growth-related plant performance.

Methodology. In this greenhouse experiment, we investigated responses of the legume *Lupinus perennis* and the C₃ grass *Agropyron repens* to a factorial combination of 50% reductions in watering frequency and watering volume. Watering treatments were designed based on 10-yr climate records from where these species co-occur. For both species, we measured leaf senescence, above- and belowground biomass accumulation, leaf net photosynthesis, and stomatal conductance. The leaf water potential and the proportion of N derived from symbiotic N₂ fixation of *L. perennis* were also measured.

Pivotal results. Reduced watering frequency had similar effects on both *A. repens* and *L. perennis*, including a 21% reduction in total biomass. However, effects of the reduced-volume treatment were largely species specific. For example, total biomass accumulation decreased and leaf senescence increased only in *L. perennis*. The proportion of N from symbiotic fixation in *L. perennis* was reduced only when water volume was also reduced, but overall leaf N remained constant in all treatments. Instantaneous prewatering gas exchange measurements showed that species maintained leaf net photosynthesis but with reduced stomatal conductance across all water availability manipulations.

Conclusions. This study provides new insights into differential and species-specific effects of changes in water frequency and volume. Moreover, it suggests that trying to understand plant responses to changing or heterogeneous precipitation regimes based solely on a single parameter of water availability (often mean annual rainfall) might mask important dynamics governing these phenomena.

Keywords: climate change, gas exchange, greenhouse, nitrogen fixation, rainfall frequency, rainfall volume, *Agropyron repens*, *Lupinus perennis*.

Introduction

Rainfall patterns are one of several climate factors expected to change substantially in the coming decades. In particular, climate models suggest an increase in frequency and magnitude of extreme rainfall events at both regional (Winter and Eltahir 2012; Huang et al. 2013) and global (Jentsch and Beierkuhnlein 2008) scales. In much of the continental United States and other parts of the world, such patterns are already being reported (Groisman et al. 2004; Knapp et al. 2008). These spatial

and temporal changes in rainfall may be independent of shifts in annual precipitation means, which have also been observed in recent decades (Wentz et al. 2007; Rapacciuolo et al., forthcoming).

Perturbations in water regimes at regional and landscape scales may dramatically affect plant growth, as water availability is a common limiting factor of plant growth and vegetation distributions (Stephenson 1990). In North America, rainfall gradients are a key determinant of ecosystem type, species composition, and primary productivity, particularly among grasslands (Knapp et al. 2001; Fay et al. 2003; Sandel et al. 2010). In addition to total precipitation, water availability is affected by soil type and texture (Selmants and Hart 2008; Looney et al. 2012), as well as temporal distributions of precipitation such as event frequency or seasonality. All these factors interact to influence local vegetation (Stephenson 1990).

Experimental studies have evaluated the effects of precipitation patterns on plant growth and physiology across numerous geographic and temporal scales (Knapp et al. 2002,

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2006; Huxman et al. 2004; Cotrufo et al. 2011; Craine et al. 2011). In Kansas grasslands, rainfall variability plays a larger role than mean soil water content in determining aboveground net primary productivity (ANPP; Knapp et al. 2001). Moreover, supplemental water during times of drought can increase ANPP of both forbs and grasses, a trend also found in Mediterranean woodlands (Knapp et al. 2002; Cotrufo et al. 2011). In spite of evidence showing the importance of rainfall distribution, few studies have compared multiple independent parameters of water availability (e.g., precipitation frequency, the amount of precipitation per event, or seasonality). Yet, such studies are needed in order to tease apart the relative effects of these parameters (Beier et al. 2012).

An ongoing field manipulation study of North American tallgrass prairie plots has found that reducing the frequency of rainfall events independent of water volume reduced soil water content as much as a reduction in total volume over the course of a growing season. Reduced rainfall frequency resulted in increased root-to-shoot ratios, reductions in ANPP, and a reduction in flowering in some species but not others compared to ambient conditions (Fay et al. 2002, 2003, 2011). A recent survey of 36 community types at 14 sites across North America found that seasonality of precipitation explained variation in ANPP better than annual averages (Robinson et al. 2012). However, more research is needed to relate these findings to other plant growth parameters, functional groups, and ecosystems. Moreover, while field studies allow a natural setting for observation, experiments in controlled conditions may contribute to our mechanistic understanding by reducing confounding or complicating factors (e.g., differences in microclimate or interspecific interactions) and permit collection of data not available in the field, such as measurements of belowground plant growth.

We sought to address this gap in knowledge by determining the relative and interactive effects of reduced watering frequency and reduced watering volume on individual plant growth in a greenhouse setting. Many parameters define a given precipitation regime; however, we focused on summer rainfall frequency and rainfall volume for two main reasons. First, they are the most commonly used parameters in previous studies (albeit individually rather than in combination) and, second, they are frequently considered in models of climate change and corresponding biotic responses (Crimmins et al. 2011; Morelli et al. 2012). Specifically, we were motivated by three questions: (1) How do differential watering frequencies and volumes impact soil moisture? (2) Over time, do these two forms of water reduction have different or interacting effects on plant growth related parameters? (3) Do species respond differently to these water regimes and in ways that can be linked to their unique functional attributes?

To address these questions, we investigated the responses of two contrasting mesic grassland species—the legume *Lupinus perennis* and the naturalized C_3 grass *Agropyron repens*—to four water availability treatments: a well-watered control, a 50% reduction in watering frequency, a 50% reduction in total water volume, and a combination of 50% reductions in both watering frequency and volume. Both species were selected because of their abundance in upper Midwest grasslands and their use in ongoing large-scale multifactorial field studies (Lee et al. 2003, 2011; Reich 2009; Reich and Hobbie 2013). In

particular, *L. perennis* was chosen in part because of its unique functional role as a nitrogen-fixing legume and its potential to therefore play an important role in community-level responses to changing precipitation patterns. *Agropyron repens* was chosen based on its contrasting functional role as a C_3 grass. Moreover, nonnative but naturalized species such as this one are more likely to benefit disproportionately and become more ecologically dominant as a result of climate change than are native species and therefore warrant increased study (Dukes and Mooney 1999; Willis et al. 2010).

Material and Methods

Experimental Design and Setup

The experimental design was a completely randomized full factorial arrangement of two species, two water volume treatments, and two watering frequency treatments, with 11 replicates of each of the eight species/treatment combinations. Seeds of *Agropyron repens* (L.) P. Beauv. and *Lupinus perennis* L. were germinated in handmade 3.0-L (30 cm tall \times 11.2 cm diameter) cylindrical PVC pots. The bottom of each pot was capped with a vinyl screen and filter paper to allow water drainage. *Lupinus perennis* seed was purchased from Prairie Moon Nursery (Winona, MN), and *A. repens* seed was purchased from V and J Seed Farm (Woodstock, IL). Both of these seed sets were collected from single populations at their respective production fields.

Soil type and texture are known to affect plant growth and biomass allocation in greenhouse drought experiments (Lindsey and Kilgore 2013), and native microflora of the soil can affect drought tolerance in C_3 grasses (Worchel et al. 2013). Therefore, to better approximate natural conditions, plants were grown in soil collected directly from grasslands at Cedar Creek Ecosystem Science Reserve (East Bethel, MN; lat. 45°24'N, long. 93°11'W). The soil is an entisol in the Nymore sand series, derived from a glacial outwash sandplain, 94% sand, acidic (pH = 5.5), and nitrogen poor (total soil N = 0.04%). Before planting, we homogenized the soil and removed larger organic debris using a 2-mm (no. 10) mesh. These species, and the use of natural soil, were chosen in part to parallel and inform a larger field experiment at Cedar Creek Ecosystem Science Reserve in which *L. perennis* and *A. repens* are among the five most abundant species.

Plants were thinned to two individuals per pot immediately following germination and kept well watered under standard greenhouse conditions. Day/night temperature controls were set for 20°–24°/17–21°C with a 14-h photoperiod (ambient sunlight augmented with $700 \pm 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation at plant height from 4:00 to 10:00 a.m.) and relative humidity ranging from 30% to 50%. When seedlings had developed two true leaves, they were thinned to one per pot and watering treatments were begun and continued for 14 wk. To control for microclimatic effects of position within the greenhouse, the arrangement of pots was initially randomized and then shuffled once every 6 d.

Watering Treatments

The design of the watering treatments was based on a pilot greenhouse study and field data from Cedar Creek. The pilot

study was conducted to measure the water-holding capacity of the field-collected soil and its rate of drying under standard greenhouse conditions. To determine water-holding capacity, several 1-kg units of soil were oven-dried for 48 h at 80°C, placed into pots, and watered to field capacity and the mass was retaken to determine the gravimetric water-holding capacity (mean \pm SE, 28.2% \pm 0.3%). Empirically determining the soil density (1.35 \pm 0.01 g cm⁻³) allowed us to convert this figure to a volumetric water-holding capacity and to ultimately determine the amount of water needed to raise the soil moisture content to target levels in each pot.

Target pre- and postwatering soil moisture levels were determined based on summer (May–August) measurements of soil moisture in experimental grassland plots at Cedar Creek between 2000 and 2009. Control water availability conditions (C) were designed to be comparable to average nondrought summer conditions in the experimental grassland plots. Specifically, 300 mL of water was applied to control pots on average every 6 d. Given the size of the pots, this is comparable to a weekly precipitation event of 3.0 cm.

Water availability was manipulated by reducing the total volume of water applied during the experiment by 50% (–V), reducing the frequency of watering events by 50% (–F), or both (–F/–V; table 1). In –F pots, plants were watered once every 11 d on average with 600 mL of water to maintain the same ultimate volume of water received. In –V pots, plants were watered every 6 d with only 150 mL, and in –F/–V pots, plants were watered once every 11 d with the reduced volume of 150 mL. Our –F treatments repeatedly provided periods of no rainfall comparable to the longest of such periods at Cedar Creek. On average, periods without a >0.5-cm rain event lasting 11 d or more occurred at the field site only once every 2 yr. Our watering treatments resulted in a schedule in which every 6 d half the plants were watered (C and –V treatments) and every 11 d all plants were watered (table 1). To control for differences in dissolved minerals or other chemicals found in tap water, plants were watered using melted snow collected on the University of Wisconsin–Eau Claire campus.

Measurements

To quantify the impact of our watering treatments on soil moisture (and, by extension, water availability), volumetric percent soil moisture was measured in all pots immediately before and 1 d following any watering event using a TH₂O portable soil moisture probe (Dynamax, Houston, TX). To determine the effects of these treatments on plant growth, the number of leaves per plant that were expanding, expanded, or in senescence (de-

fining as those senescing plus those entirely senesced) were counted during weeks 5, 7, and 13. After 14 wk, ultimate plant biomass accumulation and distribution were determined by harvesting the root and shoot tissue of each plant separately and immediately drying at 60°C for 48 h.

To determine the effects of watering treatments on physiological responses of both species in situ, rates of leaf CO₂ and water vapor exchange were taken during week 11, 1 d before all pots were watered. Measurements of young to midaged upper fully expanded leaves were taken between 9:00 a.m. and 4:00 p.m. using a LI-6400 portable infrared gas exchange analyzer (LicOR Environmental, Lincoln, NE). Controlling chamber temperature (25°C block temperature) and CO₂ concentration (400 ppm) resulted in a mean (\pm SE) vapor pressure deficit of 2.12 \pm 0.02 kPa and a mean relative humidity of 42.4% \pm 0.7%. These settings were determined following measures of ambient greenhouse conditions and used to maintain comparable leaf environmental conditions throughout the day. A red/blue LED light source was used to control photosynthetically active radiation (PAR) levels at 1200 μ mol m⁻² s⁻¹, which is within the range of light saturation found for these species (data not shown; Lee et al. 2011). Gas exchange rates were used to determine leaf net photosynthesis (μ mol CO₂ m⁻² s⁻¹) and stomatal conductance to water vapor (mol H₂O m⁻² s⁻¹), which also allowed us to calculate instantaneous water use efficiency (μ mol CO₂/mol H₂O). Data were analyzed for potential midday stomatal closure and for any systematic time of day effect by looking for interactions between treatment and time of day and by comparing net photosynthesis and stomatal conductance values at different times of day. There were no significant effects of or interactions with time of day (data not shown).

Leaf water potential measurements were also taken at this time using a model 600 pressure chamber (PMS Instrument, Albany, OR). The available configuration of the pressure chamber limited water potential measurements to *L. perennis* leaves only. The single leaf per plant used for these measurements was dried, and its specific leaf area was measured. This tissue was added to the corresponding shoot biomass at final harvest. Only one time point of gas exchange and water potential measurements considerably limits our ability to evaluate how these species were responding to the watering treatment over the course of their development; however, this snapshot of plant performance on mature plants at a distinguishing time in their watering treatment cycle provides some information concerning how these plants respond physiologically to varying kinds of water limitations.

Because of the important nitrogen-fixing role that *Lupinus*

Table 1

Volume and Frequency of Water Treatments Given to Each 3.0-L Pot of *Agropyron repens* or *Lupinus perennis* Regularly for 14 wk

Treatment	Abbreviation	Average days between watering	Volume per event (mL)
Control	C	5.7	300
Reduced frequency	–F	11.4	600
Reduced volume	–V	5.7	150
Reduced frequency + reduced volume	–F/–V	11.4	300

and other legumes play in grasslands, we estimated symbiotic nitrogen fixation responses of *L. perennis* to the watering treatments using stable isotope analysis (for a complete description of this technique, see Vose and Victoria 1986). Each pot was fertilized four times throughout the experiment with 1.70 mg of NH_4NO_3 enriched with ^{15}N and dissolved in water (Isotec, Miamisburg, OH; atom% ^{15}N of label = 0.3850%). This is equivalent to the natural rate of N deposition ($1 \text{ g m}^{-2} \text{ yr}^{-1}$) over 3 mo at Cedar Creek (data not shown). Aboveground dried biomass of a subset of both *L. perennis* and *A. repens* individuals was finely ground and analyzed for ^{15}N atom% and overall leaf N concentration (Europa Scientific Integra isotope ratio mass spectrometer, University of California, Davis, Stable Isotope Facility). Estimates of the proportion of N derived by symbiotic N_2 fixation (N_{dfa}) were calculated according to the following equation (Vose and Victoria 1986):

$$N_{\text{dfa}} = 1 - \frac{N_s - 0.3663}{N_r - 0.3663},$$

where N_s and N_r are the atom fraction ^{15}N in the sample and reference, respectively, with a standard background ^{15}N fraction of 0.3663. *Agropyron repens* plants were used as the reference to represent N derived from the soil and fertilizer only. This approach integrates symbiotic N_2 fixation with whole-plant growth over the growth interval and has been successfully used in a number of grassland experiments (Lee et al. 2003; West et al. 2005). One limitation of this approach is that it relies on an important assumption that the legume and its control plant assimilate fertilizer and soil N in the same proportion (Vose and Victoria 1986). With this limitation in mind, we consider only the relative effects of the water treatments on the amount of N derived from symbiotic N_2 fixation rather than make conclusions dependent on absolute amounts.

Data Analysis

Two-way ANOVAs were used to test the effects of our full factorial arrangement of watering treatments, species, and their interactions on plant response. Water volume, watering frequency, and species were treated as separate factors and considered fixed effects. For soil moisture content, total number of leaves, and the proportion of leaves in senescence, repeated-measures ANOVAs were used. In these cases, the watering treatments were considered fixed effects, and the replicate pots were considered random effects. Biomass, stomatal conductance, and water use efficiency data were natural log transformed to better fit normality and homoscedasticity assumptions. Post hoc Tukey HSD tests on least squares means were used to determine significant differences among the treatment combinations. Statistical analyses were performed using JMP statistical software (ver. 8.0.2.2; SAS Institute, Cary, NC).

Results

Soil Moisture

Our watering treatments strongly affected volumetric soil moisture in each pot over time (fig. 1; table 2). Soil moisture in *Lupinus perennis* pots was lower compared to pots con-

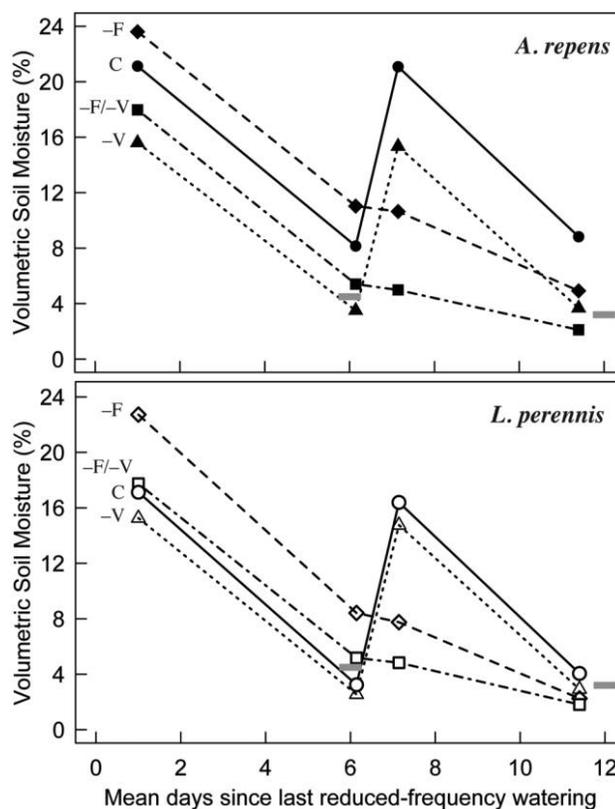


Fig. 1 Mean volumetric soil moisture (%) of pots containing single individuals of *Agropyron repens* and *Lupinus perennis* grown under standard greenhouse conditions. Treatments consisted of a well-watered control (C; circles with solid lines), a 50% reduction in watering frequency (-F; diamonds with dashed lines), a 50% reduction in watering volume (-V; triangles with dotted lines), or 50% reductions in frequency and volume (-F/-V; squares with dash-dotted lines; see table 1). Soil moisture was measured to a depth of 6 cm in all pots immediately before and 1 d after any watering event. Points represent the mean \pm SE of 11 replicates over eight watering cycles ($N = 88$). Gray bars indicate mean soil moisture from field data at 6 and 12 d after a >1-cm rain event in experimental grasslands at Cedar Creek Ecosystem Science Reserve in east-central Minnesota (May–July 2000–2009).

taining *Agropyron repens*, but both watering treatments more strongly affected soil moisture in pots containing *A. repens* than in ones containing *L. perennis* (table 2). Reductions in watering frequency reduced water availability by 17% in pots containing *A. repens* but only 7% in pots containing *L. perennis*. Similarly, reduced volume caused a 37% reduction in soil moisture in pots containing *A. repens* but only 26% in *L. perennis* (species \times frequency and species \times volume, $P < 0.016$; table 2). In -F and especially -F/-V pots, plants showed visible signs of water stress by the end of the watering cycle: leaves were curled and wilted. Within 24 h of watering, the leaves regained turgidity.

Whole-Plant Growth

Reduced-watering-frequency treatments reduced overall biomass accumulation across species by 21% (table 2; fig. 2),

Table 2

ANOVA Probabilities ($P < F$) for Main Effects and Interactions of 50% Reduced Watering Frequency and 50% Reduced Watering Volume on Individuals of *Agropyron repens* and *Lupinus perennis*

Response	Frequency		Volume		Frequency × volume		Species		Species × frequency		Species × volume		Species × frequency × volume	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Soil moisture	24.69	<u><.0001</u> (−13%)	166.5	<u><.0001</u> (−30%)	1.22	.273	47.98	<u><.0001</u>	6.09	<u>.016</u>	28.50	<u><.0001</u>	2.87	<u>.094</u>
Total biomass ^a	16.49	<u>.0001</u> (−21%)	15.48	<u>.0002</u> (−21%)	.18	.674	32.81	<u><.0001</u>	.065	<u>.799</u>	38.56	<u><.0001</u>	1.60	<u>.210</u>
Root-to-shoot ratio	5.61	<u>.020</u> (−22%)	3.61	<u>.061</u> * (+22%)	.15	.704	54.63	<u><.0001</u>	4.25	<u>.043</u>	4.26	<u>.042</u>	.0005	.983
Total no. leaves	3.4	<u>.067</u> * (−7%)	.35	.557	.82	.366	418.23	<u><.0001</u>	1.09	<u>.297</u>	.86	.355	1.81	.180
Proportion of leaves in senescence	11.26	<u>.0009</u> (+14%)	11.94	<u>.0006</u> (+14%)	1.73	.189	64.04	<u><.0001</u>	.12	<u>.734</u>	5.78	<u>.017</u>	.79	.374
Specific leaf area	.26	.618	.053	.82	.52	.478	.32	<u>.576</u>	1.70	<u>.204</u>	3.28	<u>.082</u> *	1.06	.204
Leaf net photosynthesis	2.30	.142	1.60	.217	.19	.665	13.61	<u>.0011</u>	.24	<u>.630</u>	.43	<u>.517</u>	2.32	.140
Stomatal conductance ^a	6.42	<u>.018</u> (−37%)	10.37	<u>.0035</u> (−45%)	1.60	.217	30.98	<u><.0001</u>	.085	<u>.773</u>	.031	.863	.53	.475
Instantaneous water use efficiency ^a	1.31	.263	5.09	<u>.033</u> (+38%)	.55	.467	7.57	<u>.0109</u>	.14	<u>.716</u>	2.28	.144	.87	.360

Note. Plants grown for 14 wk under standard greenhouse conditions. The magnitudes of all significant main effects are indicated in parentheses as percent change in the least squares mean from the control to the treatment. Underlined *P* values are those considered significant at $\alpha = 0.05$. For all analyses $df = 1$.

* *P* values considered significant at $\alpha = 0.10$.

^a Data ln transformed for analysis.

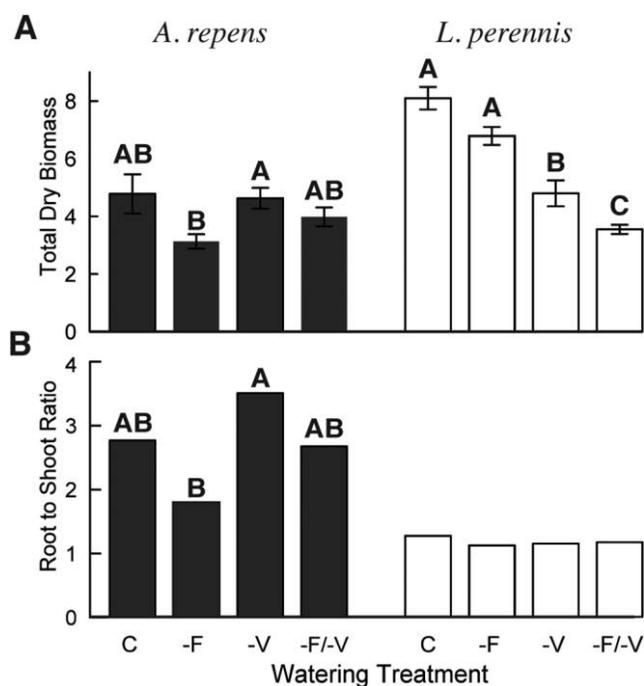


Fig. 2 Mean total dry biomass (\pm SE; A) and root-to-shoot biomass ratios (B) of *Agropyron repens* (filled bars) and *Lupinus perennis* (open bars) individuals grown for 14 wk under four watering regimes: a well-watered control (C), a 50% reduction in watering frequency (-F), a 50% reduction in watering volume (-V), or 50% reductions in frequency and volume (-F/-V; see table 1). Treatments within the same species not connected by the same letter are significantly different (Tukey HSD, $P < 0.05$). Watering treatments did not significantly affect the root-to-shoot ratio of *L. perennis*. Biomass data were natural log transformed for analysis.

while reduced water volume significantly reduced biomass accumulation in *L. perennis* only (species \times volume, $P < 0.0001$; table 2; fig. 2A). Compared to conspecific control plants, mean total biomass for *L. perennis* was 41% less for plants grown in the -V treatment and 56% less in the -F/-V treatment. The effects of reduced watering frequency were similar across species regardless of the water volume supplied (volume \times frequency, $P = 0.67$).

The effects of watering treatments on the relative distribution of root biomass to shoot biomass were also species specific (table 2). Mean root-to-shoot ratios (R : S) of *L. perennis* were similar across watering treatments, but R : S of *A. repens* were variable (fig. 2B). No treatment group differed significantly from the controls, although for *A. repens*, the -V group had a significantly higher R : S than the conspecific -F group (Tukey HSD, $P < 0.05$; fig. 2B). This variability in R : S was driven by changes in root growth and not shoot growth. Treatment effects on shoot biomass were not significant when total biomass was treated as a covariate, and results from a similar analysis of root biomass did not differ from the pattern shown in figure 2 (data not shown).

The differences in total accumulated biomass of each species in different water regimes were not caused by changes in the number of leaves produced; no significant differences were

found in the total number of leaves produced in response to water treatments (table 2; fig. 3A). Moreover, specific leaf area was not affected by any treatment or by species. Reduced watering frequency increased the proportion of leaves in senescence by 14% across species ($P = 0.0009$; table 2; fig. 3B). However, the effects of reduced water volume on leaf senescence were species specific. Both groups of *L. perennis* grown with reduced water volume (-V and -F/-V) showed a significantly greater proportion of leaves in senescence, but the increased leaf senescence among *A. repens* individuals was not statistically significant (species \times volume, $P = 0.017$; table 2; fig. 3B). For any given treatment *L. perennis* showed a greater proportion of leaves in senescence than *A. repens*, both in absolute terms and relative to conspecific control plants.

Plant Function

Prior to receiving their respective watering treatment, mature leaves of *L. perennis* were found to have lower net rates of

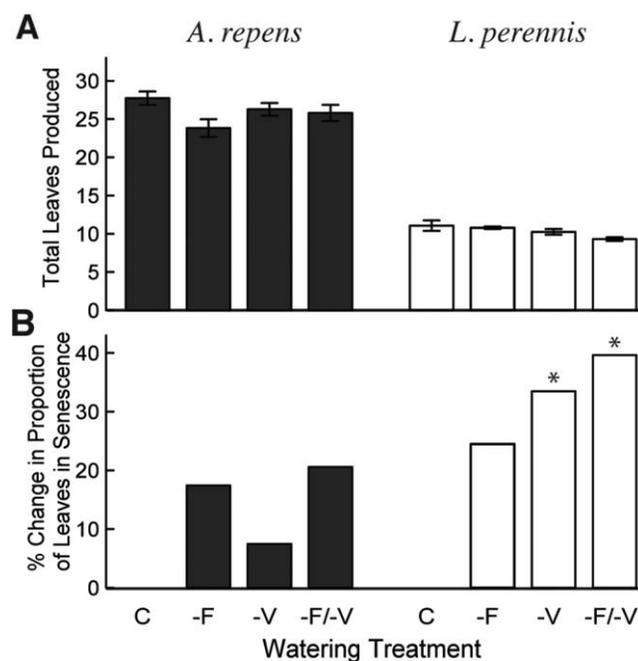


Fig. 3 A, Total number of leaves produced by individual plants of *Agropyron repens* (filled bars) and *Lupinus perennis* (open bars) and grown for 14 wk under four watering regimes: a well-watered control (C), a 50% reduction in watering frequency (-F), a 50% reduction in watering volume (-V), or 50% reductions in frequency and volume (-F/-V; see table 1). Reduced water availability treatments did not significantly affect the number of leaves in either species (Tukey HSD, $P < 0.05$). B, Percent change in proportion of leaves that are senesced or senescing with respect to control plants. Leaves were counted at weeks 5, 7, and 13 and categorized as senesced, senescing, mature, or expanding. Leaves were considered in senescence if they were scored as senesced or senescing. Mean proportions of leaves in senescence were calculated for each species in each watering treatment group ($n = 11$) across the three time points. Asterisks indicate a significant difference between treatments and the control (Tukey HSD, $P < 0.05$). The -F, -V, and -F/-V treatment groups did not significantly differ from each other in either species.

photosynthesis and stomatal conductance compared to those of *A. repens* on a per-area basis. However, photosynthetic rates were not significantly impacted by any of the reduced water availability treatments in either species ($P > 0.14$; table 2; fig. 4A). Across species, plants grown under the -F treatment had 37% lower conductance ($P < 0.018$) and those under the -V treatments had 45% lower conductance ($P < 0.004$) compared to control plants (table 2; fig. 4B). These responses combined to increase instantaneous water use efficiency (CO_2 assimilation per unit water loss) in plants grown under reduced-volume treatments by 38% across species, with *A. repens* showing the greatest increase in water use efficiency (92%) when grown under the combined reduced watering frequency and volume treatment compared to the control (fig. 4C).

At the time these measurements were taken, the mean mid-day leaf water potential among *L. perennis* control plants was -0.87 ± 0.19 MPa (least squares mean \pm SE, data not shown). Reduced water volume resulted in 65% lower *L. perennis* leaf water potentials compared to control plants ($P = 0.007$; table 3), but reduced water frequency had no significant effect. Plants grown under both the -V and -V/-F treatments had leaf water potentials of -1.7 ± 0.2 MPa (data not shown).

Leaf N concentration of *L. perennis* individuals was not impacted by the reduced water treatments (table 3; fig. 5A). However, the proportion of nitrogen derived from symbiotic N_2 fixation in *L. perennis* was negatively impacted by reductions in water volume. Decreasing the watering frequency did not have a significant effect unless the total volume was also decreased (frequency \times volume, $P = 0.015$; table 3; fig. 5B). Compared to a proportion of N derived from fixation of 0.97 ± 0.02 in control plants, *L. perennis* grown under the -F regime showed no significant change, but there was a 14% reduction in plants grown under the -V regime (0.83 ± 0.01) and a 22% reduction in the -F/-V regime (0.76 ± 0.02 ; fig. 5B).

The effects of our water treatments on the proportion N derived from fixation combined with their effects on growth paralleled the effects of these treatments on total aboveground N content. Reductions in water volume significantly reduced the total aboveground N accumulation by 31% ($P = 0.011$; fig. 5C), while the negative effects of reduced watering frequency were not significant. Curiously, *L. perennis* grown under the -V regime showed the largest reduction in total accumulated N compared to the control (-49%; fig. 5C), as reduced frequency and volume showed a marginally significant antagonistic interaction ($P = 0.06$; table 3).

Discussion

Our results suggest three key findings. First, changes in rainfall frequency or total rainfall volume can independently affect soil moisture profiles, with reduced watering volume more strongly impacting soil moisture over time compared to reduced watering frequency. Second, changes in both water volume and watering frequency affected plant growth, but in different ways depending on the species. For example, *Agropyron repens* was not limited by reduced water availability as long as low levels of water were supplied more regularly. Moreover, several measures of plant function (e.g., water use efficiency, *Lupinus perennis* leaf water potential, and *L. perennis* pro-

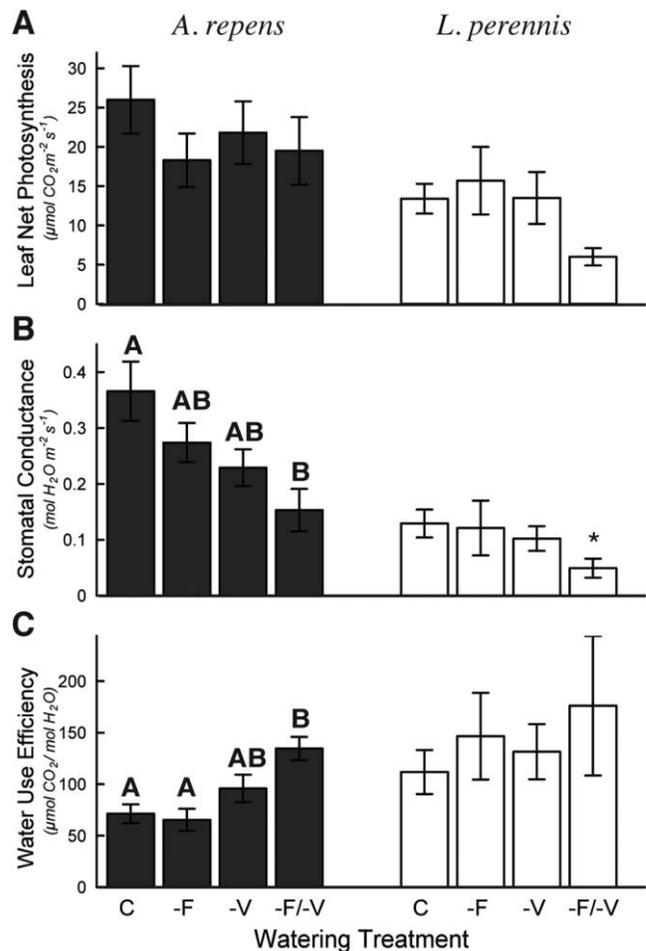


Fig. 4 Leaf net photosynthetic rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; A) and leaf stomatal conductance rates ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$; B) of *Agropyron repens* (filled bars) and *Lupinus perennis* (open bars) individuals grown under four watering regimes: a well-watered control (C), a 50% reduction in watering frequency (-F), a 50% reduction in watering volume (-V), or 50% reductions in frequency and volume (-F/-V; see table 1). The quotient of leaf net photosynthesis and stomatal conductance was calculated to determine the instantaneous water use efficiency, defined as CO_2 assimilation per unit water loss ($\mu\text{mol CO}_2 / \text{mol H}_2\text{O}$; C). Bars represent means \pm SE ($n = 3-6$). Treatments within the same species not connected by the same letter are significantly different (Tukey HSD, $P < 0.05$). Bars with no letters within a species are not significantly different ($P < 0.05$). An asterisk indicates a mean that is significantly different from all other treatment means ($P < 0.1$). Stomatal conductance and water use efficiency data were natural log transformed for analysis.

portion of N derived from fixation) were affected by reduced water volume but not reduced watering frequency. Third, functional traits of both species likely contributed to these differences. How our data inform these three findings is discussed in further detail below.

The lower overall soil water content of *L. perennis* compared to *A. repens* suggests differences in water use between the two species. However, the effects of both the reduced-frequency and reduced-volume treatments were stronger in pots con-

Table 3
Two-Way ANOVA Probabilities ($P < F$) for Main Effects and Interactions of 50% Reduced Watering Frequency and 50% Reduced Watering Volume on Individuals of *Lupinus perennis*

Response	Frequency		Volume		Frequency × volume	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Leaf water potential	.69	.425	10.97	<u>.007</u> (−65%)	.66	.433
Proportion N derived from fixation	3.35	.092* (−3%)	134.42	<u><.0001</u> (−18%)	8.04	<u>.015</u>
Leaf % N	.86	.373	.90	.363	.22	.651
Total shoot N	.29	.603	9.20	<u>.011</u> (−30%)	4.34	.061*

Note. Plants grown for 14 wk under standard greenhouse conditions. The magnitudes of all significant main effects are indicated in parentheses as percent change in the least squares mean from the control to the treatment. Underlined *P* values are those considered significant at $\alpha = 0.05$. For all analyses $df = 1$.

* *P* values considered significant at $\alpha = 0.10$.

taining *A. repens*. This may be related to total plant size, as *L. perennis* plants had greater dry biomass than *A. repens*, but to a lesser degree in reduced-water-availability treatments. Vegetation type or community composition can and does influence abiotic factors such as soil moisture (Ehrenfeld et al. 2005; Aalto et al. 2013), forming potentially complex relationships between climate, soil, and species composition of an area. A complete mechanistic explanation for these patterns exceeds the scope of our study; however, they do show the importance of considering species identity in modeling system responses to changing water availability.

Reduced watering frequency and water volume often affected these two species differently, particularly in terms of total biomass, biomass allocation, and leaf senescence. The differential impact of these two parameters is consistent with patterns observed in several field experiments (Fay et al. 2002, 2003, 2011; Robinson et al. 2012; Zhang et al. 2013). Although reduced watering frequency negatively impacted overall growth of both species, reduced water volume decreased growth only in *L. perennis*, suggesting that the growth of this species is more impacted by changes in total water volume.

Plants are commonly known to respond to suboptimal resource availability by changing resource allocation to minimize limitations on growth. Therefore, a limited water supply would be expected to promote increased growth of structures involved in water uptake, namely, roots. This is a classic drought avoidance strategy (Chaves et al. 2003), though such responses are not ubiquitous. For example, root growth of grassland plants increased in a different reduced rainfall frequency manipulation (Fay et al. 2003) but decreased during a natural drought (Hayes and Seastedt 1987). We found that watering treatments did not affect R : S of *L. perennis* but that *A. repens* was able to adjust biomass allocation, with reductions in frequency and volume having opposing effects on R : S. However, given that both species are perennials, the 14 wk of this experiment may not have been long enough to capture changes in R : S relevant over the lifetime of the plants.

Increased leaf senescence is an alternative drought avoidance strategy observed in a wide range of other plants, including economic crops, wild perennials, and several other lupine species (Rodrigues et al. 1995; Borell et al. 2000; Munné-Bosch et al. 2001; Chaves et al. 2003; Rood et al. 2003). Old leaves are shed as they become a net liability for the plant due to

low carbon fixation and high water loss but only after many of the nutrients are recycled back into the plant (Hensel et al. 1993; Heckathorne and DeLucia 1994; Munné-Bosch and Alegre 2004; Wright et al. 2004). We found that reduced watering frequency increased leaf senescence in both species but only *L. perennis* was affected by reduced watering volume, mirroring our observations of decreased biomass. However, in spite of the short-term reduction in total photosynthetic tissue induced by elevated leaf senescence, this strategy has been shown to confer several adaptive advantages on plants, particularly by increasing drought tolerance and survival (Munné-Bosch and Alegre 2004; Pérez-Ramos et al. 2013).

In contrast to species-specific effects at the whole-plant level, leaf-level gas exchange measurements show similar effects of reduced volume and reduced water frequency on *A. repens* and *L. perennis*, though these conclusions are based on a single observation. Both species had lower stomatal conductance under both reduced water availability treatments but no change in leaf photosynthesis, though differences in total biomass and leaf senescence likely translate into meaningful differences at the whole-plant scale. Fay et al. (2002) also found conductance and carbon fixation to be correlated with soil moisture in a forb but not in the C_4 grass *Agropyron gerardii*. Studies of C_3 plants have consistently identified stomatal closure as a key regulatory mechanism during mild droughts. This often leads to a reduction in photosynthetic rates (reviewed in Medrano et al. 2002); however, some evidence suggests an ability for plants to acclimate their photosynthetic rate to repeated water deficits (Matthews and Boyer 1984). Had we been able to capture gas exchange responses of plants following the application of watering treatments, we may have been able to determine whether this occurred in our study. However, diminished gas exchange is not the only physiological process that affects growth in times of water stress. Given the reduction in leaf water potential for *L. perennis*, growth may also have been inhibited due to limited water for cell elongation or protein synthesis (Lambers et al. 2006).

The unique functional attributes of *A. repens* and *L. perennis* may help explain some of the observed species-specific effects. Grasses often dominate plant communities found in drier areas and therefore are likely better adapted to drought than forbs (Fay et al. 2003; Sandel et al. 2010), though grasses in the driest locations typically possess the C_4 metabolic pathway.

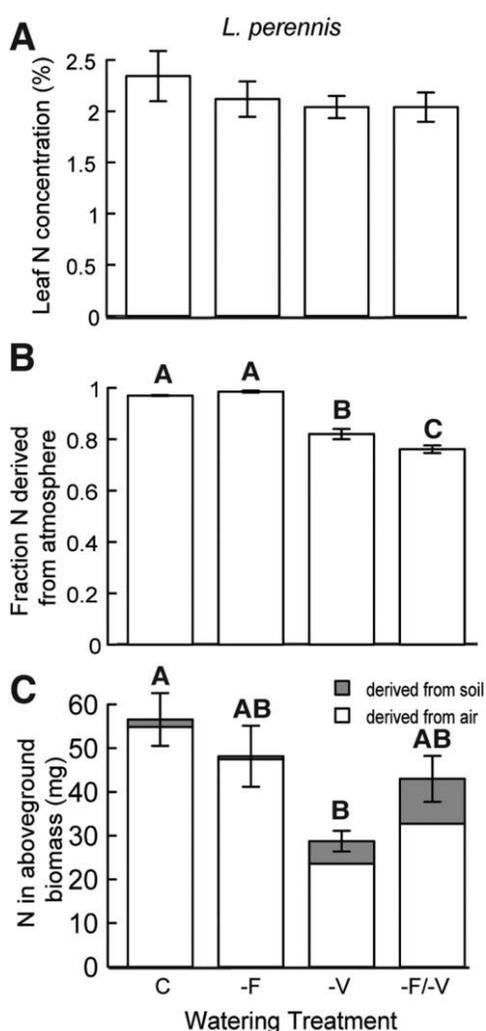


Fig. 5 Leaf nitrogen concentrations ($\text{g g}^{-1} \times 100\%$; A) and fraction of plant nitrogen derived from the atmosphere (B) of *Lupinus perennis* individuals grown under four watering regimes: a well-watered control (C), a 50% reduction in watering frequency (-F), a 50% reduction in watering volume (-V), or 50% reductions in frequency and volume (-F/-V; see table 1). These measurements, along with the aboveground biomass, were used to estimate total shoot nitrogen (mg plant^{-1} ; C). Bars represent means \pm SE ($n = 3-4$), with white portions indicating shoot N derived from soil and gray portion indicating N derived from the air via symbiotic N_2 fixation. Treatments within the same species not connected by the same letter are significantly different (Tukey HSD, $P < 0.05$). Leaf N concentration did not vary significantly between any treatments ($P < 0.05$).

Agropyron repens, a C_3 grass, has been described as drought sensitive in comparison (Tillman and Downing 1994). However, following severe drought, C_4 grasses are slow to recover their photosynthetic capability, unlike C_3 grasses (Heckathorne and DeLucia 1994; Ripley et al. 2010). In a broad sense, this may explain why *A. repens* maintained similar total dry biomass across watering treatments but *L. perennis* showed reduced biomass in the -V and -F/-V treatments.

Like total dry biomass, the proportion of N derived from

symbiotic N_2 fixation in *L. perennis* and total shoot nitrogen significantly decreased in response to volume but not frequency. It is well established that N fixation and accumulation by legumes are sensitive to drought, though research has been mostly limited to agricultural species (Serraj et al. 1999). However, overall leaf N concentration remained constant across treatments in spite of reduced symbiotic N_2 fixation in low water treatments, consistent with the findings of Rodrigues et al. (1995) in *Lupinus alba*. This may have helped prevent significant reductions in net photosynthetic rates and changes to R : S in *L. perennis* given that nitrogen concentrations are known to directly influence plant photosynthetic capacity and R : S (Heckathorne and DeLucia 1997; Ågren and Franklin 2003; Pilbeam 2011).

In natural systems, the presence of legumes can increase N concentrations and photosynthetic rates in nearby plants (Lee et al. 2003). The reduction in total N in aboveground biomass in *L. perennis* when total water volume is reduced suggests a potential reduction of total N inputs from this source and possible indirect effects in natural systems under various reduced water regimes. In this way, differential responses of functional groups may have cascading effects with changing water regimes.

To our knowledge, this is one of the first greenhouse studies to compare and contrast the simulated effects of two dimensions of precipitation, reduced rainfall frequency and reduced rainfall volume, on whole-plant growth and physiology. These results are consistent with prior studies that have shown differential growth effects from multiple aspects of rainfall (Fay et al. 2002, 2003, 2011; Robinson et al. 2012; Zhang et al. 2013). However, most of these studies are long-term field manipulations or observations that have shed light on larger-scale effects of changing water volume and frequency, such as net primary productivity and overall growth, whereas the particular physiological mechanisms that result in this altered whole-plant growth remain less clear. By monitoring individual plants in a greenhouse setting, we were able to evaluate season-long growth, distribution of biomass to roots versus shoots, leaf senescence, and legume symbiotic N fixation of individual plants grown under varied water regimes, along with a snapshot of plant gas exchange responses under these treatments, thereby better revealing species-specific differences and possible links to functional attributes.

As a whole, the differences in species responses to our four water availability treatments suggest that trying to understand plant growth and function based solely on a single parameter of water availability (often mean annual rainfall) is overly simplistic. These results underscore the importance of efforts to consider various parameters of water availability both independently and in combination, such as total rainfall volume and summer rainfall frequency, when attempting to describe a plant's hydrological regime, model vegetation responses to climate change, or reconstruct past distributions.

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Literature Cited

- Aalto J, PC le Roux, M Luoto 2013 Vegetation mediates soil temperature and moisture in arctic-alpine environments. *Arct Antarct Alp Res* 45:429–439.
- Ågren GI, O Franklin 2003 Root : shoot ratios, optimization and nitrogen productivity. *Ann Bot* 92:795–800.
- Beier C, C Bierkuhnlein, T Wohlgemuth, J Penuelas, B Emmett, C Körner, H de Boeck, et al 2012 Precipitation manipulation experiments: challenges and recommendations for the future. *Ecol Lett* 15:899–911.
- Borell AK, GL Hammer, ACL Douglas 2000 Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence. *Crop Sci* 40:1026–1037.
- Chaves MM, JP Maroco, JS Pereira 2003 Understanding plant responses to drought—from genes to whole plant. *Funct Plant Biol* 30:239–264.
- Cotrufo MF, G Alberti, I Inglema, H Marjanovic, D LeCain, A Zaldei 2011 Decreased summer drought affects plant productivity and carbon dynamics in a Mediterranean woodland. *Biogeosciences* 8:2729–2739.
- Craine JM, JB Nippert, AJ Elmore, AM Skibbe, SL Hitchinson, NA Brunsell 2011 Timing of climate variability and grassland productivity. *Proc Natl Acad Sci USA* 109:3401–3405.
- Crimmins SM, SZ Dobrowski, JA Greenberg, JT Abatzoglou, AR Mynsberge 2011 Changes in climatic water balance drive downhill shifts in plant species' optimum elevations. *Science* 331:324–327.
- Dukes JS, HA Mooney 1999 Does global change increase the success of biological invaders? *Trends Ecol Evol* 14:135–139.
- Ehrenfeld JG, B Ravit, K Elgersma 2005 Feedback in the plant-soil system. *Annu Rev Environ Res* 30:75–115.
- Fay PA, JM Blair, JB Nippert, JD Carlisle, AK Knapp 2011 Relative effects of precipitation variability and warming on tallgrass prairie ecosystem function. *Biogeosciences* 8:3053–3068.
- Fay PA, JD Carlisle, BT Danner, MS Lett, JK McCarron, C Stewart, AK Knapp, JM Blair, SL Collins 2002 Altered rainfall patterns, gas exchange, and growth in grasses and forbs. *Int J Plant Sci* 163:549–557.
- Fay PA, JD Carlisle, AK Knapp, JM Blair, SL Collins 2003 Productivity responses to altered rainfall patterns in a C₄-dominated grassland. *Oecologia* 137:245–251.
- Groisman PY, RW Knight, TR Karl, DR Easterling, B Sun, JH Lawrimore 2004 Contemporary changes of the hydrological cycle over the contiguous United States: trends derived from in situ observations. *J Hydrometeorol* 5:64–85.
- Hayes DC, TR Seastedt 1987 Root dynamics of tallgrass prairie in wet and dry years. *Can J Bot* 65:787–791.
- Heckathorne SA, EH DeLucia 1994 Drought-induced nitrogen retranslocation in perennial C₄ grasses of tallgrass prairie. *Ecology* 75:1877–1886.
- 1997 The contribution of drought-related decreases in foliar nitrogen concentration to decreases in photosynthetic capacity during and after drought in prairie grasses. *Physiol Plant* 101:173–182.
- Hensel LL, V Grbic, DA Baumgarten, AB Blecker 1993 Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in *Arabidopsis*. *Plant Cell* 5: 553–564.
- Huang P, S-P Xie, K Hu, G Huang, R Huang 2013 Patterns of the seasonal response of rainfall to global warming. *Nat Geosci* 6:357–361.
- Huxman TE, KA Snyder, D Tissue, AJ Leffler, K Ogle, WT Pockman, DR Sandquist, DL Potts, S Schwinning 2004 Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. *Oecologia* 141: 254–268.
- Jentsch A, C Beierkuhnlein 2008 Research frontiers in climate change: effects of extreme meteorological events on ecosystems. *C R Geosci* 340:621–628.
- Knapp AK, C Beier, DD Briske, AT Classen, Y Luo, M Reichstein, MD Smith, et al 2008 Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience* 58:811–821.
- Knapp AK, JM Briggs, JK Koelliker 2001 Frequency and extent of water limitation to primary production in a mesic grassland. *Ecosystems* 4:19–28.
- Knapp AK, CE Burns, RWS Fynn, KP Kirkman, CD Morris, MD Smith 2006 Convergence and contingency in production-precipitation relationships in North American and South African C₄ grasslands. *Oecologia* 149:456–464.
- Knapp AK, PA Fay, JM Blaire, SL Collins, MD Smith, JD Carlisle, CW Harper, BT Danner, MS Lett, JK McCarron 2002 Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298:2202–2205.
- Lambers H, FS Chapin, TL Pons 2006 *Plant physiological ecology*. Springer, New York.
- Lee TD, SH Barrott, PB Reich 2011 Photosynthetic responses of 13 grassland species across 11 years of free-air CO₂ enrichment is modest, consistent and independent of N supply. *Glob Change Biol* 17: 2893–2904.
- Lee TD, PB Reich, MG Tjoelker 2003 Legume presence increases photosynthesis and N concentration of co-occurring non-fixers but does not modulate their responses to carbon dioxide enrichment. *Oecologia* 137:22–31.
- Lindsey AJ, JS Kilgore 2013 Soil type affects *Pinus ponderosa* var. *scopulorum* (Pinaceae) seedling growth in simulated drought experiments. *Appl Plant Sci* 1:1300031.
- Looney CE, BW Sullivan, TE Kolb, JM Kane, SC Hart 2012 Pinyon pine (*Pinus edulis*) mortality and response to water addition across a three million year substrate age gradient in northern Arizona, USA. *Plant Soil* 357:89–102.
- Matthews MA, JS Boyer 1984 Acclimation of photosynthesis to low leaf water potentials. *Plant Physiol* 74:161–166.
- Medrano H, JM Escalona, J Bota, J Gulias, J Flexas 2002 Regulation of photosynthesis of C₃ plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann Bot* 89:895–905.
- Morelli TL, AB Smith, CR Kastely, I Mastroserio, C Mortiz, S Besinger 2012 Anthropogenic refugia ameliorate the severe climate-related decline of a montane mammal along its trailing edge. *Proc R Soc B* 279:4279–4286.
- Munné-Bosch S, L Alegre 2004 Die and let live: leaf senescence contributes to plant survival under drought stress. *Funct Plant Biol* 31: 203–216.
- Munné-Bosch S, T Jubany-Marí, L Alegre 2001 Drought-induced senescence is characterized by a loss of antioxidant defences in chloroplasts. *Plant Cell Environ* 24:1319–1327.
- Pérez-Ramos IM, F Voltaire, M Fattet, A Blanchard, C Roumet 2003 Trade-offs between functional strategies for resource use and drought survival in Mediterranean rangeland species. *Environ Exp Bot* 87:126–136.
- Pilbeam DJ 2011 The utilization of nitrogen by plants: a whole plant perspective. *Annu Plant Rev* 42:305–351.

- Rapacciuolo G, SP Maher, AC Schneider, TT Hammond, MD Jabis, KJ Iknayan, GK Walden, MF Oldfather, DD Ackerly, SR Beissinger Forthcoming Beyond a warming fingerprint: individualistic biogeographic responses to heterogeneous climate change in California. *Glob Change Biol*.
- Reich PB 2009 Elevated CO₂ reduces losses of plant diversity caused by nitrogen deposition. *Science* 326:1399–1402.
- Reich PB, SE Hobbie 2013 Decade-long soil nitrogen constraint on the CO₂ fertilization of plant biomass. *Nat Clim Change* 3:278–282.
- Ripley BS, K Frole, ME Gilbert 2010 Differences in drought sensitivities and photosynthetic limitations between co-occurring C₃ and C₄ (NADP-ME) panicoid grasses. *Ann Bot* 105:493–503.
- Robinson TMP, KJ La Pierre, MA Vadeboncoer, KM Byrne, ML Thomey, SE Colby 2012 Seasonal, not annual precipitation drives community productivity across ecosystems. *Oikos* 122:727–738.
- Rodrigues ML, CMA Pacheco, MM Chaves 1995 Soil-plant water relations, root distribution and biomass partitioning in *Lupinus albus* L. under drought conditions. *J Exp Bot* 46:947–956.
- Rood SB, JH Braatne, FMR Hughes 2003 Ecophysiology of riparian cottonwoods: stream flow dependency, water relations, and restoration. *Tree Physiol* 23:1113–1124.
- Sandel B, LJ Goldstein, NJB Kraft, JG Okie, MI Shuldman, DD Ackerly, EE Cleland, KN Suding 2010 Contrasting trait responses in plant communities to experimental and geographic variation in precipitation. *New Phytol* 188:565–575.
- Selmants PC, SC Hart 2008 Substrate age and tree islands influence carbon and nitrogen dynamics across a retrogressive semiarid chronosequence. *Glob Biogeochem Cycles* 22:1–13.
- Serraj R, TR Sinclair, LC Purcell 1999 Symbiotic N₂ fixation response to drought. *J Ex Bot* 50:143–155.
- Stephenson NL 1990 Climatic control of vegetation distribution: the role of the water balance. *Am Nat* 135:649–670.
- Tillman D, JA Downing 1994 Biodiversity and stability in grasslands. *Nature* 367:363–365.
- Vose PB, RL Victoria 1986 Re-examination of the limitations of nitrogen-15 isotope dilution technique for the field measurement of dinitrogen fixation. Pages 23–41 *in* RD Hauck, RW Weaver, eds. Field measurement of dinitrogen fixation and denitrification. Soil Science Society of America, Madison, WI.
- Wentz FJ, L Ricciardulli, K Hilburn, C Mears 2007 How much more rain will global warming bring? *Science* 317:233–235.
- West JB, J HilleRisLambers, TD Lee, SE Hobbie, PB Reich 2005 Legume species identity and soil nitrogen supply determine symbiotic nitrogen-fixation responses to elevated atmospheric [CO₂]. *New Phytol* 167:523–530.
- Willis CG, BR Ruhfel, RB Primack, AJ Miller-Rushing, JB Losos, CC Davis 2010 Favorable climate change response explains non-native species' success in Thoreau's woods. *PLoS ONE* 5:e8878. doi: 10.1371/journal.pone.0008878.
- Winter JM, EAB Eltahir 2012 Modeling the hydroclimatology of the Midwestern United States. 2. Future climate. *Clim Dynam* 38:595–611.
- Worchel ER, HE Glauque, SN Kivlin 2013 Fungal symbionts alter plant drought response. *Microb Ecol* 65:671–678.
- Wright IJ, PB Reich, M Westoby, DD Ackerly, Z Baruch, F Bongers, J Cavender-Bares, et al 2004 The worldwide leaf economics spectrum. *Nature* 428:821–827.
- Zhang B, J Cao, Y Bai, X Zhou, Z Ning, S Yang, L Hu 2013 Effects of rainfall amount and frequency on vegetation growth in a Tibetan alpine meadow. *Clim Change* 118:197–212.