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Albina Khasanove

Jeremy J. James

Rebecca E. Drenovsky

John Carroll University, rdrenovsky@jcu.edu

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Impacts of drought on plant water relations and nitrogen nutrition in dryland perennial grasses

Albina Khasanova · Jeremy J. James ·
Rebecca E. Drenovsky

Abstract

Background and aims Extensive worldwide dryland degradation calls for identification of functional traits critical to dryland plant performance and restoration outcomes. Most trait examination has focused on drought tolerance, although most dryland systems are water and nutrient co-limited. We studied how drought impacts both plant water relations and nitrogen (N) nutrition.

Methods We grew a suite of grasses common to the Intermountain West under both well-watered and drought conditions in the greenhouse. These grasses represented three congener pairs (*Agropyron*, *Elymus*, *Festuca*) differing in their habitat of origin (“wetter” or “drier”). We measured growth, water relations, N resorption efficiency and proficiency and photosynthetic N use efficiency in response to drought.

Results Drought decreased growth and physiological function in the suite of grasses studied, including a negative impact on plant N resorption efficiency and proficiency. This effect on resorption increased over the course of the growing season. Evolutionary history constrained species responses to treatment, with genera varying in the magnitude of their response to drought conditions. Surprisingly, habitat of origin influenced few trait responses.

Conclusions Drought impacted plant N conservation, although these responses also were constrained by evolutionary history. Future plant development programs should consider drought tolerance not only from the perspective of water relations but also plant mineral nutrition, taking into account the role of phylogeny.

A. Khasanova · R. E. Drenovsky (✉)
Biology Department, John Carroll University,
1 John Carroll Blvd.,
University Heights, OH 44118, USA
e-mail: rdrenovsky@jcu.edu

J. J. James
USDA-Agricultural Research Service, Eastern Oregon
Agricultural Research Center,
67826-A Hwy 205,
Burns, OR 97720, USA

Present Address:

A. Khasanova
USDA-Agricultural Research Service, Grassland Soil Water
and Research Laboratory,
808 East Blackland Rd,
Temple, TX 76502, USA

Present Address:

J. J. James
University of California Sierra Foothill Research
& Extension Center,
8279 Scott Forbes Road,
Browns Valley, CA 95918, USA

Keywords Drought tolerance · Gas exchange ·
Nitrogen use efficiency · Resorption efficiency ·
Resorption proficiency

Abbreviations

N_{eff} , %	Nitrogen resorption efficiency
N_{prof} , g kg ⁻¹	Nitrogen resorption proficiency
A, $\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$	Photosynthetic assimilation

PNUE, $\mu\text{mol CO}_2$ $\text{mol N}^{-1} \text{s}^{-1}$	Photosynthetic nitrogen use efficiency
g_s , $\text{mmol m}^{-2} \text{s}^{-1}$	Stomatal conductance
Ψ_w , MPa	Water potential
WUE, μmol $\text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$	Water use efficiency

Introduction

Drylands cover 40 % of the Earth's land surface, support over 2 billion people and house one third of the hotspots for global biodiversity (Millennium Ecosystem Assessment 2005; Myers et al. 2000). Despite their importance, dryland ecosystems also are some of the regions most susceptible to degradation and climate change (Reynolds et al. 2007). While restoration to combat dryland degradation is a top international priority (UNCCD 2012), water and nutrient co-limitations strongly impede the recovery of functional plant communities (Drenovsky and Richards 2004; James et al. 2005). Researchers have focused heavily on selection and development of plant functional traits that may improve dryland restoration outcomes (Jones 2003; Jones and Monaco 2009). Most of this work, however, has focused on selection and development of traits related to drought tolerance and growth rate with little emphasis on traits that increase long-term nutrient conservation. Nutrient conservation, which is realized through a suite of plant traits that limit nutrient losses to the environment (e.g., proficient resorption, long mean retention times, high nutrient use efficiency), is expected to influence population growth rate and restoration outcomes of nutrient-limited systems (Berendse 1994; James et al. 2011). In the process of selecting and developing plant material for dryland restoration it is critical to evaluate the relationship between drought tolerance and nutrient conservation traits.

While a general relationship between stress tolerance and tissue longevity has been well established (Coley et al. 1985; Lambers et al. 2008; Wright and Westoby 2003), the specific impacts of drought on nutrient conservation, particularly that of nitrogen (N) are complex. Under drought conditions, plants alter metabolic and physiological function to minimize negative impacts and maximize survival (Thapa et al. 2011). In response to drying soils, plants alter gene expression for proteins involved in drought tolerance (Lambers et al. 2008;

Thapa et al. 2011). At the same time, plants decrease physiological rates and alter growth and allocation patterns (Drenovsky et al. 2012; Flexas and Medrano 2002). Although reduced stomatal conductance decreases plant water loss, it also limits carbon assimilation (Casper et al. 2006), which, compounded with decreased cell turgor, can limit growth. Additionally, allocation patterns shift due to shedding of older leaves in response to water deficit (Ludlow 1989) and increased root mass allocation (Creelman et al. 1990; Ribaut et al. 2009). Although these changes help minimize cellular damage and water loss, they also have important implications for whole plant mineral nutrition. As soils dry, N uptake by roots may be limited due to decreased N supply via mass flow and diffusion (Dunham and Nye 1973) and reduced root N interception due to lower root elongation rates (Lambers et al. 2008). However, N nutrition and drought tolerance are interrelated, with increased external N supply improving physiological status and growth in response to low soil water availability (Drenovsky et al. 2012; Saneoka et al. 2004). Thus, under drought conditions, plants require nitrogen to support changes in cellular and whole plant processes and to maintain function, but they are limited in their ability to access soil N due to decreased N supply and uptake rates. Thus, N resorption, the withdrawal of N-containing biomolecules from senescing tissues, and internal plant N stores should play a critical role in drought tolerance (Yuan and Li 2007). However, efficient transport of recycled nutrients through the phloem requires adequate water recycling from the xylem, which may be limited under drought conditions (Ruehr et al. 2009). As such, N resorption responses under water-limiting conditions may be complex.

The impact of drought on N resorption has varied in the literature, with studies documenting drought increasing (Pavon et al. 2005), decreasing (Drenovsky et al. 2012; Minoletti and Boerner 1994; Wright and Westoby 2003), or not affecting (Diehl et al. 2008; Drenovsky et al. 2010) N resorption. A suite of factors may influence N resorption including (but not limited to) the timing of drought relative to phenology, the evolutionary history of the organism (i.e., phylogeny), as well as the severity of the drought (Killingbeck 1996, 2004; Marchin et al. 2010; Silla and Escudero 2006). Thus, understanding the relationship between a species' drought tolerance and its ability to conserve important internal N reserves is critical and requires controlled studies that isolate one or more co-varying factors influencing resorption.

The aim of this study was to examine if species that maintain greater growth and physiological function under drought (i.e., species that are more drought tolerant) also maintain greater N conservation under drought. For this study we included a suite of grasses widely used in dryland restoration in the western United States. The grass species used in this study represent three congener pairs, with one species in each pair being distributed largely in drier sites and the other species more common in wetter sites. Although we expected that drought would decrease growth and physiological responses in all species, we hypothesized that grass species distributed predominately in drier sites would better maintain trait responses under drought compared to congener species that typically dominate wetter sites. We also predicted that phylogeny would constrain physiological and growth responses, leading to variation among the genera studied. Lastly, we sought to understand how phenological processes and drought interact to influence the amount of N lost during senescence. We predicted that N resorption efficiency and proficiency would be greater during the fall than during the summer due to other physiological changes occurring during seasonally-cued aboveground senescence during the fall. However, we expected that drought would limit the amount of N resorbed from leaves, leading to greater plant N losses, regardless of season.

Materials and methods

Study species

The selection of genera broadly represents those used in some of the largest dryland restoration projects in the United States. Congener pairs within these perennial grass genera were used for study. Species selected represent cultivars that have been developed for restoration and vegetation management projects throughout the Intermountain West. In each pair, one species tends to be naturally distributed as well as planted in drier regions while the other species tends to be more dominant, as well as planted, in wetter regions (T.A. Jones, personal communication). Species more common in drier regions include: *Agropyron fragile* (Roth.) P. Candargy (cultivar Stabilizer), *Elymus elymoides* (Raf.) Swezey (cultivar Rattlesnake GP), and *Festuca*

idahoensis Elmer; species more common to wetter regions were: *A. desertorum* (Fisch. ex. Link) Schult. (cultivar 'Douglas'), *E. glaucus* Buckley (cultivar 'Arlington'), and *F. roemerii* (Pavlick) Alexeev. Seeds were obtained from T.A. Jones, USDA-ARS, Logan UT.

Experimental design

In April 2011, prior to germination, seeds were imbibed in water for 24 h. Following imbibition, seeds were transferred to Petri dishes moistened with 10 % strength modified Hoagland's solution (Epstein 1972). Once radicals appeared, seeds were transferred into pots (10 cm wide, 30.5 cm deep; Treepots, Stuewe and Sons, Tangent OR) with two seeds per pot and placed in the John Carroll University greenhouse under ambient light conditions. All pots were watered twice per week with 25 % modified Hoagland's solution to promote seedling growth. After 5 weeks, pots were thinned to one plant per pot, and plants were assigned to a randomized block design with eight blocks. Two watering treatments (control and drought) were applied. Control plants received water two to three times per week, or as necessary to maintain field capacity (approximately 20 % volumetric soil water content). Soils in the drought treatment were allowed to dry down to 7–8 % water content. Sufficient water was added to the pots to re-wet the soil to 10 % soil water content, and then allowed to dry again. Soil water content was measured using a soil moisture probe (Hydrosense, Campbell Scientific Inc., North Logan, Utah) three times per week. Plants were grown in the greenhouse from April to December 2011. To encourage natural senescence processes during the fall (October-December), greenhouse temperatures were allowed to cool to a minimum temperature of 4 °C. Daytime temperature during this period averaged ≈ 10 °C.

Physiological measurements and harvest

Physiological measurements were made during July 2011, 2 months after treatments were initiated. Predawn plant water potential was assessed using a Scholander pressure bomb following the procedures of Turner (1988) to minimize transpirational water loss. Photosynthetic assimilation and stomatal conductance were measured using a Li-Cor 6400 infrared gas

analyzer (Li-Cor Biosciences, Lincoln Nebraska). Three sub-sample measurements were made at 10 s intervals once conditions had equilibrated inside the chamber. Water use efficiency (WUE, $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) was calculated as photosynthetic assimilation (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) divided by stomatal conductance (g_s , $\text{mmol m}^{-2} \text{ s}^{-1}$). All physiological measurements (except predawn water potential, which was measured from 3:20 a.m. to 5:10 a.m.) were made from 9:45 a.m. to 12:15 p.m. during four sunny days with $790 \mu\text{mol m}^{-2} \text{ s}^{-1}$ average photosynthetically active radiation. Green leaves were collected for each sample for tissue elemental analysis at this time. Additionally, all leaves that senesced during the course of the experiment were collected, once the watering treatments were initiated. Leaves that senesced during the active growing season were collected separately from those that senesced at the end of the growing season to assess how phenology influences the resorption process.

Total aboveground and belowground biomass was determined by harvesting all tissues at the end of the experiment, separating live, green leaves, senesced leaves and roots. All biomass was oven-dried at 65°C and then weighed. Subsamples of green and senesced leaves were ground with a ball mill and then weighed for total N concentration by micro Dumas combustion on a CN analyzer (Costech Analytical, Valencia California). Leaves collected during the summer were used to estimate green leaf N concentration and to determine photosynthetic N use efficiency (PNUE, $\mu\text{mol CO}_2 \text{ mol N}^{-1} \text{ s}^{-1}$), which indicates the amount of carbon fixed per unit of leaf N. N resorption efficiency (N_{eff}) was determined as the proportion of nutrients resorbed from senesced leaves compared to green leaves (Lajtha and Klein 1988). N resorption proficiency (N_{prof}) was assessed as senesced leaf N concentration (sensu Killingbeck 1996). Leaves senescing during the course of the experiment were analyzed separately from those senescing in the last month of the experiment (December 2011), when plants were undergoing end-of-season senescence.

Statistical analysis

Means among congener pairs and drought treatments were compared using analysis of variance (ANOVA) for the following response variables: predawn leaf water potential; A ; g_s ; WUE; number of tillers; total

biomass; root mass ratio; summer green leaf N concentration; and PNUE. Main effects in our model were: taxonomic group (i.e., genus); habitat of origin (hereafter, "habitat"); watering treatment (control or drought); and block. Explicitly including taxonomic group into the model accounts for potential phylogenetic effects or constraints on traits (Harvey and Pagel 1991), rather than assuming that environmental effects are the sole drivers of trait responses. To determine whether resorption was influenced during drought and at the end of the growing season, we assessed N_{eff} and N_{prof} in summer and fall with repeated measures ANOVA (RM-ANOVA). The between-subject effects included the same main effects and interaction terms as for the univariate models. The within-subjects effects included the same main effects as for the univariate models, with the addition of time into the model; the interaction terms included those in the univariate models as well as their interaction with time. Since our goal was to understand how resorption patterns changed over time, only within-subjects effects are presented. Normality within the data was assessed using the Shapiro Wilks test. Levene's test was used to test for equal variance among treatment groups. If variances were not equal, the ANOVA models were weighted by the inverse of the variance of the factor or factors violating this assumption (Neter et al. 1990). All data were analyzed with SAS v9.2.

Results

Physiological measurements

Drought treated plants had 2.2-fold more negative predawn water potentials (ψ_w) compared to controls (Fig. 1, Table 1). There was a significant three-way interaction of treatment*genus*habitat (Fig. 1, Table 1). Regardless of their habitat of origin, both *Agropyron* species showed similar declines in predawn ψ_w under drought conditions. In contrast, *E. glaucus*, which typically grows in wetter soils, had a 1.6-fold less negative predawn ψ_w under control conditions but a 1.7-fold more negative predawn ψ_w under drought conditions than *E. elymoides*, which typically grows in drier soils. This trend was not observed in the third congener pair, in which both *Festuca* species had similar predawn ψ_w under control conditions, but *F. idahoensis*, which generally grows

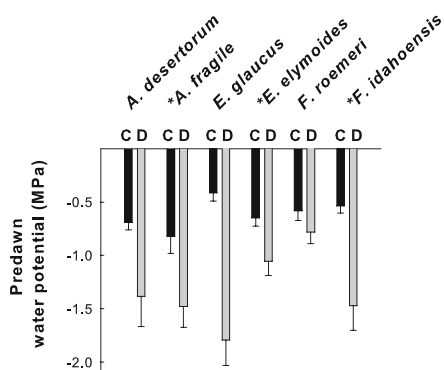


Fig. 1 Predawn water potential in response to soil water availability in grasses differing in their habitat of origin. Data are means + S.E. ($n=7-8$). Species from drier environments are indicated by an asterisk. Other abbreviations include: C control, D drought

in drier soils, had a more negative predawn ψ_w under drought conditions than *F. roemeri*, which generally grows in wetter soils. All other main effects and interaction terms were not significant (Table 1).

Photosynthetic rates (A) and stomatal conductance (g_s) were 1.3 and 2.5-fold higher for control relative to drought treated plants, respectively (Fig. 2a–b, Table 1); as a result, instantaneous water use efficiency (WUE) was 4.5-fold higher in drought-treated plants compared to controls (Fig. 2c, Table 1). There was also a difference between genera for A and g_s (Table 1). *Festuca* species had the highest and *Elymus* species had the lowest photosynthetic rates. Similarly, g_s was 1.4-fold higher for *Festuca* and *Agropyron* than for *Elymus*. There was also a significant difference among blocks for A and WUE (Table 1), but none of the other main effects or their interactions were significant (Table 1).

Growth and biomass allocation

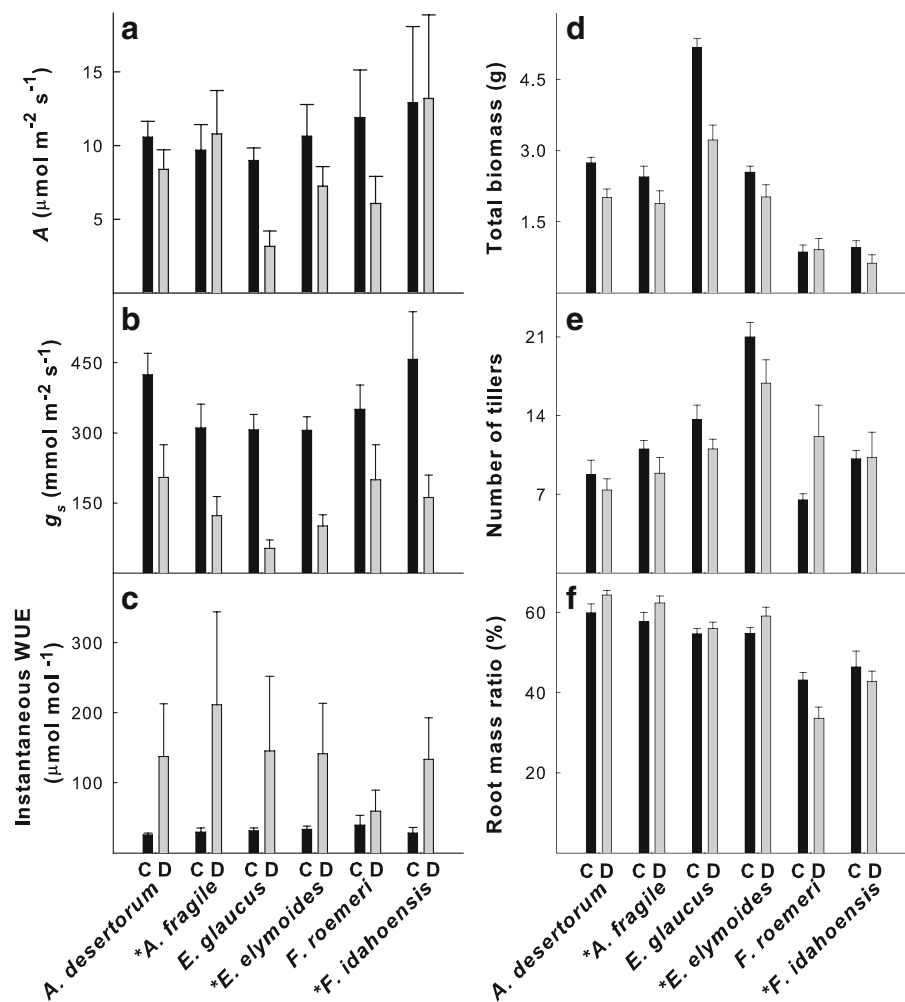
Compared to the physiological responses, growth and biomass allocation responses were more complex, with multiple, significant two and three-way interactions observed for tiller production, total biomass, and root mass ratio (Fig. 3a–c; Table 1). As a result, only the trends resulting from the highest interaction term(s) are described for each trait.

The only significant three-way interaction among treatment*genus*habitat was observed for total biomass (Table 1). In both the *Elymus* and *Agropyron* congener pairs, the species typically growing in wetter soils had a

Table 1 Results of univariate ANOVAs for response variables measured. Significant F -ratios with their associated degrees of freedom for the numerator and denominator ($d/n, d/d$) and P -values are reported in *bold*

Trait	Treatment $F_{d/n, d/d}, P$	Genus $F_{d/n, d/d}, P$	Habitat $F_{d/n, d/d}, P$	Block $F_{d/n, d/d}, P$	Genus* treatment $F_{d/n, d/d}, P$	Genus* habitat $F_{d/n, d/d}, P$	Habitat* treatment $F_{d/n, d/d}, P$	Genus* habitat* treatment $F_{d/n, d/d}, P$
Predawn Ψ_w (MPa)	54.3 , _{1,76} , <0.0001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	6.5 , _{2,76} , 0.002
A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	14.9 , _{1,71} , 0.0002	4.9 , _{2,71} , 0.01	n.s.	6.5 , _{7,71} , <0.0001	n.s.	n.s.	n.s.	n.s.
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	48.7 , _{1,71} , <0.0001	5.4 , _{2,71} , 0.006	n.s.	ns	n.s.	n.s.	n.s.	n.s.
WUE ($\mu\text{mol mol}^{-1}$)	11.6 , _{1,71} , 0.0001	n.s.	n.s.	6.7 , _{7,71} , <0.0001	n.s.	n.s.	n.s.	n.s.
Total biomass (g)	35.3 , _{1,77} , <0.0001	151.2 , _{2,77} , <0.0001	42.5 , _{1,77} , <0.0001	n.s.	7.7 , _{2,77} , 0.0009	27.0 , _{2,77} , <0.0001	n.s.	5.6 , _{2,77} , 0.005
Tiller number	n.s.	29.6 , _{2,77} , <0.0001	16.4 , _{1,77} , <0.0001	4.3 , _{5,77} , 0.0004	5.9 , _{2,77} , 0.004	5.3 , _{2,77} , 0.0007	n.s.	n.s.
Root mass ratio (%)	n.s.	90.7 , _{2,77} , <0.0001	n.s.	2.3 , _{7,77} , 0.03	7.9 , _{2,77} , 0.0008	3.8 , _{2,77} , 0.03	n.s.	n.s.
Green leaf N (g kg^{-1})	5.9 , _{1,74} , 0.02	29.4 , _{2,74} , <0.0001	n.s.	ns	6.1 , _{2,74} , 0.003	n.s.	n.s.	n.s.
PNUE ($\mu\text{mol mol}^{-1} \text{s}^{-1}$)	n.s.	n.s.	n.s.	15.4 , _{7,70} , <0.0001	6.1 , _{2,70} , 0.004	5.5 , _{1,70} , 0.02	n.s.	n.s.

Fig. 2 Gas exchange and growth and allocation traits, including photosynthetic rate (A) (panel a), stomatal conductance (g_s) (panel b) instantaneous water use efficiency (WUE) (panel c), total biomass (panel d), number of tillers (panel e), and root mass ratio (panel f) in response to soil water availability in grasses differing in their habitat of origin. Data are means \pm S.E. ($n=6-8$). Abbreviations follow those in Fig. 1



stronger and more negative biomass response to drought conditions than the species typically growing in drier soils. However, this trend was reversed in the *Festuca* congener pair, with *F. roemeri*, which typically grows in wetter soils, producing a similar amount of biomass under both control and drought conditions, while *F. idahoensis*, which generally grows in drier soils, produced 1.5-fold more biomass under control relative to drought conditions. Although the 3-way interaction term was not significant for either tiller number or root mass ratio (Table 1), there were significant genus*treatment and genus*habitat interactions observed for these traits. *Agropyron* and *Elymus* produced fewer tillers under drought compared to control conditions; additionally, both genera allocated slightly more biomass belowground when exposed to drought. In contrast, averaged across the two species, *Festuca* produced more tillers

and allocated less biomass belowground under drought than control conditions. Averaging across drought treatments, species from drier sites tended to produce more tillers than species from wetter habitats, but the number of tillers produced also was driven by genus, with the *Elymus* species tending to produce more tillers than the other two genera. Likewise, although the genus*habitat interaction was significant for root mass ratio, this pattern was strongly driven by the two *Festuca* species, which showed the largest difference in RMR based on habitat. Although a similar pattern was observed in the *Elymus* species (i.e., slightly higher RMR in the more drought tolerant *F. idahoensis*), *Agropyron* species showed similar allocation patterns, regardless of habitat of origin. The block effect was significant for both tiller number and root mass ratio (Table 1). All other main effects and interaction terms were not significant (Table 1).

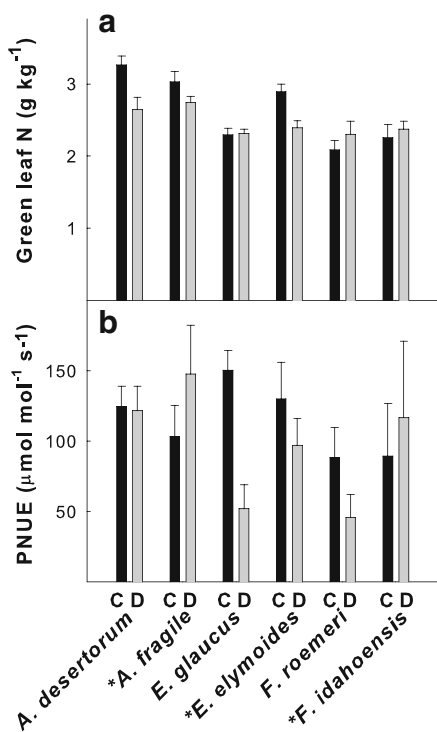


Fig. 3 Green leaf N concentration (panel a) and photosynthetic N use efficiency (PNUE) (panel b) in response to soil water availability in grasses differing in their habitat of origin. Data are means + S.E. ($n=6-8$). Abbreviations follow those in Fig. 1

Nutrient use, allocation, and conservation

Similar to the growth and biomass allocation traits, significant 2-way interactions were observed for green leaf N concentration and photosynthetic N use efficiency (PNUE) (Table 1). The genus*treatment interaction was significant for both traits (Fig. 3a–b, Table 1). For example, green leaf N concentration was similar among control and drought-treated *Festuca* plants but tended to be higher in control than drought-treated *Agropyron* plants. In contrast, PNUE was similar in control and drought treated *Agropyron* and *Festuca* plants but greater in *Elymus* control plants. The treatment*habitat interaction also was significant for photosynthetic N use efficiency (PNUE). Species from wetter sites had 1.6-fold higher PNUE under control relative to drought conditions, but species from drier sites had higher PNUE under drought conditions (Table 1). There were also significant differences among blocks for PNUE (Table 1). All other main effects and interaction terms were not significant (Table 1).

Repeated measures ANOVA revealed that both N_{eff} and N_{prof} was poorer in summer compared to fall (i.e., lower proportion of leaf N resorbed and higher senesced leaf N concentration in summer compared to fall; Table 2, Fig. 4a–d). These resorption responses through time also were influenced by treatment (Table 2). For both N_{eff} and N_{prof} , differences in resorption between control and drought-treated plants were small in summer but increased significantly during fall. The genera also varied in their resorption responses across the two seasons (Table 2), and the interaction of time*habitat was significant for N_{prof} (Table 2). However, the three way time*genus*habitat interaction also was significant for N_{eff} and N_{prof} (Table 2). In the fall, N_{eff} was similar among all genera, irrespective of habitat of origin. In contrast, the patterns varied among the three genera in summer based on habitat of origin. Both *Agropyron* species had similar N_{eff} in summer. However, *E. glaucus* (typically from wetter sites) had higher N_{eff} in summer than *E. elymoides* (typically from drier sites), while the pattern was reversed for the *Festuca* species, with *F. roemerii* (typically from wetter sites) having lower N_{eff} than *F. idahoensis* (typically from drier sites). In general, *E. elymoides* and *A. fragile* (both from drier sites) had poorer N_{prof} in summer and fall relative to the *E. glaucus* and *A. desertorum* (both from wetter sites). However, N_{prof} was similar for *Festuca* species at both time periods.

Discussion

In this study, we assessed the impacts of drought, evolutionary history, and habitat of origin on plant growth and physiology. We predicted that drought would decrease growth and physiological responses, but that evolutionary history and/or habitat of origin would constrain plant responses. As predicted, the experimental drought treatment had a significant, negative effect on physiology and growth responses in the six grasses included in our study, although these responses, in some cases, were also influenced by phylogenetic effects (i.e., significant effects of genus) and the plant's habitat of origin. Under drought conditions, instantaneous measurements such as predawn water potential, photosynthetic rate, and stomatal conductance showed strong declines. In contrast, seasonally integrated measurements, such as tiller production,

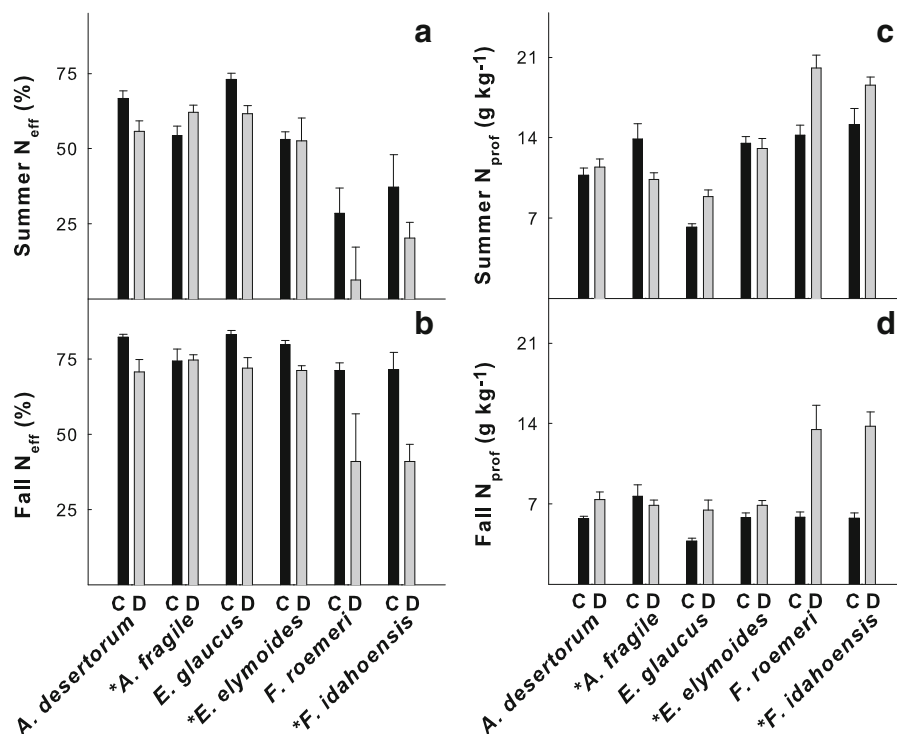
Table 2 Results of within-subjects repeated measures ANOVAs for N resorption efficiency (N_{eff}) and proficiency (N_{prof}). Significant F -ratios with their associated degrees of freedom for the numerator and denominator (dfn , $dfdb$) and P -values are reported in *bold*

Trait	Time	Time* treatment	Time* genus	Time* habitat	Time* block	Time* genus* treatment	Time* genus* habitat	Time* habitat* treatment	Time* genus* habitat* treatment
	$F_{dfn, dfdb}$, P	$F_{dfn, dfdb}$, P	$F_{dfn, dfdb}$, P	$F_{dfn, dfdb}$, P	$F_{dfn, dfdb}$, P	$F_{dfn, dfdb}$, P	$F_{dfn, dfdb}$, P	$F_{dfn, dfdb}$, P	$F_{dfn, dfdb}$, P
N_{eff} (%)	232.5 _{1,74} , < 0.0001	4.7 _{1,74} , 0.03	15.2 _{2,74} , < 0.0001	n.s.	n.s.	n.s.	5.6 _{2,74} , 0.005	n.s.	n.s.
N_{prof} (g kg ⁻¹)	347.8 _{1,74} , < 0.0001	9.6 _{1,74} , 0.003	8.6 _{2,74} , 0.0004	5.1 _{1,74} , 0.03	n.s.	n.s.	7.0 _{2,74} , 0.002	n.s.	n.s.

total biomass, and root mass ratio were more variable among species in their response to drought treatments, and the magnitude of the responses were often smaller. Similar declines in water potential (Guenni et al. 2004; Signarbieux and Feller 2012; Zhang et al. 2011), gas exchange (Casper et al. 2006; Mukherjee et al. 2011), and growth (Geber and Dawson 1997; Kimball et al. 2012; Yin et al. 2005) have been observed under both greenhouse and field conditions in a variety of functional groups.

Extending the well-described effects of drought on plant growth, in this study we also demonstrated that low soil water availability can directly impact whole plant mineral nutrition, although these responses also were modulated by phylogeny and/or habitat of origin. In the present study, green leaf N concentration tended to be higher in well watered *Agropyron* plants versus drought treated plants, although responses to treatment were minimal for *Festuca* plants. In a study of semi-arid grasslands of Inner Mongolia, China, plants from irrigated plots had higher leaf N concentration than plants from unwatered control plots (Lu and Han 2010). These differences may be related to higher plant transpiration rates and thus increased mass flow of N through the soil under well-watered conditions (Dunham and Nye 1973). In contrast, other authors have observed higher leaf N under drought conditions (Drenovsky et al. 2012; Huang et al. 2009; Wright et al. 2001). This variability may be related to how plant growth and N allocation are influenced by water availability. Increased water availability can lead to biomass dilution of plant nutrients due to increased growth rates under well watered conditions (e.g., Drenovsky et al. 2012), and higher N investment in leaf tissue can promote greater WUE under drought conditions (Wright et al. 2001). Additionally, although PNUE typically declines under drought conditions (Drenovsky et al. 2012; Yang et al. 2011), we observed no consistent differences in PNUE among control and drought plants. Instead, these responses were influenced by the interaction of genus*treatment and genus*drought tolerance effects. These data suggest that although phylogeny may constrain PNUE responses, environmental selection pressures also drive how this trait is impacted by drought. In our species, we observed that *Elymus* species responded more strongly to drought treatments, with respect to PNUE, than *Agropyron* and *Festuca* species. However, species from wetter sites tended to have higher PNUE under control relative to drought

Fig. 4 N resorption efficiency (N_{eff} , panels a–b) and N resorption proficiency (N_{prof} , panels c–d) in grasses differing in their habitat of origin in response to soil water availability. Data for both summer and fall resorption are presented. Data are means + S.E. ($n=7-8$). Abbreviations follow those in Fig. 1



conditions, supporting the typically observed trade-off between PNUE and WUE in response to drought (Wright et al. 2001). In contrast, plants from drier sites tended to have higher PNUE under drought conditions. In a recent study comparing drought tolerance among European beech populations, the most drought tolerant populations showed the smallest declines in PNUE in response to drought, and this trait was considered to be a key factor in drought tolerance in this study (Sanchez-Gomez et al. 2013).

Surprisingly, habitat of origin, in many cases, was not associated positively or negatively with plant responses to drought, although the cultivars used in this experiment are selected for use in large-scale restoration projects based on their expected drought tolerance, as well as site conditions. It is possible that more chronic or severe drought, which plants may experience under field conditions, pose a stronger environmental filter than was possible in our greenhouse study. Typically, more drought tolerant species are able to maintain or show smaller reductions in performance-related traits such as biomass and tiller production when faced with declining soil moisture availability (Couso and Fernandez 2012). In our study, most species produced less biomass under drought conditions, and these reductions were similar across a priori classifications of

drought tolerance or susceptibility, as predicted by habitat of origin. However, in two of the three genera studied, species from drier sites tended to produce more tillers, regardless of treatment. Although the ability to maintain shoot growth under drought conditions can be used as criterion for drought tolerance (Lu et al. 2012), slow growing species with decreased leaf area tend to be more stress tolerant (Ludlow 1989). Thus, drought tolerance may be conferred by various combinations of traits related to biomass and its allocation. One important difference among the congeners was the ability of the species from drier sites to maintain PNUE under drought conditions, which may be a key functional trait supporting drought tolerance (Sanchez-Gomez et al. 2013).

As hypothesized, phylogeny had a significant influence on physiology and growth responses, as evolutionary relationships can constrain plant responses to environmental variation. As a result, responses to drought varied across the three genera studied. Similar to our work, in a study comparing drought tolerance among eleven tree species, there was significant variation in functional trait responses to how drought tolerance was achieved among the five genera included in the study. *Quercus* species tended to alter total leaf area and biomass in response to drought, whereas *Pinus*,

Arbutus, and *Viburnum* tended to change biomass allocation patterns and photochemistry (Valladares and Sanchez-Gomez 2006). In our study, differences among genera were observed among traits related to gas exchange, growth, biomass allocation, and nutrient allocation and conservation. These phylogenetic effects may offer insight as to why nutrient resorption responses to drought are not consistent in the literature, as previous studies have not conducted their work within a phylogenetic framework. Future work should investigate correlations among these traits and drought tolerance from a phylogenetic perspective.

Phenology also played an important role in internal plant N recycling. Across treatments, poorer N_{eff} and N_{prof} were observed in summer compared to fall, particularly among the *Festuca* species. Additionally, drought resulted in poorer N_{eff} and N_{prof} , with the magnitude of difference between control and drought plants increasing by the end of the growing season. These data indicate three critical aspects of the resorption process. First, low soil water availability limits plant nutrient resorption. During the senescence process, mobile nutrients are retranslocated to storage tissues via the phloem, and water availability influences this process (Ruehr et al. 2009). Although not as well studied as the influence of soil nutrient availability, other authors have observed decreased resorption in response to drought (Huang et al. 2009; Marchin et al. 2010; Wright and Westoby 2003 but see Sanz-Perez et al. 2009). Second, the impacts of drought on resorption may depend on seasonal timing and duration of the drought. We observed a stronger negative impact of drought on resorption later in the growing season, when plants were also undergoing natural senescence processes, than during the summer growing season, suggesting that the impacts of drought on plant nutrient budgets may be amplified over time. Future research should assess the relative impacts of short-term versus long-term drought effects, as well as the impact of drought severity on the resorption process. At a global scale, N resorption is more efficient and proficient with decreases in mean annual precipitation (Yuan and Chen 2009a, b). However, in our study, we observed poorer resorption in plants experiencing drought, relative to control plants. Thus, patterns predicted at the global scale were not observed in our suite of co-occurring species. At smaller spatial extents, variability in local environmental pressures, such as interannual precipitation differences and

variation in soil type (which influences soil moisture availability) may obscure predicted patterns in plant resorption responses to drought (Drenovsky et al. 2010). Third, phenological differences in resorption are likely linked to the relative importance of growth versus storage at the time of leaf drop. Although not measured in our study, during end-of-season whole plant senescence, other critical physiological processes are occurring, such as storage of non-structural carbohydrates in root tissues. Sink strength is considered to be one of the most critical controls over the resorption process, as it influences phloem transport rates (Chapin and Moilanen 1991). Thus, greater N resorption during fall may be associated with other, concurrent storage processes.

Survival patterns in arid systems are heavily driven by plant size, with larger individuals typically having greater survival rates (Toft and Fraizer 2003). Restoration research programs have placed major emphasis on screening and breeding varieties for size and vigor under drought conditions with the overall aim of producing plant materials more capable of establishing and restoring some aspects of ecosystem function in heavily degraded, often novel, conditions (Toft and Fraizer 2003). Here, we show that beyond the direct effects of drought on growth and plant N supply, drought also can have a negative impact on internal N cycling processes. While the impacts of drought on internal N cycling processes did not necessarily correspond with expected differences based on habitat of origin, in five of our six study species drought inhibited the ability of plants to remobilize N from senescing leaves, as assessed by both N_{eff} and N_{prof} . In some of our species, drought prevented 30 to 50 % of the leaf N that could have been remobilized from senescing leaves and stored internally, as compared to control plants. The large variation in response among the genera and the large variation in this trait among study species suggest an important role of evolutionary history in these responses as well as a strong potential for selection on this important trait. Thus, while selection and development of varieties with increased drought tolerance will remain central in arid land restoration programs, our study provides an argument for also simultaneously assessing N cycling traits and trait variation of improved varieties.

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