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Evolutionary responses of invasive grass species to variation in precipitation and soil nitrogen

Monica A. Nguyen[1]*, Amy E. Ortega[1], Kurt Q. Nguyen[1], Sarah Kimball[2], Michael L. Goulden[3] and Jennifer L. Funk[1]

[1]Schmid College of Science and Technology, Chapman University, Orange, CA, USA; [2]Center for Environmental Biology, University of California, Irvine, CA, USA; and [3]Department of Earth System Science, University of California, Irvine, CA, USA

Summary

1. Global climate models suggest that many ecosystems will experience reduced precipitation over the next century and the consequences for invasive plant performance are largely unknown. Annual invasive species may be able to quickly evolve traits associated with drought escape or tolerance through rapid genetic changes.

2. We investigated the influence of 5 years of water and nitrogen manipulations on trait values in a southern California grassland system. Seeds from two annual grass species (*Avena barbata* and *Bromus madritensis*) were collected from experimental plots and grown in a common environment over two generations. We measured 14 physiological, morphological, phenological and reproductive traits.

3. Both species displayed phenotypic differences depending on the water treatment from which they were collected, but not depending on the nitrogen treatment. Both species displayed trait values characteristic of drought escape (e.g. earlier flowering in *A. barbata* and *B. madritensis*, lower water-use efficiency in *B. madritensis*) when grown from the seeds collected from plots that experienced five years of reduced precipitation. Furthermore, *A. barbata* individuals grown from the seeds collected from drought plots had higher reproductive output and higher photosynthetic performance than individuals grown from water addition plots, with individuals grown from ambient plots displaying intermediate trait values. Notably, we found no phenotypic variation among treatments for six root traits.

4. *Synthesis*. Trait differences were observed following two generations in a common garden, suggesting that treatment differences were genetically based. This suggests that populations were responding to selection over the 5 years of water manipulations, a remarkably short time period. The rapid evolutionary responses observed here may help these two widespread invasive grass species thrive under reduced precipitation scenarios, which could have important implications for fire dynamics, invasive species management and native plant restoration in communities invaded by annual grasses.

Key-words: annual species, *Avena barbata*, *Bromus madritensis*, drought escape invasion ecology, invasive species, phenology, photosynthesis, root traits, water-use efficiency

Introduction

Global change, including increases in temperature, atmospheric CO_2 concentration and nitrogen (N) input from anthropogenic activities, is one of the most significant threats to natural ecosystems. Further, global climate models have predicted the alterations in inter- and intra-annual precipitation in many regions (Melillo, Richmond & Yohe 2014). Biological invasion is another important agent of global change with significant effects on global biodiversity (Millennium Ecosystem Assessment, 2005). A meta-analysis of 1041 cases of invasion found that alien invasive plants decrease the abundance and diversity of native resident plant species by 43.5% and 50.7%, respectively (Vilà *et al.* 2010). Significant impacts on native resident species' abundance and diversity are more likely to occur if the invading species is an annual grass (Pyšek *et al.* 2012). Refining our understanding of how invasive plants respond to environmental variation will

*Correspondence author: E-mail: nguyen.an.monica@gmail.com

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improve our ability to predict their effects on natural plant communities in the face of global change. In this study, we explore the adaptive responses of two annual invasive grass species to declines in precipitation and increased N deposition in a southern California grassland.

There are two major strategies a plant can employ to cope with reduced water availability: drought tolerance and drought escape. Drought tolerance, characterized by low photosynthetic activity, high water-use efficiency (WUE, the rate of carbon assimilation relative to transpiration) and slow growth, allows an individual to conserve resources while continuing to be active during the drought period (Sherrard & Maherali 2006; Franks 2011). Conversely, drought escape involves the completion of an individual's life cycle before the drought reaches its most extreme state. Drought escape is characterized by high photosynthetic rates, low WUE and early flowering (Sherrard & Maherali 2006; Franks 2011). An increase in N allocation to photosynthetic enzymes and chlorophyll content in leaves may act to increase light harvesting (Terashima & Evans 1988; Evans 1989) and, consequently, carbon fixation over a shorter period of time. Thus, increased N availability resulting from human activities may enhance the growth in species displaying a drought escape strategy.

Will invasive species benefit from projected arid, N-rich conditions? High soil N concentrations can increase invasive species performance (Huenneke *et al.* 1990; Davis, Grime & Thompson 2000; Ostertag & Verville 2002; Gross, Mittelbach & Reynolds 2005); however, the effects of N addition are complex and will depend on soil water availability, competition and the form of available N (Everard *et al.* 2010; Ross, Ehrenfeld & Patel 2011; Eskelinen & Harrison 2014). Furthermore, an analysis of several U.S. data bases suggests that invasive species tend to initiate leaves and flower earlier in the growing season compared to native species (Wolkovich & Cleland 2011). This phenological pattern, consistent with drought escape, suggests that many annual invasive species may be well suited to increasingly arid conditions.

Many studies have shown that invasive species can be phenotypically plastic in response to changes in water, light and nutrient availability (e.g. Funk 2008; Davidson, Jennions & Nicotra 2011); however, fewer studies have examined the potential for invasive species to adapt to environmental variation through genetic changes. In a study comparing flowering times of annual *Brassica rapa* before and after a four-year drought in southern California, Franks, Sim & Weiss (2007) found that seeds collected from wet and dry environments post-drought flowered significantly earlier than those collected before the drought, which is characteristic of a drought escape response. Further, Franks (2011) suggested that populations of *B. rapa* escape drought through reduced WUE, which allows for rapid development and earlier flowering. A study of the annual invasive grass *Avena barbata* grown in wet and dry environments also found strong evidence that earlier flowering was adaptive under drought (Sherrard & Maherali 2006).

Despite evidence of rapid evolution in the flowering times of *B. rapa* and *A. barbata*, adaptive responses to climate change may be slower or lacking in other traits and for other species (Franks, Sim & Weiss 2007). Anatomical and physiological characteristics of roots will influence both water and N acquisition, yet few studies have examined root adaptation along a precipitation gradient. Studies using congeneric and conspecific pairs occurring in high and low rainfall sites suggest that low specific root length (SRL, m g^{-1}), large root diameter or slow root elongation rates may be favoured under drier conditions, reflecting an investment in thick, longer-lived roots that more efficiently transport water (Wright & Westoby 1999; Nicotra, Babicka & Westoby 2002; Heschel *et al.* 2004).

We searched for evolutionary responses to N and water manipulations in two invasive grass species (*A. barbata* and *Bromus madritensis*) that are widespread throughout much of the west coast of North America. We subjected populations of both species to altered precipitation and N availability for 5 years, collected seeds from multiple maternal plants and grew them in a common environment over two generations. We measured a suite of above- and below-ground traits that are associated with water and N use to address this question: 'Did invasive species exhibit trait differences in response to the 5-year environmental manipulations that persisted after two generations in a common environment?' If so, our results would suggest that the trait differences were genetic and, thus, the result of rapid evolution in response to environmental change. Answering this question is a critical first step in understanding how invasive species may respond to future climate change.

Materials and methods

STUDY SITE AND SEED SOURCE

The study site was located in a grassland community within the Irvine Ranch Conservancy in Orange County, California (Potts *et al.* 2012; Allison *et al.* 2013; Kimball *et al.* 2014). Orange County has a Mediterranean climate characterized by hot, dry summers and cool, wet winters. We focused on two invasive grass species, *A. barbata* and *B. madritensis*, which were abundant at our site. These species are native to the Mediterranean Basin and, due to repeated introductions, have widespread global distributions. In California, where our study was conducted, *A. barbata* and *B. madritensis* have overlapping but slightly different geographic distributions, with *B. madritensis* occurring in more arid environments including the Mojave Desert (DeFalco *et al.* 2003; Steers, Funk & Allen 2011).

Twenty-four experimental plots (6.7 m × 9.3 m) extending over an area roughly one acre in size were imposed on existing vegetation, with each plot randomly exposed to one of three precipitation treatments (ambient, water addition or water reduction, $n = 8$ per treatment, Fig. 1). Each plot was divided lengthwise with each half receiving one of two N treatments (ambient or N addition). The water reduction treatment received approximately 51% less water than the ambient water treatment, while the water addition treatment received approximately 33% more over the five-year manipulation period (March 2007–May 2012) (Kimball *et al.* 2014). Clear, retractable roofs were deployed during a subset of winter storms to control water input into the reduction plots. Rainfall collected from reduction plots was stored in opaque polyethylene tanks and used to supplement

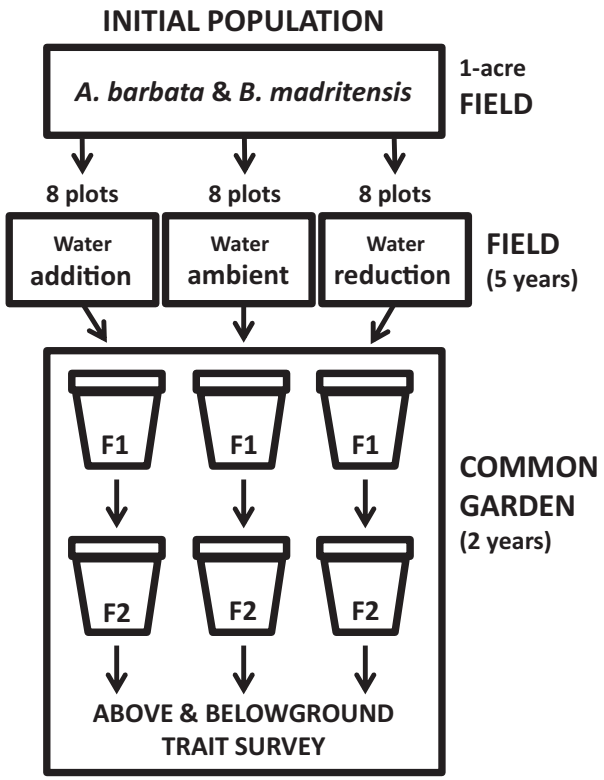


Fig. 1. Following 5 years of water manipulations in the field (Irvine, CA), seeds were collected from experimental plots and grown in a common environment over two generations. Physiological, morphological and phenological traits were measured on the second generation grown in a common environment.

water addition plots through a system of gasoline-powered pumps connected to drip tubing (Kimball *et al.* 2014). Baseline N deposition at our study site is approximately 1.5 g m^{-2} $year^{-1}$ (Kimball *et al.* 2014). Nitrogen addition plots received 6 g N m^{-2} $year^{-1}$, a value chosen to simulate a site approaching N saturation after years of continuous deposition (Kimball *et al.* 2014). Supplemental N was given in two applications: 2 g of quick-release calcium nitrate following the first storm of the season and 4 g of slow-release calcium nitrate following the first month of the growing season (Kimball *et al.* 2014).

In May 2012, the seeds were collected from randomly selected maternal plants of *A. barbata* and *B. madritensis* and transported to Chapman University, which is located 16 km from the field site and has similar weather conditions. To ensure the representation of the entire population, and minimize the effect of genetic drift, we tried to sample individuals from all plots ($n = 8$) per watering treatment. However, some plots did not contain individuals of both species. When a species was present in a given plot, we collected the seeds from one to eight maternal plants in that plot. Only seeds mature enough to be removed with a gentle pull of the spikelet were collected. Separate envelopes were used to ensure that offspring from each mother plant remained isolated. On 3 December 2012, we germinated the seeds on moist filter paper at 4 °C for 96 h. After germination, only one seedling from each mother plant was used; thus, each replicate had a different mother. Because later analyses showed that there was no significant effect of N on any of the traits measured, individuals from the two N treatments were grouped within each water treatment. We had at least nine replicates per water treatment for *B. madritensis* and at least 20 replicates per water treatment for *A. barbata* (Table 1). Following germination, seedlings were transferred to 4.1-L pots filled with moistened potting soil (Sunshine Mix #1, Sun Gro Horticulture). Plants were grown in full sun at Chapman University and watered daily to saturation.

Table 1. The number of individuals of *Avena barbata* and *Bromus madritensis* sampled for each water treatment. The number of different plots that maternal plants originated from is given in parentheses. Because there was no significant effect of N on any trait, individuals from the two N treatments were grouped within their respective water treatments

	A. barbata	*B. madritensis*
Reduction	26 (7)	18 (8)
Ambient	21 (8)	15 (5)
Addition	20 (8)	9 (3)

Because adaptation cannot be assumed based on the phenotypes displayed in one generation (Turner, Hufbauer & Rieseberg 2014), common garden experiments must occur over multiple generations in order to differentiate between genetic and epigenetic effects, including maternal effects (Moran & Alexander 2014). Maternal effects refer to a situation in which an individual's phenotype is determined not only by its genotype and current environment, but also by the environment experienced by its mother (Lacey 1998). We controlled for maternal effects by growing seeds collected from the field (F1) and their offspring (F2) in a common environment (see Fig. 1). Any maternal effects present in the F1 generation should have been eradicated in the F2 generation because F1 plants were grown under identical environmental conditions. Thus, treatment differences observed in the F2 generation should be the result of genetic differences rather than maternal effects. We collected the seeds from each F1 mother plant and germinated them as described above so that each F2 replicate had a different mother. Because of some mortality during germination and establishment, we had slightly fewer replicates in the F2 generation ($n = 67$ *A. barbata*, $n = 42$ *B. madritensis*) than in the F1 generation ($n = 72$ *A. barbata*, $n = 43$ *B. madritensis*) across treatments.

ABOVE-GROUND MEASUREMENTS

Collection of physiological measurements began 70 days after germination of the F2 generation (February 2014). We conducted gas-exchange measures with a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). We maintained constant chamber conditions that approximated growing conditions, including CO_2 at 400 μL L^{-1}, light at 1800 μmol photon m^{-2} s^{-1}, chamber temperature at 25 °C and relative humidity at 52–62%. Measures included photosynthetic rate, stomatal conductance, transpiration rate and instantaneous WUE (photosynthetic rate/transpiration rate). When leaves were too small to fill the chamber, the leaf area was determined and used to correct gas-exchange data.

We measured leaf chlorophyll content with a portable chlorophyll SPAD-502 meter (Spectrum Technologies, Plainfield, IL, USA). Three leaves were harvested following gas-exchange and chlorophyll measures to determine leaf dry mass per unit area (LMA) and leaf N concentration. The leaves were scanned to determine total leaf area, dried at 60 °C for 72 h and weighed. The dried leaves were then ground in a Wiley mill with a 40-mesh screen, and leaf N was determined with an elemental analyser (Costech 4010, Pioltello, Italy).

We assessed the plant size by measuring canopy cover prior to flowering, estimated as the product of plant width along two

perpendicular axes. We checked plants daily and recorded date of flowering, defined as the first appearance of florets. While both species largely reproduce by selfing in natural populations (Johansen-Morris & Latta 2006; Grossman & Rice 2014), outcrossing does occur; thus, plants from different treatments were grown in the same area but with physical barriers between them to avoid cross-fertilization. Though spatially separated, all treatments received similar light and water levels daily. From March to June 2014, mature seeds were collected, air-dried and weighed to determine total seed biomass as a metric of reproductive fitness.

ROOT MEASUREMENTS

To obtain root measures for the F2 generation, seeds from a subset of F1 plants from the above-ground trait survey were germinated using the procedure described above and transferred to 4.1-L pots filled with a moistened sand–perlite–vermiculite (1:1:1) mixture. Plants were randomly selected within plots and, as with above-ground measures, we sampled from all plots ($n = 8$) per watering treatment where possible. We had eight replicates per watering treatment for each species. Seedlings were grown for 5 weeks, receiving a total of 0.05 g N, 0.05 g P and 0.05 g K (Miracle-Gro) over the first 2 weeks. The plants were watered daily to saturation except on days when pots received fertilizer.

After 5 weeks, the plants were harvested and the roots were washed and scanned using the WinRHIZO image analysis system (Regent Instruments Inc., Quebec, QC, Canada) to determine total root length. Root growth rate (cm per day), root length density (root length per volume of soil) and fine root ratio [the ratio of fine root length (diameter <0.5 mm) to total root length] were calculated. We separated below- and above-ground biomass, dried them at 60 °C for 72 h and weighed them to determine root-to-shoot biomass ratio (R:S) and specific root length (SRL, m g^{-1}). Biomass was combined, ground and analysed for N concentration as described above. Plant N uptake rate (g N per day) was calculated as [(plant biomass × plant N)/growing day number].

STATISTICAL ANALYSES

Box–Cox transformations of the data were used where necessary to meet the assumptions of normality for statistical analyses. We performed analysis of variance (ANOVA) with water and N treatment as fixed effects and plot as a random effect to compare trait values between treatments within each species. The significance of fixed effects was assessed using Satterthwaite approximated degrees of freedom. We used type III sums of squares ANOVA for a mixed-effects model in the LME4 package in R (http://www.r-project.org, v.3.2.0). Following Moran (2003), all *P*-values are reported and sequential Bonferroni corrections for multiple statistical tests were not conducted. *Post hoc* analyses were performed using Tukey's honestly significant difference.

Results

There was no significant effect of N on any trait; thus, data from the two N treatments were grouped within a water treatment and N as a main effect was excluded from all subsequent analyses. Water availability affected several above-ground traits in *A. barbata* as well as first flowering date and water-use efficiency in *B. madritensis* (Table 2). With respect to physiological traits, photosynthetic rate and leaf chlorophyll content in *A. barbata* differed across water treatments with a trend towards higher trait values in the water reduction treatment relative to the ambient and added precipitation treatments (Table 2, Fig. 2). Photosynthetic rate and chlorophyll content did not differ across treatments in *B. madritensis* (Table 2, Fig. 3). Water-use efficiency was lower in plants from the reduced precipitation plots in *B. madritensis* compared to the ambient and water addition treatments (Table 2, Fig. 3). There was no difference in WUE across the treatments in *A. barbata* (Fig. 2). The pattern observed in *B. madritensis* was driven by higher transpiration rates in plants from reduced precipitation plots (data not shown). Water had no significant effect on leaf N content in either species (Table 2, Fig. 2).

Table 2. *F*-values calculated from one-way anova for second-generation (F2) plants of two invasive grass species with water treatment as a fixed factor and plot as a random factor. Numerator and denominator degrees of freedom for *F*-values are presented in parentheses. Significant effects ($P < 0.05$) are in boldface type

	Avena barbata		*Bromus madritensis*	
	F	*P*	*F*	*P*
Above-ground traits				
Photosynthetic rate	3.82 (2,18)	**0.041**	0.44 (2,40)	0.645
Leaf chlorophyll	5.09 (2,67)	**0.009**	2.64 (2,41)	0.084
WUE	0.18 (2,24)	0.835	5.46 (2,40)	**0.008**
Leaf N content	2.62 (2,64)	0.081	1.55 (2,41)	0.224
LMA	0.23 (2,66)	0.793	1.05 (2,42)	0.360
Canopy cover	3.32 (2,67)	**0.042**	1.50 (2,42)	0.235
Flowering date	8.04 (2,67)	**<0.001**	11.36 (2,41)	**<0.001**
Seed biomass	8.92 (2,67)	**<0.001**	2.83 (2,42)	0.070
Below-ground traits				
Root growth rate	0.49 (2,24)	0.617	0.55 (2,42)	0.586
R:S	0.30 (2,24)	0.747	1.13 (2,24)	0.339
Root length density	0.47 (2,24)	0.632	0.66 (2,24)	0.527
Fine root ratio	0.93 (2,12)	0.423	0.57 (2,24)	0.575
Specific root length	0.57 (2,17)	0.576	0.29 (2,24)	0.748
N uptake rate	0.62 (2,24)	0.545	0.55 (2,24)	0.583

WUE, Water-use efficiency; LMA, leaf mass per area; R:S, root-to-shoot biomass ratio.

With respect to growth and reproductive traits, flowering date was earlier in the water reduction and ambient water treatments relative to the water addition treatment in both *A. barbata* and *B. madritensis* (Figs 2 and 3; Table 2). Plants from the water reduction plots in *A. barbata* also produced greater seed biomass relative to the addition plots, with plants from the ambient plots displaying intermediate seed biomass (Fig. 2). There was a significant effect of water on canopy cover in *A. barbata* with higher canopy cover in water reduction plots relative to water addition plots (Table 2, Fig. 2). However, there was no difference in seed biomass or canopy cover across watering treatments in *B. madritensis* (Fig. 3). LMA did not differ among treatments for either species (Figs 2 and 3).

There was no significant effect of water on root traits of either species in the F2 generation (Table 2, Fig. S1 in Supporting Information).

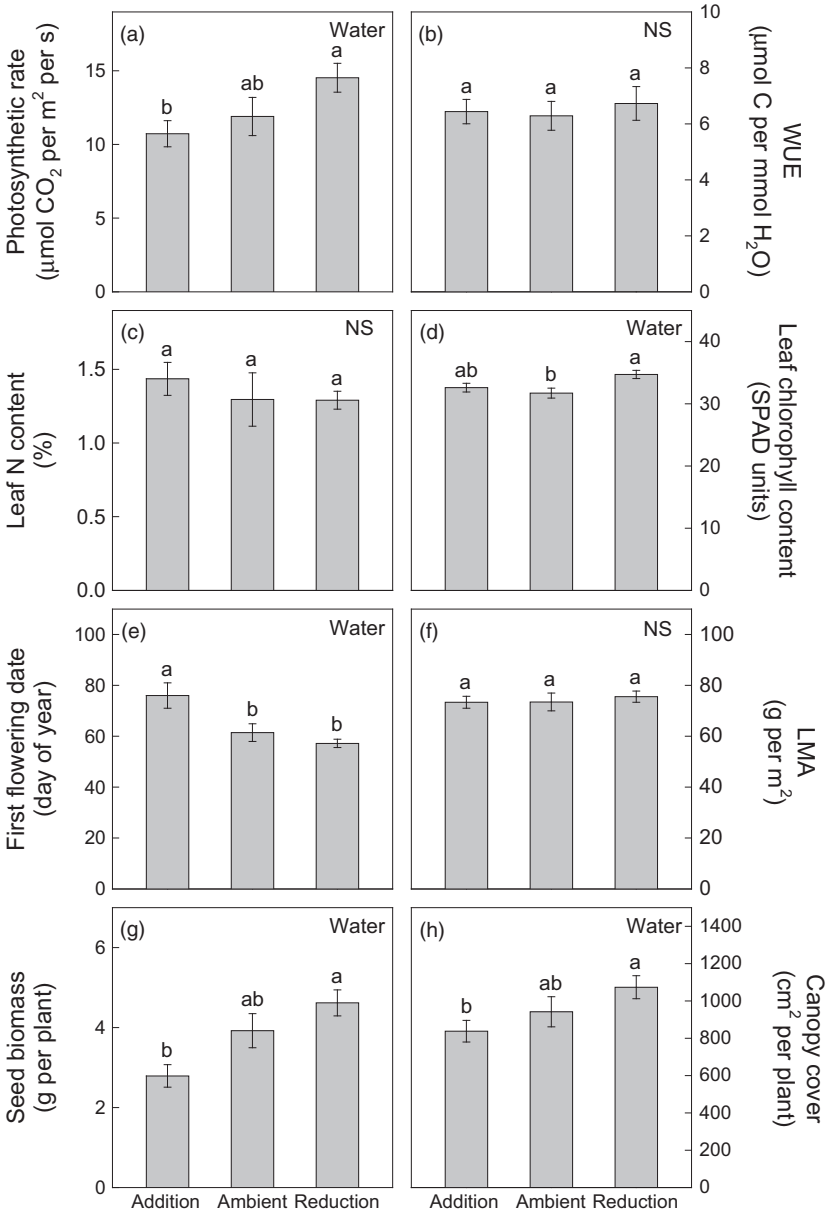


Fig. 2. Photosynthetic rate (a), water-use efficiency (b), leaf nitrogen content (c), leaf chlorophyll content (d), first flowering date (e), leaf mass per area (f), seed biomass (g) and canopy cover (h) for *Avena barbata* from three watering treatments grown in a common environment (F2 generation). Means and standard errors of water addition, ambient and reduction treatments are shown. Different letters denote significant differences among means as determined through *post hoc* Tukey's HSD tests. Where applicable, significant effects of water are noted.

Discussion

Our results suggest that invasive species may exhibit a rapid evolutionary change following 5 years of global change manipulations. Trait differences occurred in response to altered water availability, but not in response to the changes in available N. Earlier flowering (*A. barbata* and *B. madritensis*) and lower WUE (*B. madritensis*) in the reduced water treatment are in line with a drought escape response as found in previous work (Sherrard & Maherali 2006; Franks, Sim & Weiss 2007; Franks 2011). *Avena barbata* from water reduction plots also had significantly higher total reproductive biomass than individuals from water addition plots, and photosynthetic rate and leaf chlorophyll content showed trends towards higher values in water reduction plots than in ambient water plots, suggesting that these physiological responses may be advantageous in drought-prone regions. High leaf chlorophyll content may allow for increased light harvesting over shorter periods of time, as evidenced by higher photosynthetic rates, and thus enable an increased reproductive output. Higher reproductive output may also have resulted from an earlier flowering time or a shift in allocation from vegetative to reproductive structures (Wolfe & Tonsor 2014) and could increase the number of successful offspring in arid environments, where establishment is difficult.

Notably, we found that field manipulations did not alter root traits, which is largely unstudied outside of model organisms (reviewed in Comas *et al.* 2013). Previous studies of root adaptation in natural systems have focused on conspecific or congeneric comparisons of species from different rainfall environments, which reflect evolution over potentially long time-scales (Wright & Westoby 1999; Nicotra, Babicka & Westoby 2002; Heschel *et al.* 2004). It is possible that our

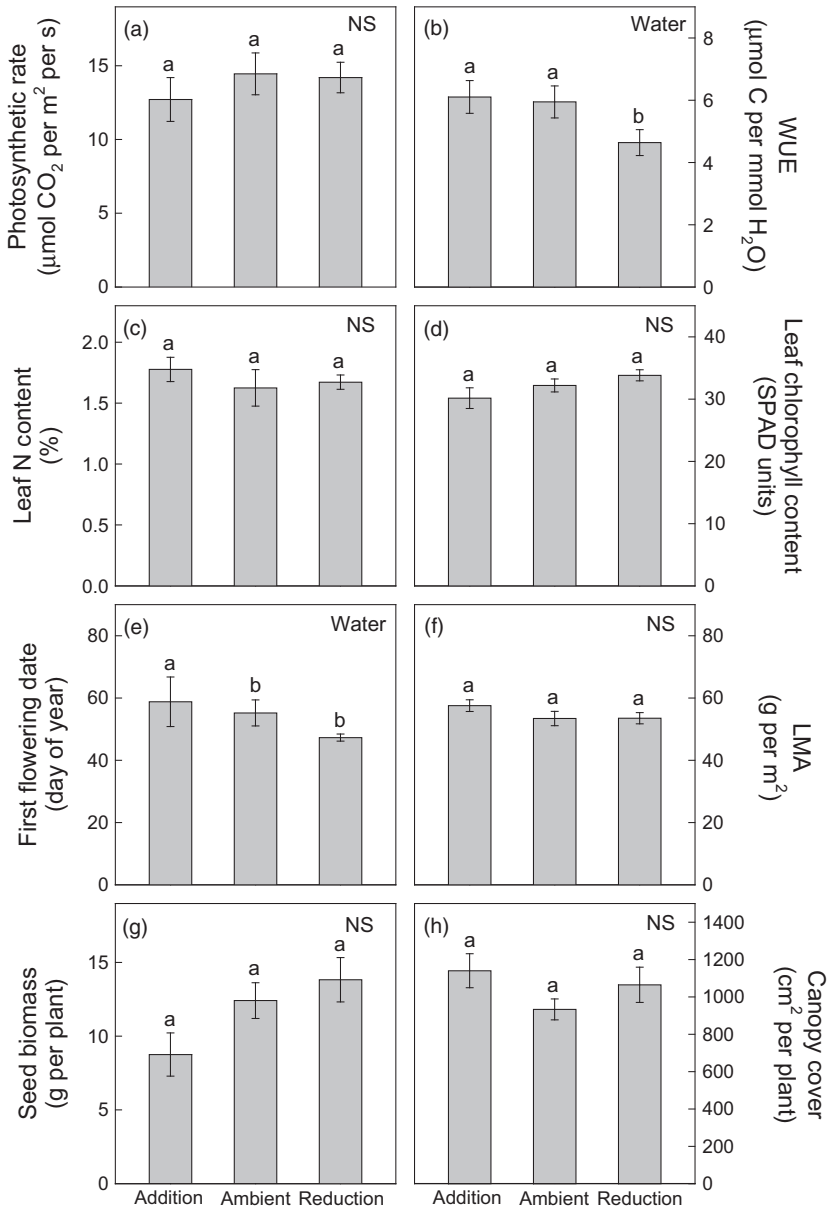


Fig. 3. Photosynthetic rate (a), water-use efficiency (b), leaf nitrogen content (c), leaf chlorophyll content (d), first flowering date (e), leaf mass per area (f), seed biomass (g) and canopy cover (h) for *Bromus madritensis* from three watering treatments grown in a common environment (F2 generation). Means and standard errors of water addition, ambient and reduction treatments are shown. Different letters denote significant differences among means as determined through *post hoc* Tukey's HSD tests. Where applicable, significant effects of water are noted.

five-year experiment was too short to observe the differences in root traits. In the absence of rapid genetic responses, plasticity in root traits may be an important mechanism for responding to environmental changes on short time-scales. In response to the reductions in water availability, individual plants can increase root biomass, root elongation rate and SRL to maximize water acquisition (e.g. Williams & Black 1994; Padilla *et al.* 2009; Drenovsky, Khasanova & James 2012; Larson & Funk 2016). Several of these responses (rapid elongation, high SRL) are contrary to strategies found in species adapted to low rainfall environments (Wright & Westoby 1999; Nicotra, Babicka & Westoby 2002). More data are needed to understand the relative role of plasticity and genetic adaptation in below-ground trait responses to the variation in water availability.

In contrast to water manipulation, nitrogen addition over a five-year period did not result in detectable trait differences. Previous work from these field plots suggested that N addition only increased grassland productivity when combined with water addition, and only in some years (M. L. Goulden, unpubl. data). It is possible that the proximity of the subplots (ambient and N addition within a single plot) may explain why N addition failed to alter traits in either species. Cross-pollination or seed dispersal may have contributed to gene flow among subplots. While many studies have found that exotic annual grasses respond positively to N addition (e.g. Huenneke *et al.* 1990; Davis, Grime & Thompson 2000; Gross, Mittelbach & Reynolds 2005), growth rates may saturate or decline at high soil N (e.g. Padgett & Allen 1999; Mattingly & Reynolds 2014). Our site is relatively fertile (0.19% N and 628.3 mg P kg^{-1}; J. L. Funk, unpubl. data), likely resulting from historic cattle grazing and N deposition in the Orange County area. Thus, N manipulations may yield different results if conducted in more N-limited environments.

One novel implication of our study is that it may be possible for invasive plant species to adapt to environmental change over just 5 years or five generations. Although there are examples of rapid evolution in other weed species (Franks, Sim & Weiss 2007; Frenck *et al.* 2013; Sultan *et al.* 2013; Grossman & Rice 2014), 5 years is a remarkably short time period based on data from other studies. In a review of evolutionary responses of invasive species to novel environmental conditions, Moran & Alexander (2014) suggested that significant evolutionary changes in relevant traits are not likely to be observed in <25 generations. For example, Turner, Hufbauer & Rieseberg (2014) confirmed that rapid evolution in growth and reproductive output has occurred in populations of *Centaurea diffusa* in the invaded range, which were separated from native populations by 100 years, or approximately 50 generations. Similarly, Dlugosh & Parker (2008) found evidence for rapid evolution of growth rate, date of first flowering and size of *Hypericum canariense* in what they predicted to be 25 generations following introduction. Our results suggest that populations of annual invasive grasses can show significant changes in response to environmental conditions in as little as five generations.

Higher rates of carbon assimilation, earlier phenology and higher reproductive output in weedy species under conditions of reduced precipitation could have important implications at the community level. In Mediterranean climate ecosystems, such as our southern California site, rainfall occurs almost exclusively during the winter months (November to May) and drought often brings reduced rainfall and/or a shorter rainfall period. Invasive species that are able to complete their life cycle during a shorter wet season while maintaining or, in our case, enhancing reproductive output (*A. barbata*) will have an advantage over species that have longer life cycles and are adversely affected by drought. Although *A. barbata* may be more evolutionarily responsive to water manipulation than *B. madritensis* (displayed more phenotypic responses), the differences in absolute trait values suggest that *B. madritensis* was better at executing a drought escape strategy than *A. barbata* (lower LMA and WUE, higher total reproductive biomass and leaf N content compared to *A. barbata*; Figs 2 and 3). Consequently, *B. madritensis* was one of the most common species in reduced precipitation plots after 5 years (Goulden *et al.*, unpubl. data). While we did not measure trait values in co-occurring native species, the plot-level community composition data suggest that native grassland species may have a limited potential to respond to drought on short time-scales. Future research should test for adaptive evolution in co-occurring native and invasive species to assess the potential impact that rapid evolution may have on community-level processes and ecosystem function.

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Data accessibility

F2 generation root traits figure: see Figure S1, Supporting Information.

F2 above- and below-ground trait data: Zenodo doi: 10.5281/zenodo.46049 (Funk 2016).

References

Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K. & Martiny, J.B.H. (2013) Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology*, **94**, 714–725.

Comas, L.H., Becker, S.R., Cruz, V.M.V., Byrne, P.F. & Dierig, D.A. (2013) Root traits contributing to plant productivity under drought. *Frontiers in Plant Science*, **4**, 442.

Davidson, A.M., Jennions, M. & Nicotra, A.B. (2011) Do invasive species show higher phenotypic plasticity than native species and if so, is it adaptive? A meta-analysis. *Ecology Letters*, **14**, 419–431.

Davis, M.A., Grime, J.P. & Thompson, K. (2000) Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*, **88**, 528–534.

DeFalco, L.A., Bryla, D.R., Smith-Longozo, V. & Nowak, R.S. (2003) Are Mojave Desert annual species equal? Resource acquisition and allocation for the invasive grass *Bromus madritensis* subsp. *rubens* (Poaceae) and two native species. *American Journal of Botany*, **90**, 1045–1053.

Dlugosh, K.M. & Parker, I.M. (2008) Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters*, **11**, 701–709.

Drenovsky, R.E., Khasanova, A. & James, J.J. (2012) Trait convergence and plasticity among native and invasive species in resource-poor environments. *American Journal of Botany*, **99**, 629–639.

Eskelinen, A. & Harrison, S. (2014) Exotic plant invasions under enhanced rainfall are constrained by soil nutrients and competition. *Ecology*, **95**, 682–692.

Evans, J.R. (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, **78**, 9–19.

Everard, K., Seabloom, E.W., Harpole, W.S. & de Mazancourt, C. (2010) Plant water use affects competition for nitrogen: why drought favors invasive species in California. *The American Naturalist*, **175**, 85–97.

Franks, S.J. (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *New Phytologist*, **190**, 249–257.

Franks, S.J., Sim, S. & Weiss, A.E. (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 1278–1282.

Frenck, G., van der Linden, L., Mikkelsen, T.N., Brix, H. & Jørgensen, R.B. (2013) Response to multi-generational selection under elevated [CO_2] in two temperature regimes suggests enhanced carbon assimilation and increased reproductive output in *Brassica napus* L. *Ecology and Evolution*, **3**, 1163–1172.

Funk, J.L. (2016) Data from "Evolutionary responses of invasive grass species to variation in precipitation and soil nitrogen", Zenodo, DOI: 10.5281/zenodo.46049, http://zenodo.org/record/46049.

Funk, J.L. (2008) Differences in plasticity between invasive and native plants from a low resource environment. *Journal of Ecology*, **96**, 1162–1174.

Gross, K.L., Mittelbach, G.G. & Reynolds, H.L. (2005) Grassland invasibility and diversity: responses to nutrients, seed input, and disturbance. *Ecology*, **86**, 476–486.

Grossman, J.D. & Rice, K.J. (2014) Contemporary evolution of an invasive grass in response to elevated atmospheric CO_2 at a Mojave Desert FACE site. *Ecology Letters*, **17**, 710–716.

Heschel, M.S., Sultan, S.E., Glover, S. & Sloan, D. (2004) Population differentiation and plastic responses to drought stress in the generalist annual *Polygonum persicaria*. *International Journal of Plant Sciences*, **165**, 817–824.

Huenneke, L.F., Hamburg, S.P., Koide, R., Mooney, H.A. & Vitousek, P.M. (1990) Effects of soil resources on plant invasion and community structure in California serpentine grassland. *Ecology*, **71**, 478–491.

Johansen-Morris, A.D. & Latta, R.G. (2006) Fitness consequences of hybridization between ecotypes of *Avena barbata*: hybrid breakdown, hybrid vigor, and transgressive segregation. *Evolution*, **60**, 1585–1595.

Kimball, S., Goulden, M., Suding, K. & Parker, S. (2014) Altered water and nitrogen input shifts succession in a Southern California coastal sage community. *Ecological Applications*, **24**, 1390–1404.

Lacey, E.P. (1998) What is an adaptive environmentally induced parental effect? *Maternal Effects as Adaptations* (eds T. Mousseau & C. Fox), pp. 54–66. Oxford University Press, Oxford, UK.

Larson, J.E. & Funk, J.L. (2016) Seedling root responses to soil moisture and the identification of a belowground trait spectrum across three growth forms. *New Phytologist*, **210**, 827–838.

Mattingly, W.B. & Reynolds, H.L. (2014) Soil fertility alters the nature of plant–resource interactions in invaded grassland communities. *Biological Invasions*, **16**, 2465–2478.

Melillo, J.M., Richmond, T.C. & Yohe, G.W. (2014) Climate Change Impacts in the United States: The Third National Climate Assessment. pp. 841. U.S. Global Change Research Program, doi:10.7930/J0Z31WJ2.

Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-Being: Biodiversity Synthesis*. World Resources Institute, Washington, DC, USA.

Moran, M.D. (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos*, **100**, 403–405.

Moran, E.V. & Alexander, J.M. (2014) Evolutionary responses to global change: lessons from invasive species. *Ecology Letters*, **17**, 637–649.

Nicotra, A.B., Babicka, N. & Westoby, M. (2002) Seedling root anatomy and morphology: an examination of ecological differentiation with rainfall using phylogenetically independent contrasts. *Oecologia*, **130**, 136–145.

Ostertag, R. & Verville, J.H. (2002) Fertilization with nitrogen and phosphorus increases abundance of non-native species in Hawaiian montane forests. *Plant Ecology*, **162**, 77–90.

Padgett, P.E. & Allen, E.B. (1999) Differential responses to nitrogen fertilization in native shrubs and exotic annuals common to mediterranean coastal sage scrub of California. *Plant Ecology*, **144**, 93–101.

Padilla, F.M., Miranda, J.D., Jorquera, M.J. & Pugnaire, F.I. (2009) Variability in amount and frequency of water supply affects roots but not growth of arid shrubs. *Plant Ecology*, **204**, 261–270.

Potts, D.L., Suding, K.N., Winston, G.C., Rocha, A.V. & Goulden, M.L. (2012) Ecological effects of experimental drought and prescribed fire in a southern California coastal grassland. *Journal of Arid Environments*, **81**, 59–66.

Pyšek, P., Jarosik, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U. & Vila, M. (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Global Change Biology*, **18**, 1725–1737.

Ross, K.A., Ehrenfeld, J.G. & Patel, M.V. (2011) The effects of nitrogen addition on the growth of two exotic and two native forest understory plants. *Biological Invasions*, **13**, 2203–2216.

Sherrard, M.E. & Maherali, H. (2006) The adaptive significance of drought escape in *Avena barbata*, an annual grass. *Evolution*, **60**, 2478–2489.

Steers, R.J., Funk, J.L. & Allen, E.B. (2011) Can resource-use traits predict native vs. exotic plant success in carbon amended soils? *Ecological Applications*, **21**, 1211–1224.

Sultan, S.E., Horgn-Kobelski, T., Nichols, L.M., Riggs, C.E. & Waples, R.K. (2013) A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. *Evolutionary Applications*, **6**, 266–278.

Terashima, I. & Evans, J.R. (1988) Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. *Plant and Cell Physiology*, **29**, 143–155.

Turner, K.G., Hufbauer, R.A. & Rieseberg, L.H. (2014) Rapid evolution of an invasive weed. *New Phytologist*, **202**, 309–321.

Vilà, M., Basnou, C., Pysek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A., Roy, D., Hulme, P.E. & Partners, A.D. (2010) How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment *Frontiers in Ecology and the Environment*, **8**, 135–144.

Williams, D.G. & Black, R.A. (1994) Drought response of a native and introduced Hawaiian grass. *Oecologia*, **97**, 512–519.

Wolfe, M.D. & Tonsor, S.J. (2014) Adaptation to spring heat and drought in northeastern Spanish *Arabidopsis thaliana*. *New Phytologist*, **201**, 323–334.

Wolkovich, E.M. & Cleland, E.E. (2011) The phenology of plant invasions: a community ecology perspective. *Frontiers in Ecology and the Environment*, **9**, 287–294.

Wright, I.J. & Westoby, M. (1999) Differences in seedling growth behaviour among species: trait correlations across species, and trait shifts along nutrient compared to rainfall gradients. *Journal of Ecology*, **87**, 85–97.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Effects of water availability on root traits.