Journal of Vegetation Science 20: 860–870, 2009
© 2009 International Association for Vegetation Science

Plant responsiveness to variation in precipitation and nitrogen is consistent across the compositional diversity of a California annual grassland

St. Clair, Samuel B.[1]*; Sudderth, Erika A.[2]; Castanha, Cristina[3,4]; Torn, Margaret S.[3,5] & Ackerly, David D.[2,3,6]

[1]*Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT 84602, USA;* [2]*Department of Integrative Biology, University of California, Berkeley, CA 94720, USA; Email sudderth@berkeley.edu;* [3]*Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA;* [4]*Email castanha@nature.berkeley.edu;* [5]*Email mstorn@lbl.gov;* [6]*Email dackerley@berkeley.edu;*
**Corresponding author; Fax +1 801 422 0008; E-mail stclair@byu.edu*

Abstract

Question: How does responsiveness to water and Nitrogen (N) availability vary across the compositional and functional diversity that exists in a mesic California annual grassland plant community?

Location: Northern California annual grassland.

Methods: A mesocosm system was used to simulate average annual precipitation totals and dry and wet year extremes observed in northern California mesic grasslands. The effects of precipitation and N availability on biomass and fecundity were measured on three different vegetation types, a mixed grass forb community, and a forb and a grass monoculture. The treatment effects on plant community composition were examined in the mixed species community.

Results: While growth and seed production of the three vegetation types was inherently different, their responses to variation in precipitation and N were statistically similar. Plant density, shoot biomass, and seed production tended to increase with greater water availability in all vegetation types, with the exception of a consistent growth reduction in high precipitation (1245 mm) plots in the first year of the study. Shoot biomass responded positively to N addition, an effect that increased with greater water availability. Nitrogen addition had little effect on plant density or seed production. In the mixed grass-forb community, biomass responsiveness to water and N treatments were consistently driven by the shoot growth of *Avena barbata*, the dominant grass species.

Conclusions: Vegetation responses to changes in precipitation and N availability were consistent across a range of composition and structural diversity in this study. Plant growth and seed production were sensitive to both increased and decreased precipitation totals, and the magnitude of these responses to N availability varied depending on soil moisture conditions. Our results suggest the impacts of changing precipitation regimes and N deposition on annual productivity of California grasslands may be predictable under different climate scenarios across a range of plant communities.

Keywords: Annual grassland; *Avena*; Climate change; Drought; Fecundity; Soil moisture; Water deficit.

Introduction

Human activities are altering the earth's climate (IPCC 2007). These changes can have important impacts on plant productivity and ecosystem function (Nemani et al. 2003). Precipitation patterns are an important controller of plant community composition (Knapp & Smith 2001; Weltzin et al. 2003) and a primary characteristic defining the Earth's biomes (Gurevitch et al. 2006). Soil moisture is a critical factor that integrates ecosystem responses to climate change (Weltzin et al. 2003). For example, one of the major influences of warming on ecosystem processes occurs when increased rates of evapotranspiration reduce soil moisture (Harte et al. 1995; Calanca et al. 2006). The effects of other global change factors including elevated CO_2 and N deposition on plant productivity vary depending on water availability (Schimel et al. 1997; Poorter & Perez-Soba 2001).

Nitrogen is the element that most frequently limits biological productivity of terrestrial ecosystems (Vitousek 2004), including grasslands (Harpole et al. 2007a; LeBauer & Treseder 2008). Rates of Nitrogen deposition have doubled as a

result of human activity (Vitousek et al. 1997a; Galloway et al. 2004), which can have negative consequences on ecosystem function (Vitousek et al. 1997b; Fenn et al. 2003a). The size and flux of soil N pools, as influenced by atmospheric inputs (Fenn et al. 2003b), microbial activity (Stark & Firestone 1995), and losses are largely determined by precipitation events (Schimel et al. 1997). It is therefore critical to understand how N impacts on ecosystem function will be influenced by future precipitation patterns.

Grasslands have been shown to be responsive to variability in precipitation, (Duncan & Woodmansee 1975; Knapp & Smith 2001). Several studies conducted in California annual grasslands have demonstrated that precipitation addition experiments have neutral to positive effects on plant biomass accumulation (Dukes et al. 2005; Harpole et al. 2007b; Suttle et al. 2007). In these same studies, water additions were found to have negative (Suttle et al. 2007) to neutral effects (Harpole et al. 2007b) on plant diversity. Far fewer studies have examined grassland community responses to drier conditions because of the difficulty of excluding precipitation in field studies. However, recent climate models suggest that Mediterranean grasslands are more likely to experience increases in drought, particularly during the winter growing season (Hayhoe et al. 2004; IPCC 2007). Intra-annual variation in precipitation patterns are projected to increase, with longer periods of droughts interspersed with more intense rainfall events (Easterling et al. 2000; Groisman et al. 2005; Sun et al. 2007).

While plant communities generally respond positively to greater water and N availability, the degree of their responsiveness can vary depending on the composition and structure of the plant community (Knapp & Smith 2001; LeBauer & Treseder 2008). For example, greater species and functional trait diversity increase the responsiveness of perennial grassland productivity to N amendments (Reich et al. 2001a, b). Wang et al. (2007) observed that drought sensitivity among a diversity of plant communities was positively correlated with biomass. While several studies have shown that water and N availability can cause shifts in annual grassland community composition (Zavaleta et al. 2003; Suttle et al. 2007), few studies have examined how variation in species composition and functional traits that are inherent in annual grassland communities influence responsiveness to variation in water and N availability. In addition, it is not clear whether shifts in species composition resulting from competitive interactions are driven by plant mortality, reductions in seed production, or low seed viability.

The dramatic shift in the species composition of 9 million hectares of California grasslands from native grasses to exotic annual grasses during the 19th century has been attributed to grazing practices and changing climate conditions (Heady 1977). Jackson (1985) argued that pre-adaptation of exotic annuals to California's Mediterranean climate conditions was an important determinant of their invasion success. *Avena fatua* and *A. barbata* are among the most common and ecologically important of the non-native annual grasses that have invaded California's grasslands (Heady 1977; Jackson 1985). California's Mediterranean climate appears to be ideal for *Avena* spp. functional strategy, which respond to ample winter moisture and then employ a strategy of drought avoidance prior to onset of the dry summer season (Jackson 1985). However, *Avena* spp. do appear to be sensitive to winter drought (Sherrard & Maherali 2006), which could impact success under future climates.

The central objective of this study was to characterize plant productivity in relation to variation in annual precipitation and N across a portion of the compositional and functional diversity inherent in an annual grassland system. A particular focus was placed on better understanding the impacts of soil moisture extremes during the growing season, which is projected to be an increasingly common feature of the Mediterranean climate. A repeated 21-day watering cycle at three levels (low, ambient, or high), applied over 11 days (pulse period), followed by a dry period of 10 days, was implemented in a greenhouse mesocosm experiment. Precipitation and N treatments were applied across three different vegetation types: a mixed grass-forb community typical of northern California annual grasslands, a monoculture of a dominant grass (*Avena barbata*), and a forb (*Erodium botrys*) monoculture that naturally form monospecific stands. We measured how variation in water and N influenced species composition (mixed community only), and vegetative growth, seed production, and viability in each of the three vegetation types. The following hypotheses were tested: (1) plant growth and seed production are responsive to precipitation extremes; (2) structural and compositional differences among the three vegetation types result in differential sensitivity to variation in water and N availability; and (3) reduced water availability dampens plant responsiveness to N addition.

Materials and Methods

Soil collection and mesocosm construction

In the spring of 2005, soil was collected at the Hopland Research and Extension Center, located in Mendocino County in the Coast Range of northwest California. The site was prepared for soil collection by removing vegetation. The soil was excavated by horizon, using a grader, and transported to a greenhouse facility in Richmond California, where the experiment was conducted. The soil was classified as a Typic Haplustept, with A, B1, and B2 horizons, each of which was on average 20-cm deep. Field bulk densities of 1.15, 1.4, and 1.6 g cm^{-3} for the A, B1, and B2 horizons, respectively, were determined. Each soil horizon fraction was then sieved to roughly 8 mm using a large screen.

Cylindrical mesocosms, 57 cm in diameter and 66-cm tall, were constructed from thick wall polyvinyl chloride (PVC). A screen and fine sand were placed at the bottom of each mesocosm above a drainage hole to ensure adequate drainage. In the mesocosms, the native soil profile was reconstructed by packing each 20-cm horizon in its original position. To achieve the target bulk density, we put the same mass of a given horizon soil into each mesocosm, and packed it to the correct volume. Twice, 10 mm of water were applied to the soil surface of each mesocom prior to initiation of the experiment to induce germination and for subsequent removal of the seed bank. The soils were then allowed to air-dry in the greenhouse for several weeks prior to initiation of the experiment.

Experimental design

The experiment was conducted over two growing seasons (November 2005-May 2007) in a climate-controlled greenhouse in Richmond, CA (37°54′50″N, 122°19′40″W). We used a randomized complete block design with three main treatment factors in five blocks. Independent variables were three different vegetation types, three annual precipitation totals, and two nitrogen levels. Dependent variables were species composition of the mixed community, plant density, plant growth (measured as aboveground biomass), seed production, and seed viability of all three vegetation types.

Vegetation types

Seeds for each plant species were collected at the Hopland Research and Extension Center in June 2005 and 2006, in the vicinity of the soil collection site. The first vegetation type was a mixed grass-forb community consisting of five grasses and two forbs sown at the following densities (seeds m^{-2}): *Avena barbata* (Pott.) (1500), *Bromus hordeaceus* (L.) (1500), *Briza maxima* (L.) (1000), *Hordeum murinum* (L.) (1000), *Aegilops triuncialis* (L.) (500), *Erodium botrys* (cav.) Bertol. (250), and *Amsinckia douglasiana* (A.DC.) (250). The second and third vegetation types were *Avena* and *Erodium*, which are a dominant grass and forb species, grown in monoculture communities at densities of 4000 and 2000 seeds m^{-2}, respectively. Sowing densities and species proportions were based on composition and density studies conducted in the vicinity of the Hopland Research and Extension Center (Pitt & Heady 1978; Corbin et al. 2007). The vegetation types were designed to be representative of a typical mixed northern California annual grassland community, and monocultures of *Avena barbata* and *Erodium botrys* that naturally form monospecific stands. If response to water and N availability vary among vegetation types, it will be most apparent by observing extremes in composition and functional traits. Therefore, the vegetation types were chosen to represent extremes in composition (mixed versus monoculture), and functional and structural diversity (forbs versus grasses) inherent in annual grasslands of northern California.

A litter addition was included in the second year because it is an important feature of annual grasslands (Amatangelo et al. 2008). One hundred grams of homogenized plant biomass without seeds (within each of the three vegetation types) were added as litter. A standard amount of litter was placed on each mesocosm so we could examine 2007 treatment effects independent of differences in 2006 litter production. One hundred grams represented about 35% of the total aboveground biomass produced in each of the vegetation types during the 2005-2006 growing season.

Precipitation and N application regime

Prior to initiating the precipitation treatments, and after the seeds had been placed on the soil surface, all mesocosms received a uniform amount of water (27 mm water over an 11-day period) to ensure uniform seed germination. During the 2005-2006 season, the three annual precipitation totals were 315, 675, and 1245 mm yr^{-1}, representative of the annual precipitation observed during dry, average, and wet years, respectively, at Hopland Research and Extension Center from 1989 to 2005.

During the 2006-2007 season, the three precipitation treatments were adjusted slightly to 297, 657, and 987 mm yr^{-1}. We adjusted the high precipitation totals in 2007 down to 987 mm yr^{-1} (from 1245 mm) as a target for a high precipitation optimum for growth. From the 2006 data it was evident that a threshold point was reached and excessive water had a negative effect on growth.

The pattern of water application over the course of the growing season was determined based on an analysis of climate data from the Hopland station. We found that variation in total rainfall among years was primarily due to the number of rainy days, intervals between rain events, and duration of the season, whereas the average precipitation per rainy day did not vary between dry and wet years. According to this pattern, each 1-day watering event was held constant at 15 mm. Cumulative annual precipitation differences that define the precipitation treatments were the result of differences in the number of 15-mm precipitation events (high precipitation = 10 (2005-2006) or 8 (2006-2007), ambient = 6, low = 3) applied within an 11-day watering period of the 21-day repeating water cycle. At the end of the 11-day watering period, all three precipitation treatments experienced a 10-day drydown period. The total number of 21-day repeating water cycles experienced over the growing season also varied between the watering treatments (high = 8, ambient = 7, low = 6). The precipitation treatments were applied using an automated watering system designed to uniformly apply 15 mm of water across the soil surface during each irrigation event. Computer-controlled electronic relays operated programmable water pumps and a series of solenoids to precisely fill the water reservoirs associated with each mesocosm. Irrigation tubing connected the water reservoirs to two concentric rings of drip irrigation tubing on the soil surface of each mesocosm, through which water was slowly released.

Nitrogen was added in the form of ammonium nitrate (dissolved in water) and applied at a rate of 2 g N m^{-2}, four times during the spring of 2007 (during water cycles 4-6) to give a total of 8 g N m^{-2}. Other studies have shown that California annual grasslands are responsive to N additions at this level (Dukes et al. 2005).

Greenhouse conditions

Environmental sensors and a data logging system were used to measure and record air temperature, humidity, and light intensity at 15-min intervals. During the 2007 growing season, mean temperature in the greenhouse ranged from 10°C to 19°C, with an average of 15°C, and relative humidity ranged from 48% to 88%, with an average of 70%. A lighting system with high-pressure sodium bulbs was used to balance daytime light gradients, but artificial light did not extend beyond the natural photoperiod. Photosynthetic photon flux density (PPFD) varied throughout the day, with a maximum of 1800 $\mu mol \cdot photons \cdot m^{-2} \cdot s^{-1}$ and an average of 701 $\mu mol \cdot photons \cdot m^{-2} \cdot s^{-1}$.

Vegetation measurements

Aboveground plant biomass was harvested at soil level in mid-May 2006 and 2007. Plants were sorted by species, counted, and then height was measured. During the competitive growth phase, significant thinning can occur in grasslands, which results in mortality of some plants. Throughout the rest of the manuscript, we refer to this dead plant biomass as the "litter" fraction. Plant material was then dried at 60°C for a minimum of 1 week until plant tissues were dry, at which point they were weighed to obtain dry mass. Plant density was determined as the number of plant stems per unit area of each mesocosm. Seeds from each mesocosm were collected by species as they matured. At the end of the season, the seeds were dried at 40°C for 3 days and weighed to obtain a measure of seed mass per species in each plot. Seeds were dried at 40°C to preserve their viability for a subsequent seed viability experiment described below.

Leaf nutrient analysis

During the first week of March 2006 and during water cycles 4 and 6 (February, April) in 2007, leaves were harvested for N and phosphorus (P) analysis. Sampling occurred on the youngest fully expanded leaves on one or two plants in each mesocosm. For 2006, leaf samples of *Avena* and *Erodium* were analyzed in the grass and forb monoculture plots, respectively, while in 2007 sampling occurred in all plots. Measurements in the mixed plots in 2007 were conducted on *Avena* leaf tissue only. Samples were frozen in liquid nitrogen, ground using a ball mill, and divided into fractions for analysis. One fraction was dried at 60°C, ashed at 550°C for 7 h, dissolved in 0.1 N HCl and analyzed for P content spectrophotometrically (Murphy & Riley 1962). A second fraction was freeze-dried, weighed and analyzed for total N using an elemental analyzer. National Institute of Standards and Technology (NIST) peach

leaves were used as a plant reference standard to assure quality control.

Seed viability experiment

In the spring of 2008, the effects of rainfall and N treatments on the viability of seeds collected from the three vegetation types were tested. The experiment was conducted in the Jane Grey greenhouses at the UC Berkeley Botanical Garden. Daytime temperatures in the greenhouse averaged 25°C. For species that produced sufficient seed in 2007, 27 seeds collected from each mesocosm were randomly selected for the germination experiment. Seedling flats were filled to a depth of ~5 cm with soil collected from the Hopland Research Center. The seeds were planted ~5-mm deep in a nine by 18 grid pattern, with each species planted in three rows of nine seeds. All flats were watered to saturation on 13 February 2008, and watered daily for the remainder of the experiment. The germination date of each seed was recorded. Growth rates of the grass species were determined by recording the date that each seedling reached 4 and 8 cm in height. Forb growth rates were determined by counting the number of leaves in each seedling on two different dates.

Statistical analysis

The main and interactive treatment effects on the measured response variables were assessed using analysis of variance (ANOVA). Vegetation type and precipitation and N levels were treated as fixed effects, and block was included as a random effect. A multivariate analysis of variance (MANOVA) model was used to test treatment effects on shoot and seed biomass for the combination of all seven plant species in the mixed vegetation assemblage in 2007. The MANOVA model used for the 2006 data tested the effects of precipitation treatments on the combination of only five species because data were insufficient to include *Erodium* and *Amsinckia*. Dependent variables were tested for normality using Shapiro-Wilk W statistic to determine the goodness of fit of the data plotted in normal quantile plots. A Box-Cox transformation was applied to data when they did not satisfy the assumptions of normality. Statistical significance was defined as $\alpha \leq 0.05$. Statistical analysis was performed using JMP (Version 7.0) statistical software (SAS institute, Cary, NC, USA).

Table 1. *F*-values from ANOVA model testing the main effects and the interaction of precipitation (Ppt) and vegetation type (Veg) on plant productivity, seed mass and plant density from the 2006 season. Significance designated as $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Factors	*df*	Plant density	Shoot mass	Seed mass
Ppt	2	69.23***	31.18***	32.49***
Veg	1,2	2.94	15.21**	84.92***
Ppt×Veg	2,4	1.31	1.42	1.71

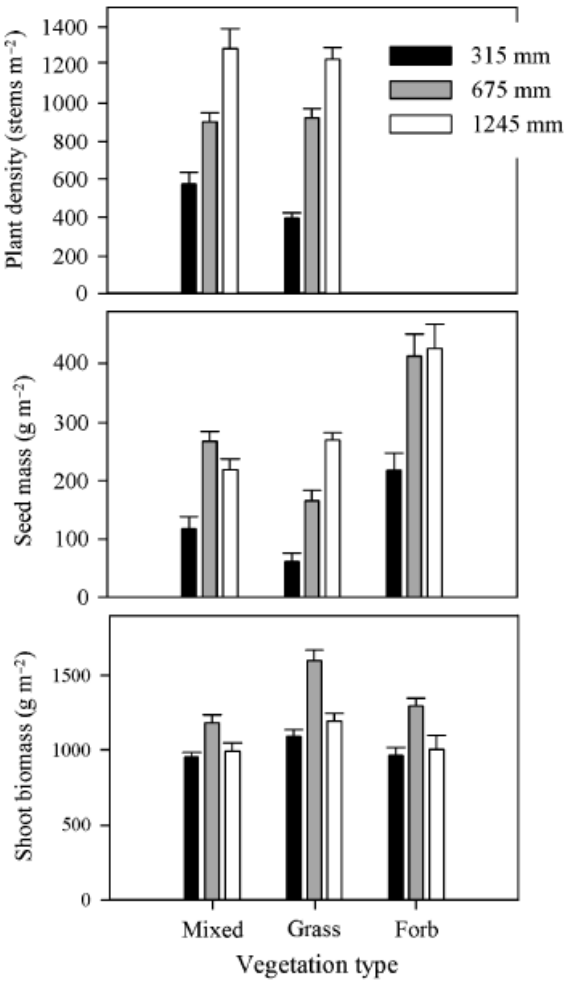

Fig. 1. Influence of low (315 mm), ambient (675 mm), and high (1245 mm) annual water inputs on the productivity of mixed, grass monoculture, and forb monoculture plant communities during the 2005-2006 growing season. Bars are means values ($n = 5$) presented with SE.

Results

2006 plant growth and seed production

The three plant communities differed significantly in shoot and seed biomass but the mixed

Table 2. Means, SE, and *F*-statistics indicating the influences of precipitation level (P1 = 315 mm, P2 = 675 mm, P3 = 1245 mm) on shoot and seed biomass of individual species as measured in May of 2006 in the mixed plant community. Means ($n = 5$) presented with SE. Significance designated as $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

	Total	Litter	*Avena*	*Aegilops*	*Hordeum*	*Briza*	*Bromus*	*Erodium*	*Amsinckia*
Shoot biomass									
P1	1069 ± 45	715 ± 20	148 ± 25	13.4 ± 3.7	61 ± 9.8	8.0 ± 5.2	3.2 ± 0.9	1.0 ± 0	2.8 ± 1.1
P2	1450 ± 68	591 ± 28	358 ± 32	34 ± 5.9	143 ± 12	6.0 ± 1.9	4.6 ± 1.1	1.0 ± 0	30 ± 19
P3	1213 ± 62	468 ± 30	326 ± 26	63 ± 5.5	79 ± 8.1	12.9 ± 4.2	3.3 ± 0.7	1.5 ± 0.56	7.0 ± 2.1
Ppt	8.11^{*}	60.00^{***}	12.86^{**}	17.73^{**}	19.18^{***}	0.44	0.31	–	–
Seed biomass									
P1			26 ± 7.4	6.9 ± 1.5	76 ± 13	5.3 ± 3.0	2.0 ± 0.3	1.0 ± 0	1.5 ± 0.3
P2			89 ± 11	16 ± 2.8	147 ± 13	3.8 ± 0.8	5.9 ± 0.8	1.0 ± 0	7.1 ± 4.5
P3			119 ± 6.0	31 ± 2.6	62 ± 12	4.7 ± 2.3	1.3 ± 0.5	1.0 ± 0	1.2 ± 0.2
Ppt			42.89^{***}	27.25^{***}	8.93^{**}	0.40	0.99	–	–

Table 3. *F*-values from ANOVA model testing the main effects and interactions of precipitation (Ppt), N addition (N). and vegetation type (Veg) on total plant community density, above ground biomass (AGB) and seed mass during the 2007 season. Significance designated as $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Source of variance	*df*	Plant density	Shoot biomass	Seed biomass
Ppt	2	62.12^{***}	60.02^{***}	75.14^{***}
N	1	0.03	117.39^{***}	0.09
Veg	1,2	749.14^{***}	18.94^{***}	185.66^{***}
Ppt×N	2	2.05	5.72^{*}	1.33
Ppt×Veg	2,4	1.36	0.82	0.38
N×Veg	2	0.59	1.61	0.86
Ppt×N×Veg	4	1.10	1.45	3.02^{*}

community and *Avena* monoculture did not differ in plant density (Table 1, Veg; Fig. 1). Mixed community and grass monoculture densities increased concomitantly with water availability (Table 1, Ppt; Fig. 1). Across all three plant communities, shoot biomass exhibited consistent negative effects under low (315 mm) and high (1245 mm) water levels relative to ambient water conditions (675 mm) (Table 1, Fig. 1). Ambient and high water plots produced significantly more seeds than low precipitation plots in all three plant vegetation types (Table 1, Fig. 1).

Diminished shoot and seed production in the low precipitation plots of the mixed communities were primarily driven by reductions in *Avena, Aegilops*, and *Hordeum*, the three dominant grass species. Lower shoot biomass in high precipitation plots was primarily a function of reductions in *Avena*, *Hordeum*, and litter (Table 2). Among the dominant grass species, greater water availability tended to promote seed production, with the exception of *Hordeum* (Table 2). The precipitation effect on the combination of *Avena*, *Hordeum*, *Aegilops*, *Briza*, and *Bromus* in the MANOVA model was significant for shoot biomass ($F = 8.34$, $P < 0.0001$) but not seed biomass ($F = 1.97$, $P = 0.15$).

2007 plant growth and seed production

The three plant vegetation types differed significantly in density, biomass, and seed production in 2007 (Table 3, Veg; Fig. 2). The grass monoculture had a significantly higher plant density but much lower seed production than the mixed or forb communities (Fig. 2). Plant density, shoot biomass, and seed production increased with greater water availability, an effect that was statistically significant across plant vegetation types (Table 3, Ppt; Fig. 2). In 2007, N addition significantly increased shoot biomass across all three plant vegetation types (Fig. 2), but had no effect on plant density or seed production (Table 3, N). The Ppt×N interaction was significant for shoot biomass (Table 3), as a function of N stimulating shoot growth more strongly under ambient and high water conditions (Fig. 2). At low and ambient water levels, N addition had a negative effect on seed production in the mixed community but not in the monoculture plots (Table 3, significant Ppt×N×Veg).

At the species level, shoot biomass of *Avena, Briza*, and *Erodium* responded positively to increasing water availability, while *Aegilops, Hordeum, Bromus*, and *Amsinckia* showed no significant response (Table 4). Only *Avena* and *Bromus* demonstrated positive shoot growth in response to N addition (Table 4). The responsiveness of *Avena* to N increased with greater water availability (Table 4, Ppt×N). A similar trend was seen for litter production (Table 4, Ppt×N). The MANOVA model testing treatment effects on shoot biomass for the combination of all seven species indicated that both precipitation ($F = 3.90$, $P < 0.001$) and N ($F = 3.55$, $P = 0.015$) were statistically significant, but that the interaction term Ppt×N ($F = 0.71$, $P = 0.75$) was not. A similar pattern was seen in the MANOVA model results for seed biomass, with the exception that the main effect for N was not significant: Ppt

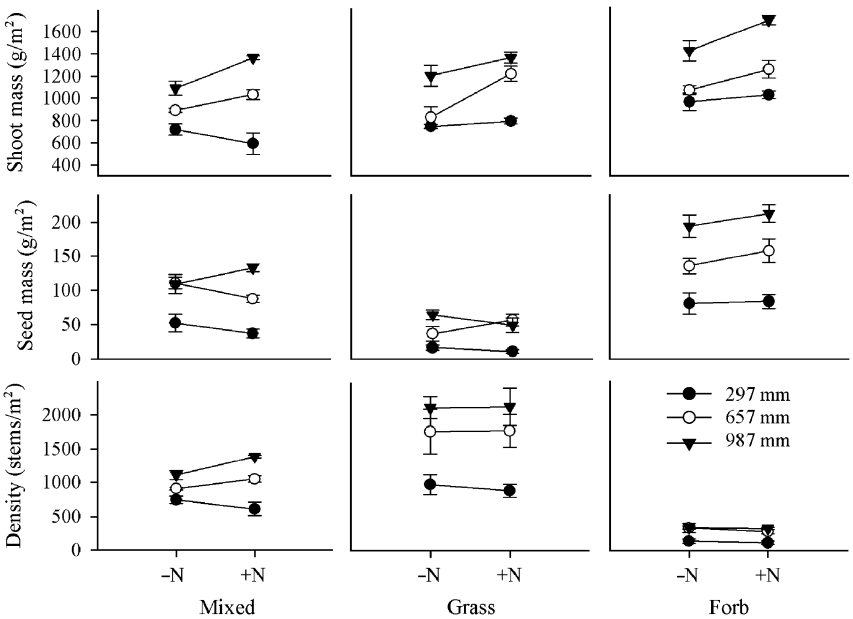

Fig. 2. Influence of low (297 mm), ambient (657 mm), and high (987 mm) seasonal water totals and N availability on the productivity of mixed grass-forb, grass (*Avena* monoculture), and forb (*Erodium* monoculture) communities during the 2006-2007 growing season. Symbols are mean values ($n = 5$) presented with SE.

Table 4. Means, SE, and *F*-statistics indicating the influences of precipitation (P1 = 297 mm, P2 = 657 mm, P3 = 987 mm) and N (N1 = control, N2 = N addition) levels on shoot and seed biomass of individual species as measured in May of 2007 in the mixed plant community. Means ($n = 5$) presented with SE. Significance designated as $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

	Total	Litter	*Avena*	*Aegilops*	*Hordeum*	*Briza*	*Bromus*	*Erodium*	*Amsinckia*
Shoot biomass									
P1N1	721 ± 51	446 ± 23	131 ± 12.5	47 ± 16	25.0 ± 9.0	0.6 ± 0.3	0.30 ± 0.1	1.3 ± 1.0	19.0 ± 2.6
P2N1	891 ± 19	495 ± 30	207 ± 9.3	45 ± 4.1	11.5 ± 4.0	11.4 ± 2.3	1.0 ± 0.2	2.2 ± 0.6	7.3 ± 2.9
P3N1	1090 ± 63	646 ± 45	236 ± 18.8	37 ± 4.7	18.3 ± 0.8	21 ± 8.6	1.1 ± 0.5	11.4 ± 4.0	10.2 ± 2.3
P1N2	594 ± 96	356 ± 95	137 ± 15.9	33 ± 7.8	17.1 ± 5.6	.07 ± 0.07	0.6 ± 0.2	1.9 ± 1.6	12.9 ± 2.2
P2N2	1033 ± 45	602 ± 45	270 ± 32	32 ± 4.9	22.6 ± 2.7	4.0 ± 0.9	2.0 ± 0.8	2.2 ± 1.7	10.7 ± 4.9
P3N2	1366 ± 18	774 ± 36	364 ± 12.9	39 ± 5.4	23.6 ± 3.9	17.4 ± 4.9	2.0 ± 0.4	4.7 ± 1.8	11.8 ± 3.5
Ppt	40.41***	11.78**	63.69***	0.07	0.32	10.83**	3.05	5.98*	2.77
N	3.52	1.39	17.36*	1.31	0.38	1.16	7.11*	1.26	0.00
Ppt×N	10.09**	4.35*	5.42*	0.36	2.54	0.47	0.28	1.72	0.88
Seed mass									
P1N1			18.0 ± 5.8	17.0 ± 6.5	11.5 ± 1.7	0.2 ± 0.2	0.1 ± 0.1	5.1 ± 2.9	0.5 ± 0.3
P2N1			51 ± 5.4	37.1 ± 2.3	12.9 ± 2.3	8.2 ± 1.9	0.5 ± 0.2	1.2 ± 0.3	0.0 ± 0.0
P3N1			42 ± 4.6	27.5 ± 5.7	18.9 ± 1.7	19.0 ± 6.8	0.6 ± 0.4	1.3 ± 0.5	0.4 ± 0.2
P1N2			14.3 ± 3.9	9.1 ± 3.4	8.4 ± 0.8	0.0 ± 0.0	0.03 ± 0.03	4.8 ± 2.8	0.2 ± 0.1
P2N2			43 ± 5.3	24.0 ± 2.9	16.9 ± 2.7	1.6 ± 0.3	0.7 ± 0.3	0.4 ± 0.2	0.5 ± 0.2
P3N2			68 ± 8.2	31 ± 3.1	22.3 ± 2.5	8.8 ± 2.2	1.6 ± 0.5	1.3 ± 1.0	0.8 ± 0.3
Ppt			24.39***	15.36**	11.70**	13.07**	4.71*	4.73*	2.56
N			0.34	2.45	2.36	4.02	2.68	0.03	1.29
Ppt×N			29.32***	2.39	2.51	1.65	3.04	0.03	2.02

($F = 3.59$, $P < 0.001$), N ($F = 1.77$, $P = 0.15$), Ppt×N ($F = 1.61$, $P = 0.11$).

The seed production of all five grass species tended to increase with greater water availability. In the two forb species, greater water availability had no effect on *Amsinckia* or a negative effect on *Erodium* (Table 4). We observed no significant effect of N addition on seed production in any of the species. However, N did significantly increase *Avena* seed production in high precipitation plots (Table 4, Ppt×N).

Leaf nutrients

In 2006, the watering treatment significantly affected foliar N concentration ($F = 7.79$, $P = 0.016$),

with high water treatments having 20% lower N concentrations relative to low or ambient plots. However, even leaves collected from the high precipitation plots averaged 3.5% N. In 2007, the watering treatments again had a significant effect on foliar N concentrations ($F = 7.22$, $P = 0.026$). Leaf samples from low precipitation plots had on average 12% more N than samples in ambient or high precipitation plots. There were no significant differences in foliar N among the vegetation types in either year. Foliar N concentrations were 35% lower in 2007 than in 2006 (2.65% versus 4.00% dry weight) across all treatments. Treatment variables had no significant influence on foliar P concentrations in 2006. In 2007, foliar P concentrations were significantly different among vegetation types, with the monoculture grass and monoculture forb communities having 11% and 24% less foliar P, respectively, than the mixed grass community ($F = 16.24$, $P < 0.0001$). The low precipitation plots had significantly lower (15%) foliar P concentrations than ambient or high precipitation plots ($F = 6.27$, $P = 0.002$).

Seed viability results

Germination percentage for all species was typically above 80% across all treatment categories, with no strongly significant effects of precipitation or N on germination success. Mean days to germination ranged from 6 (*Hordeum)* to 15 (*Briza* and *Amsinkia*) days, with treatment condition having no significant influence on germination lag time. For all species, early seedling growth rates were approximately 0.6-0.9 cm day^{-1}, with no significant treatment effects on seedling growth rates. *Avena* seeds harvested from low precipitation plots in mixed and *Avena* monoculture communities were 25% smaller than seeds in ambient and high precipitation plots ($F = 20.37$, $P < 0.0001$), but this did not translate to lower seed germination or early seedling growth rates.

Discussion

Plant responses to precipitation extremes

Results from both seasons generally supported our first hypothesis that variability in annual precipitation can have significant influences on grassland communities. Relative to average moisture conditions, both vegetative and seed biomass fractions increased with greater water availability, consistent with other California annual grassland water addition experiments (Dukes et al. 2005; Suttle et al. 2007), and declined markedly in the low precipitation treatment. The exception to this pattern was a shoot biomass reduction in the high water plots that was consistent across all three vegetation types during the 2006 growing season. This result suggests that somewhere between 987 and 1245 mm a threshold point was reached where excess water had approximately the same biomass reducing effect as the water deficit condition (Fig. 1). At the Hopland field station (where the soils for this experiment were collected), cumulative annual precipitation of 1245 mm was exceeded in 1995, 1996, and 1998, so water inputs in this range are not an uncommon phenomenon. Chou et al. (2008) showed a linear increase in aboveground plant biomass in a California grassland study with precipitation of up to 1300 mm yr^{-1}.

The negative growth effect of high cumulative water in our study may be related to timing and magnitude of the water applications. Our watering regime was patterned according to a future climate scenario, in which there are periods of intense water inputs followed by intermittent dry periods. Intense water inputs during wet periods can leach N from the root zone (Ineson et al. 1998). We observed that high precipitation tended to reduce foliar N concentrations relative to foliar N in ambient and low precipitation plots. Volumetric soil moisture content in the high precipitation plots was maintained at 40-45% during the entire 11-day watering period of each water cycle (data not shown). Growth capacity of dryland plant species including *Avena* can be sensitive to root hypoxia under intense periods of water input (Drew 1997; Kato-Noguchi 2002; Kato-Noguchi & Kugimiya 2002).

While shoot biomass was inhibited in high water plots in 2006, seed production on a mass basis was not (Fig. 1), indicating that seed output was not simply a function of vegetative productivity. Water deficit in the low precipitation plots consistently lowered shoot biomass and seed production in both years (Figs 1 and 2), but that did not translate to a reduction in seed viability, suggesting that preadaptation to environmental stresses can minimize maternal effects (Riginos et al. 2007). Taken together, these results suggest that shifts in plant community composition in response to precipitation extremes are more likely to be driven by changes in seed production rather than maternal effects on seed viability or early seedling fitness.

Vegetation effects

Based on inherent structural differences of the three vegetation types (St.Clair et al. 2009), we made

two predictions. First, mixed communities would show stronger responses to both precipitation and N addition compared to monocultures because variation in competitive strategies (complementarity) among the species would allow greater resource use (Gross et al. 2007). Second, we expected reduced water inputs to have less of an impact on forb-dominated plots because their longer root architecture would presumably allow greater access to deeper water sources (Nippert & Knapp 2007). Despite the inherent structural differences among the three plant communities, their responses to variation in water and N availability in this study were comparable, as indicated by the lack of significant "Vegetation" interaction terms in the ANOVA models (Tables 1 and 3). This result, while inconsistent with our two predictions, is consistent with observations that patterns of rain use efficiency (biomass production/precipitation) converge under water limitation across the functional diversity of desert, grassland, and forest biomes (Huxman et al. 2004). Wang et al. (2007) showed that responsiveness to moisture conditions is biomass-dependent. While multiple characteristics varied among the three vegetation types in our study, shoot biomass was remarkably similar in both years of the study (Figs 1 and 2), which may partially explain the consistency in response to variations in water and N among the three vegetation types.

Precipitation×N interactions

Decreasing water availability did place constraints on plant responsiveness to N, as outlined in our third hypothesis. The dependence of N stimulation of plant productivity on soil moisture conditions has been noted in water addition experiments in annual grassland studies (Zavaleta et al. 2003; Harpole et al. 2007b). Our study is consistent with these results and extends the patterns to show that N effects are neutral to negative under drier conditions (Fig. 2). The dry-down periods imposed in the experiment resulted in 50% reductions in soil moisture content, which in low precipitation plots led to extended periods with $<10\%$ soil moisture content (St. Clair et al. 2009). Ambient and high precipitation plots also experienced significant reductions during the dry-down period, but their soil moisture content values were more than twice as high as in low precipitation plots. The lack of plant response to N additions under lower precipitation conditions suggests that plants were more water-limited than N-limited and/or low soil moisture constrained mass transport of N (Ingestad & Agren 1995), although the latter is not supported by our foliar N data, which was 12% higher in low precipitation plots.

Avena was the only species that showed seed production increases in response to N addition, but the response was dependent on high water conditions (Table 4). While most species in our study showed strong vegetative growth and/or seed mass sensitivity to water availability, *Avena* was the only species that had a consistent response to N, either directly or through interactions with water availability (Table 4). Among 12 annual species growing in a water and N addition experiment in a more xeric California grassland experiment, *Avena fatua* was the only species that had both positive growth responses to additions of water and N and an additive increase when the treatments were applied in combination (Harpole et al. 2007b). In our study, *Avena barbata* showed consistent shoot biomass and seed reductions in response to water deficits over both years (Tables 2 and 4), which is consistent with results showing that *A. barbata* is drought-sensitive (Sherrard & Maherali 2006). Low precipitation years and periods of intermittent drought during the growing season are likely to have negative impacts on *Avena*, which may create competitive advantages for more drought-tolerant forbs and native grasses (Seabloom et al. 2003).

Conclusions

The results from this experiment suggest that plant growth and fecundity are sensitive to precipitation extremes projected under future climate scenarios. Furthermore, we found consistency in the pattern of response to water availability across the inherent structural and functional diversity simulated in our study. Since variability in water and N are projected to increase, and their effects on plant function are important and often interdependent, our understanding of grassland function in future climates should be informed by a clear understanding of precipitation-nitrogen interactions. The responsiveness of *Avena barbata* to water and N resources in this study appear consistent with explanations of its successful invasion of California grasslands (Jackson 1985). Extended drought periods during the growing season are likely to have negative effects on *Avena* while greater moisture and N inputs will increase its competitive advantage.

Acknowledgements: We gratefully acknowledge the assistance of Markus Kleber and Alex Morales in soil

collection and reconstruction, and Melissa Crago, Tara Macomber, Julia Shams, Stephanie Bernard, Paul Cook, and Kallista Bley in maintenance of the watering system and data collection. This study was supported by the Program for Ecosystem Research, Office of Science, U.S. Department of Energy, under Contract No. DE-AC02-05CH11231.

References

Amatangelo, K.L., Dukes, J.S. & Field, C.B. 2008. Responses of a California annual grassland to litter manipulation. *Journal of Vegetation Science* 19: 605–612.

Calanca, P., Roesch, A., Jasper, K. & Wild, M. 2006. Global warming and the summertime evapotranspiration regime of the Alpine region. *Climatic Change* 79: 65–78.

Chou, W.W., Silver, W.L., Jackson, R.D., Thompson, A.W. & Allen-Diaz, B. 2008. The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. *Global Change Biology* 14: 1382–1394.

Corbin, J., Dyer, A.R. & Seabloom, E.W. 2007. Competitive interactions. In: D'Antonio, C., Corbin, J. & Stromberg, M. (eds.) *Ecology and management of California grasslands*. pp. 156–168. University of California Press, Berkeley.

Drew, M.C. 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 223–250.

Dukes, J.S., Chiariello, N.R., Cleland, E.E., Moore, L.A., Shaw, M.R., Thayer, S., Tobeck, T., Mooney, H.A. & Field, C.B. 2005. Responses of grassland production to single and multiple global environmental changes. *PLoS Biology* 3: 1829–1837.

Duncan, D.A. & Woodmansee, R.G. 1975. Forecasting forage yield from precipitation in California's annual rangeland. *Journal of Range Management* 28: 327–329.

Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R. & Mearns, L.O. 2000. Climate extremes: observations, modeling, and impacts. *Science* 289: 2068–2074.

Fenn, M.E., Baron, J.S., Allen, E.B., Rueth, H.M., Nydick, K.R., Geiser, L., Bowman, W.D., Sickman, J.O., Meixner, T., Johnson, D.W. & Neitlich, P. 2003a. Ecological effects of nitrogen deposition in the western United States. *Bioscience* 53: 404–420.

Fenn, M.E., Haeuber, R., Tonnesen, G.S., Baron, J.S., Grossman-Clarke, S., Hope, D., Jaffe, D.A., Copeland, S., Geiser, L., Rueth, H.M. & Sickman, J.O. 2003b. Nitrogen emissions, deposition, and monitoring in the western United States. *Bioscience* 53: 391–403.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R. & Vorosmarty, C.J. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70: 153–226.

Groisman, P.Y., Knight, R.W., Easterling, D.R., Karl, T.R., Hegerl, G.C. & Razuvaev, V.A.N. 2005. Trends in intense precipitation in the climate record. *Journal of Climate* 18: 1326–1350.

Gross, N., Suding, K.N., Lavorel, S. & Roumet, C. 2007. Complementarity as a mechanism of coexistence between functional groups of grasses. *Journal of Ecology* 95: 1296–1305.

Gurevitch, J., Scheiner, S.M. & Fox, G.A. 2006. *The ecology of plants*. 2nd ed. Sinauer, Sunderland.

Harpole, W.S., Goldstein, L. & Aicher, R. 2007a. Resource limitation. In: D'Antonio, C., Corbin, J. & Stromberg, M. (eds.) *Ecology and management of California grasslands*. pp. 119–127. University of California Press, Berkeley.

Harpole, W.S., Potts, D.L. & Suding, K.N. 2007b. Ecosystem responses to water and nitrogen amendment in a California grassland. *Global Change Biology* 13: 2341–2348.

Harte, J., Torn, M.S., Chang, F.R., Feifarek, B., Kinzig, A.P., Shaw, R. & Shen, K. 1995. Global warming and soil microclimate – results from a Meadow-warming experiment. *Ecological Applications* 5: 132–150.

Hayhoe, K., Cayan, D., Field, C.B., Frumhoff, P.C., Maurer, E.P., Miller, N.L., Moser, S.C., Schneider, S.H., Cahill, K.N., Cleland, E.E., Dale, L., Drapek, R., Hanemann, R.M., Kalkstein, L.S., Lenihan, J., Lunch, C.K., Neilson, R.P., Sheridan, S.C. & Verville, J.H. 2004. Emissions pathways, climate change, and impacts on California. *Proceedings of the National Academy of Sciences of the United States of America* 101: 12422–12427.

Heady, H. 1977. Valley grassland. In: Barbour, M. & Major, J. (eds.) *Terrestrial vegetation of California*. pp. 491–514. Wiley, New York.

Huxman, T.E., Smith, M.D., Fay, P.A., Knapp, A.K., Shaw, M.R., Loik, M.E., Smith, S.D., Tissue, D.T., Zak, J.C., Weltzin, J.F., Pockman, W.T., Sala, O.E., Haddad, B.M., Harte, J., Koch, G.W., Schwinning, S., Small, E.E. & Williams, D.G. 2004. Convergence across biomes to a common rain-use efficiency. *Nature* 429: 651–654.

Ineson, P., Taylor, K., Harrison, A.F., Poskitt, J., Benham, D.G., Tipping, E. & Woof, C. 1998. Effects of climate change on nitrogen dynamics in upland soils. 1. A transplant approach. *Global Change Biology* 4: 143–152.

Ingestad, T. & Agren, G.I. 1995. Plant nutrition and growth – basic principles. *Plant and Soil* 168: 15–20.

IPCC. 2007. *Working group 1, fourth assessment report*. Cambridge University Press, Cambridge.

Jackson, L.E. 1985. Ecological origins of California's Mediterranean grasses. *Journal of Biogeography* 12: 349–361.

Kato-Noguchi, H. 2002. Ethanol sensitivity of rice and oat coleoptiles. *Physiologia Plantarum* 115: 119–124.

Kato-Noguchi, H. & Kugimiya, T. 2002. Anoxia tolerance and ethanol sensitivity of rice and oat coleoptiles. *Acta Physiologiae Plantarum* 24: 73–77.

Knapp, A.K. & Smith, M.D. 2001. Variation among biomes in temporal dynamics of aboveground primary production. *Science* 291: 481–484.

LeBauer, D.S. & Treseder, K.K. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89: 371–379.

Murphy, J. & Riley, J. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31–36.

Nemani, R.R., Keeling, C.D., Hashimoto, H., Jolly, W.M., Piper, S.C., Tucker, C.J., Myneni, R.B. & Running, S.W. 2003. Climate-driven increases in global terrestrial net primary production from 1982 to 1999. *Science* 300: 1560–1563.

Nippert, J.B. & Knapp, A.K. 2007. Linking water uptake with rooting patterns in grassland species. *Oecologia* 153: 261–272.

Pitt, M.D. & Heady, H.F. 1978. Responses of annual vegetation to temperature and rainfall patterns in northern California. *Ecology* 59: 336–350.

Poorter, H. & Perez-Soba, M. 2001. The growth response of plants to elevated CO_2 under non-optimal environmental conditions. *Oecologia* 129: 1–20.

Reich, P.B., Knops, J., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M., Lee, T., Wedin, D., Naeem, S., Bahauddin, D., Hendrey, G., Jose, S., Wrage, K., Goth, J. & Bengston, W. 2001a. Plant diversity enhances ecosystem responses to elevated CO_2 and nitrogen deposition. *Nature* 410: 809–812.

Reich, P.B., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M.G., Knops, J., Wedin, D., Naeem, S., Bahauddin, D., Goth, J., Bengtson, W. & Lee, T.D. 2001b. Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO_2 and N availability regimes? A field test with 16 grassland species. *New Phytologist* 150: 435–448.

Riginos, C., Heschel, M.S. & Schmitt, J. 2007. Maternal effects of drought stress and inbreeding in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany* 94: 1984–1991.

Schimel, D.S., Braswell, B.H. & Parton, W.J. 1997. Equilibration of the terrestrial water, nitrogen, and carbon cycles. *Proceedings of the National Academy of Sciences of the United States of America* 94: 8280–8283.

Seabloom, E.W., Harpole, W.S., Reichman, O.J. & Tilman, D. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences of the United States of America* 100: 13384–13389.

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

Stark, J.M. & Firestone, M.K. 1995. Mechanisms for soil-moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology* 61: 218–221.

St. Clair, S.B., Sudderth, E.A., Fischer, M.L., Torn, M.S., Stuart, S.A., Salve, R., Eggett, D.L. & Ackerly, D.D. 2009. Soil drying and nitrogen availability modulate carbon and water exchange over a range of annual precipitation totals and grassland vegetation types. *Global Change Biology* doi: 10.1111/j.1365-2486.2009.01862.x.

Sun, Y., Solomon, S., Dai, A.G. & Portmann, R.W. 2007. How often will it rain? *Journal of Climate* 20: 4801–4818.

Suttle, K.B., Thomsen, M.A. & Power, M.E. 2007. Species interactions reverse grassland responses to changing climate. *Science* 315: 640–642.

Vitousek, P. 2004. *Nutrient cycling and limitation: Hawaii as a model system*. Princeton University Press, Princeton.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H. & Tilman, D.G. 1997a. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7: 737–750.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. 1997b. Human domination of Earth's ecosystems. *Science* 277: 494–499.

Wang, Y.F., Yu, S.X. & Wang, J. 2007. Biomass-dependent susceptibility to drought in experimental grassland communities. *Ecology Letters* 10: 401–410.

Weltzin, J.F., Loik, M.E., Schwinning, S., Williams, D.G., Fay, P.A., Haddad, B.M., Harte, J., Huxman, T.E., Knapp, A.K., Lin, G.H., Pockman, W.T., Shaw, M.R., Small, E.E., Smith, M.D., Smith, S.D., Tissue, D.T. & Zak, J.C. 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. *Bioscience* 53: 941–952.

Zavaleta, E.S., Shaw, M.R., Chiariello, N.R., Thomas, B.D., Cleland, E.E., Field, C.B. & Mooney, H.A. 2003. Grassland responses to three years of elevated temperature, CO_2, precipitation, and N deposition. *Ecological Monographs* 73: 585–604.

Received 25 November 2008;
Accepted 27 April 2009.
Coordinating editor: A. Acosta.