

Biogeochemical and ecological feedbacks in grassland responses to warming

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Plant growth often responds rapidly to experimentally simulated climate change^{1,2}. Feedbacks can modulate the initial responses³, but these feedbacks are difficult to detect when they operate on long timescales⁴. We transplanted intact plant–soil mesocosms down an elevation gradient to expose them to a warmer climate and used collectors and interceptors to simulate changes in precipitation. Here, we show that warming initially increased aboveground net primary productivity in four grassland ecosystems, but the response diminished progressively over nine years. Warming altered the plant community, causing encroachment by species typical of warmer environments and loss of species from the native environment—trends associated with the declining response of plant productivity. Warming stimulated soil nitrogen turnover, which dampened but did not reverse the temporal decline in the productivity response. Warming also enhanced N losses, which may have weakened the expected biogeochemical feedback where warming stimulates N mineralization and plant growth^{1,5,6}. Our results, describing the responses of four ecosystems to nearly a decade of simulated climate change, indicate that short-term experiments are insufficient to capture the temporal variability and trend of ecosystem responses to environmental change and their modulation through biogeochemical and ecological feedbacks.

Rising greenhouse-gas concentrations are expected to increase global mean temperature by 2–7 °C this century with concomitant changes in precipitation worldwide⁷. Predicting future ecosystem carbon stocks and feedbacks to climate change requires an understanding of the dynamics of climate change effects on long-term plant productivity. Evidence for progressive impacts of global change on terrestrial ecosystem processes⁸ includes studies in which the stimulation of tree growth by increased CO₂ and N addition^{9,10} and of soil CO₂ production by warming¹¹ diminished over time. Long-term responses of aboveground net primary productivity (ANPP) to warming and altered precipitation are less clear. Short-term responses of ANPP to warming are generally neutral to positive^{1,2}, whereas increased precipitation usually stimulates ANPP and decreased precipitation reduces ANPP (ref. 2). However, most experiments have been of insufficient duration to establish a clear trajectory of change with time^{1,2}. Ecological interactions and biogeochemical feedbacks including progressive nutrient limitation^{12,13} and shifts in species composition^{3,14} that may occur only after many years could modulate the initial productivity response.

We examined the response of ANPP to climate change treatments (warming with decreased precipitation, warming with ambient precipitation and warming with increased precipitation) in four grass-dominated ecosystems along an elevation gradient in northern Arizona (Table 1 and Supplementary Table S1). In

2002, we initiated warming treatments by transplanting intact plant–soil mesocosms to adjacent lower and warmer elevations and transplanting control mesocosms into their native climates, so that treatments and controls experienced the same disturbance. We also manipulated precipitation using interceptors (–30%) and collectors (+50%, see Methods).

Across all ecosystems, warming initially increased ANPP and the effect varied over time (Fig. 1); the effects of the precipitation treatments were mostly non-significant (Supplementary Table S2). All combinations of warming and precipitation treatment initially increased ANPP, indicating that the positive effects of warming dominated the response. The stimulation of ANPP was positive for all years, but declined progressively over time (Fig. 1, Supplementary Tables S2, S3); by the ninth year, the effect of warming on ANPP was no longer significant (95% bootstrapped confidence interval, –7% to 24%).

Low ANPP in 2002 when the experiment was initiated was associated with a severe drought¹⁵, the most severe on record for this region¹⁶. Thus, the patterns we observed may reflect an unusual convergence of disturbance by drought and transplanting interacting with simulated climate change in unexpected ways, but we submit that these patterns provide a unique window into the functioning of semi-arid grassland ecosystems in a warmer and drier climate. We therefore assessed the temporal response of ANPP to warming in several ways to test its robustness. First, we found that the progressively declining relative effect of warming on ANPP (P_r) was apparent even after excluding the anomalously low-productivity drought year (2002) and the subsequent year (2003) (Supplementary Table S4). Second, we found that the absolute effect of warming on ANPP (P_a) declined over time as well (Supplementary Table S4), though the responses varied among ecosystems (Supplementary Table S5). Third, we found that the decline in the response of ANPP to warming was not caused by the sensitivity of plant growth to interannual variability in precipitation¹⁷: ANPP normalized by annual precipitation still exhibited a progressively declining response over nine years of climate change treatments (Supplementary Fig. S1). Droughts such as that experienced in 2002 are projected to occur more frequently in the southwestern USA, coinciding with higher temperatures¹⁶. Thus, the dynamics of ANPP that we observed throughout the course of this experiment may be representative of the transition to a drier and hotter southwestern USA (refs 16,18), amplifying the natural dynamics that occur with alternating drought (lower productivity) and recovery (higher productivity) periods.

We investigated three mechanisms that may have influenced the ANPP response to warmer temperatures. First, biogeochemical feedbacks. Warming can stimulate ANPP by enhancing soil nutrient mineralization^{1,11,19,20}, a mechanism that explains sustained increases in productivity in response to warming⁵. Alternatively,

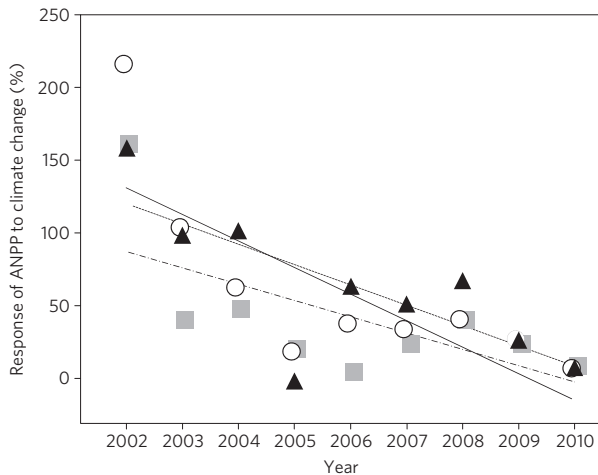
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Table 1 | Site characteristics of four grassland ecosystems along the C. Hart Merriam elevation gradient.

	Latitude	Longitude	Elevation (m)	Mean annual temperature* (°C)	Mean annual precipitation* (mm)
High desert grassland	35.58° N	−111.57° W	1,760	13.0	190.8
Pinyon-juniper woodland	35.50° N	−111.62° W	2,020	10.5	282.1
Ponderosa pine forest	35.42° N	−111.67° W	2,344	9.1	520.6
Mixed conifer forest	35.35° N	−111.73° W	2,620	6.6	661.2

*Based on weather station records at each site and gap-filled data from nearby weather stations for winter precipitation data from 2002 to 2010.

**Figure 1 | The relative responses of ANPP to climate change treatments from 2002 to 2010.** Responses are expressed as R_e (see Methods).

Responses declined by 11% per year for the warming with increased precipitation treatment (black triangle, $r^2 = 0.42$, solid line), by 14% per year for the warming with decreased precipitation treatment (grey square, $r^2 = 0.57$, dot-dash line) and by 18% per year for the warming with ambient precipitation treatment (white circle, $r^2 = 0.60$, dashed line). Bootstrapped estimates of confidence intervals for these slopes did not overlap zero (Supplementary Table S3), indicating significantly declining trends.

increased nutrient mineralization can promote N losses²¹, reducing N availability and ANPP over time²². To evaluate whether a warming-induced increase in soil N cycling redistributed N from soils to plants, increased losses, or both, we measured N and tracer ^{15}N (applied during year two of the experiment; see Methods) in plants and soils for all mesocosms in year ten. Second, changes in species composition. Shifts in species composition could contribute to the declining response of ANPP over time if more-productive species are replaced by less-productive species³ in response to warming. We evaluated changes in species composition and calculated the number of species lost and retained from the native community (see Methods). Third, reduced species richness. Plant productivity often declines as species richness declines^{23–25}, possibly because of niche complementarity and positive interactions among species^{24,25}. Such declines are most

apparent at low species richness²⁶, typical of the grasslands studied here, indicating the possibility that a decline in species richness could cause the progressively diminishing responses of ANPP that we observed. To evaluate these mechanisms, we included metrics of each in a series of multiple regressions and selected the best model using an information theoretic approach (see Methods, Table 2 and Supplementary Table S6).

The multiple regression results supported both biogeochemical and plant-community drivers of the progressive responses of ANPP to warming (Table 2, Supplementary Tables S5 and S6). More rapid soil N turnover, plant ^{15}N uptake and ^{15}N loss were associated with a slower decline in the ANPP response to long-term warming (Table 2 and Supplementary Fig. S2). Specifically, the positive response of ANPP to warming declined more slowly over time in treatments where warming reduced soil ^{15}N and soil N (Fig. 2a), as indicated by the negative coefficients in the regression model (Supplementary Table S6). Furthermore, where warming increased plant ^{15}N content, the positive response of ANPP to warming also declined more slowly over time (positive model coefficient for plant ^{15}N , Supplementary Table S6). These results are consistent with warming increasing rates of N cycling and thus promoting N availability and plant growth, as observed in a hardwood forest⁵. But in the herbaceous ecosystems studied here, low productivity and the absence of woody vegetation may have limited the capacity to take up mineralized N in plant biomass, reducing the strength of this positive feedback. If the increased N losses we observed (Supplementary Fig. S2) are sustained, the positive effect of temperature on increased N availability and plant growth should weaken further and may even reverse in the longer term (Fig. 3).

Biogeochemical feedbacks through N cycling had stronger statistical support for modulating the temporal response of ANPP to warming, but ecological feedbacks through altered community composition were also associated with the dynamics of the response. In all warmed ecosystems, plant species composition shifted towards communities typical of the warmer environments (Supplementary Fig. S3). These changes in community composition hastened the declining response of ANPP to warming, an influence opposing that of the biogeochemical mechanism discussed above. Specifically, the declining response of ANPP to warming was more apparent where species native to the original site were lost (Fig. 2b and Table 2) and where species richness declined (Supplementary Table S6). Communities of warmed ecosystems were significantly different from the control communities and shifted towards those

Table 2 | Multiple regression model describing the P_a response of ANPP to warming ($n = 62$).

Response	Driver	Coefficient	p -value	Adjusted R^2	Akaike weight (w_i)
P_a response of ANPP	Constant	1.44	0.022	0.95	0.438
	Plant ^{15}N	20.26	<0.001		
	Soil ^{15}N	−0.40	<0.001		
	Species loss	−1.48	0.046		

Linear coefficients (slopes), constants (intercepts) and associated p -values are shown, along with the adjusted R^2 and the w_i (see Methods).

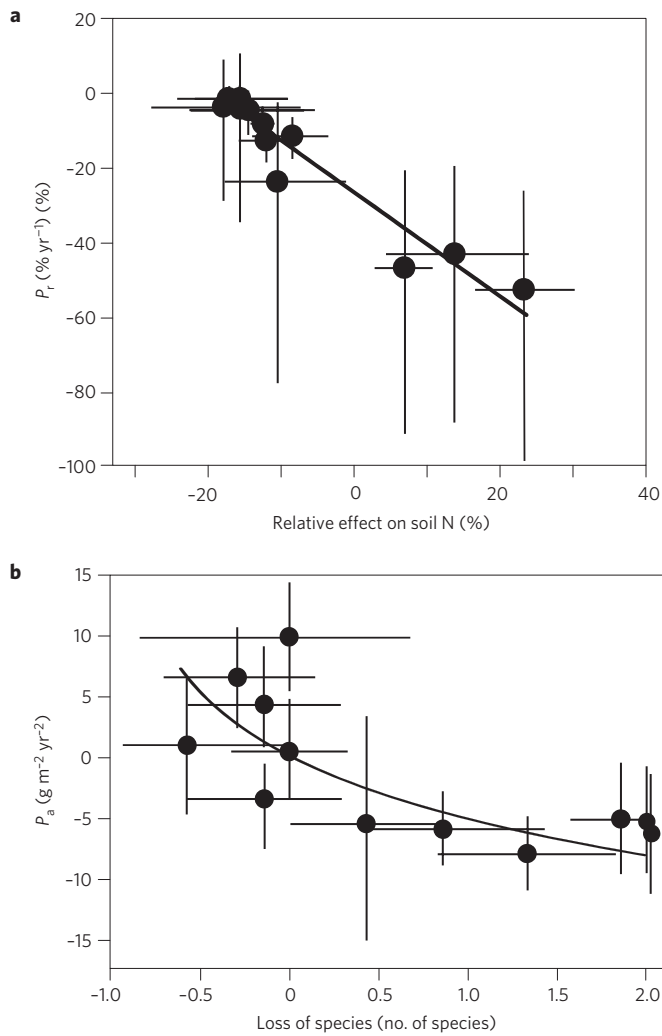


Figure 2 | Variables explaining the response of ANPP to warming treatments over time. **a**, The relative effect of the climate change treatments on soil N was inversely related to the P_r of the climate change treatments on ANPP (see Supplementary Table S6): $P_r = 139X - 27\%$, where X is the relative effect of treatment on soil N ($r^2 = 0.92$). This relationship was recapitulated in the P_a on soil ^{15}N and ANPP (Table 2). **b**, Effect of warming on loss of species native to the control site was associated with a declining P_a of ANPP (Table 2): $P_a = -7.31\ln(L + 0.97)$, where L is the number of species lost ($r^2 = 0.51$).

typical of the new environment (Supplementary Figs S3 and S4). Loss of species present in the native community could have been driven by changes in the abiotic environment such as soil moisture²⁷ or by biotic interactions²⁸. However, communities transplanted to lower-elevation sites still differed from communities native to the transplanted sites after nine years (Supplementary Figs S3 and S4), indicating that soil properties, microbial communities, or other factors influenced species composition. Although the mechanisms of community change remain unclear, our results indicate that where warming caused a loss of native species and reduced species richness, the positive response of ANPP to warming declined more rapidly (Fig. 2b). Our findings are consistent with past work invoking altered community composition as a key driver of ecosystem-scale responses to climate change^{4,14,29}.

More-rapid N cycling slowed the decline in the positive effects of warming on ANPP. That warming-enhanced N cycling did not sustain the initial increase in ANPP may have been caused by N losses from the plant–soil system and the low capacity of the

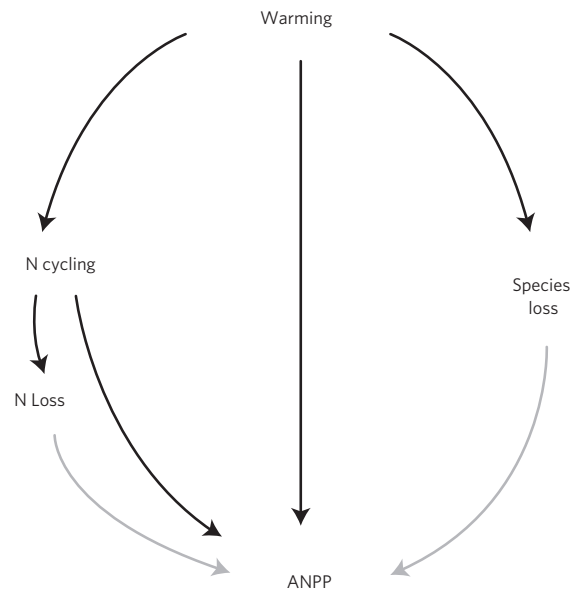


Figure 3 | Mechanisms accounting for progressive responses of ANPP to warming. Warming directly stimulates ANPP, but the response is subsequently modified by indirect effects through biogeochemistry (left side) and changes in the plant community (right side). Black arrows indicate positive effects and grey arrows indicate negative effects.

vegetation to capture and retain this extra N. Where warming did not stimulate soil N cycling and ^{15}N losses, the reduction over time in the stimulation of ANPP was more rapid, possibly indicating progressive N limitation³⁰. Thus, in contrast to predictions by some models^{6,31} that warming increases N availability and causes a sustained increase in plant productivity, our results indicate that faster N turnover could stimulate N losses and therefore potentially limit, or possibly even reverse, the early positive effect of warming on plant productivity. Loss of native species and declines in species richness also seemed to contribute to the declining response of ANPP over time, though support for these mechanisms was weaker than that for the biogeochemical feedbacks (Fig. 3). Together our results are consistent with the notion that biogeochemical feedbacks can manifest rapidly in climate change experiments, whereas ecological feedbacks such as species reordering tend to operate more slowly⁴. Thus, long-term climate change experiments are essential for understanding the strength, direction and relative importance of these feedbacks to ecosystem responses to climate change.

Methods

Mesocosm transplant experiment. Our study sites are located along the C. Hart Merriam elevation gradient in northern Arizona (Table 1 and Supplementary Table S1). Grass-dominated areas were selected in each of four ecosystems. In 2002, 40 mesocosms from each site were placed intact into 30 cm diameter \times 30 cm deep PVC cylinders, 20 were transplanted downslope to the next lower (and thus warmer) site as the warming treatment, whereas the other 20 mesocosms were transplanted within the native site as control groups (Supplementary Fig. S5). The precipitation treatments at each site consisted of a 50% increase, ambient and a 30% reduction of annual precipitation ($n = 6$ or 7) spanning projections of precipitation changes over North America³². For each mesocosm, precipitation was reduced using two clear acrylic channels (covering 30% of the mesocosm area) to intercept and drain precipitation away from the mesocosm. Precipitation inputs were increased using a plastic rain collector (50% of the mesocosm area) mounted outside the mesocosm and connected by polyethylene tubing to redirect intercepted rain to the mesocosm. We also added a rain collector for each transplanted mesocosm to compensate for lower annual precipitation with the warming treatment. Soil-moisture condition was not different between transplanted (warmed with ambient precipitation) and native (control) mesocosms (Supplementary Fig. S6).

ANPP and species composition. We took overhead photographs of each microcosm using a digital camera (Canon PowerShot A620, Canon USA, Lake Success, New York, USA) in October 2002, March 2003, from September to October

in 2003, from March to September in 2004, from April to September in 2005, April and September 2006, May and September 2007, from April to October in 2008, from May to August in 2009 and from May to September in 2010. We calculated the total percentage cover (fraction of the microcosm surface area covered by vegetation) from 2002 to 2010 and total percentage cover of individual species in 2010, using Adobe Photoshop (version 10.0.1, Adobe Systems, San Jose, California, USA). We estimated ANPP as the sum of early and late peak aboveground biomass using a total-percentage-cover—biomass regression (Supplementary Fig. S7).

Progressive effects. To estimate the effects of climate change treatments on ANPP, we calculated relative and absolute effect sizes to compare the treatment effects across years. The relative effect size (R_c) was calculated as:

$$R_c = (T - C)/C \times 100\%$$

where T and C are the means of treatment and control groups, respectively. The absolute effect size (A_e) was calculated as:

$$A_e = T - C$$

We calculated progressive effects as the slope of the relationship between treatment year and the effect of the climate change treatment on ANPP. P_t was calculated as the slope of the relationship between R_c and time, whereas P_a was calculated as the slope between A_e and time. Mean slopes and 5% and 95% confidence limits were determined using bootstrapping (1,000 iterations, Resampling Stats for Excel 4.0, Statistics.com LLC, Arlington, Virginia, USA).

N content. In 2003, 69 mg of $^{15}\text{NH}_4$ ($1 \text{ g } ^{15}\text{N m}^{-2}$) was sprayed with a handheld plant sprayer evenly over each mesocosm. In 2011, we harvested aboveground biomass and collected soil samples (0–20 cm depth) from mesocosms at all sites. Plant and soil samples were oven dried for 24 h (105°C for soil, 60°C for plant) and $\delta^{15}\text{N}$ and %N were determined on a Carlo Erba NC2100 Elemental Analyser connected to a Thermo Electron Delta V isotope-ratio mass spectrometer.

Species composition. We determined species composition in each mesocosm in 2010. Species native to the original site were determined as those present in the control mesocosms. The number of species lost in the warming treatments was calculated for each mesocosm as:

$$\bar{N}_c - N_i$$

where \bar{N}_c is the median number of species in the control treatment and N_i is the number of species native to the control site still present in the climate change treatment. The number of new species gained from the new environment was also enumerated for each transplanted mesocosm. Species richness was determined in all mesocosms and the relative response calculated as above.

Regression and model selection. We examined biogeochemical and plant-community drivers of the response of ANPP through time, including soil N, soil ^{15}N , plant N, plant ^{15}N , species richness, native species loss and new species gain. We calculated A_e for all response variables. We calculated R_c where possible, for all variables except species loss and species gain, where the value in the control treatment was zero. We built regression models for all possible combinations of driver variables to explain the P_a and P_t responses of ANPP to warming over time and evaluated them using information-theoretic model selection procedures³³ (see Supplementary Information).

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Author contributions

B.A.H. conceived the study. Z.W. and P.D. collected the data. Z.W. and B.A.H. analysed the data and drafted the paper. P.D. and G.W.K. helped with result interpretation and writing.

Additional information

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