

RESEARCH REVIEW

Global-change drivers of ecosystem functioning modulated by natural variability and saturating responses

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Abstract

Humans are altering global environment at an unprecedented rate through changes in biodiversity, climate, nitrogen cycle, and land use. To address their effects on ecosystem functioning, experiments most frequently explore one driver at a time and control as many confounding factors as possible. Yet, which driver exerts the largest influence on ecosystem functioning and whether their relative importance changes among systems remain unclear. We analyzed experiments in the Patagonian steppe that evaluated the aboveground net primary production (ANPP) response to manipulated gradients of species richness, precipitation, temperature, nitrogen fertilization (N), and grazing intensity. We compared the effect on ANPP relative to ambient conditions considering intensity and direction of manipulations for each driver. The ranking of responses to drivers with comparable manipulation intensity was as follows: biodiversity > grazing > precipitation > N. For a similar intensity of manipulation, the effect of biodiversity loss was 4.0, 3.6, and 1.5, times larger than N deposition, decreased precipitation, and increased grazing intensity. We interpreted our results considering two hypotheses. First, the response of ANPP to changes in precipitation and biodiversity is saturating, so we expected larger effects when the driver was reduced, relative to ambient conditions, than when it was increased. Experimental manipulations that reduced ambient levels had larger effects than those that increased them. Second, the sensitivity of ANPP to each driver is inversely related to the natural variability of the driver. In Patagonia, the ranking of natural variability of drivers is as follows: precipitation > grazing > temperature > biodiversity > N. So, in general, the ecosystem was most sensitive to drivers that varied the least. Comparable results from Cedar Creek (MN) support both hypotheses and suggest that sensitivity to drivers varies among ecosystem types. Given the importance of understanding ecosystem sensitivity to predict global-change impacts, it is necessary to design new experiments located in regions with contrasting natural variability and that include the full range of drivers.

Keywords: biodiversity loss, climate change, ecosystem sensitivity, land-use change, nitrogen deposition

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Introduction

Ecosystems are exposed to human-induced drivers of global change, such as increased temperature and nitrogen (N) deposition as well as alterations in biodiversity and land use (Steffen *et al.*, 2015). These drivers exert different influences on the ecosystems, and the ranking of the importance of drivers of change differs across biomes (Sala *et al.*, 2000). Which driver exerts the largest influence on the functioning of each ecosystem type is relevant for understanding the consequences of

global change and ultimately designing adaptation strategies (Scheffer *et al.*, 2015). Or, in other words, which ecosystems are most sensitive to any single driver?

To address the effects of global-change drivers on ecosystem functioning, experiments most frequently explore one driver at a time and control as many other potential confounding factors as possible. For example, experiments simulate N deposition by fertilizing natural plots and using unfertilized plots as controls (Chung *et al.*, 2007). Similarly, changes in precipitation are commonly simulated by precipitation interception (Yahdjian & Sala, 2006) combined with irrigation (Heisler-White *et al.*, 2009; Reichmann *et al.*, 2013). The

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effects of changes in biodiversity are usually assessed by planting experimental plots with different number of species (Tilman *et al.*, 1996) or through species removal (Flombaum & Sala, 2008). Multifactorial experiments that manipulate several variables (Reich *et al.*, 2001; Shaw *et al.*, 2002; Boyero *et al.*, 2014) are more powerful although less frequent than single-variable experiments. Analysis of single-variable experiments that were performed in the same location complement results from multifactorial approaches. To disentangle which driver of global change exerts the largest influence on ecosystem functioning, it is necessary to compare the results of different experimental manipulations. A way to achieve this comparison has been to perform syntheses of ecological experiments by reviewing, integrating, and summarizing a large number of independent studies (Wu *et al.*, 2011; Hooper *et al.*, 2012), so the different drivers of global change can be compared using a statistical approach (Arnqvist & Wooster, 1995; Hedges *et al.*, 1999; Crain *et al.*, 2008). However, issues of variation among ecosystems cannot be controlled, and the different experimental methodologies involved in each individual study usually limit the strength of the results of meta-analysis (Fraser *et al.*, 2012). For instance, the differences between the manipulation intensity of each driver among experiments (e.g., 90% species losses against 30% precipitation reductions) are usually not controlled in meta-analyses, introducing a potential unaccounted source of uncertainty when different experiments are compared (Hooper *et al.*, 2012).

Another alternative for assessing the effects of multiple drivers has been the comparison of different experiments performed in the same site, with the advantage of sharing the same natural conditions (i.e., climate, soil, and plant species; (Tilman *et al.*, 2012; Hautier *et al.*, 2015). For instance, the comparison of different global-change experiments performed in Cedar Creek (MN, USA) revealed that biodiversity loss had larger effects on primary production than N addition and drought (Tilman *et al.*, 2012). Reduction in species richness from 16 to one species had the largest effect among all other treatments, followed by the addition of 95 kg N ha⁻¹ yr⁻¹ (Tilman *et al.*, 2012).

The question that remains unanswered is the generality of these results. Specifically, is the hierarchy of drivers related to the intensity and direction of the manipulation? Does the hierarchy of drivers vary among ecosystem types or is it universal? Are differences among the responses of drivers related to the natural variability of each driver in each ecosystem?

Grasslands are affected by four drivers of global change: climate change, land-use change, biodiversity change, and increased reactive nitrogen (Sala *et al.*,

2000). Grasslands, shrublands, and savannas cover 40% of the Earth's land surface and are characterized by low levels of precipitation that ranges from 150 to 1200 mm yr⁻¹ (Reynolds *et al.*, 2007). Along this precipitation gradient, interannual variability of precipitation decreases from the arid to the humid end (Knapp *et al.*, 2015). Projections of climate change in grasslands include increases in temperature and alterations of the precipitation regime, with decreases or increases depending on the region (Hartmann *et al.*, 2013). Land-use change in grasslands varies along a precipitation gradient with humid grasslands usually being replaced by crops and affected by fire (Briggs *et al.*, 1998), whereas arid grasslands, which cannot support agriculture and show small fuel accumulation, are affected by grazing of different intensities (Golluscio *et al.*, 2015). Biodiversity change in grasslands results in the local extinction of native species that can or cannot be accompanied by invasion of alien species (Milchunas & Lauenroth, 1993). Finally, grasslands are subjected to increases in N availability as a result of anthropogenic N deposition, which varies according to the location of sites relative to pollution sources (Reay *et al.*, 2008).

Grasslands are the ideal model ecosystem for global-change research because of the small size and short life span of grasses that made experimental approaches feasible. Manipulative experiments explored the effect of changes in precipitation, temperature (see Wu *et al.*, 2011 for a revision of these experiments), CO₂, N deposition (Reich *et al.*, 2001; Shaw *et al.*, 2002), and biodiversity (Tilman *et al.*, 1996; Hector *et al.*, 1999; Flombaum & Sala, 2008; Wilsey *et al.*, 2009). To address which global-change driver is the most influential, a single site with multiple experimental manipulations can provide key evidence as mentioned earlier for Cedar Creek (Tilman *et al.*, 2012). The Patagonian steppe shares with Cedar Creek the dominant plant life forms and a long history of manipulative experiments in global-change drivers (Adler *et al.*, 2005; Yahdjian & Sala, 2006; Flombaum & Sala, 2008; Yahdjian *et al.*, 2014), but has lower annual precipitation.

The objectives of this study were (i) to evaluate the relative effect of the main global-change drivers on the functioning of the Patagonian steppe ecosystem and (ii) to explore the mechanisms behind the different ecosystem sensitivity to each driver. First, we proposed two hypotheses to explain the response of ecosystem functioning to global-change drivers. Second, we synthesized existing manipulative field experiments performed in a single study site in the Patagonian steppe. We compared the ecosystem responses taking into account the magnitude of the manipulation imposed and using averaging tools from meta-analysis. We compared the effect of reduced and increased

precipitation (Yahdjian & Sala, 2006), reduced biodiversity (Flombaum & Sala, 2008), increased N availability (Yahdjian *et al.*, 2014), increased grazing intensity (Adler *et al.*, 2005), and increased temperature (Roset, 2000) on aboveground net primary production (ANPP) of the Patagonian steppe (Table 1). We used a framework that included the intensity of both ecosystem responses and global-change drivers. To compare the effects on ANPP, we used the response ratio (the ratio of mean outcome in the manipulated group to that in the control group; lnRR) (Hedges *et al.*, 1999). To compare the intensity of the manipulation of each driver, we used the manipulative ratio (the ratio of the treatment level to that in the control; lnMR). To assess the ecosystem sensitivity to drivers relative to the natural variability of each driver, we used available time series for climate drivers and assessments of the spatial variability for all other drivers. Finally, we compared our results against those reported from a similar synthesis from a mesic grassland in the Cedar Creek experimental site (Tilman *et al.*, 2012).

Hypotheses for the effects of global-change drivers

The effect of increasing global-change drivers such as precipitation and biodiversity relative to ambient conditions is smaller than the effect of reducing those drivers

Grassland ANPP shows a saturating response to precipitation, biodiversity, and N, with a steep response at low levels of the driver and gradually diminishing responses at high levels (Aber *et al.*, 1989; Tilman *et al.*, 1997; Huxman *et al.*, 2004; Sala *et al.*, 2012). Under extreme conditions, water logging can cause a decrease in ANPP yielding a hump-shaped relationship between

precipitation and ANPP. This phenomenon occurs in rare locations and occasions in grasslands (O. Mckenna & O.E. Sala, under review). Temperature and grazing depict a unimodal relationship with optimal levels at which ANPP and plant growth are maximal (Milchunas & Lauenroth, 1993; Sage & Kubien, 2007). Thus, resources (water and N) and biodiversity experiments that reduce the level of a driver (e.g., drought, or species loss experiments) have higher probability to be included in the steep portion of the ANPP/driver relationship, while those increasing the level of a driver (e.g., irrigation experiments) have higher probability to be included in the flat portion of the curve. Biodiversity experiments typically reduce the number of species relative to the ambient condition, while temperature and N deposition increase ambient conditions through warming and fertilization experiments. Finally, precipitation change experiments include both (irrigation and drought).

Global-change drivers with the highest impact are those that experience lowest level of natural variability

This hypothesis is based on the idea that the natural variability of global-change drivers varies among drivers and among ecosystem types. Ecosystem made up of species that evolved under stable conditions for one driver and variable conditions for others would be most sensitive to changes in the driver that historically has been the most stable. The rationale for this mechanism is that organisms that evolved under a changing environment form communities that are more resilient than those communities composed of species that evolved under a constant environment. High environmental variability in space or time will allow survival of species adapted to broad environmental conditions.

Table 1 Manipulative experiments in the Patagonian steppe used in this study. The manipulated level underlined is the ambient condition that was used as the control treatment

Global-change driver	Type of manipulative experiment	Variable used to quantify the intensity (units)	Ambient condition	Manipulated levels	Replicates per level	Year	Source
Climate: precipitation	Rain-out shelter	Annual precipitation (mm yr ⁻¹)	150	-80%, -50%, -30%, <u>0%</u>	10	2001	Yahdjian & Sala (2006)
	Watering		170	+50%, <u>0%</u>	5	2002	
Biodiversity	Plant-species removal	Plant-species richness (number)	6	1, 2, 4, <u>6</u> species	18, 30, 30, 6	2003	Flombaum & Sala (2008)
Nitrogen deposition	Fertilization	Nitrogen availability (g N m ⁻²)	5.4	<u>Ambient</u> , +5 g N	10	2006	Yahdjian <i>et al.</i> (2014)
Land use: grazing intensity	Natural gradient	Forage consumption (g m ⁻² yr ⁻¹)	0	High, low, <u>exclosure</u>	6	2000–01	Adler <i>et al.</i> (2005)
Climate: temperature	Open-top chambers	Annual temperature (°C)	8.0	<u>Ambient</u> , +T (0.58 °C)	10	1999	Roset (2000)

For example, enhanced precipitation variability results in high plant-species diversity because the differential nonlinear response of plant species to precipitation (Chesson *et al.*, 2004). So, a hypothetical community from a high precipitation location may include species adapted to wet and dry conditions. This diverse community, from the point of view of traits that confer drought tolerance and ability to utilize wet periods, will be more resilient to changes in precipitation. However, traits associated with precipitation variability not always confer resilience to other environmental factors. This hypothetical community, which is diverse in water availability traits, may not be diverse in terms of traits associated with N availability. Finally, increased diversity results in an increased resilience in what is known as the portfolio effect (Tilman *et al.*, 1997). Sensitivity to global-change drivers among ecosystem types will be inversely related to the natural variability of each factor in each ecosystem type. Evidence suggests that ecosystem types respond differently to the variability of precipitation, temperature, and cloudiness (Seddon *et al.*, 2016).

Study site and manipulative experiments

The study site, the Rio Mayo experimental station in the Patagonian steppe, Argentina (lat 45°41'S, long 70°16'W), is a long-term ecological research site representing a broad ecosystem distributed along the southern portion of Argentina. The climate is semiarid, with mean annual precipitation of 170 mm and mean annual temperature of 8 °C. Vegetation is dominated by six species, three grasses and three shrubs, which account for 95% of ANPP (Jobbágy & Sala, 2000) and evolved under a short history of grazing with almost absence of large herbivores (Adler *et al.*, 2005).

Biodiversity: plant-species richness

The biodiversity gradient consisted in 1, 2, 4, and 6 plant species with 6, 15, 15, and 1 possible species combination replicated 3, 2, 2, and 6 times totalizing 84 plots (Flombaum & Sala, 2008). All 84 plots initially contained the six dominant plant species. The gradient was generated by species removal while maintaining constant biomass across treatments. Species richness and composition treatments were randomly assigned, and target species were removed. To control for vegetation cover as a confounding factor, the experiment initially equalized this variable along the plant-species richness gradient by removing portions of the species remaining in the plots. Thus, plots differed in the number and composition of species, but had the same initial cover and physical environment characteristics

(Flombaum & Sala, 2008). Plots were 25 m² and contained four fixed parallel lines of 5 m on which vegetation cover per species was recorded yearly. Vegetation cover was estimated using the line-intercept method, recording the crowns that intercepted the line to the nearest 1 cm. Species cover was converted to biomass employing specific allometric-calibration curves constructed for this study site (Flombaum & Sala, 2007). Vegetation cover by species at peak biomass is a good estimator of aboveground net primary production (ANPP) in ecosystems with pronounced seasonality (Sala & Austin, 2000). For further detail, see Flombaum & Sala (2008).

Climate: precipitation

Two experimental precipitation gradients were included in this study. The first was a drought experiment performed during two consecutive years where ambient precipitation was reduced by -80, -50, -30, and 0% (control) in natural vegetation plots using rain-out shelters (Yahdjian & Sala, 2006). Rain-out shelters intercepted different percentages of precipitation with different number of transparent acrylic shingles. The transparent acrylic bands were located over the canopy and intercepted a negligible amount of light (Yahdjian & Sala, 2002). The number of replicates per treatment was 10, and response variables were measured in the center of 3.8-m² plots. The second experiment was a watering experiment, where annual precipitation was increased by 0 (control) and 50% with two pulse water additions during the growing season (Yahdjian & Sala, 2006). The watering experiment was performed in the same set of plots than the drought experiment; thus, 0 and 50% watering treatments were overimposed over the four drought treatments in a factorial design. Here, we considered only the watering treatments that were overimposed on 0% interception drought plots to avoid confounding legacy effects, and consequently, for the watering experiment, there were five replicates (Yahdjian & Sala, 2006). Experimental precipitation plots were 3.8 m² and were centered on an average-size shrub (*Mulinum spinosum*), and all contained dominant tussock grasses. Vegetation cover was estimated during peak biomass in two 2.7-m perpendicular lines and later converted to ANPP using calibration regressions performed for each dominant species in this study site as explained above (Flombaum & Sala, 2007). For further detail, see Yahdjian & Sala (2006).

Climate: temperature

Temperature increase was achieved with open-top chambers (2.6 m² and 30 cm height) of transparent

acrylic (Roset, 2000; Henry & Molau, 1997). The experiment included ten replicates for increased temperature and control plots of similar area. The plots contained a mean-size tussock of the dominant species, *Pappostipa speciosa*. Temperature was measured in five plots using thermocouples NTS at 5 cm above soil surface avoiding direct solar radiation. Aboveground net primary production was estimated using a radiometer (SKYE SKR 100/110) and converted to green biomass with specific regressions developed for the study site. Measurements were obtained at the peak of green biomass (January). For further details, see Roset (2000).

Nitrogen deposition: fertilization

The N fertilization experiment was performed during two consecutive years. N deposition usually increases N availability, which was simulated by a N fertilization experiment. The experiment included two treatment levels, plots fertilized with 5.0 g N m⁻² yr⁻¹ and controls, with ten 9-m² plots per level. Fertilizer was applied as NH₄NO₃ diluted in 2 l of water (equivalent to a 0.22 mm rain pulse) uniformly distributed on the soil surface with a sprayer in three application events during the growing season, in October, December, and January. Nonfertilized plots received similar amount of water only, applied with the same protocol. Plots were 9 m² and contained two parallel 3-m lines on which vegetation cover was recorded during peak biomass and later converted to ANPP with the same methodology described above. Plots contained natural vegetation that included the dominant shrubs and grasses of the study site. For further detail, see Yahdjian *et al.* (2014).

Land use: grazing intensity

Sheep grazing is the major land use in the Patagonian steppe. Sheep spatial distribution and hence grazing intensity are controlled by the location of drinking water, which could be natural or artificial. The longer the distance to the water source, the lower the grazing intensity. The experiment we included here used a natural grazing intensity gradient defined by the proximity to water sources (Adler *et al.*, 2005). The experiment included three levels of grazing intensity, high, low, and no grazing located at 500 m, 1000 m, and inside an enclosure (no livestock). Here, we used forage consumption as an indicator of grazing intensity (Table 1). We considered the enclosure as the control level because of the low grazing history of Patagonia (Adler *et al.*, 2005). Three independent and permanent water sources were used in the study site and enclosures older than 20 years. For each grazing intensity level, forage consumption was evaluated in 100-m² plots as

the difference of production in temporally ungrazed plots minus grazed. ANPP was estimated by direct harvesting within the plots. For further details, see Adler *et al.* (2005).

Response and manipulative ratios

We estimated the response ratio as the natural logarithm of the ratio between ANPP of the manipulated level and its control (lnRR) (Hedges *et al.*, 1999). Similarly, the manipulative ratio (lnMR) was estimated as the natural logarithm of the ratio between the manipulated level and its control (Table 1). The lnMR and lnRR indexes are set under the same logic that is to use the value for the control treatment as the reference. The lnRR calculates the response of ANPP under a treatment relative to ANPP under ambient conditions. The lnMR evaluates the intensity and direction of the treatment (e.g., reduced precipitation) relative to ambient conditions (e.g., ambient precipitation). The use of lnMR and lnRR allowed us to compare changes in ANPP among different experimental studies in a unit-less scale and in a common framework that considered the intensity and direction of the manipulation relative to a control treatment. To estimate the ranking of responses to drivers, we compared the mean absolute change in lnRR among drivers. We used the absolute change to avoid confounding differences in lnRR sign as a result of reducing or increasing ambient levels, with difference in response as a result of the sensitivity of ANPP to the driver. Specifically, for each driver, we averaged the module of lnRR across manipulated levels and estimated its variance as the sum of the variance of lnRR for each manipulated level. Finally, differences among lnRR were tested using the Tukey–Kramer test. For each driver, the variance of lnRR for the manipulated level-*i* was calculated as the sum of two terms: (i) ANPP variance-*i* divided by ANPP mean-*i* and sample size-*i* and (ii) the same ratio for the control (Eqn 1) (Hedges *et al.*, 1999).

$$\text{varlnRR}_i = (\text{varANPP}_i / \text{meanANPP}_i n_i) + (\text{varANPP}_{\text{control}} / \text{meanANPP}_{\text{control}} n_{\text{control}}) \quad (\text{Eqn 1})$$

Natural variability of global-change drivers

To estimate the natural variability of the drivers, we used the coefficient of variation (CV) that compares variability independently of mean values. Mean and standard deviation for annual precipitation and temperature were obtained from data from an automated

meteorological station during 1984–2005 adjacent to the experimental site. Mean and standard deviation for plant-species richness were obtained from 24 1-m² plots reported in Adler *et al.* (2005). Adler *et al.* (2005) calculated forage consumption based on stocking rates and reported a range for forage consumption that we assumed represented a 95% confident interval. Nitrogen CV was estimated for the sum of soil nitrate and ammonium availability for the top 5 cm using 10 replicates (Yahdjian *et al.*, 2006). We performed six pairwise *t*-test with $\alpha' = 0.008$ to keep a global error level of $\alpha = 0.05$. Climate data for Cedar Creek were available from meteorological station for the period 1963–2011 (www.lter.edu).

Ranking of responses to drivers

The ranking of responses to global-change drivers among the studies from the Patagonian steppe was as follows: biodiversity > grazing > precipitation > nitrogen (Fig. 1). Biodiversity change was the driver that exerted the largest effect on ANPP, followed by grazing, precipitation, N deposition, and temperature (Fig. 1). Statistical analysis ($\alpha = 0.05$) shows that biodiversity has a larger lnRR than grazing, and grazing than precipitation, N and temperature although the latter three were not different. For a similar reduction of the driver ($\ln\text{MR}_{2 \text{ species}} = -1.10$; $\ln\text{MR}_{\text{high grazing}} = -0.81$; $\ln\text{MR}_{-80\% \text{ precipitation}} = -1.25$), the effect on ANPP for

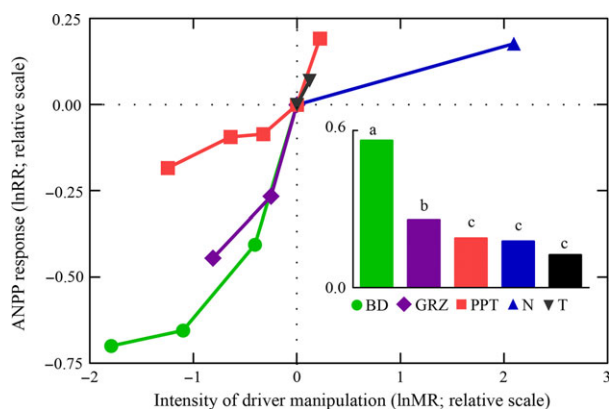


Fig. 1 Relative effect of global-change drivers on aboveground net primary production (ANPP) in the Patagonian steppe. The intensity of the manipulation was calculated as the natural logarithm of the ratio between the manipulated level and its control ($\ln\text{MR}$). The ANPP response under manipulated level conditions and its control was calculated as the natural logarithm of the ratio between ANPP under manipulated conditions and control ($\ln\text{RR}$). The inset shows the mean of the absolute $\ln\text{RR}$ for biodiversity change (BD), grazing intensity (GRZ), precipitation (PPT), N fertilization (N), and temperature (T). Different letters indicate statistical differences (*t*-test; $\alpha' = 0.005$).

biodiversity was 1.5 and 3.6 times higher than for grazing intensity and precipitation ($\ln\text{RR}_{2 \text{ species}} = -0.65$; $\ln\text{RR}_{\text{high grazing}} = -0.44$; $\ln\text{RR}_{-80\% \text{ precipitation}} = -0.18$). Comparing similar intensities of driver changes but with opposite direction ($\ln\text{MR}_{1 \text{ species}} = -1.79$; $\ln\text{MR}_{+5\text{gN}} = 2.10$), the effect of biodiversity reductions on ANPP was 4.0 times higher than that of N addition ($\ln\text{RR}_{1 \text{ species}} = -0.70$; $\ln\text{RR}_{+5\text{gN}} = 0.18$). Nitrogen was the driver most intensely manipulated in the selected studies; however, its effect was relatively low (Fig. 1). The intensity of reducing species richness from six to one would be comparable to an 84% reduction in annual precipitation with a probability of occurrence of 0.02% for this site (based on meteorological data). The increased temperature had a negligible effect on ANPP ($\ln\text{RR}_{+T} = 0.07$), but experimental change in temperature was the smallest ($\ln\text{MR}_{+T} = 0.13$), representing only 7.3% above ambient condition.

Saturating responses

Our interpretation of the ranking of responses to global-change drivers is based on the two hypotheses described above. The saturating response of ecosystem processes to some drivers explains, in our case, why ANPP responses to reduction in biodiversity and precipitation had a larger effect than increases in nitrogen. As a consequence, drivers that experienced a reduction relative to the ambient condition had higher chance to produce an effect on ANPP, while the driver that experienced an increment relative to the ambient condition had lower chance to have an effect. Experiments assessing the effect of biodiversity change on ANPP usually compare control conditions vs. treatments with lower number of species (Flombaum & Sala, 2008; Hooper *et al.*, 2012; Tilman *et al.*, 2012). In the Patagonian steppe, biodiversity loss was simulated by reducing from six to one plant-species richness. This large manipulative reduction was accompanied by a large decrease in ANPP (Flombaum & Sala, 2008). On the contrary, change in N availability was simulated by a fertilization experiment, where ambient condition was increased 8.1 times (Yahdjian *et al.*, 2014). Even if the manipulation of plant-species richness and fertilization had been similar in intensity, the effect of N addition on ANPP was much lower than that of loss of biodiversity (Fig. 1).

Biodiversity loss also had a higher effect than N addition in a N-limited grassland, the Cedar Creek experimental site, USA (Tilman *et al.*, 2012). Biodiversity reduction from 16 to one plant species was similar in treatment intensity as adding 95 kg N ha⁻¹ yr⁻¹ ($\ln\text{MR}_{1 \text{ species}} = -2.77$; $\ln\text{MR}_{+95 \text{ kg N}} = 2.82$); however, the effect of plant-species richness on ANPP was 1.76

higher than N fertilization ($\ln RR_{1 \text{ species}} = -0.95$; $\ln RR_{+95 \text{ kg N}} = 0.54$; Fig. S1) (Tilman *et al.*, 2012). Patagonia and Cedar Creek presented lower effects on ANPP by increasing than reducing levels of the global-change drivers, biodiversity, N, and precipitation (Table 2). In both ecosystems, the average intensities of addition and subtraction were similar, but the effects on ANPP were nearly half for experimental increases than reductions of ambient conditions (Table 2). Together, these results suggest that biodiversity may have larger effect on ANPP than other drivers in part because the reduction of species reflected the steep portion of the saturating curve to ANPP (Tilman *et al.*, 1996; Hector *et al.*, 1999; Flombaum & Sala, 2008).

Natural variability

Ecosystem responses to the different drivers may be the result of differences in the natural variability of each environmental factor. The ranking of variability of global-change drivers in the Patagonian steppe is precipitation > grazing > temperature > biodiversity > nitrogen (Fig. 2). The CV for mean annual precipitation was 1.3 and 2.3 times higher than for sheep forage consumption and plant-species richness ($CV_{\text{precipitation}} = 25\%$; $CV_{\text{grazing}} = 17.9\%$; $CV_{\text{biodiversity}} = 10.6\%$; Fig. 2). Among drivers that were subjected to experimental reductions (biodiversity, grazing, and precipitation), precipitation had the highest natural variability and showed the lowest ecological sensitivity (i.e., the higher CV and the lower $\ln RR$). On the contrary, biodiversity was the driver with lowest natural variability and the one that had the highest ecological sensitivity (Figs 1. and 2). Grazing intensity was intermediate in both aspects. Among the drivers that were subjected to additions, N had low CV and low $\ln RR$ suggesting that the saturating response can be much important than the response to natural variability.

In the Patagonian steppe, most grasses and all shrubs are perennial and long-lived conferring low temporal

and spatial variability to species richness (Aguilar & Sala, 1994, 1999). The low natural variability of plant-species composition could be the result of the low number of redundant species suggested by the highly differentiated use of resources in space and time (Sala *et al.*, 1989; Flombaum & Sala, 2012); and consequently, the loss of species left unused resources with a significant reduction in ANPP (Flombaum & Sala, 2008). In contrast, annual precipitation in the Patagonian steppe is highly variable with a coefficient of variation of 25% based on 20 years of data. Plant species are adapted to scarce and variable water resources, and, as a result, ANPP was buffered to changes in precipitation (Yahdjian & Sala, 2006). The species adaptation to variable water availability resulted in a lower than expected effect of dry and wet years on ANPP (Yahdjian & Sala, 2006; Sala *et al.*, 2012).

A prediction of this hypothesis is that the sensitivity of ecosystems to precipitation change increases with decreasing long-term natural variability, which in turn tend to increase with mean annual precipitation (Knapp *et al.*, 2015). Therefore, we expect mesic grasslands like Cedar Creek to be more sensitive to changes in precipitation than an arid ecosystem like the Patagonian steppe. In fact, in Cedar Creek, an extreme drought that reduced average precipitation by 50% had a larger effect than a reduction of biodiversity of the same magnitude (Tilman *et al.*, 2012). In contrast, in the Patagonian steppe where precipitation variability is naturally large, a similar 50% reduction for these same drivers showed a larger effect of biodiversity than drought on ANPP (Fig. S1).

Conclusions

The saturating hypothesis highlights the necessity to consider the intensity and direction of manipulation when comparing multiple drivers. This hypothesis can potentially account for an unrecognized source of variation when trying to identify which driver exerts the

Table 2 Effect on ANPP of increased vs. reduced environmental conditions relative to ambient. Pooled experiments for the Patagonian steppe (Argentina) and Cedar Creek (MN, USA, Tilman *et al.*, 2012). For similar manipulation ratios, the response ratio when the driver was reduced was almost double of the response ratio for increased values for both study sites. Values represent mean \pm 1 SE.

Study site	Changes from ambient condition	Driver	Manipulative ratio ($\ln MR$)	Response ratio ($\ln RR$)
Patagonia	Increased	N deposition, temperature, precipitation	0.80 ± 1.13	0.16 ± 0.04
	Reduced	Biodiversity, precipitation, grazing intensity	-0.82 ± 0.53	-0.35 ± 0.24
Cedar Creek	Increased	N deposition, temperature, CO ₂ concentration, precipitation	1.57 ± 1.11	0.35 ± 0.15
	Reduced	Biodiversity, precipitation	-1.73 ± 0.89	-0.60 ± 0.24

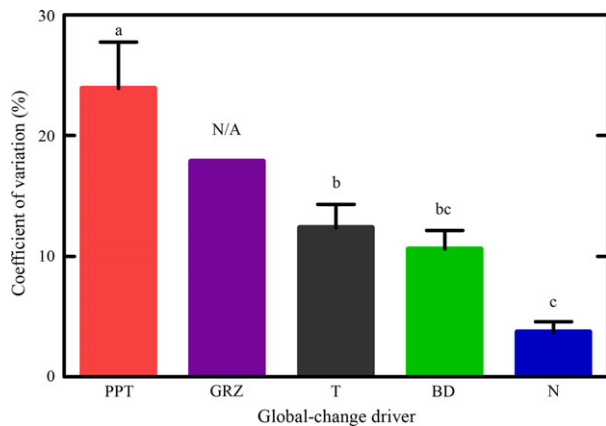


Fig. 2 Comparison of natural variability among global-change drivers in the Patagonian steppe. The variability was standardized by the mean using the coefficient of variation. References: precipitation PPT; grazing intensity GRZ; temperature T; biodiversity BD; nitrogen deposition N. Letters indicate significant differences (*t*-test, $\alpha = 0.008$); N/A = nonavailable data.

largest influence on ecosystem functioning. Most of the experiments are constrained by the feasibility of manipulations. Therefore, there are many more experiments in which nitrogen has been added than subtracted and there are more studies of species deletions than additions (Yahdjian *et al.*, 2011; Tilman *et al.*, 2012; Isbell *et al.*, 2015). For precipitation change, there are more studies of irrigation than precipitation reduction (Wu *et al.*, 2011). In addition, our understanding of interactions among drivers is limited by the number of multifactorial experiments (Reich *et al.*, 2001; Shaw *et al.*, 2002; Boyero *et al.*, 2014). These experimental constraints have yielded an unbalance understanding of the response surface of most ecosystem processes to changes in global-change drivers. We are hopeful that the ingenuity of the ecological community will come up with new experimental designs that solve the current experimental imbalance.

Our suggestions about sensitivity to global-change drivers are based on results from experiments from two sites with different patterns of resource availability and variability. Further test of the sensitivity hypothesis will be necessary using sites that naturally have complementary patterns of resource variability. The influence of climate variability on ecosystem functioning has been recently recognized as a relevant control on primary production (Gherardi & Sala, 2015a,b), and together with the variability of temperature and cloudiness influence the sensitivity of primary production (Seddon *et al.*, 2016). Strategic location of experiments in variability space or the direct manipulation of this aspect will allow for testing of this hypothesis.

The impact of global change on ecosystem functioning results from the rate of change in

global-change drivers and ecosystem sensitivity to each driver (Sala *et al.*, 2015). Therefore, predictions about the future of ecosystems and their ability to provide goods and services depend on our understanding of both sensitivity and rate of change. The former is the domain of ecological disciplines, while the latter falls in the territory of atmospheric and social sciences. Results of this article contribute directly to addressing the ecosystem sensitivity to global-change drivers.

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Supporting Information

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

Figure S1. Relative effect of global-change drivers on above-ground net primary production in the Patagonian steppe (Argentina) and Cedar Creek (MN, USA).