Ecological Monographs, 87(3), 2017, pp. 457–469
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Exacerbated nitrogen limitation ends transient stimulation of grassland productivity by increased precipitation

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Abstract. Given that plant growth is often water-limited in grasslands, it has been proposed that projected increases in precipitation could increase plant productivity and carbon sequestration. However, the existing evidence for this hypothesis comes primarily from observational studies along natural precipitation gradients or from short-term manipulative experiments. It remains unclear whether long-term increased precipitation persistently stimulates grassland productivity. In the world's largest remaining temperate grassland, we found that experimentally increased precipitation enhanced net primary production, soil-available nitrogen and foliar nitrogen concentrations during the first six years, but it ceased to do so in the following four years, unless nitrogen was simultaneously added with water. The ^{15}N enrichment of plant and soil nitrogen pools in later years indicates increased nitrogen losses, which exacerbated nitrogen limitation and ended the stimulation of productivity by increased precipitation. Changes in species abundance might have contributed little to the changes in water treatment effects. Our study demonstrates that the long-term response of grassland ecosystems to increased precipitation will be mediated by nitrogen availability. Our results also point to a shift from co-limitation by water and nitrogen early to perhaps limitation by nitrogen only later in this temperate grassland, highlighting significant variations in the type of resource limitation induced by climate change.

Key words: increased precipitation; nitrogen deposition; nitrogen losses; nitrogen stable isotopes; plant productivity; plant species abundance.

Introduction

Anthropogenic carbon (C) emissions from fossil fuel combustion and land use changes are expected to increase global atmospheric CO_2 concentrations and surface temperatures significantly (IPCC 2014), and consequently alter the global hydrological cycle (Vörösmarty and Sahagian 2000). Various patterns of world and/or local precipitation change have long been discussed and predicted. For instance, precipitation averaged over the mid-latitude land areas of the Northern Hemisphere will likely increase, and interannual variation in precipitation amount and the frequency of large and extreme events (e.g., storm or prolonged drought) will likely increase (IPCC 2014). Extreme precipitation events can have large impacts on terrestrial ecosystems (Easterling et al. 2000), causing significant economic costs and profound consequences for human welfare (Meehl et al. 2000). However, an increase in precipitation amount may enhance plant productivity, which could possibly enhance food production and/or ecosystem C storage in water-limited ecosystems (Luo et al. 2008, Chen et al. 2009), thereby dampening future climate change.

Grasslands cover about 41% of the Earth's land surface (White et al. 2000), and can be sensitive to precipitation change (Xiao et al. 1995, Knapp and Smith 2001). The mean precipitation in most grasslands of the northern hemisphere, and some grasslands of the southern hemisphere (including the prairie of South America, most savanna of South Africa, and some savanna and prairie regions in northern Australia) will likely increase by the end of the 21st century (2081–2100) relative to 1986–2005 (IPCC 2014). Precipitation controls on grassland plant productivity have been observed across natural precipitation gradients over multiple sites (Sala et al. 1988, Knapp and Smith 2001, Guo et al. 2012, La Pierre et al. 2016, Schubert et al. 2016) or across multiple years in the same site (Bai et al. 2004, Brookshire and Weaver

Manuscript received 10 September 2016; revised 28 February 2017; accepted 10 March 2017. Corresponding Editor: Sara Vicca.

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2015, Estiarte et al. 2016). However, the results from these approaches may be confounded by other covarying factors, such as soil nutrient availability and plant species composition. Manipulative experiments control for such confounding factors while allowing simultaneous consideration of multiple climate change scenarios (Beier et al. 2012, Gherardi and Sala 2015). Consistent with the results from many observational studies, a meta-analysis of 34 precipitation experiments showed that increased precipitation enhanced aboveground productivity (Wu et al. 2011). However, most precipitation manipulation experiments are short in duration (five years or less) and the enhanced productivity could be a transient effect. Thus, long-term experiments are needed to determine whether increased precipitation will persistently stimulate plant productivity. Nutrient limitation can increasingly constrain plant productivity responses to elevated CO_2 (Reich and Hobbie 2013), since C and nitrogen (N) are sequestered in long-lived plant biomass and soil organic matter, progressively decreasing the mineral N available for plant uptake in the long term (Luo et al. 2004). Similarly, we hypothesize that increased precipitation could stimulate N cycling and consequently increase N losses, which in the end may further constrain plant productivity responses to changes in water availability.

With concerns over the effects of global increasing deposition of reactive N, numerous N manipulation experiments have been conducted in grasslands (Elser et al. 2007). However, the interactions between N addition and other key environmental drivers, like water, remain unknown. Therefore, we designed a long-term field experiment to explore the temporal trend in the effects of water and N addition on net primary production (productivity) in the world's largest remaining temperate grassland, which is in northern China. Specifically, we aimed to address two fundamental questions: (1) How different are the short-term vs. long-term responses of grassland productivity to water addition? (2) What controls the long-term response of productivity to water addition? By crossing treatments of N and water addition, we tested our hypothesis that long-term water addition would eventually decrease N availability, which would thereby limit plant productivity.

Methods

Study site and experimental design

This long-term experiment was conducted at Duolun Ecosystem Research Station in Inner Mongolia, northern China (116°17′ E, 42°02′ N; elevation 1,324 m). Annual precipitation averages 371 mm (1953–2014) with 80% of inputs from June to September. Mean annual temperature is 2.1°C with monthly mean temperature ranging from 18.9°C in July to −17.5°C in January. The soil is classified as chestnut according to the Chinese classification, or Haplic Calcisols according to the FAO classification. Total C and total N in the 0–10 cm mineral soil in the control averaged 1.79% ± 0.04% (mean ± SE) and 0.18% ± 0.003%, respectively, from 2005 to 2014 (Appendix S1: Table S1). The plant community in the study site is mainly dominated by two perennial grasses, *Stipa krylovii* and *Agropyron cristatum*, and one perennial forb, *Artemisia frigida*. The growing season in this temperate grassland runs from early April to late September. The study site has never been cultivated, and had been used for livestock grazing until it was fenced in 2000. Woody species have seldom been found at the study site, presumably because the previous intensive grazing limited growth of woody species (Macias et al. 2014).

In April 2005, seven 107 × 8 m blocks containing naturally assembled communities were set up in a homogeneous and flat field with a split-plot design. Each block was divided into two main plots, which were randomly assigned to the ambient and water addition treatment. Each main plot was divided into six 8 × 8 m plots, and two of six plots were treated as two N treatments (ambient or addition of 10 g $N \cdot m^{-2} \cdot yr^{-1}$). The N treatment presented in this study is a subset of several nutrient addition treatments included in the multi-factor experiment. There was a 1-m buffer zone between plots. In the water addition plots, 15 mm of groundwater was applied weekly by sprinkling irrigation from June to August, and thus a total of 180 mm water was added each year (approximately 50% of the long-term mean annual precipitation in the study area). The amount of water added is larger than the projected increases in precipitation (10–20%) in the grasslands of northern China, including our study area, by the end of this century (IPCC 2014). However, this amount is comparable to the difference in annual precipitation between dry and wet years (Appendix S1: Fig. S3A), and an increase in precipitation of similar magnitude is expected to occur more frequently in this study region (Sun and Ding 2010). Nitrogen (10 g $N \cdot m^{-2} \cdot yr^{-1}$) was applied in the form of urea, with one-half the annual amount applied on each of two occasions each year, during early May and July (the early and peak stages of the growing season, respectively, equal amount applied each time), in order to alleviate N limitation of plant growth.

Plant and soil sampling and biogeochemistry measurements

We assessed both above- and belowground plant biomass, foliar N concentration and soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations, soil moisture, and pH for every plot over the 10-year sampling period (2005–2014). Aboveground biomass was harvested by clipping a 15 × 200 cm strip just above the soil surface in late August. To avoid unwanted effects of repeatedly clipping at the same location on plant and soil, each year we clipped at a new location within plots. All living vascular plants were sorted by species, dried, and weighed. All aboveground biomass dies during the winter season and

the standing crop of the steppe community reaches the annual peak biomass in late August; thus, our measured peak community biomass approximates the annual aboveground net primary production (ANPP) of this ecosystem. The biomass from prior years that has not decomposed was separated and not included in ANPP estimates. Belowground net primary production (BNPP) was estimated by the root in-growth method. In early May of each year, we excavated two 40 cm deep cylindrical holes using an 8 cm diameter soil core sampler in each plot. Roots can reach a depth of 100 cm in our study site, but 91% of roots typically occurred in the uppermost 40 cm. The soils were refilled in the same hole after removing roots via 2-mm sieves. We retrieved root in-growth samples in late September by using a 6 cm diameter soil core sampler at the center of the original root in-growth holes. The dry mass of roots was determined by oven-drying at 70°C to constant mass. This sample size has been used previously for root biomass studies in temperate grassland ecosystems (e.g., Chen et al. 2009, Wan et al. 2009, Wei et al. 2013). Ecosystem net primary production was the sum of ANPP and BNPP. Relative abundance for each species was calculated as the ratio of its aboveground biomass to ANPP.

The leaves of six studied species from August harvests were collected, ground, and analyzed for determining N concentrations using the Auto-Kjeldahl method (Kjeltec System 1,026 distilling unit, Foss Tecator, Höganäs, Sweden) in each plot every year. The six sampled species included three dominant species, *Agropyron cristatum*, *Artemisia frigida*, and *Stipa krylovii*, and three other common species, *Cleistogenes squarrosa*, *Melilotoides ruthenica*, and *Potentilla tanacetifolia*, which in total made up 60–83% of aboveground net primary production in the control during the 10 year of the study. We sampled leaves based on the presence/absence and abundance of each species. The legume species, *Melilotoides ruthenica*, disappeared in a few N addition plots during the later years of the study, preventing us from collecting leaves of all species in each of the seven replicate plots. Thus, leaves were collected from five of the seven replicate plots in which all six plant species were sufficiently abundant during all years of the study. The aggregated values of foliar N concentration were the means of these six species weighted by relative biomass. Foliage samples collected in years 4, 8, and 9 of the experiment were also analyzed for the ratio of stable N isotopes (expressed by $\delta^{15}N$) with an Isotope-Ratio Mass Spectrometer (IRMS, Isoprime 100; Isoprime, Stockport, UK) coupled with an Elementary Analyzer (Elementar vario MICRO cube; Elementar, Langenselbold, Germany). Three international standards (IAEA N1, +0.4‰; USGS 25, −30.4‰; USGS 26, +53.7‰) and one laboratory standard were used to calibrate the measured $\delta^{15}N$ values. The aggregated values of foliar $\delta^{15}N$ were also the means of these six species weighted by relative biomass.

The 0–10 cm mineral soil samples were collected by mixing five 3 cm diameter cores in each plot in late August each year. We sieved (2 mm) soil cores and extracted 10 g fresh soil sample with 50 mL of 2 mol/L KCl. The KCl extracts were analyzed for NO_3^- and NH_4^+ concentrations with a continuous flow spectrophotometer (FIAstar 5000 Analyzer; Foss Tecator, Hillerød, Denmark). The KCl extracts were also analyzed for $\delta^{15}N$ and stable oxygen isotopes ($\delta^{18}O$) of NO_3^- and $\delta^{15}N$ of NH_4^+ in year 9 of the experiment, in order to examine if denitrification occurred. Nitrate isotopes were determined by the modified azide method (Tu et al. 2016). The measured results for $^{15}N/^{14}N$ of NO_3^- were calibrated using three international standards (IAEA N3, +4.7‰; USGS 32, +180‰; USGS 34, −1.8‰), and $^{18}O/^{16}O$ of NO_3^- using international standards (IAEA N3, 25.6‰; USGS 34, −27.9‰; USGS 35, 57.5‰). $^{15}N/^{14}N$ of NH_4^+ was determined with the method that combined hypobromite oxidation and hydroxylamine reduction (Liu et al. 2014), and was calibrated using international standards (IAEA N1, +0.4‰; USGS 25, −30.4‰; USGS 26, +53.7‰). Briefly, NH_4^+ and NO_3^- were chemically converted to N_2O, separately, and isotopes of the produced N_2O were determined by an IRMS (Isoprime 100) coupled with a trace-gas pre-concentrator and an auto-sampler (Liu et al. 2014, Tu et al. 2016). The air-dry soil samples collected in years 8 and 9 of the experiment and the urea applied in years 4, 8, and 9 were also measured for $\delta^{15}N$ using the EA-IRMS as for foliar samples.

We measured soil moisture biweekly from June to September in each year, by taking two 10 cm deep soil cores in each plot and then oven drying (48 h, 105°C). Growing season mean values of soil moisture in the present study were calculated from the monthly mean values, which were first averaged from all measurements in the same month. Soil pH was measured using a pH meter in soil water suspension (soil : water = 1:2) in September each year. Daily precipitation and air temperature data were obtained from the eddy tower set by the Institute of Botany, the Chinese Academy of Sciences, and the tower was about 100 m away from our study site. To indicate water deficit or drought conditions, the Standardized Precipitation Evapotranspiration Index (SPEI) was determined by the difference between precipitation and potential evapotranspiration, according to the equation of Thornthwaite (1948).

Data analyses

We used mixed-effects models to test treatment effects and temporal trends while accounting for both the split-plot design and the repeated measurements. To estimate mean effects of water addition on productivity, soil-available N, and foliar N concentration, we also used the response ratio (Hedges et al. 1999), which is the ratio of these variables in water treatment to the control. Linear regression was used to determine the relationships between the productivity response to water addition and soil-available N and foliar N concentration response to water addition. We also regressed the ecosystem

productivity response against precipitation, air temperature and SPEI reflecting water deficit, to examine whether grassland responses to water addition were dependent on climatic factors. All statistical analyses were performed in R 3.1.1 (Team 2015).

For the mixed effects models, we fit response variables (including productivity, soil-available N, foliar N concentration, and N isotope ratios of leaves and soil for various N forms) with the lme function in the nlme package of R as

$$\begin{aligned} &lme(Y \sim Water * Nitrogen * Time - 1, random \\ &\quad = \sim 1|Side/Block/Plot, correlation \\ &\quad = corAR1(, form = \sim 1|Side/Block/Plot)), \end{aligned}$$

where Y was the response variable, Water was binary (0 = ambient, 1 = addition), Nitrogen was binary (0 = ambient, 1 = addition), Time was a factor with two levels (early = 2005–2010, late = 2011–2014), Side was a factor with two levels (east or west main plot), and Block was an integer (1–7 for spatial blocks from north to south). In other words, we tested all main effects and interactions for the fixed effects of Water, Nitrogen, and Time; we fit a random intercept for each plot nested within each block for the nitrogen treatment, within each side of the field (water treatment); and we assumed a first-order autoregressive temporal covariance structure. We obtained similar results with less parsimonious (i.e., larger Akaike information criterion) alternative models that assumed linear temporal trends (i.e., where Time was an integer experimental year), or that assumed an alternative temporal error covariance structure (i.e., compound symmetry). The denominator degrees of freedom for main effects and interactions in these models reflect sample sizes for main plots (two sides, to which Water treatments were randomized), plots (28 plots, to which Nitrogen treatments were randomized), and observations (280 observations = 28 plots × 10 yr, which include repeated measurements in plots for testing Time effects and interactions).

We used structural equation modeling (SEM) to estimate the contributions of water, N, and species abundance to the productivity response. The primary advantage of SEM is to evaluate complex causality between variables by translating the hypothesized causal relationships into a pattern of expected statistical relationships in the data (Grace 2006). In the model, we assumed that water addition had the potential to alter productivity directly, as well as indirectly through changing species abundance and N availability. We used the chi-square test to judge the fit of the model. The model has a good fit when $0 \leq \chi^2 \leq 2$ and $0.05 < P \leq 1$. Here, a large P value (>0.05) indicates that the covariance structure of the data does not differ significantly from the expected model (Grace 2006). All variables were log-transformed to meet the assumptions of normality. SEM analyses were performed using AMOS 18.0 (Amos Development Company, Greene, Maine, USA).

Results

Plant productivity in the control plots was positively correlated with growing season precipitation across the entire study period ($r^2 = 0.59$, $P = 0.006$; Fig. 1C), indicating that productivity at the study site was water limited. At such a water-limited site, water addition only transiently increased plant productivity in the first six years ($P = 0.008$), whereas the stimulation of productivity by water addition ceased to occur from the seventh year on, unless N was added with water (Fig. 1A; Appendix S1: Table S2). At increased N supply, water addition persistently promoted productivity throughout all years of the experiment. In the first six years of the experiment, water addition increased productivity by 164 $g \cdot m^{-2} \cdot yr^{-1}$ at ambient N and by 140 $g \cdot m^{-2} \cdot yr^{-1}$ at increased N (Fig. 1B). These changes corresponded to 57% stimulation of productivity by water addition at ambient N, compared with 32% stimulation at increased N. In the following four years, however, water addition reduced productivity by 12 $g \cdot m^{-2} \cdot yr^{-1}$ (or 2%) at ambient N level but enhanced productivity by 119 $g \cdot m^{-2} \cdot yr^{-1}$ (or 32%) at increased N level (Fig. 1B). Similar response patterns were found when we examined above- or belowground productivity separately (Appendix S1: Fig. S1, Table S2).

The aboveground biomass of the six studied species responded differently to water and N treatments over time (Fig. 2). Water addition increased the biomass of *Agropyron cristatum* during the entire experimental period ($P = 0.079$; Appendix S1: Table S3), whereas this water stimulation was smaller at ambient than at enriched N supply (Fig. 2A). Water addition had the contrasting effects on the biomass of *Artemisia frigida* in early (first six years) vs. later (next four years) periods ($P = 0.0005$ for the water × period interaction; Appendix S1: Table S3; Fig. 2B). The significant water × N interaction on the biomass of *Stipa krylovii* ($P = 0.041$; Appendix S1: Table S3) showed the dependence of its biomass response to water addition on N supply, and this dependence was more pronounced in later years than in early years ($P = 0.0003$ for the water × N × period interaction; Appendix S1: Table S3; Fig. 2C). Compared with these dominant species, water addition did not significantly affect the biomass of two other common species, *Cleistogenes squarrosa* and *Potentilla tanacetifolia* (Fig. 2D, E), but water and N interactively affected the biomass of *Melilotoides ruthenica* ($P < 0.0001$; Appendix S1: Table S3; Fig. 2F). Additionally, water addition marginally significantly enhanced relative abundance of one dominant species *Agropyron cristatum* ($P = 0.081$), but reduced those of the other two dominant species, *Artemisia frigida* and *Stipa krylovii* (both $P < 0.10$; Appendix S1: Table S4, Fig. S2). For three other common species, relative abundance responses to the temporal water treatment were similar to aboveground biomass responses (Appendix S1: Fig. S2).

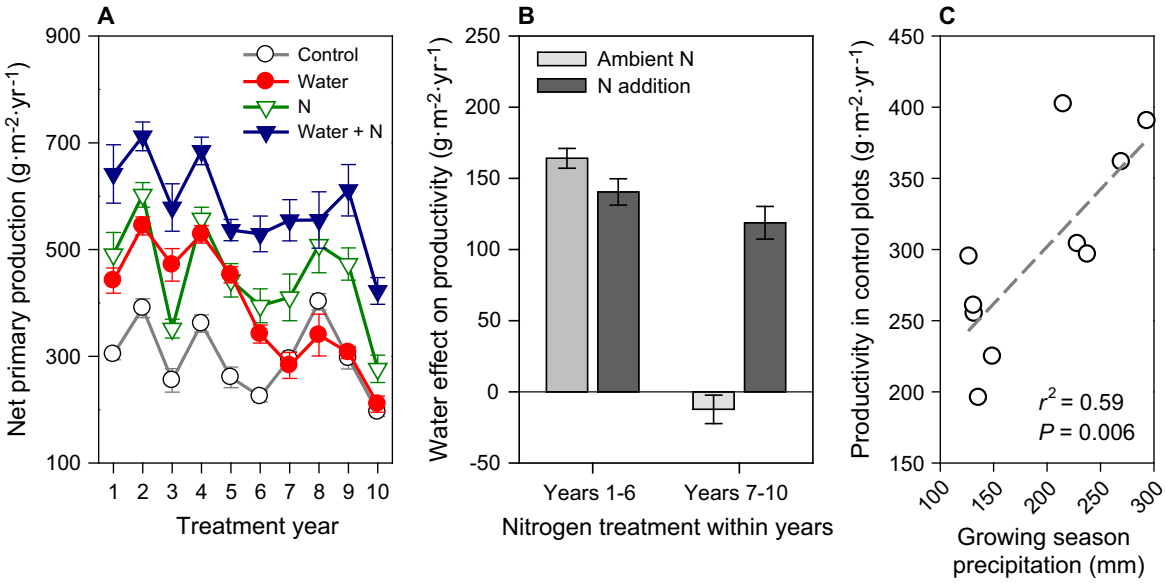

FIG. 1. (A) Effects of water and N addition on total net primary production calculated as the sum of above- and below-ground productivity. (B) The relative effects of water addition on productivity at ambient and increased N levels during early (2005–2010) and late (2011–2014) periods of the study. The relative effects were calculated as ([productivity with water addition] − [productivity with ambient water]) at each N treatment level. (C) Relationship between productivity in the control and growing season precipitation over the 10-year experimental period. Error bars show one standard error of the mean.

We examined the potential role of the background climate in determining the change in productivity responses from early to later years, but did not find evidence that any climate extreme, such as severe drought, periods of heavy rainfall, or heat waves, explained these trends (Appendix S1: Fig. S3A–C). None of the relationships between precipitation amount, precipitation frequency, air temperature, or water deficit and the productivity response to water addition were significant (all $P > 0.05$; Appendix S1: Fig. S3D–G). During the later period of the study, we did not observe that water addition increased plant productivity in either wet or dry years (Fig. 1A).

Similar to the response of plant productivity, water addition significantly enhanced soil N availability and foliar N concentration during the first six years of the study, but not thereafter, unless N was also added (Figs. 3A, 4A and Appendix S1: Fig. S4A–F, Table S5). During the first six years of the study, soil N availability and foliar N concentration were respectively 54% and 22% higher under water addition than in the control. During the last four years, water addition did not increase soil N availability and foliar N concentration at ambient N supply, but increased soil and foliar N at enriched N supply (Figs. 3B, 4B). Thus, water addition increased productivity most during the years when it most strongly increased soil-available N and foliar N concentrations, which was also evident by significantly positive correlations between the productivity response and both the soil-available N response ($r^2 = 0.84$, $P = 0.0002$; Fig. 3C) and the foliar N response ($r^2 = 0.70$, $P = 0.003$; Fig. 4C) to water addition.

To examine whether water-induced decreases in soil-available N and foliar N concentrations in later years were caused by increased N losses, we determined ^{15}N both in plants and soils. Compared to controls, water addition did not increase foliar $\delta^{15}N$ in year 4, but significantly increased foliar $\delta^{15}N$ in years 8 and 9 of the experiment (Fig. 5A and Appendix S1: Fig. S4G–L, Table S6). Water addition enhanced $\delta^{15}N$ of soil total N in years 8 and 9 (when $\delta^{15}N$ for soils was first measured; Fig. 5B; Appendix S1: Table S6). In year 9, we further measured N isotopic composition for soil-available N and observed an increase of 2‰ in $\delta^{15}N$ of soil NH_4^+ (Fig. 5C) in response to water addition, compared to the control. We also found an increase of 9‰ in $\delta^{15}N$ and 5‰ in $\delta^{18}O$ of soil NO_3^- under water addition relative to controls (Fig. 5D, E). Furthermore, the $\delta^{18}O$ and $\delta^{15}N$ of soil NO_3^- were highly positively correlated across all treatments, with a slope of about 0.56 ($r^2 = 0.77$, $P < 0.0001$; Fig. 5F). Together, these results suggest that long-term water addition increased N losses, e.g., probably through denitrification. In addition, we examined the effects of N addition on the $\delta^{15}N$ of plants and soils. The $\delta^{15}N$ values of the urea added during the entire experiment were consistent and averaged −3.4‰ (Appendix S1: Table S1). Foliar $\delta^{15}N$ was lower in two N-addition treatments than in the control in year 4 (Fig. 5A), suggesting an uptake of the added N which was more ^{15}N-depleted than the N sources in the control. However, foliar $\delta^{15}N$ became higher in N-addition treatments than in controls in later years, due to increased $\delta^{15}N$ of soil-available N (i.e., NH_4^+ and NO_3^-, Fig. 5C, D).

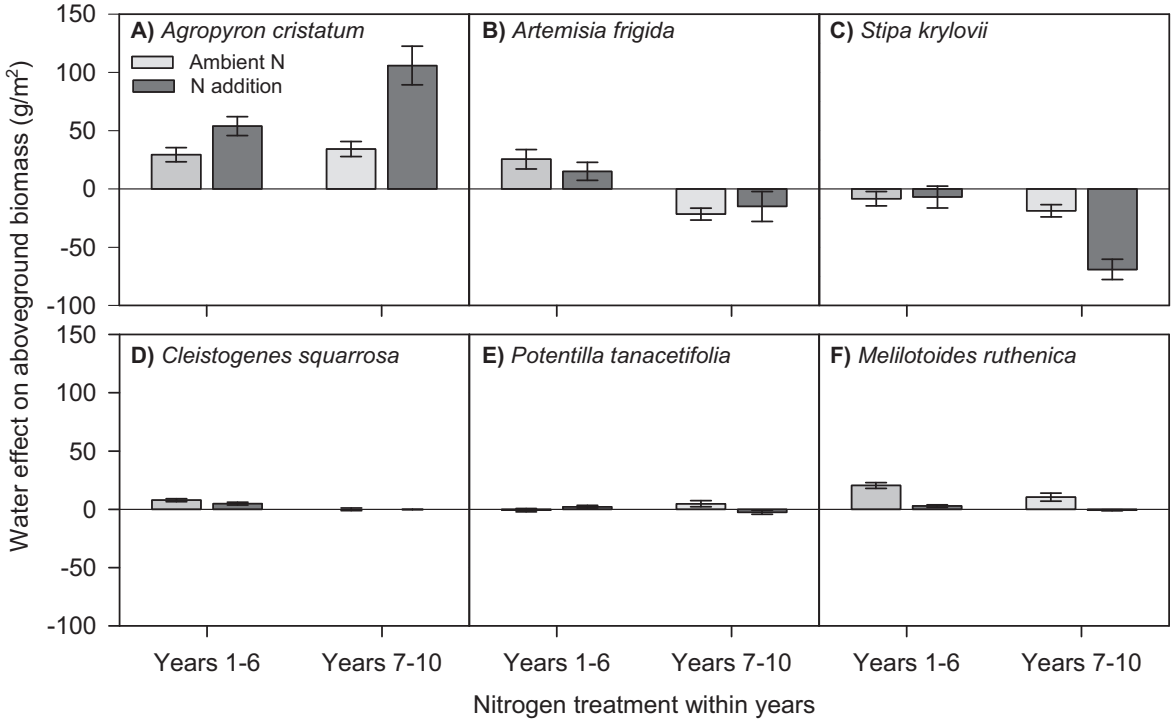

FIG. 2. The relative effects of water addition on aboveground biomass at ambient and increased N levels during early (2005–2010) and late (2011–2014) periods of the study, for (A–C) dominant species and (D–F) other common species. The relative effects were calculated as ([species biomass with water addition] − [species biomass with ambient water]) at each N treatment level. Error bars show one standard error of the mean.

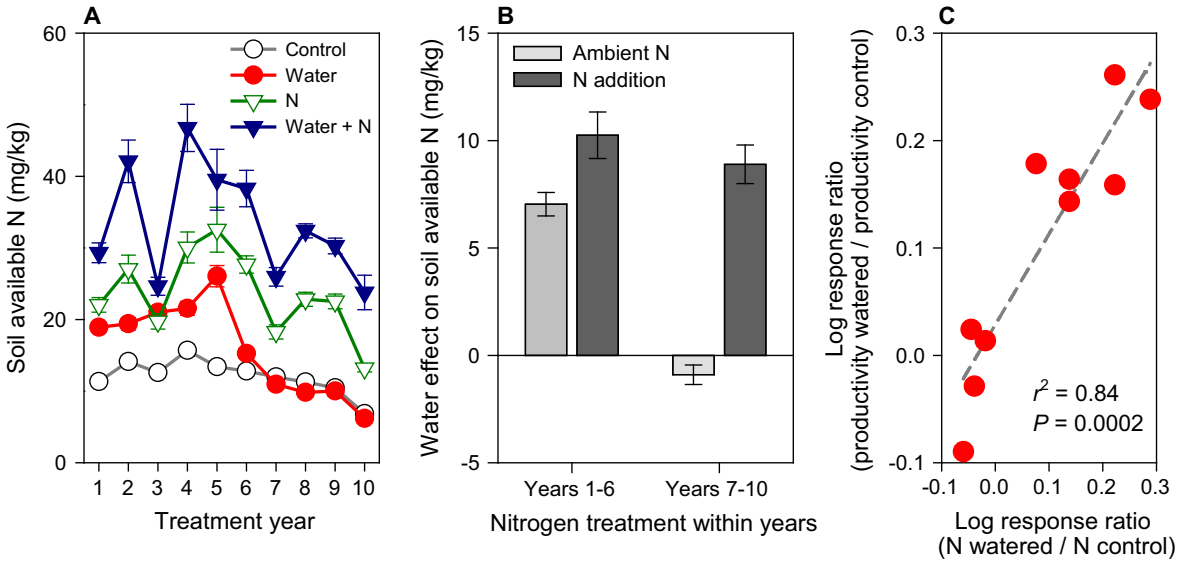

FIG. 3. (A) Effects of water and N addition on soil N availability (nitrate plus ammonium). (B) The relative effects of water addition on soil-available N at ambient and increased N levels during early (2005–2010) and late (2011–2014) periods of the study. The relative effects were calculated as ([available N with water addition] − [available N with ambient water]) at each N treatment level. (C) Relationships between the productivity response and the soil N availability response to water addition. Water effects were estimated as the response ratio, which is the ratio of the variables in water treatment to control. Error bars show one standard error of the mean.

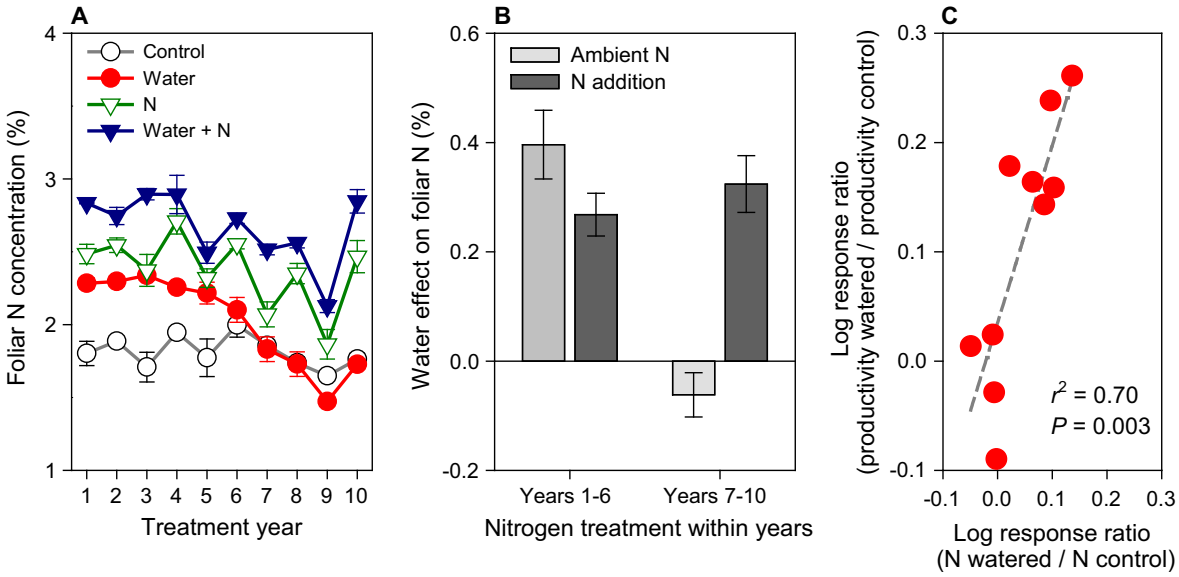

FIG. 4. (A) Effects of water and N addition on foliar N concentration. (B) The relative effects of water addition on foliar N at ambient and increased N levels during early (2005–2010) and late (2011–2014) periods of the study. The relative effects were calculated as ([foliar N with water addition] − [foliar N with ambient water]) at each N treatment level. (C) Relationships between the productivity response and the foliar N response to water addition. Water effects were estimated as the response ratio, which is the ratio of the variables in water treatment to control. Error bars show one standard error of the mean.

DISCUSSION

Responses of plant productivity to increased precipitation

A number of short-term studies (five years or less) have shown that increased precipitation stimulated plant productivity (Wu et al. 2011). For example, in the 1970s, Lauenroth et al. (1978) reported that water addition during the growing season increased plant productivity based on a four-year experiment conducted in a semiarid shortgrass prairie. Suttle et al. (2007) found that water addition in both spring and winter enhanced plant productivity in the first three years of a five-year experiment in a California grassland. These findings are consistent with our results from the first six years of the experiment.

Our results also show the magnitude of the effect of water treatment on plant productivity in the early years is similar to the extent of the influence of annual variability in natural precipitation on productivity. For instance, the growing season precipitation was 294 mm in year 2 and 132 mm in year 3 of the study, and this annual variation (162 mm) is about as much as the amount we added under the water treatment (180 mm). In control plots, plant productivity was 53% greater in year 2 than in year 3 (390 $g \cdot m^{-2} \cdot yr^{-1}$ in year 2 and 255 $g \cdot m^{-2} \cdot yr^{-1}$ in year 3), which was similar to the 57% increase in productivity induced by the water addition treatment (Fig. 1 and Appendix S1: Fig. S3).

Consistent with our hypothesis, even at this water-limited site where plant productivity in the control increased significantly with increasing natural precipitation (Fig. 1C), positive effects of water addition on productivity vanished from the seventh year on. To our knowledge, our study is the first to report this phenomenon. In contrast to our findings, water addition was found to consistently increase aboveground productivity in a 19-year manipulation experiment conducted in a tallgrass prairie (Collins et al. 2012, Knapp et al. 2012). That study found that changes in identity of dominant grass species, rather than N availability, affected the long-term productivity response to water. We suspect the differences between the results of that study and ours can be partly explained by the fact that the soil nutrient pools are much smaller in our grassland (1.79% C and 0.18% N) than in that tallgrass prairie (2.70% C and 0.24% N, McCulley et al. 2009). In our grassland, increased N losses under water addition may further deplete the relatively small soil nutrient pool, exacerbating N limitation and consequently ceasing to produce productivity responses to water addition during the later years of the study. The different response patterns between our semiarid steppe and the mesic tallgrass prairie highlight that the differences in short- and long-term responses to chronic resource manipulations may be ecosystem-dependent and suggest that increased precipitation may more easily lead to N depletion in arid regions than in less arid regions.

Mechanisms by which increased precipitation influences productivity over time

Several mechanisms were likely to potentially explain the observed changes over time in the response of plant productivity to increased precipitation. First, water

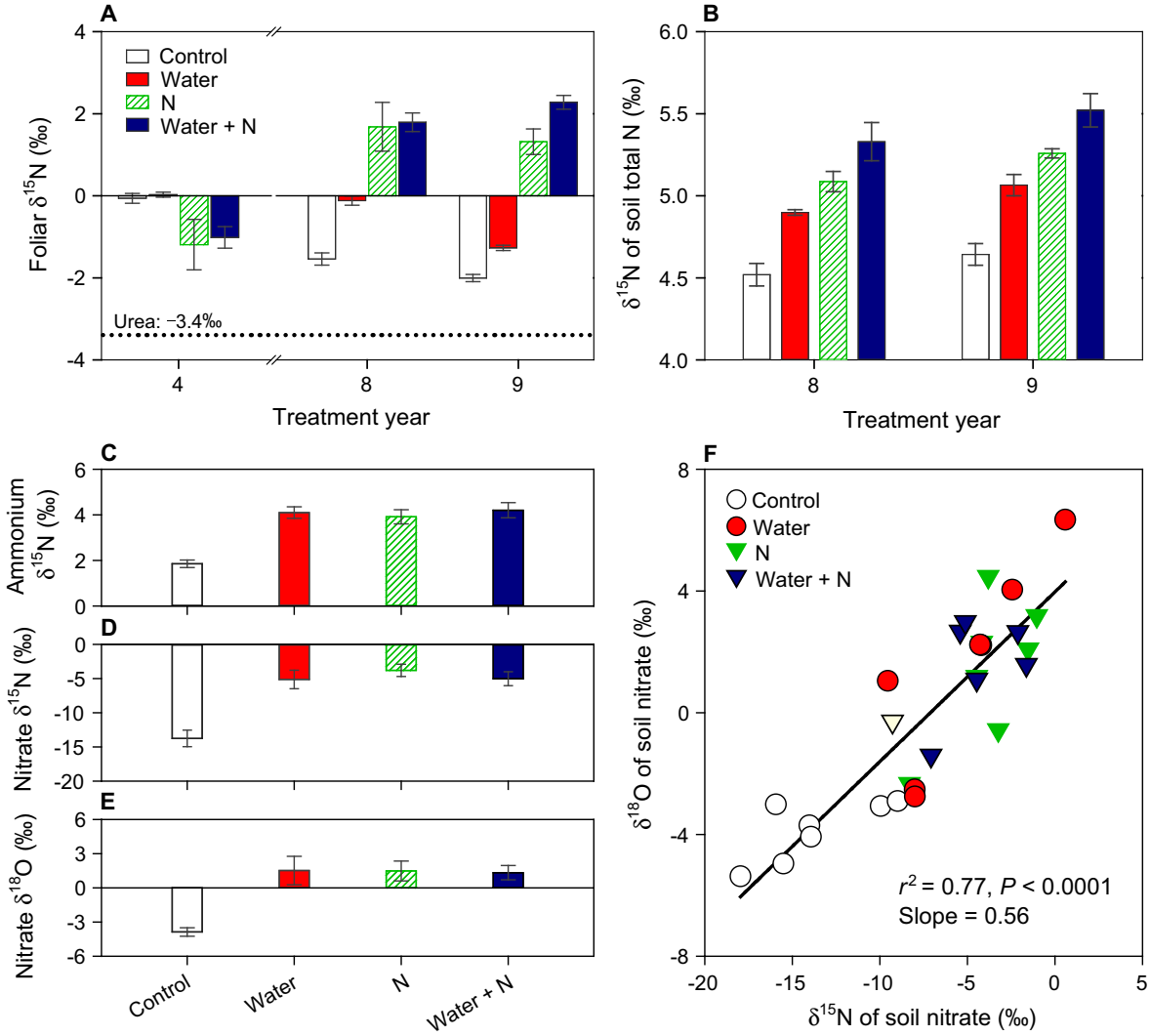

FIG. 5. (A, B) Effects of water and N addition on the $\delta^{15}N$ signatures of foliage and soil total N. (C–E) Effects of water and N addition on $\delta^{15}N$ and $\delta^{18}O$ of soil-available N in year 9 of the study. (F) Relationship between soil nitrate $\delta^{18}O$ and $\delta^{15}N$ across all treatments. Error bars show one standard error of the mean. The $\delta^{15}N$ of urea (the form of N applied) is −3.4‰.

addition can alleviate water limitation of plant growth. For instance, added precipitation has been shown to reduce drought severity and lengthen the growing period in a California grassland when drought occurred late in the growing season of 2001 (Dukes et al. 2005). Second, water addition can influence productivity through changes in soil N availability. Almost all studies performed in arid and semiarid ecosystems indicate that water addition (pulse) after an extended drought enhanced immediately soil N mineralization and N availability (Austin et al. 2004). Third, water addition can alter plant phenology and physiology directly by increased water availability and/or indirectly through the mediation of soil N availability, which in turn affects photosynthesis and productivity. For example, high precipitation increased flowering stalk production of the dominant species and consequently enhance grassland productivity (La Pierre et al. 2016). Finally, water addition can result in species changes that consequently affect productivity. After 10 years of water addition, species reordering occurred, as one C_4 tallgrass species (*Panicum virgatum*) became dominant instead of another C_4 grass (*Andropogon gerardii*) in a lowland community (Collins et al. 2012).

In the present study, we suggest that the second mechanism, changes in soil N availability, is the most likely factor responsible for the observed pattern of plant productivity following water addition treatment. Monthly addition of 60 mm water in our study stimulated both microbial and plant activities, consistent with results of previous studies (Huxman et al. 2004, Dijkstra et al. 2012), as indicated by increased soil-available N and leaf N concentrations (Figs. 3, 4). Increased leaf N

concentration can facilitate plant photosynthesis and thereby increase plant productivity. This is well supported by the close relationships of plant productivity response with both soil-available N response and leaf N response across the entire study period (Figs. 3C, 4C). These results suggest that N availability is an important factor regulating plant productivity in the study region.

In addition, we find that the magnitude of water-induced increases in productivity was similar to that of N addition in the early years (Fig. 1A), whereas the effect of water addition on leaf N concentration was smaller than that of N addition (Fig. 4A). These findings suggest that water addition enhances plant productivity principally but not solely through the change of N cycling; other mechanisms, e.g., increasing plant growth period (direct effect of water addition) and altering plant physiology, might have also played a role. Water addition has been found to reduce leaf longevity but increase leaf biomass production by producing more new leaves to replace the old leaves in the early period of our study (Ren et al. 2011). Water addition also likely increased plant productivity by enhancing specific leaf area and photosynthetic rate (Ren et al. 2011).

We further disentangle the contributions of the direct and indirect effects of water addition on productivity using structural equation modeling. The models explain 56% and 62% of the variation in productivity, respectively, during the early and later periods. We find that the direct and indirect effects of water contributed almost equally to the productivity response in the early period, as they had similar standardized path coefficients (0.44 and 0.46, respectively; both $P < 0.001$; Fig. 6). In the later period, however, the indirect effect of water through soil N availability was much stronger than the direct effect of water, as indicated by the standardized path coefficients (0.65 and 0.27, respectively; both $P < 0.001$; Fig. 6). This model demonstrates the importance of soil N availability in regulating plant productivity responses to water addition.

Water-induced shifts in species composition over time may also partly explain why water addition affected productivity more during the earlier than during the later years of our study. In particular, turnover of dominant species has been shown to be a key factor in driving ecosystem responses to resource alterations (Lauenroth et al. 1978, Smith et al. 2009). In the present study, we find that water addition consistently increased both the absolute biomass of *Agropyron cristatum* and its relative abundance, although early on, the stimulation of productivity by water was smaller under ambient than enriched N. This is probably because *Agropyron cristatum* is most productive in a moist habitat, as indicated by lower leaf $\delta^{13}C$ ($-29.0‰ \pm 0.1‰$) and thus lower water use efficiency, compared with the averaged leaf $\delta^{13}C$ ($-27.3‰ \pm 0.2‰$) of other C_3 plants (Appendix S1: Fig. S4). Water addition reduced the relative abundance and biomass of the other two dominant species, *Artemisia frigida* and *Stipa krylovii*, which may contribute to the lack of the productivity response to water addition in later years. However, our structural equation models indicate that the contribution of change in species abundance to the temporal water treatment effect was very limited (Fig. 6).

Water addition could increasingly stimulate productivity over time if water addition increases soil N availability and foliar N concentration, as observed in this study (Figs. 3, 4), by stimulating soil microbial activity (e.g., N mineralization, N nitrification, and litter decomposition; Aerts 1997, Wang et al. 2006, Schaeffer et al. 2013). However, water addition could diminishingly stimulate productivity over time if water addition results in decreased soil N availability because water addition likely increases N losses (Yahdjian and Sala 2010), e.g., through denitrification and leaching (Mudge et al. 2016). Our experiment included the combined addition of water and N, and showed that productivity was persistently increased by water only when N was also added (Fig. 1). These results further point to the role of N availability in regulating the productivity response to water addition in this temperate grassland. That is, water addition enhanced productivity when and where water addition increased N availability or when and where N did not limit productivity.

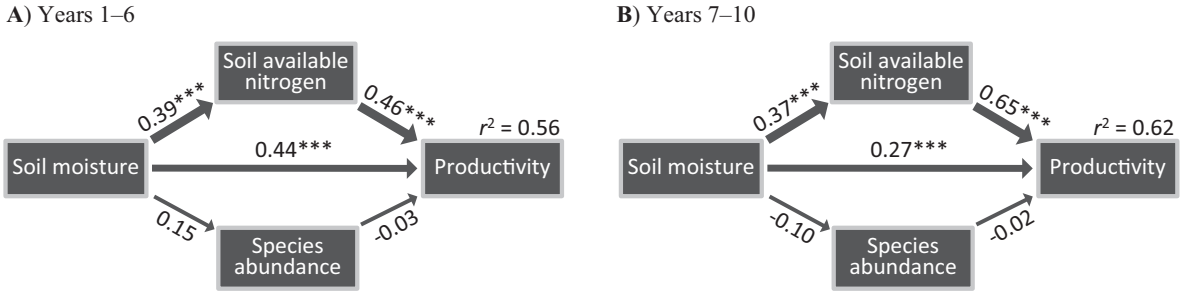

FIG. 6. Structural equation models based on the data from (A) years 1–6 and (B) years 7–10 of the study. The top and bottom arrows respectively show the indirect effects of water addition on productivity by soil N availability and species abundance. The middle arrow shows the direct effect of water addition on productivity. Numbers adjacent to arrows are standardized path coefficients, indicating the effect size of the relationship. Arrow width is proportional to the strength of the relationship. The proportion of variance explained is given as r^2. ***$P < 0.001$. Results of model fitting: (A) $\chi^2 = 0.219$, $P = 0.64$, df = 1; (B) $\chi^2 = 1.663$, $P = 0.197$, df = 1.

Why did the stimulation of soil N availability by water addition vanish in later years? We propose that water addition eventually increased N losses so that N availability consequently decreased. To assess this, we measured $\delta^{15}N$ for soil total N, soil-available N, and plants (Fig. 5). The $\delta^{15}N$ reflects the nature of ecosystem N cycling, with higher values indicating greater N losses and a more open N cycling (Högberg 1997, Robinson 2001). In the later stage of our experiment, water addition induced increases in $\delta^{15}N$ of leaves and of various soil N forms relative to controls, suggesting that water addition substantially enhanced N losses. Furthermore, denitrification might be an important N loss pathway under water addition, as indicated by strong ^{15}N enrichment of soil KCl-extracted NO_3^- and the significant correlation between $\delta^{18}O$ and $\delta^{15}N$ (See more details in Appendix S1: *Supplementary Discussion*).

We also considered other potential factors possibly affecting the lack of productivity responses to water addition during later years, such as changes in atmospheric N deposition, background climate and the reduction of BNPP, which might occur with ongoing water addition. First, our results from the N addition treatment also suggest that plant productivity at this site was N limited over the 10-year sampling period (Fig. 1). If atmospheric N deposition was significantly lower in the later four years than in the first six years, the water effect would be less pronounced in the later period. However, the N deposition rate did not decrease, and instead tended to increase over time in this Inner Mongolia grassland, and had a mean of 1.2 g N/m^2 (Jia et al. 2014). Second, there was no evidence of interannual climatic variability explaining the temporal pattern in the water treatment effect, since climatic conditions did not shift from one to another regime between the first six and last four years of the study, and none of the climatic variables, including precipitation amount and frequency, air temperature and water deficit, were associated with the productivity response to water addition (Appendix S1: Fig. S3). Third, if water addition reduces BNPP and concurrently decreases root/shoot ratio, part of the productivity response could be explained by the BNPP response. Contrary to this hypothesis, water addition decreased both BNPP and ANPP, leading to no change in root/shoot ratio in water addition treatment compared to the control ($P = 0.115$; Appendix S1: Table S2, Fig. S1). On the other hand, decreases in BNPP may further reduce N uptake by roots (Jackson et al. 1997) and thus exacerbate N limitation. Overall, plant access to and uptake of N are likely responsible for the productivity response to water addition through time.

In addition, we examined the effects of N addition on the $\delta^{15}N$ of plants and soils. We found that foliar $\delta^{15}N$ after eight years of N addition had increased relative to controls, although the urea applied had a depleted ^{15}N signature (−3.4‰). This increase could be attributed to plant uptake of soil-available N (i.e., NH_4^+ and NO_3^-) with enhanced $\delta^{15}N$, which must be caused by some N loss processes exerting high isotope fractionations, e.g., urea ammonia volatilization, nitrification, and denitrification (15–60‰, Robinson 2001). A similar result was observed in a tropical forest (Corre et al. 2010), where foliar $\delta^{15}N$ shifted from 0.2‰ in the control to 1.9‰ in the N-addition treatment after 10 years, although the urea added was depleted in ^{15}N (−2.2‰).

In contrast to the result from water addition alone, the combined addition of water and N did not reduce but enhanced N availability to plants, although it also increased plant and soil $\delta^{15}N$ and thus N losses (Fig. 5). These contrasting effects may be mainly due to the amount of N applied (10 g $N \cdot m^{-2} \cdot yr^{-1}$), which could have overwhelmed the amount of N lost from water plus N addition. We also found that water addition increased plant productivity when N was added at the level of 5 g $N \cdot m^{-2} \cdot yr^{-1}$ (Appendix S1: Fig. S5), suggesting that water-induced N loss was likely less than this amount. Compared to N addition alone, combined water and N addition has more strongly stimulated enzymatic and microbial activities at this study site (Wang et al. 2015), and resulted in more N release and higher soil N availability. On the other hand, combined water and N addition led to greater increases in biomass and N concentration of plant species compared to both water and N when added separately (Figs. 1, 4), which implies that litter with great quantity and high quality would return to soil, consequently leading to increased N availability.

Changes in limitation type induced by increased precipitation

Plant productivity in most arid and semiarid ecosystems is co-limited by N and water availability (Eskelinen and Harrison 2015). This is also true for our study site, which has a total annual precipitation of 371 mm, as indicated by the response of plant productivity to water and N treatments (Fig. 1). However, our results also suggest that this grassland would shift from co-limitation of N and water to N-limitation if annual precipitation is increased. This observation is consistent with the widely-accepted perspective that arid ecosystems are more water limited and semiarid and mesic ecosystems are more N limited, and our study provides evidence that the primary limiting factor can be altered by precipitation at a given site.

Theoretical predictions have shifted from single-resource limitation that is derived from Liebig's Law of the Minimum (Liebig 1842) toward co-limitation by multiple resources. Several studies (Harpole et al. 2011, 2016, Fay et al. 2015) highlight that resource co-limitation occurs frequently and has an important role in regulating primary production. They define co-limitation as simultaneous or independent co-limitation in which productivity responses occur if both resources are added simultaneously or individually, and distinguish these from serial limitation (a synergistic response to a second

resource only after addition of a "primary" limiting resource). For instance, a 17-year experiment in hayed grasslands shows that N addition initially increased plant biomass, while this N effect disappeared later, possibly owing to the exacerbation of potassium limitation (van der Woude et al. 1994). Similarly, our results point to the switch in the form of co-limitation from independent co-limitation by water and N early to perhaps serial limitation later (i.e., water is the primary limiting source and N is a second limitation source). The type of co-limitation seems to be sensitive to the length of the experiment, and thus more long-term experiments are needed to address the multiple mechanisms that could result in different types of co-limitation emerging over time.

Placing productivity-dependent C sequestration in context

As grassland ecosystems are a crucial component of global C cycling, potentially serving as a large sink for atmospheric CO_2 (Jones and Donnelly 2004), it is important to predict their responses to climate change. Precipitation regimes are forecasted to change regionally and globally. Global annual precipitation is anticipated to increase by 5–10% by the end of this century (IPCC 2007). At regional scales, for example, summer precipitation in North China is expected to increase by over 12% by the end of this century (Sun and Ding 2010). Model simulations (Luo et al. 2008) have predicted that increased precipitation would increase soil C sequestration in grasslands, which was only verified by short-term experiments (He et al. 2012). However, our findings suggest that previous studies have likely over-estimated the extent to which increases in precipitation could persistently enhance CO_2 sequestration by stimulating plant growth. Increased precipitation may have positive effects on some productivity-dependent ecosystem services, such as global climate regulation, but these benefits will unlikely persist in the long term.

Acknowledgments

We thank Weixing Zhu, two anonymous reviewers, and the editor (Sara Vicca) for the comments that greatly improved the manuscript. We are grateful to Shibo Fang for providing water deficit data. We thank the large number of individuals who have helped with field sampling and laboratory analysis. We also appreciate the Duolun Restoration Ecology Research Station for access permission to the study site. This research was financially supported by the National Key Research and Development Program of China (2016YFA0600800 and 2016YFC0500707), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB15020200), the National Natural Science Foundation of China (31300387, 31370009, 31422009, and 41073056), and the China Postdoctoral Science Foundation (2012M510847).

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecm.1262/full

Data Availability

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.74f3b