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Key Points:
- Increased precipitation increased ANPP and N cycling rates but did not affect root standing crops
- Total soil C and N did not change despite 25 years of extreme precipitation and high fire frequency
- Plant compositional shifts and biogeochemical pathways may be responsible for biogeochemical stability

Supporting Information:
- Supporting Information S1

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Stability of grassland soil C and N pools despite 25 years of an extreme climatic and disturbance regime

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Abstract Global changes are altering many important drivers of ecosystem functioning, with precipitation amount and disturbance frequency being especially important. Carbon (C) and nitrogen (N) pools are key contemporary attributes of ecosystems that can also influence future C uptake via plant growth. Thus, understanding the impacts of altered precipitation amounts (through controls of primary production inputs) and disturbance regimes (through losses of C and N in biomass) is important to project how ecosystem services will respond to future global changes. A major difficulty inherent within this task is that drivers of ecosystem function and processes often interact, resulting in novel ecosystem responses. To examine how changes in precipitation affect grassland ecosystem responses under a frequent disturbance regime (annual fire), we assessed the biogeochemical and ecological consequences of more than two decades of irrigation in an annually burned mesic grassland in the central United States. In this experiment, precipitation amount was increased by 31% relative to ambient and 1 in 3 years were statistically extreme relative to the long-term historical record. Despite evidence that irrigation decreased root:shoot ratios and increased rates of N cycling—each expected to reduce soil C and N with annual burning—we detected no changes in these biogeochemical pools. This surprising biogeochemical resistance highlights the need to explore additional mechanisms within long-term experiments concerning the consequences of global change impacts on ecosystems.

1. Introduction

Numerous aspects of global change are expected to impact ecosystem structure and function, with increases in the magnitude, duration, and frequency of extreme climate events and altered disturbance regimes likely to have some of the greatest effects [*Gutschick and BassiriRad*, 2003; *Knapp et al*., 2008; *Smith*, 2011; *Reichstein et al*., 2013]. Precipitation patterns are expected to be altered across the majority of ecosystems globally, resulting in chronic alterations to the overall magnitude of precipitation (wetter or drier depending on geographic location [*IPCC*, 2013; *Greve et al*., 2014]) as well as increased extreme wet and dry periods [*Greve et al*., 2014; *Cook et al*., 2015; *Knapp et al*., 2015]. Both spatial and temporal models [*Sala et al*., 1988, 2012; *Huxman et al*., 2004; *Del Grosso et al*., 2008] predict that changes in precipitation amount will affect primary productivity in most terrestrial ecosystems, with the potential to impact various ecosystem services such as the relocation of atmospheric C (i.e., CO_2) into vegetative biomass and subsequently, into the soil after plant senescence [*Scurlock and Hall*, 1998]. However, simultaneous changes in other global change drivers may interact with altered precipitation regimes, and these interactions should be taken into account to accurately predict ecosystem responses to future environmental conditions [*Sala et al*., 2000; *Eskelinen and Harrison*, 2015]. In ecosystems prone to fire, fire frequency will likely increase with more frequent, intense droughts and heat waves [*Easterling et al*., 2000] and may be maintained through feedbacks such as plant community shifts to biota well adapted to fire [*Mutch*, 1970] and immigration by exotic species after disturbance [*Vitousek*, 1996]. Additionally, fire is used as a management tool in many grasslands around the world [*Knapp et al*., 1998b; *Freckleton*, 2004], and its use may increase in grasslands and savannas to offset the rapid encroachment of woody plants [*Briggs et al*., 2002; *Bond et al*., 2005; *Ratajczak et al*., 2012].

During fire, most carbon (C) and nitrogen (N) contained in aboveground plant tissue is volatized and lost to the atmosphere [*Seastedt*, 1988], with important consequences for both soil carbon sequestration and future plant growth (via N loss). Indeed, projections from the CENTURY ecosystem model [*Parton et al*., 1987] predict reductions of soil C and N pools over time under frequent fire regimes in productive grasslands [*Ojima et al*., 1990, 1994]. However, fire also has been shown to increase the proportion of primary production occurring

belowground [*Johnson and Matchett*, 2001], having the potential to offset these C and N losses as roots die and are incorporated into the soil (i.e., root turnover). Also, because of the high proportional contribution of root turnover to the soil C pool compared with aboveground plant litter [*Sulzman et al*., 2005; *Guzman and Al-Kaisi*, 2010; *Leppälammi-Kujansuu et al*., 2014], this offsetting effect may be large.

Chronic changes to precipitation amounts have the potential to modify fire effects on biogeochemical cycling through alterations in plant growth strategies and soil nutrient processes, especially in grasslands where growth is primarily limited by water and nitrogen [*Huenneke et al*., 1990; *Seastedt et al*., 1991; *Blair*, 1997; *Knapp et al*., 2001]. Under higher soil resource levels, decreases in root:shoot allocation can favor aboveground net primary productivity (ANPP) for increased light capture, but under more limited soil resource levels, shifts toward belowground NPP (BNPP) may facilitate greater water and/or nutrient uptake [*Bloom et al*., 1985; *Giardina et al*., 2003; *Gao et al*., 2011]. Similarly, plant community shifts may alter root:shoot ratios under chronic changes in resource availability as different species, having alternate carbon allocation strategies, shift in abundance under new soil resource conditions [*Weaver*, 1958; *D'Antonio and Mahall*, 1991; *Nippert and Knapp*, 2007]. However, results from precipitation studies conducted in U.S. shortgrass [*Wilcox et al*., 2015], northern mixed grass [*Frank*, 2007], and southern mixed grass [*Byrne et al*., 2013] have shown plant allocation responses contrasting with these hypotheses, highlighting the need for additional information pertaining to how plant growth allocation will respond to altered precipitation amounts in various ecosystems. Finally, because N cycling rates tend to increase under chronically higher soil moisture, the amount of N available for plant uptake can be expected to change with future alterations in precipitation [*Matson and Vitousek*, 1981; *Wang et al*., 2006; *Chapin et al*., 2011]. A likely scenario for many central U.S. grasslands is that precipitation amounts will be chronically increased [*IPCC*, 2013], and thus, both allocation and increased incorporation of N into plant tissue may increase the rate of C and N lost during fire events by increasing both the quality and quantity of ANPP. Limited information is currently available pertaining to the extent to which these precipitation impacts might counteract stabilizing biogeochemical mechanisms of fire.

To examine how changes in precipitation alter ecosystem processes under a frequent disturbance regime (annual fire), we assessed the biogeochemical and ecological consequences of over two decades of irrigation in annually burned mesic grassland in the central U.S. We conducted intensive sampling in 2013–2015 of both biotic and abiotic responses to this long-term manipulation (1991–2015) and assessed these data in combination with responses measured both annually and intermittently throughout the study. This experiment represents an extreme manipulation designed to broaden our predictive capabilities by pushing the system beyond current and historical environmental conditions [*Beier et al*., 2012] and in order to provide insight into potential thresholds of function and the mechanisms responsible [*Kayler et al*., 2015]. In this particular system, an annual fire regime represents the highest frequency of this disturbance possible, and 25 years of increased growing season precipitation inputs not only represent an historically unprecedented consecutive period of high precipitation years (a 31% increase over the entire 25 years) [*Knapp et al*., 1998a] but also represent a frequent occurrence of extreme wet years (1 in 3 years was statistically extreme in total amount) [*Collins et al*., 2012 in the sense of *Knapp et al*., 2015]. In this study, we address the following questions: (1) How are soil biogeochemical properties affected by long-term chronic irrigation under extreme disturbance regimes? And, (2) what are the biotic and abiotic mechanisms behind these biogeochemical impacts? We predicted that chronic irrigation would shift allocation of biomass aboveground and increase rates of N cycling due to higher levels of soil moisture. As a consequence of these responses, we predicted that an annual fire regime would reduce soil C and total soil N through continual volatilization of C and N in aboveground plant tissue.

2. Methods

2.1. Site Description

Data used in this study comes from a long-term irrigation experiment at the Konza Prairie Biological Station (KPBS). KPBS is a native tallgrass prairie preserve located in the Flint Hills region of eastern Kansas, USA (39°09′N, 96°55′W). Average annual temperature is 12.5°C (U.S. Climate Reference Network data [*Diamond et al*., 2013]), and annual precipitation averages 835 mm. Although the majority of annual rainfall typically comes between April and September [*Hayden*, 1998], precipitation generally decreases in later months of the growing season when temperatures are high [*Buis et al*., 2009], resulting in substantial plant water stress. Average

aboveground net primary productivity (ANPP) at KPBS is 536 g m^{-2}, the majority of which is made up of C_4 perennial grasses, namely, *Andropogon gerardii*, *Sorghastrum nutans*, *Schizachyrium scoparium*, and *Panicum virgatum*. However, much of the plant diversity is made up of less abundant annual and perennial forb species [*Towne*, 2002]. Soils in the experiment are silty clay loams [*Ransom et al*., 1998].

2.2. Experimental Design

From 1991 to 2015, irrigation occurred via two transects of 1 m tall sprinklers in an area burned annually since 1991 and burned periodically previous to that. The amount of water added to plots over the course of the growing season averaged 268.6 mm (max 1991: 469.0 mm, min 2008: 55.2 mm) corresponding with an average 31% increase in annual precipitation (max 1991: 77.4%, min 2008: 4.8%). The experiment spanned a slight (~7 m) topographic gradient, but we conducted intensive sampling and use long-term data only from the upland portion of the study since this area had shallower soils and potential water limitations were expected to be greatest [*Knapp et al*., 1993]. In 2013, we established 30, 1 m^2 sampling plots in the upland portion of the experiment; 10 plots were placed in ambient transects (five in each transect) and 20 plots in irrigation transects (see Text S1 and Table S1 in the supporting information for details and rational for replicate numbers). Ambient precipitation was measured at a weather station 200 m away from the experiment, and irrigation amounts were measured using two rain gauges, one in each transect. Gauges were maintained just above the plant canopy height throughout the growing season. Growing season soil moisture—integrated from 0 to 15 cm—was measured hourly in two randomly assigned plots within each treatment using time domain reflectometry (model CS616; Campbell Scientific) probes. For further experimental details, see Text S2 and *Knapp et al*. [1994].

2.3. Long-Term Sampling

In September–October from 1991 to 2012, ANPP measurements were estimated by clipping all aboveground plant biomass in six randomly placed 0.1 m^2 quadrats at each sampling location. Subplots were averaged to obtain sampling location ANPP estimates. There were four ANPP sampling locations in the upland area of the experiment in 1991 and 1992 and 11 from 1993 to 2012. Biomass samples dried at 60°C for 48 h prior to weighing. Plant species abundances were visually estimated each growing season during late July in four permanent 10 m^2 plots per treatment in 1991 and 1992 and in 11 plots from 1993 to 2012. Ammonium and nitrate concentrations were measured in 1992, 1997, and 2010 within 19.1 mm diameter, 5 cm deep soil cores taken in the middle-to-late growing season in both irrigated and ambient areas. Ten cores were aggregated and homogenized for each sampling location. Soil samples were processed through a 4 mm sieve, and additional root material was removed using forceps. Nitrate and ammonium were quantified using 1 M KCl extractions. Soil measurements were conducted at the Kansas State University Soils Testing Lab (Manhattan, KS, USA).

2.4. Intensive Sampling 2013 and 2015

In September of 2013, ANPP was estimated by clipping all aboveground vegetative biomass in two 0.1 m^2 subplots per sampling plot and averaged to obtain plot means. Samples were processed identically to those from 1991 to 2012. Belowground net primary productivity (BNPP) was estimated using two root ingrowth cores [*Persson*, 1980] per plot. Ingrowth cores were 5 cm diameter by 30 cm depth and installed mid-May through September. BNPP samples were divided into 0–15 cm ($BNPP_{0–15}$) and 15–30 cm ($BNPP_{15–30}$) categories, elutriated and washed to separate soil from root biomass, dried at 60°C for 48 h, and weighed. BNPP samples were then combusted in a muffle furnace at 450°C for 4 h to estimate ash-free dry mass. Overall treatment means of BNPP were divided by those of ANPP to estimate root:shoot NPP. Standing crop root biomass ($StC_{0–15}$ and $StC_{15–30}$) was sampled down to 30 cm in mid-September using a 5 cm diameter soil core and processed similarly to BNPP samples.

C:N was measured for root and leaf tissue from each plot via dry combustion-infrared detection of carbon (C) and thermal conductivity detection for nitrogen (N) using a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI, USA). For leaf-level C:N, 1–2 of the newest, fully emerged leaves were clipped in each plot at the peak of the growing season (early August) from each of 3–4 individuals of *Andropogon gerardii*. Samples were dried at 60°C for 48 h and ground to $<$1 mm before elemental analysis. One $BNPP_{0–15}$ sample from each plot was processed in the same way to measure root C:N. $\delta^{15}N$ and $\delta^{13}C$ were measured in the live root components of four randomly chosen $SC_{0–15}$ samples in ambient and irrigation treatments using combustion with a

CE1110 elemental analyzer (Carlo Erba Instruments, Milan, Italy) and Delta Plus mass spectrometer (Thermo Eloctron Corp., Bremen, Germany). Isotopes were measured using a ConFlo II Universal Interface (Thermo Electron Corp., Bremen, Germany). The Δ values were obtained by comparing samples to a working standard which always had a within-run standard deviation of $<$0.1‰. Soil available N was measured using two nylon 10 g anion/cation resin bags per plot. Bags were buried 10 cm deep at opposite corners of plots from early May to September. Available N (nitrate and ammonium) bound to resins was extracted using 2 M KCl and measured using an Alpkem Flow Solution 4 Automated Wet Chemistry System (O.I. Analytical, College Station, TX, USA).

In 2015 (25 years after the experiment began), we measured the total soil C and N in 19.1 mm diameter soil cores at both 0–25 cm and 0–5 cm depths in eight irrigated and eight ambient plots. Four soil cores were aggregated and homogenized at each plot for 0–25 cm samples, while 10 soil cores were aggregated and homogenized for 0–5 cm measurements. Soil samples were processed through a 4 mm sieve, and additional root material was removed using forceps. Total soil C and N were measured via dry combustion and gas chromatography using a Carlo-Erba NA 1500 C/N analyzer (CE Instruments Ltd., Wigan, United Kingdom). See Text S3 for more information concerning sampling and processing protocols.

2.5. Statistical Analyses

ANPP from 1991 to 2012 was compared between irrigated and ambient plots using a repeated measures mixed effects analysis of variance (ANOVA) with autoregressive covariance matrix, chosen using corrected Akaike information criterion (AIC) (AICc), and transect as a random effect to account for pseudo-replication of plots along transects [*Millar and Anderson*, 2004]. Soil C and total N were compared between treatments and depths using a mixed effects model with transect as a random effect. Soil ammonium and nitrate were analyzed using a repeated measures mixed effects ANOVA with transect as a random effect and year as a repeated term. Long-term community composition was analyzed to assesses yearly difference in plant communities from 1991 to 2013 using permutational multiple analysis of variance (MANOVA) in R (*adonis* function in the vegan package [*Oksanen et al.*, 2013]). Similarity percentage analysis (*simper* function in the vegan package) was used to distinguish which species were most driving differences in species composition. Differences between plant species covers in irrigated versus ambient plots from 2006 to 2013 were assessed using repeated measures mixed model ANOVA, with transect as a random factor and heterogeneous autoregressive covariance structure, based on AICc; cover values were natural log-transformed as necessary to satisfy assumptions of normality. Growing season soil moisture differences in 2013 were examined using a repeated measures ANOVA with day of year as the repeated effect and autoregressive heterogeneous covariance structure, based on AICc. Data from 2013 describing grass biomass, forb biomass, woody biomass, ANPP, BNPP, total NPP, standing crop root biomass, root C:N, leaf C:N, and soil inorganic N were compared among ambient and irrigation treatments using a mixed effects model with transect as a random effect and treatment as a fixed effect. Depth was included as an additional fixed effect in BNPP and standing crop root biomass models. Differences in δ^{15}N and δ^{13}C data were examined between ambient and irrigated plots using a mixed effects model with transect as a random effect. Permutational MANOVA and analysis of similarity percentages were run using the vegan package in R (v 3.1.2; R Foundation for Statistical Computing; Vienna, Austria), while all other analyses were conducted using SAS (v9.3; SAS Inst.; Cary, NC, USA).

3. Results

3.1. Precipitation and Soil Moisture

During the 22 years prior to sampling in 2013 (i.e., 1991–2012), water falling on irrigated plots (1101 $\pm$ 131 mm: $\mu \pm$ standard deviation) was 30.9% higher than ambient (842 $\pm$ 177 mm), and 2013 irrigation was similar to these long-term trends (Figure 1). Although 2013 season-long average soil moisture was not significantly different between irrigated and ambient plots ($F_{2,15.7} = 0.20$, $P = 0.82$)—likely due to a combination of high temporal variability as well as low replication in ambient plots ($n = 2$)—increases in soil moisture tracked irrigation events well: soil moisture conditions were maintained at elevated levels throughout the majority of the growing season, while ambient plots experienced extended periods of lower soil moisture (Figure 1). Soil moisture trends from previous years (2007–2012) were similar (Figure S1 in the supporting information).

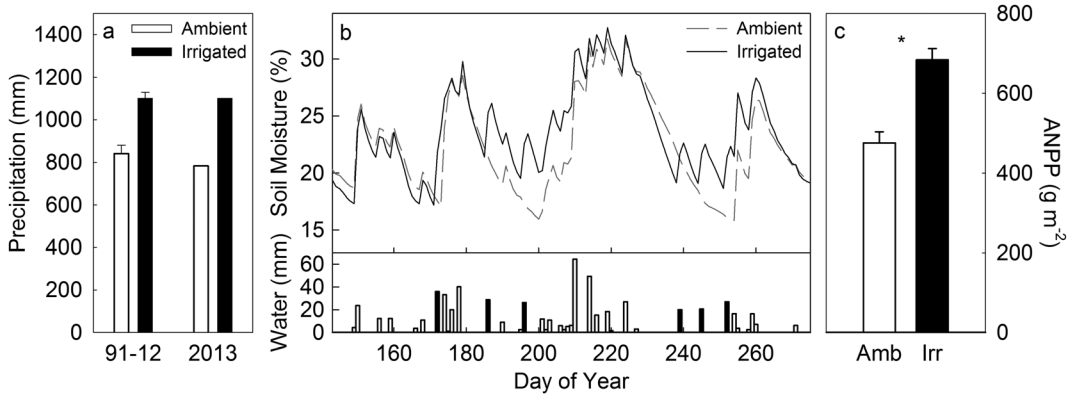

Figure 1. Precipitation, soil moisture, and aboveground net primary production (ANPP) in irrigated versus ambient plots from 1991 to 2012 compared with 2013 at the Konza Prairie Biological Station, Manhattan, KS, USA. (a) The open bars represent the average ambient annual precipitation for 1991–2012 and annual precipitation in 2013. The filled bars represent the ambient annual rainfall + irrigation during the same time periods. (b) The upper graph shows the daily volumetric soil moisture 0–15 cm in ambient (dashed) and irrigated (solid) plots during the 2013 growing season. The lower graph shows the ambient rainfall (open bars) and irrigation amounts (filled bars). (c) Average ANPP in ambient (open bars) and irrigated (filled bars) plots from 1991 to 2012. The asterisk represents the significant differences between treatments at $\alpha = 0.05$, and the error bars represent the standard error.

3.2. Plant Species Composition

Species composition was not significantly different between ambient and irrigated treatments from 1991 to 1995. However, starting in 1996, irrigated and ambient communities began to diverge based on permutational MANOVA. By 2006, communities were consistently different throughout the remainder of the experiment (Table S2). Pooling species abundance data after plant communities diverged (2006–2013), similarity percentage analysis identified differences in irrigated and ambient communities to be driven primarily by relative cover of *Solidago canadensis* (Amb:6.0%, Irr:22.0%), *Andropogon gerardii* (Amb:38.0%, Irr:29.1%), *Panicum virgatum* (Amb:11.3%, Irr:15.8%), *Sorghastrum nutans* (Amb:15.3%, Irr:10.1%), and *Amorpha canescens* (Amb:9.5%, Irr:12.2%; ordered by level of contribution and collectively explaining 73% of the variance in species composition between treatments; Table S3). When analyzed by year, these same species were consistently important; in every year during and after the community shift, the species above were always identified within the top six species contributing to community differences (Table S4). Univariate analysis of individual species relative cover in 2006–2013 indicated a significant increase in *S. canadensis* ($F_{1,20.2} = 21.4$, $P < 0.01$), a moderate increase in *A. canescens* ($F_{1,20.4} = 3.18$, $P = 0.09$), and a decrease in *S. nutans* ($F_{1,44.4} = 6.85$, $P = 0.01$) in irrigated versus ambient plots (Figure 4b). *A. gerardii* and *P. virgatum* were not indicated as significantly different by the univariate models.

3.3. Biogeochemical Pools

Both soil C and total soil N in 2015 were significantly higher in 0–5 cm than in 0–25 cm soil layers (C: $F_{1,26} = 325.0$, $P < 0.01$; N: $F_{1,26} = 135.9$, $P < 0.01$). Surprisingly, however, after 25 years of chronic irrigation and high-frequency fire, we found no differences between irrigation treatments in total soil C or N at either depth (Table 1) and no significant interaction between soil depth and treatment (C: $F_{1,26} = 0.43$, $P = 0.52$; N: $F_{1,26} = 1.33$, $P = 0.26$).

Table 1. Soil C and Total Soil N Measurements[a]

	Depth (cm)	Ambient (g g^{-1})	Irrigated (g g^{-1})
Soil C	0–5	4.55 ± 0.26	4.47 ± 0.24
	0–25	3.36 ± 0.32	3.37 ± 0.16
Soil N	0–5	0.31 ± 0.02	0.30 ± 0.02
	0–25	0.25 ± 0.02	0.25 ± 0.01

[a]Soil C and total soil N in soil samples taken in irrigated and ambient treatments at Konza Prairie Biological Station in June 2015. Data are summarized as $\mu \pm 1$ standard deviation.

Ammonium and nitrate concentrations in soil from 0 to 5 cm taken in 1992, 1997, and 2010 showed ammonium concentrations significantly higher in irrigated (4.53 ± 5.14 μg g^{-1} of soil) versus ambient plots (1.91 ± 1.48 μg g^{-1}; $F_{1,3} = 22.05$, $P = 0.02$; Figure 2). Conversely, nitrate was not significantly different in irrigated

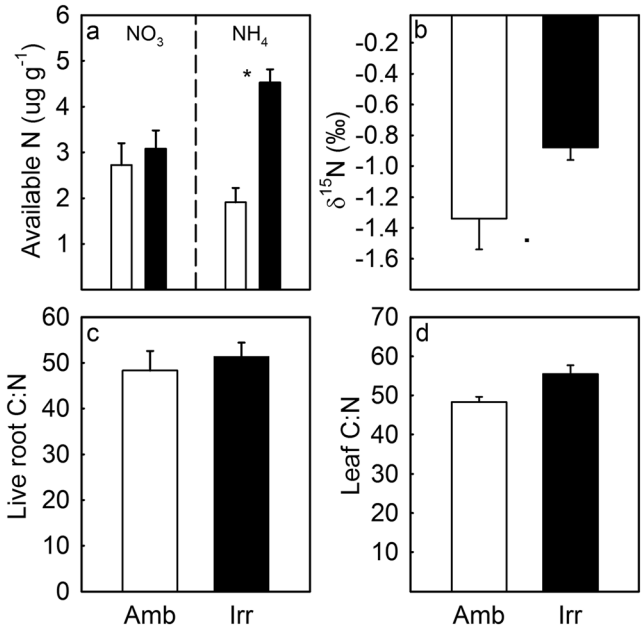

Figure 2. Biogeochemical characteristics of ambient (open bars) and irrigated (filled bars) plots at the Konza Prairie Biological Station, Manhattan, KS. (a) Nitrate and ammonium concentrations were measured on 0–5 cm deep soil samples taken in 1992, 1997, and 2010—values shown are averaged over all years. (b) δ^{15}N and (c) live root C:N were measured using live root samples taken in early September 2013, while (d) leaf C:N (from *A. gerardii*) was measured using samples collected during peak growing season in 2013. The asterisk represents the significant differences at $\alpha = 0.05$, and the "full stop" indicates the differences at $\alpha = 0.1$. The error bars represent the standard error from the mean.

($3.08 \pm 3.97\ \mu g\ g^{-1}$) and ambient plots ($2.72 \pm 2.65\ \mu g\ g^{-1}$; $F_{1,14.2} = 0.01$, $P = 0.92$; Figure 2). In 2013, we found similar trends using resin bag data: no difference was detected for cumulative (i.e., season-long) nitrate concentrations in irrigated ($0.27 \pm 0.16\ \mu g/10\ g$ resin bag) versus ambient plots ($0.25 \pm 0.16\ \mu g$; $F_{1,27} = 0.27$, $P = 0.61$), while ammonium concentrations bound to resin bags were marginally greater in irrigated ($0.98 \pm 0.82\ \mu g$) versus ambient plots ($0.69 \pm 1.1\ \mu g$; $F_{1,27} = 3.33$, $P = 0.08$). Additionally, we found marginally significant differences in δ^{15}N between treatments: roots from irrigated plots had higher values of δ^{15}N (i.e., were more enriched in ^{15}N; -0.88 ± 0.16‰) than ambient plots (-1.34 ± 0.40‰; $F_{1,6} = 4.49$, $P = 0.08$; Figure 2), suggesting greater rates of N transformation and associated losses in irrigated soils [*Hart et al.*, 1994; *McCulley et al.*, 2009]. Yet we found no evidence of differences of C:N in live root tissue obtained from root cores ($F_{1,26} = 0.33$, $P = 0.57$) or in leaf tissue of the dominant plant species present, *A. gerardii* ($F_{1,3.19} = 1.58$, $P = 0.29$) between irrigated (root: 51.4 ± 13.1; leaf: 55.5 ± 10.0) and ambient plots (root: 48.3 ± 13.5; leaf: 48.3 ± 3.8; Figure 2). We also found no significant difference between δ^{13}C values in irrigated (-14.8 ± 1.31‰) versus ambient (-17.2 ± 2.16‰; $F_{1,2} = 2.21$, $P = 0.28$) live roots sampled from 0 to 15 cm in the soil.

3.4. Primary Productivity and Biomass

From 1991 to 2012, ANPP was 43.9% higher in irrigated plots ($683.7 \pm 152.9\ g\ m^{-2}$) versus ambient ($475.1 \pm 117.4\ g\ m^{-2}$; $F_{1,2.05} = 25.9$, $P = 0.03$; Figure 1c), and the effect varied among years ($F_{21,41.3} = 7.36$, $P < 0.01$). In 2013, we found no significant difference of ANPP in irrigated ($557.2 \pm 139.3\ g\ m^{-2}$) versus ambient ($551.0 \pm 163.7\ g\ m^{-2}$; $F_{1,3} = 0.01$, $P = 0.93$; Figure 3). The lack of ANPP response in 2013 is not unusual in the upland portion of the experiment; in 11 out of 22 years between 1991 and 2012, ANPP responses were not significantly different between treatments at $\alpha = 0.05$ (6 out of 22 years not significant at $\alpha = 0.1$). Despite this lack of significant difference of ANPP in 2013, BNPP was 20.6% lower in irrigated plots ($414.3 \pm 111.3\ g\ m^{-2}$) relative to ambient ($521.6 \pm 154.5\ g\ m^{-2}$; $F_{1,27} = 4.63$, $P = 0.04$). This may reflect a difference in root:shoot ratios instead of irrigation causing lower levels of primary production, a long-term trend we consider unlikely. In support of this, standing crop root biomass—a multiyear integrative measure of BNPP used previously in tallgrass prairie to assess long-term root production responses to environmental change [*Kitchen et al.*, 2009]—was not different between irrigated ($982.1 \pm 200.9\ g\ m^{-2}$) and ambient plots ($931.9 \pm 159.2\ g\ m^{-2}$; $F_{1,3} = 0.38$, $P = 0.58$; Figure 3c), which may be more indicative of long-term trends of BNPP under irrigation. We found significantly greater levels of BNPP and standing crop root biomass in shallower soils (0–15 cm; BNPP: $265.3 \pm 71.1\ g\ m^{-2}$, standing crop: $604.1 \pm 144.6\ g\ m^{-2}$) compared with deeper soils (15–30 cm; BNPP: $171.2 \pm 66.4\ g\ m^{-2}$; $F_{1,51} = 28.14$, $P < 0.01$; standing crop: $361.27 \pm 143.6\ g\ m^{-2}$; $F_{1,51} = 43.35$, $P < 0.01$), but no evidence for changes in the depth at which BNPP or standing crop occurred in irrigated versus ambient (Depth * Treatment; BNPP: $F_{2,51} = 0.42$, $P = 0.66$; standing crop: $F_{2,51} = 2.20$, $P = 0.12$). See Table S1 for full statistical model output.

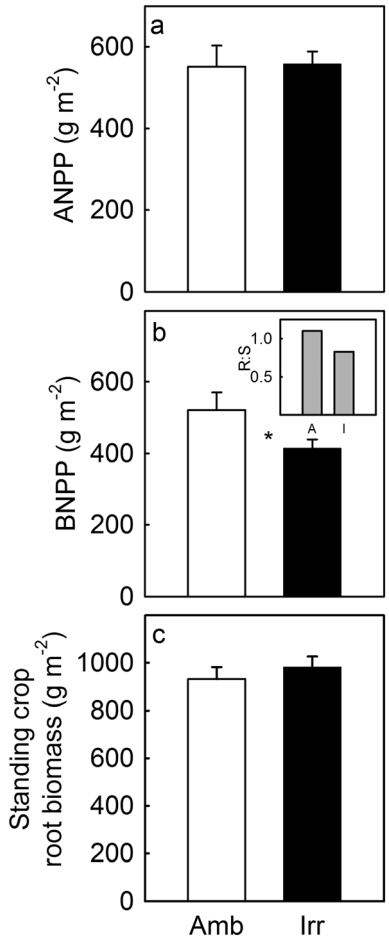

Figure 3. Net primary productivity, split into aboveground (a; ANPP) and belowground (b; BNPP) categories, and (c) standing crop root biomass in ambient (open bars) and irrigated (filled bars) plots measured in 2013 at the Konza Prairie Biological Station, Manhattan, KS, USA.Root:shoot was calculated by dividing the treatment means for BNPP by those of ANPP in 2013 (Figure 3, inset). Significant differences between irrigated and ambient plots are indicated with an asterisk for $\alpha = 0.05$ and with a full stop for $\alpha = 0.1$. The error bars represent the standard error from the mean.

4. Discussion

4.1. Stability of Biogeochemical Pools

By combining chronic increases in precipitation (with 1 of 3 years statistically extreme with regard to precipitation amount) and a severe disturbance regime (annual fire) for over two decades, this experiment created environmental conditions not experienced in the past, with the goal of quantifying responses of slowly changing, yet important, attributes of ecosystems such as plant community structure and soil C and N pools. Both the magnitude and temporal length of these manipulations make this experiment unique as a source of information to test current ecosystem models and inform new ones, a process urgently needed to accurately predict future biogeochemical cycles [*Luo et al.*, 2014]. Previous findings from modeling studies have suggested that soil C and N in this productive grassland would decrease when exposed to frequent fire events as C and N are continuously lost from the system through volatilization [*Ojima et al.*, 1990, 1994]. Indeed, we found irrigation resulted in increased long-term aboveground growth, most of which was lost annually during fire events. BNPP and standing crop root biomass were either reduced or unchanged in irrigated plots (Figure 3), suggesting that C losses via fire were not compensated by increased root growth with greater water availability, at least at shallower soil depths (≤30 cm). Using this information in conjunction with previously documented increases in soil respiration rates in irrigation plots [*Knapp et al.*, 1998a], we predicted that irrigation should reduce soil C pools in the long term. We also found evidence for greater N fluxes in irrigated soils, coinciding with previous findings of increased N mineralization and N leaching under higher soil moisture conditions (Figures 1 and S1) [*Matson and Vitousek*, 1981; *Chapin et al.*, 2011]. Based on these findings, we predicted a reduction of total soil N in irrigated plots due to N losses via leaching and/or volatilization of N in aboveground plant tissue by frequent fire. Yet after 25 years we found no differences in soil C or total soil N between irrigated and nonirrigated treatments at either 0–5 cm or 0–25 cm depths (Table 1). A number of current studies have provided evidence for the general stability of soil biogeochemical pools under recent climate in grassland ecosystems [*Hopkins et al.*, 2009; *Meersmans et al.*, 2009; *Chapman et al.*, 2013; *Chen et al.*, 2015], yet the stability of C and N pools we observed under these extreme global change scenarios was still surprising; in the following sections, we discuss potential explanations for this unexpected stability.

4.2. Potential Stabilizing Mechanisms

4.2.1. Background Variables

Background global change variables may contribute to maintenance of C and N in soils despite higher losses. Increasing levels of atmospheric CO_2 (from ~356 to 396 ppm globally over the course of the experiment) have

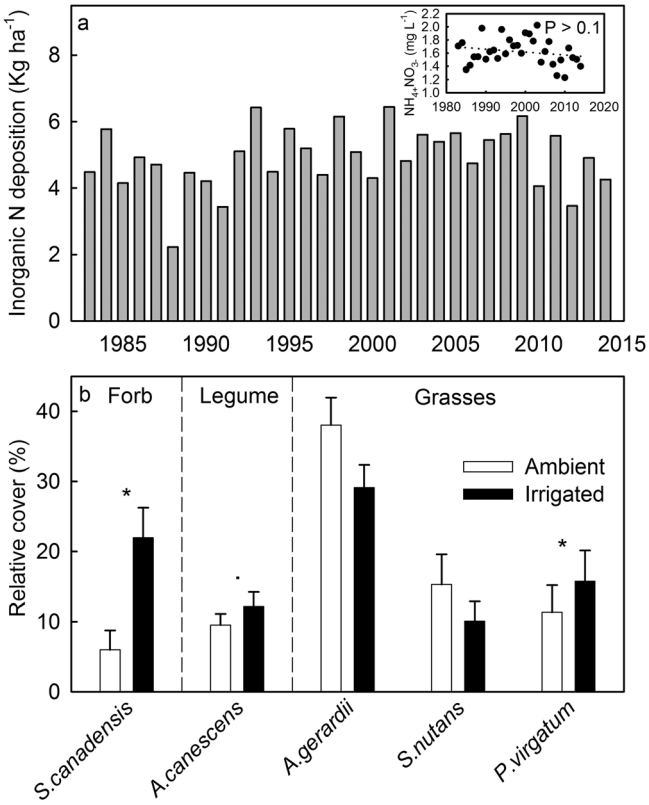

Figure 4. (a) Total annual atmospheric wet deposition of inorganic N (main panel) and concentration of nitrate and ammonium in rainfall (inset) from 1983 to 2014 at Konza Prairie Biological Station, Manhattan, KS. Data obtained from the National Atmospheric Deposition Program (NRSP-3). (b) Differences in relative cover of five species most contributing to differences between irrigated and ambient plant communities after divergence (2006–2013). The error bars represent the standard error calculated for each year and averaged across years. The asterisks represent the significant difference between cover values at $\alpha = 0.05$ and periods at $\alpha = 0.1$.

been shown to increase growth and root turnover rates [*Lichter et al.*, 2005; but see *Heath et al.* 2005]. This background response may be strong, especially given that belowground inputs of C to soil are generally more important than aboveground inputs [*Sulzman et al.*, 2005; *Leppälammi-Kujansuu et al.*, 2014]. However, as both ambient and irrigated treatments experienced the same background CO_2 levels throughout the experiment, and BNPP and standing crop root biomass were not increased in irrigated plots (Figure 3), this is likely not a major mechanism driving stability of soil C in irrigated plots. Another possibility is that N additions via deposition might have compensated for greater amounts of N volatilized during fire events. Yet when we examined total wet N deposition data collected at Konza prairie from 1983 to 2014, we found no trend of increasing deposition during the course of the experiment, either in total amount or per millimeter rainfall deposition (Figure 4a), so this is also likely not a major mechanism explaining biogeochemical stability.

4.2.2. Plant Species and Functional Composition

Changes in plant community composition can substantially impact fluxes of C and N [*Wedin and Tilman*, 1990; *St Clair et al.*, 2009; *Xu et al.*, 2015], and this may have counteracted increased losses via frequent fire. In irrigated plots, we found increases in the relative abundance of a common forb species, *Solidago canadensis*, and a legume, *Amorpha canescens*, as well as altered abundances of three dominant grass species, *Sorghastrum nutans*, *Panicum virgatum*, and *Andropogon gerardii* (Figure 4b); these shifts in species composition may have impacted rates and quality of C and/or N entering into the soil from roots having different morphological, chemical, or symbiotic properties [*Taylor et al.*, 1991; *Fornara et al.*, 2009; *Li et al.*, 2016]. Species in the *Solidago* genus and *P. virgatum* have been shown to have thicker roots than most grass species [*Craine et al.*, 2001], which might buffer soil C pools in irrigated plots through slower long-term decomposition rates [*Hobbie et al.*, 2010]. Our findings of decreased root:shoot ratios in irrigated plots may be driven in part by the increases in *S. canadensis* and *P. virgatum* abundances, as C_3 forbs and mesic grasses tend to have lower root:shoot ratios than more xeric grasses [*Tilman and Wedin*, 1991; *Craine et al.*, 2002; *Mokany et al.*, 2006]. This may be why we found altered root:shoot ratios consistent with optimal allocation theory [*Bloom et al.*, 1985; *Giardina et al.*, 2003; *Gao et al.*, 2011], while previous short-term studies did not [*Frank*, 2007; *Byrne et al.*, 2013; *Wilcox et al.*, 2015]. Additionally, increases in the abundance of *A. canescens*—found to be a nitrogen fixer in various U.S. grasslands [*Becker and Crockett*, 1976; *West et al.*, 2005]—may have partly counteracted N losses via fire in irrigated plots. Although the increase in *A. canescens* abundance was relatively small (Figure 4b), N fixers often have disproportionately large impacts compared with their abundance [*Bond*, 1994]. Based on these results, we suggest that shifts in plant species and functional composition may be an important consideration for understanding how soil C and N pools will

respond under extreme precipitation increases and disturbance regimes and that these patterns warrant further study.

4.2.3. Biogeochemical States and Pathways

Recently, there is increasing evidence that some C and N pathways may stabilize ecosystems from biogeochemical losses. In this grassland, although the majority of C in senesced aboveground plant biomass is released to the atmosphere during fire events, ~4% is left behind as pyrogenic C, which has a long turnover time in the soil [*Knicker et al*., 2012; *Soong and Cotrufo*, 2014], thus limiting C losses. Additionally, increased soil respiration in irrigated plots [*Knapp et al*., 1998a] indicates increased microbial activity in these soils. Recent findings have shown that in finely textured soils (such as those in this experiment), microbial biomass C is adsorbed quickly and in large amounts to clay and silt particles, thus providing a substantial stabilization of soil C when levels of microbial activity are high [*Cotrufo et al*., 2015]. With regards to N, a significant portion of N in aboveground plant tissue is translocated to belowground plant organs during senescence [*Hopkinson and Schubauer*, 1984; *Heckathorn and Delucia*, 1996; *Xu et al*., 2012]. As the experimental area is burned in the spring (to align with land management practice in many grassland foraging ecosystems), plants likely had ample time during senescence in the fall to move N from leaves to roots and rhizomes and thus minimize N loss under annual burning practices despite long-term irrigation-induced increases in aboveground production.

4.2.4. Additional Mechanisms

It is likely that many factors combined to stabilize biogeochemical pools in this study. Furthermore, many drivers can have multiple, sometimes contrasting, biogeochemical effects. For example, although frequent fire volatizes large amounts of aboveground C and N, it also tends to increase production and standing crop root biomass [*Johnson and Matchett*, 2001; *Kitchen et al*., 2009], which may partially offset any effects of altered precipitation on expected C losses. Indeed, findings from nearby areas with 20+ years of different burn regimes show limited and inconsistent effects of annual fire on responses of soil C and N [*Reed et al*., 2009; *Kitchen et al*., 2009; *Wilson et al*., 2009; *Soong and Cotrufo*, 2014], and fire effects on these pools were especially limited at depth [*Kitchen et al*., 2009]. As discussed above, increases in precipitation and soil moisture often result in greater soil respiration due to increased microbial activity, potentially increasing C losses, but increased microbial activity can also increase C adsorption to clay particles, increasing C retention. Also, root exudation and turnover rates vary substantially among plant species [*Grayston et al*., 1997; *Gill and Jackson*, 2000] and in stressed versus nonstressed plants [*Henry et al*., 2007]. These factors may respond to environmental drivers and impact biogeochemical pools differently than root growth. Overall, our findings highlight the substantial complexity underlying biogeochemical stability in productive grasslands. We recommend careful and holistic consideration of multiple ecosystem properties and processes when assessing how soil C and N stocks might change in the future.

4.3. Conclusions

Although we found evidence for mechanisms proposed to decrease soil C and N under frequent disturbance, we found no difference in these soil pools after 25 years of imposing extreme climatic conditions under an extreme fire disturbance regime. This lack of response of biogeochemical properties in the system may be due to any or a combination of factors identified in this study. Based on our findings, we suggest that biogeochemical stability was not a result of background increases of atmospheric CO_2 or N wet deposition, but may be due to plant compositional or functional shifts corresponding with chronic changes in rainfall, pathways that stabilize carbon in soil pools, and/or reallocation of N from aboveground to belowground biomass pools. These findings highlight the need to consider multiple global change factors and a variety of potential mechanisms associated with changes in climate and disturbance regimes when assessing future states of ecosystem processes and services. We advocate for additional efforts confronting ecosystem models with long-term experimental data to ultimately improve projections of ecosystem services under multifaceted global change scenarios.

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