**Title:** How Big is Big Enough? Surprising Responses of a Semi-arid Grassland to Increasing Deluge Size

**Associated publication:** Post, A.K. & Knapp, A.K. How big is big enough? Surprising responses of a semiarid grassland to increasing deluge size. *Glob Change Biol.* 2021; 27: 1157–1169. https://doi.org/10.1111/gcb.15479

**Abstract**: Climate change has intensified the hydrologic cycle globally, increasing the magnitude and frequency of large precipitation events, or deluges. Dryland ecosystems are expected to be particularly responsive to increases in deluge size, as their ecological processes are largely dependent on distinct soil moisture pulses. To better understand how increasing deluge size will affect ecosystem function, we conducted a field experiment in a native semi-arid shortgrass steppe (Colorado, USA). We quantified ecological responses to a range of deluge sizes, from moderate to extreme, with the goal of identifying response patterns and thresholds beyond which ecological processes would not increase further (saturate). Using a replicated regression approach, we imposed single deluges that ranged in size from 20 to 120 mm (82.3rd to > 99.9th percentile of historical event size) on undisturbed grassland plots. We quantified pre- and post-deluge responses in soil moisture, soil respiration, and canopy greenness, as well as leaf water potential, growth, and flowering of the dominant grass species (*Bouteloua gracilis*). We also measured end of season above- and below-ground net primary production (ANPP, BNPP). As expected, this water-limited ecosystem responded strongly to the applied deluges, but surprisingly, most variables increased linearly with deluge size. We found little evidence for response thresholds within the range of deluge sizes imposed, at least during a dry year. Instead, response patterns reflected the linear increase in the duration of elevated soil moisture (2-22 days) with increasing event size. Flowering of *B. gracilis* and soil respiration responded particularly strongly to deluge size (14- and 4-fold increases, respectively), as did ANPP and BNPP (~60% increase for both). Overall, our results suggest that this semi-arid grassland will respond positively and linearly to predicted increases in deluge size, and that event sizes will need to exceed historical magnitudes before responses saturate.

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**Recommended dataset citation**: Post, A.K. & Knapp, A.K. 2020. Data associated with "How Big is Big Enough? Surprising Responses of a Semi-arid Grassland to Increasing Deluge Size". Colorado State University Libraries. https://doi.org/10.25675/10217/217319

**Data collection location**: USDA-ARS Central Plains Experimental Range (40.8422, -104.7156)

**Data collection time period**: 2018-05-01 to 2018-08-28

**File information**: 10 files are included in this folder.

1. "README". Contains detailed information concerning files 2-10.
**File format**: .pdf

2. "Precipitation". Ambient precipitation during experimental period (2018-05-01 to 2018-08-20)
**File format**: .csv
**Variables:**

- Date: Date of recorded rainfall. Note: Only dates with rainfall are listed, all unlisted days received 0 mm precipitation.
- Precip (mm): Daily precipitation in mm
- Shelter: Indicates whether rain occurred when rainout shelters were installed
    - Y = Precipitation occurred when shelters were erected and did not fall on plots
    - N = Precipitation occurred when shelters were removed

3. “Soil moisture”. Soil moisture (to 20 cm depth) of plots during experimental period (2018-05-01 to 2018-08-21).
**File format**: .csv
**Variables:**
- Date: Date of measurement
- Plot: Plot where measurement was taken (Plots 5-30, 33, 34)
- Trt: Deluge treatment ID (A = 0 mm, B = 20 mm, C = 40 mm, D = 60 mm, E = 80 mm, F = 100 mm, G = 120 mm)
- VWC: Soil moisture measured as % volumetric water content (VWC), corrected for soil texture of site

4. “Soil respiration”. Soil $CO_2$ efflux and soil temperature (10 cm depth) of plots during experimental period (2018-05-08 to 2018-08-21).
**File format**: .csv
**Variables:**
- Date: Date of measurement
- Plot: Plot where measurement was taken (Plots 5-30, 33, 34)
- Trt: Deluge treatment ID (A = 0 mm, B = 20 mm, C = 40 mm, D = 60 mm, E = 80 mm, F = 100 mm, G = 120 mm)
- Precip: Size of deluge treatment (in mm)
- Efflux: $CO_2$ efflux (soil respiration) of the plot in $\mu mol\ CO_2\ m^{-2}\ s^{-1}$.
- Soil_temp: Concurrent measurement of soil temperature to a depth of 10 cm, in degrees Celsius
- VWC: Concurrent measurement of soil moisture to a depth of 20 cm measured as % volumetric water content (VWC), corrected for soil texture of site

5. “ANPP”. Aboveground net primary production of the plots, collected at the end of the experimental period (late Aug).
**File format**: .csv
**Variables:**
- Plot: Plot where measurement was taken (Plots 5-30, 33, 34)
- Trt: Deluge treatment ID (A = 0 mm, B = 20 mm, C = 40 mm, D = 60 mm, E = 80 mm, F = 100 mm, G = 120 mm)
- Rep: Experimental replication of sample (0.1 $m^2$, 2 reps/plot)
- Grass_mass: Mass of non-reproductive grass in sample, in grams
- Forb_mass: Mass of forbs in sample, in grams
- SH_mass: Mass of *B. gracilis* seedheads (flowering stalks) in sample, in grams
- SH_num: Number of *B. gracilis* seedheads (flowering stalks) in sample
- Grass_total: Total mass of grass (Grass_mass + SH_mass) in sample, in grams
- Total_biomass: Total mass of the entire sample (Grass_Total + Forb_mass), in grams
- ANPP_total: Aboveground net primary production- Total_biomass corrected for area ($g\ m^{-2}$)

6. “BNPP”. Belowground net primary production of the experimental plots. In-growth cores were installed 2018-05-10 to 2018-08-28.
**File format**: .csv
**Variables:**

- Plot: Plot where measurement was taken (Plots 5-30, 33, 34)
- Trt: Deluge treatment ID (A = 0 mm, B = 20 mm, C = 40 mm, D = 60 mm, E = 80 mm, F = 100 mm, G = 120 mm)
- Depth: Depth increment of in-growth core
    - T = Top (0-10 cm)
    - M = Middle (10-20 cm)
    - B = Bottom (20-30 cm)
- Mass: Mass of roots in sample, in grams
- BNPP: Belowground net primary production – root mass corrected for area (g $m^{-2}$)

7. “Canopy greenness”. Plot greenness from repeat digital photography, as calculated with the green chromatic coordinate (GCC) during the experimental period (2018-05-01 to 2018-08-21).
**File format**: .csv
**Variables:**

- Date: Date of measurement (Format: month_day)
- Plot: Plot where measurement was taken (Plots 5-30, 33, 34)
- Trt: Deluge treatment ID (A = 0 mm, B = 20 mm, C = 40 mm, D = 60 mm, E = 80 mm, F = 100 mm, G = 120 mm)
- Precip: Size of deluge treatment (in mm)
- GCC: Average green chromatic coordinate (greenness) of pixels in plot image

8. “Leaf area”. Number of leaves and leaf length measured on designated *B. gracilis* tillers during the experimental period (2018-06-07 to 2018-08-14).
**File format**: .csv
**Variables:**

- Date: Date of measurement
- Plot: Plot where measurement was taken (Plots 5-30, 33, 34)
- Trt: Deluge treatment ID (A = 0 mm, B = 20 mm, C = 40 mm, D = 60 mm, E = 80 mm, F = 100 mm, G = 120 mm)
- Rep: Experimental replication (3 tillers/plot, A-C)
- NumLvs: Number of green leaves per tiller
- AvgLH: Average length (in cm) of all green leaves per tiller
- CumLH: Cumulative length (in cm) of all green leaves per tiller – the length of all the green leaves were summed for each tiller
  * We multiplied this number by average *B. gracilis* leaf width (1.9 ± 0.2 mm, n = 106 leaves) to estimate live leaf area per tiller.

9. “Flowering stalk density”. Number of *B. gracilis* flowering stalks in each 1-$m^2$ plot over the course of the experimental period (2018-06-21 to 2018-08-14).
**File format**: .csv
**Variables:**

- Date: Date of measurement
- Plot: Plot where measurement was taken (Plots 5-30, 33, 34)

- Trt: Deluge treatment ID (A = 0 mm, B = 20 mm, C = 40 mm, D = 60 mm, E = 80 mm, F = 100 mm, G = 120 mm)
- Precip: Size of deluge treatment (in mm)
- num_flowers: Number of *B. gracilis* flowering stalks present in the plot

10. "Water potential". The pre-dawn and midday water potential of *B. gracilis* leaves, measured before and after the deluge treatments (2018-07-06 to 2018-08-02).
**File format**: .csv
**Variables:**
- Date: Date of measurement
- Time: Time of day the measurement was taken
    - PD = pre-dawn (~ 3-4 am)
    - MD = midday (~ 12-1 pm)
- Plot: Plot where measurement was taken (Plots 5-30, 33, 34; only 2-3 plots/treatment measured each date)
- Trt: Deluge treatment ID (A = 0 mm, B = 20 mm, C = 40 mm, D = 60 mm, E = 80 mm, F = 100 mm, G = 120 mm)
- Precip: Size of deluge treatment (in mm)
- WP: Measured water potential in MPa (averaged across 1-2 leaves per plot)

**Methods**:

***Site Description***

Research was conducted at the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) Central Plains Experimental Range (CPER) in Northeastern Colorado (40.8422, -104.7156). This undisturbed, native shortgrass steppe ecosystem has a mean annual precipitation of 321 mm, with about 70% occurring during the summer months (May-September), and a mean annual temperature of 8.6°C (Lauenroth & Sala, 1992). Average ANPP is ~100 g $m^{-2}$ (Lauenroth & Sala, 1992), and the dominant $C_4$ grass, *Bouteloua gracilis* (blue grama), can account for up to 90% of total plant cover (Milchunas et al., 1989). Soils at the experimental site are classified in the Ascalon series (Aridic Argiustolls; https://soilseries.sc.egov.usda.gov/) with a sandy clay loam texture (61% sand, 17% silt, 22% clay) and 6.4% organic matter (Soil, Water, and Plant Testing Laboratory, Colorado State University). Estimated field capacity is 27% volumetric water content (Saxton & Rawls, 2006).

***Experimental Design and Treatments***

During the 2018 growing season, we applied single deluge events of varying size (20-120 mm) to 28 1-$m^2$ plots. Plots were located in a relatively flat area previously subjected to moderate livestock grazing but that had been protected from large ungulate grazing for 7 years prior to the experiment (M. Johnston, USDA-ARS pers. comm). Plots were spaced at least 3 m apart, and aluminum roof flashing was installed 20 cm outside the perimeter of each plot to a depth of 10 cm and extending 5 cm aboveground. To prevent the confounding effects of an unpredicted natural rain event, we erected rainout shelters over all the plots 7 days prior to the deluge addition and removed them 12 days after the conclusion of the deluge treatment. The roofs were constructed from clear corrugated polycarbonate (Suntuf, Palram Americas, 2.44 m by 3.05 m) with a 0.7 to 1.0 m buffer between the roof edge and plot edge. Roofs were installed

1 m above ground-level and angled slightly to direct water drainage away from the plots. Previous research indicates that the shelters only moderately reduce transmission of photosynthetically active radiation (PAR; average reduction of 16.4 ± 5.4 %, Post & Knapp, 2020) and have minimal effects on the microclimate and plant responses (Loik et al., 2019).

The experiment employed a replicated regression design with plots randomly assigned to one of six deluge treatments, or to the ambient treatment (referred to as "0 mm") that did not receive a deluge addition (n = 4 plots/treatment). The deluge treatments included the addition of a single rain event that was either 20, 40, 60, 80, 100, or 120 mm in size. Deluges were added in the mid-growing season (Jul 10-12) when the shortgrass steppe is highly responsive to large water inputs (Post & Knapp, 2020). Our goal was to include deluge sizes representing large rain events that occur relatively frequently in this ecosystem (20 mm), as well as historically unprecedented deluges that might occur in the future as the climate changes (120 mm). Therefore, based on the historical precipitation record from a nearby NOAA weather station (1980-2017; Nunn, CO; 40.7063, -104.7833), we selected event sizes that spanned the 82.3$^{rd}$ to > 99.9$^{th}$ percentile of summer (Jun-Aug) rain event sizes for this location (Fig 1a). For this analysis, we excluded rain events less than 2 mm (Heisler-White et al., 2008); although small events can temporarily stimulate plant physiological activity (Sala & Lauenroth, 1982), they are largely ineffective at promoting plant growth (Sala et al., 1992). We then combined consecutive rain days into a single rain event because, in this region, rain days tend to be clustered (Bertolin & Rasmussen, 1969) and have an additive effect on ecosystem processes (Noy-Meir, 1973; Loik et al., 2004).

During a natural deluge event, the amount of runoff (and run-on) that occurs varies according to local topography, soil type, and soil surface characteristics, as well as plant cover, rainfall intensity, and antecedent soil moisture conditions (D'Odorico & Porporato, 2006; Liu et al., 2011; Zhao et al., 2013; Fischer et al., 2015). Our goal was to control for these variables and focus on deluges that only differed in size. Thus, we minimized runoff by applying deluge treatments over a 1-3 day period, adding a maximum of 40 mm in a single day. As a result, the 20 and 40 mm events were fully applied the first day of watering, the 60 and 80 mm events were applied over two days, and the 100 and 120 mm events were applied over three consecutive days. For each plot, deluge treatments were applied to the entire area within the flashing buffer (1.4 x 1.4 m) using a hand-held sprayer attached to a flow meter and pump. We used potable local water (McDonald Farms Enterprises, Frederick, CO) that met US EPA drinking water standards. Therefore, added water was not a significant source of nitrogen relative to typical annual atmospheric inputs (Burke et al., 2002; Burke et al., 2008) and well below nitrogen critical load estimates for regional grasslands (Symstad et al., 2019).

***Measured Responses***

Over the course of the growing season, we monitored soil moisture, soil $CO_2$ efflux (respiration), and canopy greenness, as well as leaf growth, leaf water potential, and flowering of the dominant species, *B. gracilis*. At the end of the growing season, we assessed above- and below-ground net primary production (ANPP, BNPP). Methods generally followed protocols in Post & Knapp (2020).

Soil moisture and soil respiration were measured weekly throughout the growing season (soil moisture: May 1 - Aug 21, soil respiration: May 8 - Aug 21) with more frequent measurements (every 1-3 days) directly after the deluge addition. Soil volumetric water content (VWC) was measured at the plot center and integrated over the top 20 cm of soil using a time domain reflectometry (TDR) probe (Campbell Hydrosense II). Measurements were corrected for soil texture of the site using gravimetric soil moisture measurements (Post & Knapp, 2020). To monitor soil respiration, a permanent PVC collar (10 cm diameter) was installed in each plot at the beginning of the growing season to a depth of 2.4 cm and extending 2 cm aboveground. To standardize, collars were placed in areas of bare soil between *B. gracilis* crowns. Before each measurement, any aboveground vegetation growing inside the collar was clipped and removed. We measured midday (10 am – 2 pm) soil $CO_2$ efflux at ambient $CO_2$ concentration, temperature, and humidity using a 6400-09 soil flux chamber attached to an LI-6400 (LI-COR, Lincoln, NE). Concurrently, soil temperature was measured just outside of the collar to a depth of 10 cm.

Using repeat digital photography, we assessed weekly changes in plot canopy greenness. For each image, a Sony cyber-shot digital camera (model DSC-WX100, 2496 x 1872 pixel resolution) was positioned directly above the plot at a 90° angle to capture an image of the upper right corner (0.25 $m^2$) of each plot. This location was chosen to avoid the influence of markers placed elsewhere in the plots. Images were analyzed using the R package EBImage (Pau et al., 2010) to calculate the average green chromatic coordinate (GCC) of the pixels in each photograph. See Post & Knapp (2020) and Seyednasrollah et al. (2019) for more details on this method. Because GCC is a ratio, it is robust to variations in image lighting, thus we did not observe any impact of the shelters on greenness measurements.

We monitored growth and flowering of the dominant grass species, *B. gracilis*, in response to the deluge addition. In early June, three tillers from spatially separate *B. gracilis* crowns were identified and marked in each plot. Every 1-2 weeks (Jun 7 – Aug 14), we counted and measured the live (at least 50% green) leaves on each tiller. The lengths of the green leaves were summed for each tiller to obtain a measure of total live leaf length per tiller. We then multiplied this number by average *B. gracilis* leaf width (1.9 ± 0.2 mm, n = 106 leaves) to estimate live leaf area per tiller. The three tillers within each plot were averaged to obtain a plot-level value. Starting in mid-June (before the appearance of the first *B. gracilis* flowering stalks), we counted the number of flowering stalks present in each plot to determine flowering density (# flowering stalks/$m^2$) through the end of the season (Aug 21).

We also monitored *B. gracilis* plant water status in response to the deluge treatments by measuring pre-dawn and midday leaf water potential. Measurements were taken 4 days prior to the deluge addition, the day after watering treatments concluded, and then weekly for the following three weeks. However, due to plant senescence, the 0 mm treatment was only sampled once, and the 20 mm was only sampled twice, after the deluge addition. Each time, we measured 1-2 leaves per plot from 2-3 plots per treatment using a Scholander pressure chamber (PMS instruments). Leaves were stored in the dark in humid bags and measured within 1 hour of collection.

Belowground net primary production (BNPP) was measured using 30 cm depth fine-root in-growth cores (Pérez-Harguindeguy et al., 2013). This depth accounts for 80% of roots found at this site (Sims et al., 1978; Liang et al., 1989). Methods are described in Post & Knapp (2020), but briefly, we used 2 mm fiberglass window screen formed into hollow cylinders with a 5 cm diameter. These were filled with root-free soil collected from a location adjacent to our site. One core per plot was installed in between grass crowns on May 10, 2018 to a depth of 30 cm. Cores were removed at the end of the growing season in late August and temporarily stored in plastic bags at 40° C. Each core was divided into three depth intervals (0-10 cm, 10-20 cm, 20-30 cm), which were each processed separately. Each segment was rinsed through 2 sieves (2 mm and 0.5 mm), and roots on the larger sieve were collected. Material remaining on the smaller sieve was rinsed into a bin of water and roots floating at the surface were hand-picked. Samples were dried at 60° C for two days and then weighed to the nearest 0.0001 g.

At the end of the growing season (late August), we sampled aboveground net primary production (ANPP). In each plot, all plant material from two 0.1 $m^2$ quadrats was harvested and sorted by functional group (grass, forb). *B. gracilis* flowering stalks were also separated. Biomass samples were dried at 60°C for two days, sorted to remove biomass from previous years (distinguished by grey color), and weighed to the nearest 0.01 g.

**Date of last update:** 2020-11-24

**Limits on re-use:** This data is not available for re-use until the manuscript based on it is fully published.