Final report

on the work and achievements in the K112576 projekt entitled „Ecosystem responses to droughts: experiments, observations, and synthesis"

György Kröel-Dulay, 20 December, 2023

1. Introduction

There is broad-scale consensus that climate change is happening and that it is caused by anthropogenic activity. Understanding ongoing changes and forecasting potential future changes is crucially important for a successful adaptation. Extreme events are a major element of ongoing climate change. Among extreme events, droughts are probably the most widespread and most drastic events. Studying naturally occurring drought events offer insight into actual changes in nature; but differentiating the effects of drought from parallel environmental changes can be problematic. Conversely, drought experiments can separate the effects of drought from the effects of other factors, but we cannot simulate the whole range of changes that happen during a natural drought event and field experiments limitations in terms of spatial scale. Therefore, conducting observational studies on natural drought events and experimental drought studies in combination may provide a more general understanding of the effects of droughts on ecosystems. **The overall objective of this research project was to study ecosystem responses to drought by integrating experimental and observational studies.**

Specific objectives

1. to investigate medium- and long-term ecosystem (productivity, decomposition, biomass allocation, soil moisture) and community (plant, nematode, root-colonising fungi) responses in a field experiment that combine a single extreme drought (in 2014) with recurring moderate manipulation of precipitation (drought and excess water; from 2015 onward) in a full factorial design,

2. to assess how small-scale experimental disturbances affected the sensitivity to and recovery after an extreme natural drought (2003) and subsequent weather fluctuations in a semiarid grassland based on a 20-year dataset (1998-2017),

3. to conduct a meta-analysis and compare drought experiments with observational studies on natural droughts in terms of productivity responses.

2. Major achievements

(a) We maintained a long term climate change experiments with yearly experimental manipulation and continued a 20-year monitoring study on the dynamics of sand grasslands (this is a frequently undervalued result of a short-term project)

(b) We conducted field sampling across multiple years as planned in the research proposal;

(c) We published 15 papers, with eight papers where the PI is first or last author including a first-authored paper in Nature Ecology and Evolution.

(d) We found that chronic change in precipitation is more important than extreme events in shaping ecosystm response to climate change

(e) We showed that disturbances can substantially modify grassland response to extreme events

(f) We demonstrated the drought experiments may largely underestimate ecosystem response to drought.

(f) A PhD degree was completed (Ildikó Orbán) and on ongoing PhD topic (Amira Vörös) is based on the project.

3. Work completed and results

I. Ecosystem and community responses extreme drought and chronic precipitation change (ExDRain experiment)

1. Changes in aboveground net primary production (ANPP) of different plant life forms in response to extreme drought and chronic rain manipulation

Experimental extreme drought in 2014 caused a large immediate drop in the ANPP of perennial grasses (Figure 1), and annual grasses increased their ANPP in the subsequent years (Figure 1), leading to a temporary shift in dominance from perennial grasses to annuals (Figure 2). Compared to ambient climate, under chronic dry conditions (both moderately and severely dry), the effects of extreme drought quickly disappeared (Figure 1 and 2), because chronic dry conditions alone also led to drop in ANPP and shift from perennial grasses to annuals. Here (chronic dry conditions) extreme event accelerated changes; they happened faster in extreme drought plots than in the absence of extreme event. Compared to ambient climate, under chronic wet conditions the effect of extreme drought lasted longer (Figure 1 and 2), because perennial grass ANPP increased in the absence of extreme drought; extreme drought plots could still not catch up. Here, extreme event delayed changes that took place in the absence of extreme events.

Our results suggest that extreme events may not be important in the long run. Although much emphasis is put on extreme events in climate change context, their ecological effects may often be transitory. Ecosystems may recover to their pre-event state, if environmental conditions are stable. However, if ecosystems persistently stay in their new state, it may be because mean climate has changed, and ecosystem change would have happened anyway.

But extreme events are important in the short term and in the medium term, because they modulate how ecosystems track changing climate. They may catalyse ecosystem change to unfavourable change in climate (accelerate ecosystem collapse), but may delay ecosystem response to favourable change.

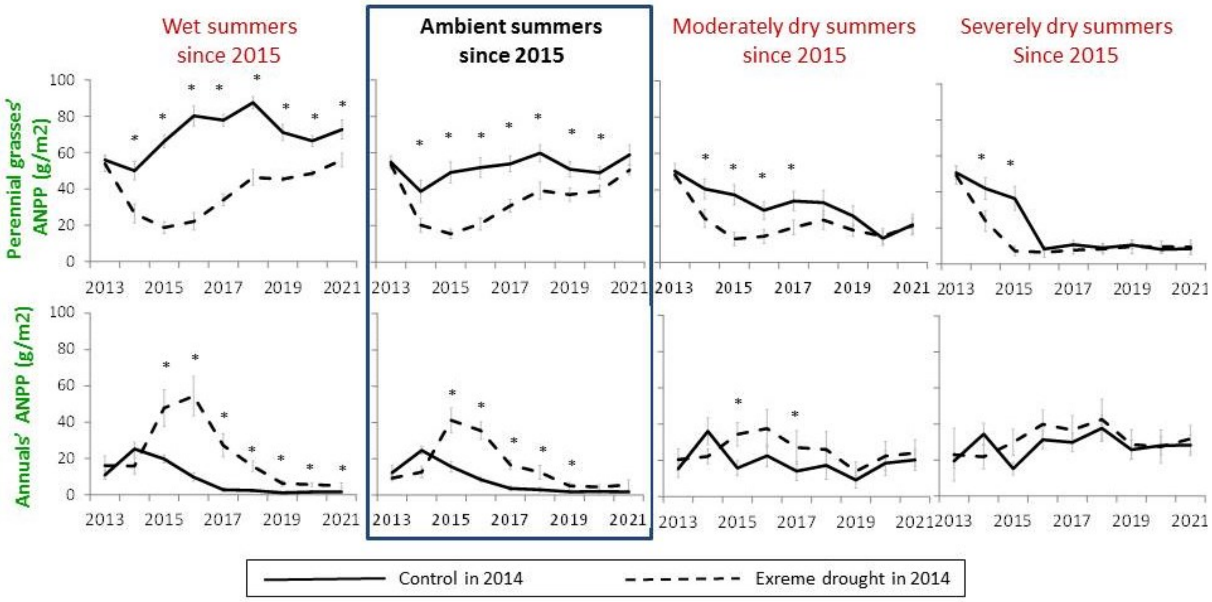


Figure 1. Changes in Perennial grass ANPP (upper panels) and annuals' ANPP (lower panels in response to an experimental extreme drought (in 2014) and to chronic changes In precipitation (wet summers, ambient summers, moderately dry summers, and severely dry summers).

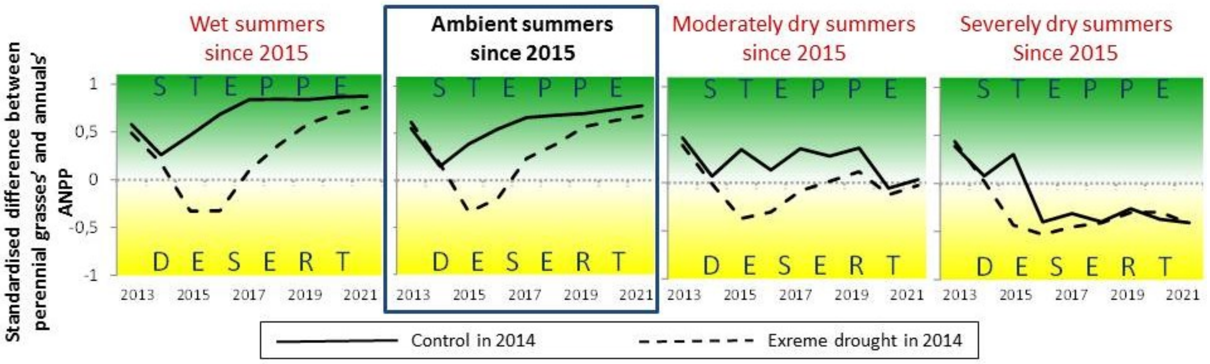


Figure 2. Change in life from dominance, expressed as the standardised difference between perennial grasses' and annuals' ANPP, in response to extreme drought and chronic change in summer precipitation.

2. Changes in aboveground biomass, belowground biomass, and biomass allocation

Droughts and drying climate have substantial effect on plant productivity and standing biomass and thus on carbon cycling. While drought effects on grassland aboveground biomass have been widely studied and are generally negative, reports on belowground biomass are much fewer and results are more variable, most likely due to changes in biomass allocation. We studied the response of aboveground biomass, belowground biomass, and root : shoot ratio to a single extreme drought and four levels of chronic precipitation change in a field experiment in a temperate grassland. We estimated aboveground biomass in each year (2013-2023), but sampled belowground biomass only in 2019, because the destructive nature of soil and root sampling does not allow for a regular sampling.

Chronic drought decreased aboveground biomass, and extreme drought negative effect was also detectable five year after the event, except for the case when severe drought followed the extreme event (Figure 3). Total belowground biomass was not affected by extreme drought (Figure 3), but was negatively affected by chronic droughts. Root : shoot ratio was not affected by extreme drought event (Figure 3), but increased in chronic drought plots, because decrease of aboveground biomass was much larger than decrease in belowground biomass.

Our results indicate that chronic change in precipitation is more important than extreme events in shaping biomass pools and allocation in grasslands, and belowground biomass is less sensitive to droughts than aboveground biomass, which underlines the need for studying both components in climate change impact studies.

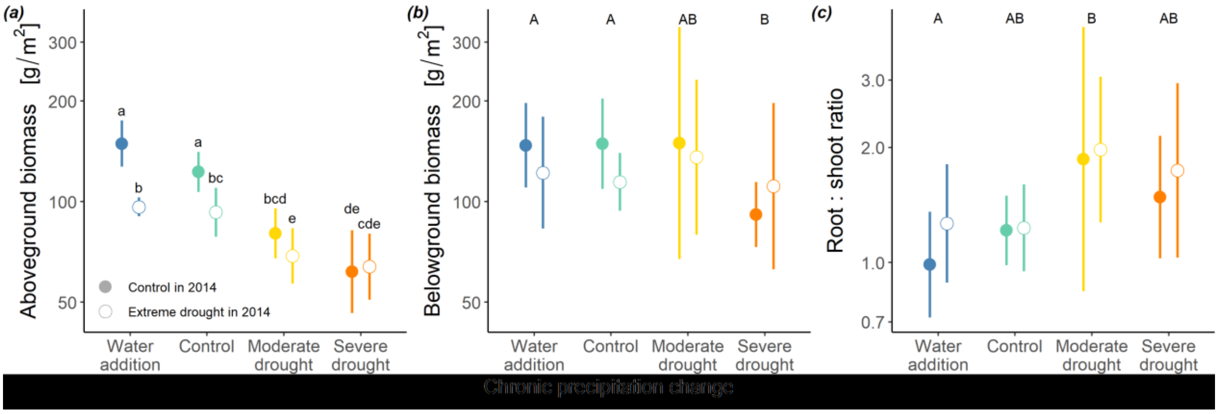


Figure 3. The biomass allocation of the vegetation by treatment combinations in the experimental plots in 2019 (five years after the extreme drought). Aboveground biomass (a), belowground biomass (b), and root : shoot ratio (c). Lower case letters indicate significant differences between treatment combinations, different capital letters mark statistically different groups among chronic precipitation treatment. (Significance level was α = 0.05.)

3. Changes in soil organic carbon content

In addition to aboveground and belowground plant biomass, soils also harbour a huge amount of organic carbon. In order to assess the sensitivity of soil organic matter content to our experiental manipulations. We sampled soil and analysed organic carbon content in 2019. We took 10 soil cores from the 0-20 cm soil layer and mixed them to reach a composite sample for all the 48 plots. After homogenisation, we determined organic carbon content with the Tyurin method (MSZ 21470-52:1983) from 0.2-1 g of soil. Single extreme drought treatment had no separate effect, while chronic changes in precipitation had a marginally significant effect (p = 0.077).

We found that extreme drought treatment had no separate effect (Figure 4), while chronic changes in precipitation had a marginally significant effect (p = 0.077; Figure 4). Although the changes are only moderate, it is surprising that we see a change in soil carbon content already after a few years of precipitation change. Also, because of the huge amount of carbon stored in the soil, relatively small changes in soil organic carbon is an important contribution to change in total amount of carbon stored in the ecosystem.

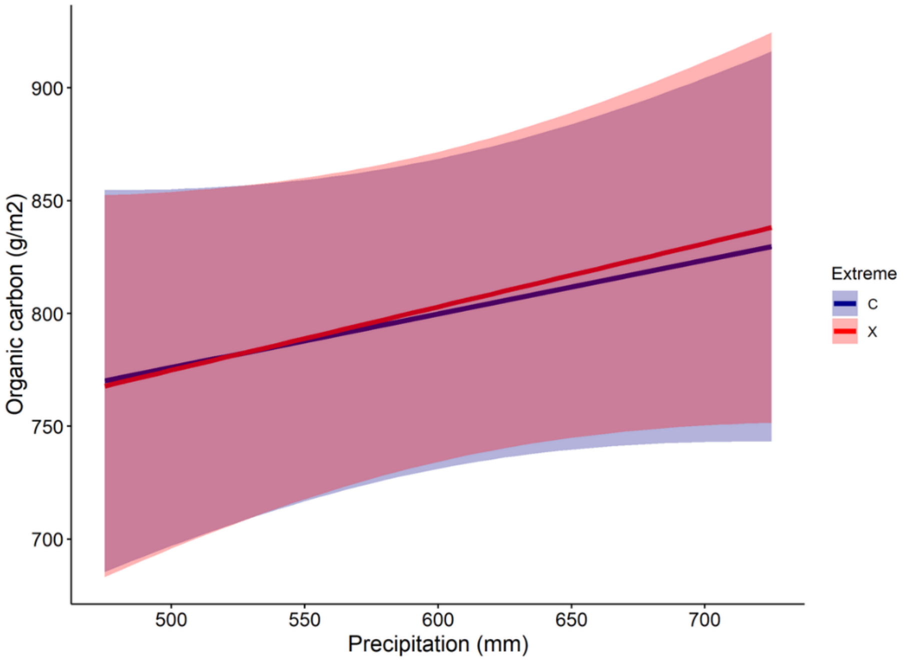
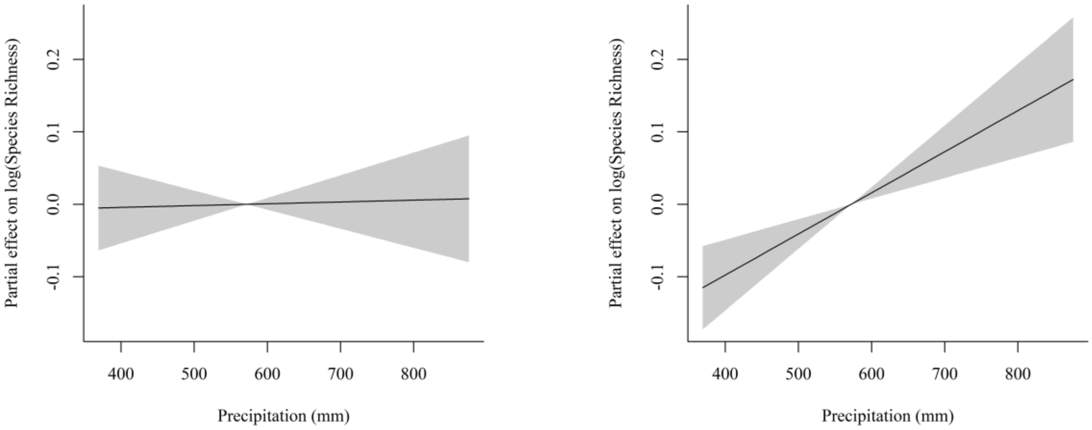


Figure 4. Changes in soil (0-20 cm) organic carbon pool as a function of precipitation in the experimental plots

4. The relationship between productivity and species richness: making use of the rainfall gradient induced by the precipitation manipulations

Despite many theories and efforts in the field, the relationship between productivity and plant species richness and the effect of disturbances on this relationship remained unclear. We investigated how the species richness of a water-limited natural grassland ecosystem responds to experimentally modified environmental productivity (chronic alteration of precipitation) with or without a previous severe disturbance event (extreme drought). We compared a bivariate analysis of the relationship between environmental productivity and species richness to a multivariate approach by structural equation modelling that can separate the direct and indirect effects of productivity.

Using a bivariate analysis, we found sharply different environmental productivity–species richness relationships in the presence and the absence of disturbance (Figure 5), whereas the multivariate analysis revealed a direct and an indirect pathway between productivity and species richness (Figure 6). The direct relationship was positive, independent of the presence of disturbance (arrow ‘a’ in Figure 6). The indirect pathway acted in the absence of disturbance, which had an immediate negative (arrow ‘c’ in Figure 6) but decreasing (arrow ‘d’ in Figure 6) effect on the biomass of the dominant species. In this case, increasing environmental productivity (simulated by the chronic precipitation treatment) enhanced the biomass of the dominant species (arrow ‘b’ in Figure 6), which decreased species richness (arrow ‘e’ in Figure 6), acting as a mediator variable. We have shown that environmental productivity has the opposite effect on plant species richness when considering either the direct or indirect pathways acting through the dominant species' biomass. A manuscript on this topic is being prepared for submission in early 2024 (Ónodi et al. in prep).



a) b)

Figure 5. Effect of the environmental productivity (precipitation) on species richness in control (a) and disturbed (b) plots. Lines show the partial effect of productivity (i.e., the effect after removing the effect of disturbance). The grey region indicates the 95% confidence interval around the regression. The relationship is significant only in disturbed plots (p-values are 0.86 and 6*10-5 in control and disturbed plots, respectively).

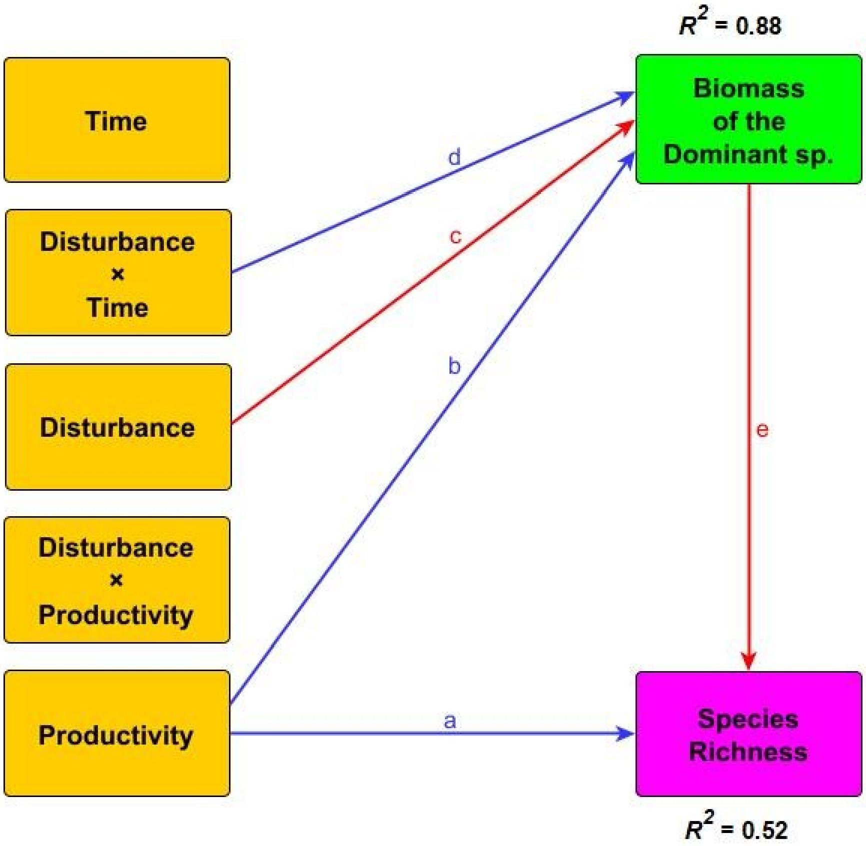


Figure 6. Structural equation model representing connections among environmental productivity (precipitation), biomass of the dominant species, species richness, disturbance, and time since disturbance. Red arrows indicate positive effects, blue arrows indicate negative effects. The exogenous variables and their interactive effects are displayed in orange boxes, the mediator variable in the green box, and the endogenous response variable in the purple box.

5. Nematode diversity response to drought

As part of this project and in line with the research plan we repeated nematode sampling of previous years (the destructive nature of sampling did not allow for a yearly sampling). Nematodes were extracted from 25 g of soil from each sample in two replicates (i.e. 2*25 g per sample). Extraction was performed with a modified Baermann-funnel technique (Figure 7) as described in Szakálas et al. (2015). After extraction, samples were counted for total number of nematodes under a stereo microscope (Olympus SZH 10) then heat-killed and fixed in a formaldehyde solution of 3-4% for genus-level identification.

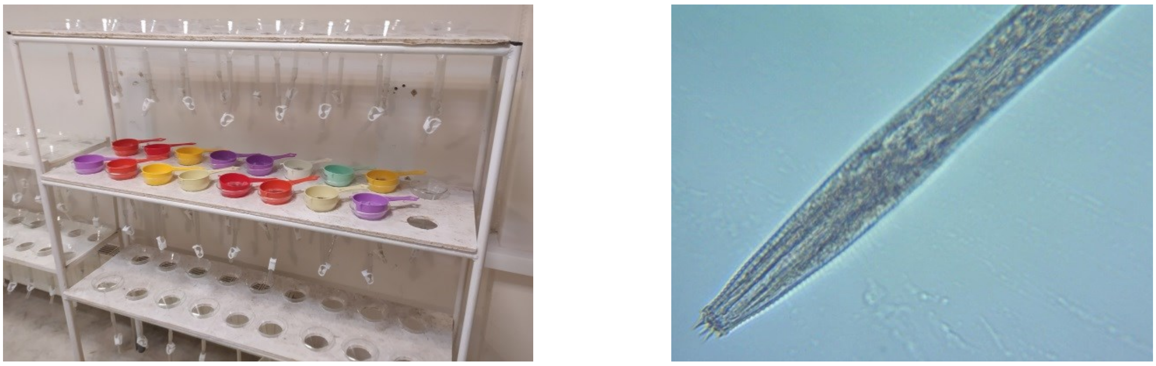


Figure 7. Nematode extraction in the lab (left) and the head of a Nothacrobeles species (right)

Our results show that drought treatments significantly decreased nematode density (ANOVA F=27.236, p<0.001). The density of the bare areas within the drought plots differed from the other treatments (Szakálas et al. in prep). Furthermore, the densities in the plant covered microhabitats of the control plots differed significantly from the dried and plant covered ones. The drought induced an increase in the densities of Nothacrobeles that was already a dominant genus before the treatments. According to the Rényi's diversity profiles, drought plots were characterized by the least structured nematode community, and the effects of the drought treatment were more pronounced in the bare soil microhabitat compared to the vegetated ones. The diversity patterns differed less between the bare and plant covered samples in the control plots than in the drought ones. The bacterial-feeding nematodes appeared to be the most sensitive group to drought, as no effects were observed on the density of the predator and omnivore nematodes.

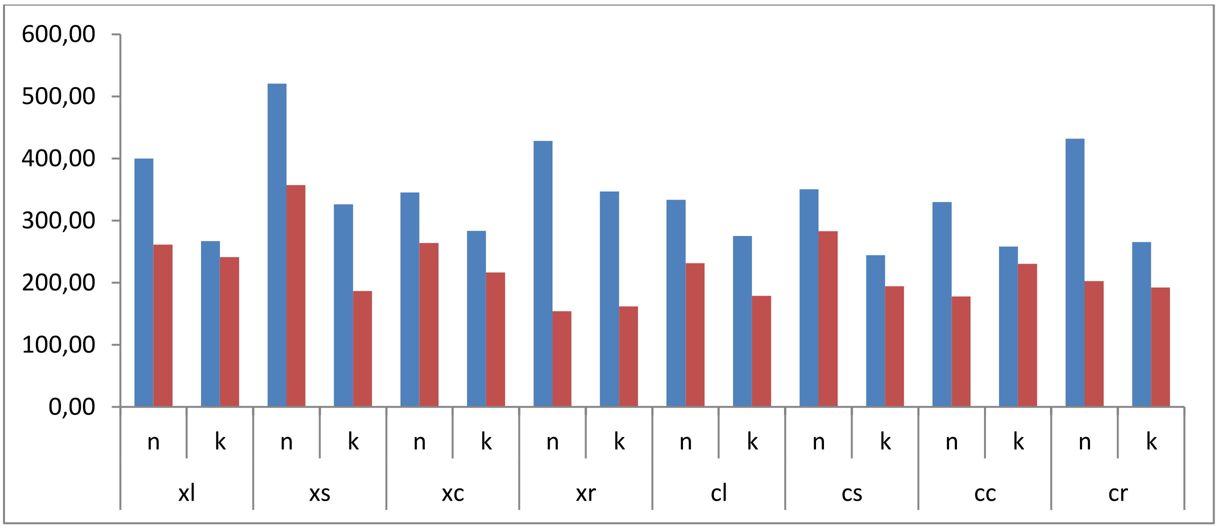


Figure 8. Nematode density values for Year 1 and Year 2 (individuals/50 g soil). Extreme drought treatment (first letter): x – extreme drought in 2014, c – control in 2014; chronic precipitation treatments (second letter): s – severe drought, l – ligt drought, r- irrigation, c-control cover: n-vegetation spots, k-bare spots

The overall dominance of genus Nothacrobeles (Allen & Noffsinger, 1971) is not surprising at all. Although relatively little information is available on this genus, there are several observations of Nothacrobeles species described or observed from deserts (De Ley et al., 1999) and from Southern Spain from volcanic sandy soil, near a salt mine (Abolafia and Peña-Santiago, 2003). So this group appears to be tolerant to extreme soil and drought conditions.

6. Changes in fungi and bacterial diversity in response to extreme drought and chronic change in summer precipitation

The soil samples from the experimental site were taken exactly before the annual manipulations in 2014 and also thereafter in every second year (2015, 2017 2019, 2021 and 2023). Altogether we collected 252 composite soil samples out which 156 have already been processed.

Community DNA was extracted from every composite soil sample with Qiagen DNeasy® PowerMax® Soil Kit. Prokaryotic 16S rRNA partial gene was amplified by the Earth Microbiome primer pair, whereas fungal nuclear rRNA ITS2 region was amplified by the ITS3_KYO2 - ITS4 primer pair. The products were analysed by Next Generation Sequencing on the Illumina platform. The raw sequences were processed and analysed using the pipeline of the SEED2 software. Soil parameters were also measured. Statistical analyses were carried out with the R software.

The 16S rRNA gene amplicon sequencing resulted in a total of 35,000 – 98,000 high-quality prokaryotic reads per sample. Whereas sequencing of fungal nuclear rRNA ITS2 region resulted in a total of 5,000 – 263,000 high-quality fungal reads per sample. We found that experimental manipulations had a significant effect on fungal community composition (Figure 9), whereas bacterial communities grouped according to their topological positions (blocks) within the sampling site (Figure 10).

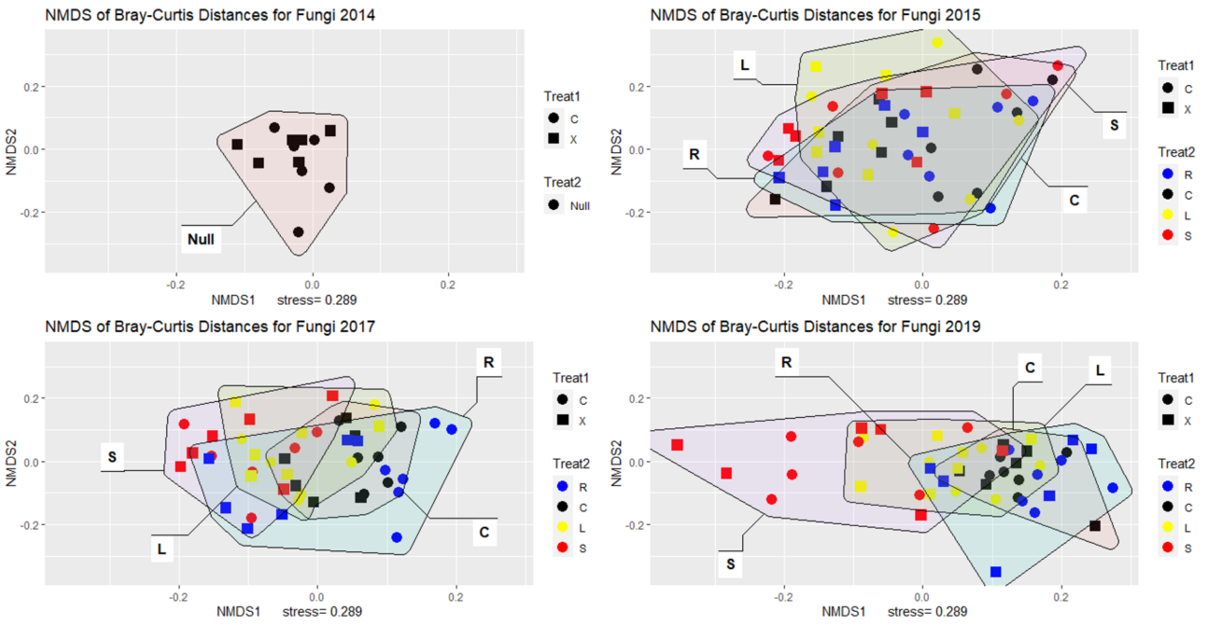


Figure 9. The effect of extreme drought (C: control in 2014; X:extreme drought in 2014) and chronic change in precipitation (R: watered; C: control; L: light drought, S: severe drought) on the composition of soil fungi communities.

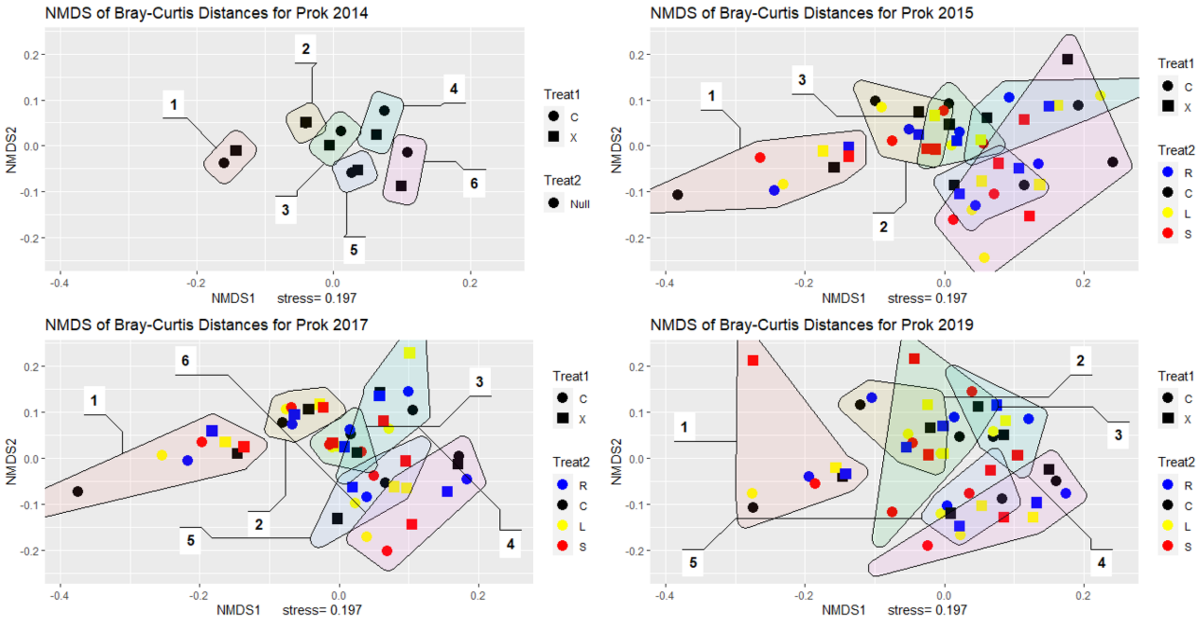


Figure 10 The effect of extreme drought (C: control in 2014; X:extreme drought in 2014) and chronic change in precipitation (R: watered; C: control; L: light drought, S: severe drought) on the composition of bacterial communities. Numbers on the graph refer to experimental blocks in the field

7. Effect of extreme drought on plant litter decomposition

Plant litter decomposition is a key ecosystem process in carbon and nutrient cycling, and is heavily affected by changing climate. While the direct effects of drought on decomposition are widely studied, in order to better predict the overall drought effect, indirect effects associated with various drought-induced changes in ecosystems should also be quantified. We studied the effect of the extreme (5-month) experimental drought on decomposition, and if this effect varies with two dominant perennial grasses, plant parts (leaves vs. roots), and soil depths (0–5 cm vs. 10–15 cm) in a semi-arid temperate grassland.

After 12 months, the average litter mass loss was 43.5% in the control plots, while only 25.7% in the drought plots (Fig. 1). Overall, mass loss was greater for leaves (44.3%) compared to roots (24.9%), and for Festuca vaginata (38.6%) compared to Stipa borysthenica (30.5%) (Figure 11). This variation was consistent with the observed differences in nitrogen and lignin content between plant parts and species. Mass loss was greater for deep soil (42.8%) than for shallow soil (26.4%) (Figure 11). Collectively, these differences in decomposition between the two species, plant parts, and soil depths were similar in magnitude to direct drought effect. Drought induces multiple changes in ecosystems, and our results highlight that these changes may in turn modify decomposition. We conclude that for a reliable estimate of decomposition rates in an altered climate, not only direct but also indirect climatic effects should be considered, such as those arising from changing species dominance, root-to-shoot ratio, and rooting depth.

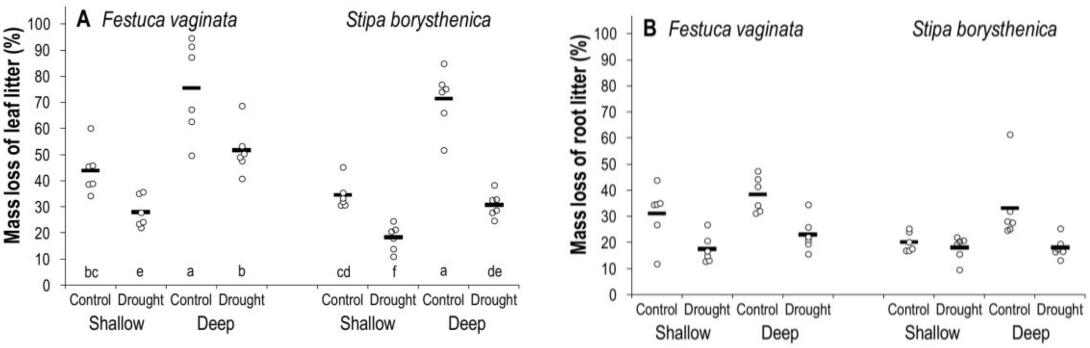


Figure 11 Mass loss (the percentage of initial mass) for (A) leaf litter and (B) root litter of Festuca vaginata and Stipa borysthenica at two soil depths of the control and drought plots at the end of the experiment (after 12 months). Data are shown as univariate scatterplots, where circles represent individual data points (n = 6 per treatment). Horizontal bars denote the mean values. Post hoc test was only performed for leaf litter, where we found a significant interaction between the explanatory variables in the linear model (species × treatment interaction; Tables 2 and S2). Different letters below the scatterplots indicate significant ($P < 0.05$) differences.

II. .The effect of small-scale experimental disturbances on sensitivity to and recovery from an extreme natural drought (2003) and subsequent weather fluctuations in a semiarid grassland based on a 20-year dataset (1998-2017),

Our study aimed to investigate how dominant species in a semiarid grassland respond to a combination of small-scale experimental disturbances and naturally occurring droughts and uncover the underlying mechanisms. We conducted a small-scale disturbance experiment in a semiarid temperate grassland and followed recovery for 18 years, including severe drought events. In 1999, we established 16 sites, eight dominated by Festuca vaginata and another eight by Stipa borysthenica, the two dominant species of sand grasslands in the region. At each site, three permanent plots were marked that received either a cutting or digging treatment or remained as controls. We monitored the cover and density changes of Festuca and Stipa annually.

In the early years following the disturbance, Festuca recovered at a similar rate under both disturbance treatments, while Stipa recovered faster in cut than in dug plots (Figure 12). When natural drought events caused major diebacks of both species, Stipa recovered very quickly and regained dominance in initially Stipa-dominated plots, and it also took over in initially Festuca-dominated control and cut plots (Figure 12). However, digging at Festuca-dominated sites delayed drought-induced Stipa colonisation and thus favoured Festuca recovery (Figure 12). We found that the poor performance of Stipa in dug plots was related to sharply reduced seedling establishment, which resulted from the low number of seeds captured by the bare soil surface after digging.

Our results demonstrate that recurring drought events may induce dominance shifts in temperate grasslands, but small-scale disturbances can modulate vegetation responses. Our findings emphasise the importance of post-disturbance regeneration patterns in drought-induced vegetation shifts and show that seed dispersal strategy may have a major effect on vegetation dynamics.

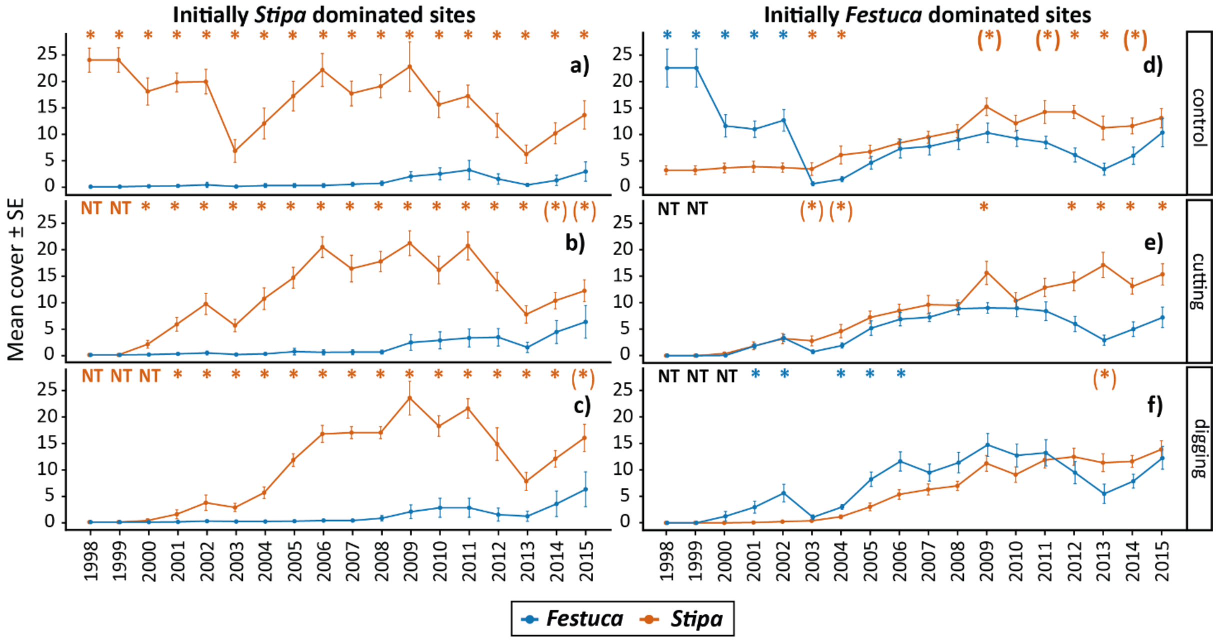


Figure 12. Comparison of the cover (mean ± SE) of dominant species in plots with initial (a)-(b)-(c) Stipa and (d)-(e)-(f) Festuca dominance. Marks above each year denote the results of one-sample Wilcoxon signed rank tests performed on the logarithm of the Stipa: Festuca ratio, separately for each year within a dominance and treatment group. P-values under 0.05 are marked with "*", and marginally significant values (0.05-0.1) are marked with"(*)". The colour of the asterisks corresponds to the dominant species. Tests were not performed in years marked with" NT" (as in" not tested").

III. Comparison of drought experiments with observational studies on natural droughts in terms of productivity responses.

To assess how climatic changes will affect ecosystems, field researchers commonly use one of two approaches: in situ observations or manipulative experiments. Observations have the advantage of being able to cover large areas and long time periods, but the links between ecosystem processes and climatic conditions are only correlational. In contrast, experiments can directly test responses to a given factor (for example, a manipulated climate variable) and isolate the effects of individual factors that often correlate with others in real-world settings.

But experiments face logistical limits to their size and duration, and manipulated variables may poorly mimic natural changes or cause unwanted side effects. Despite the differences between experiments and observations, few data syntheses compare the two types of studies.

We compared responses of AGB to experimentally applied versus observed drought events in a systematic review using hierarchical meta-analyses. We tested for effects of potential confounding factors such as drought severity (per cent reduction in annual precipitation), drought length (years) and site aridity (the ratio of mean annual precipitation (MAP) to mean annual potential evapotranspiration (PET), MAP/PET).

The estimated mean effect of drought was 53% (95% confidence interval (CI), 16% to 90%) weaker in experimental than in observational studies, after controlling for potentially confounding factors (Figure 13). Drought responses increased with increasing aridity and marginally with increasing drought severity (Figure 14) but were not significantly affected by drought length.

Our findings suggest that experiments considerably underestimate the effects of droughts in grasslands and shrublands. We conclude that while ecosystem experiments are an invaluable tool for studying the impacts of climate change, especially to distinguish among the effects of factors that change simultaneously and to unravel the mechanisms of ecosystem responses, they may underestimate the magnitude of the effects of climate change.

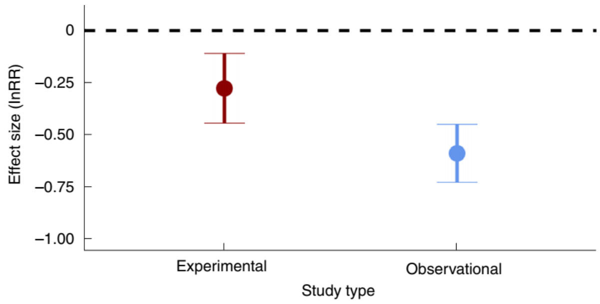


Figure 13. Response of aboveground biomass to drought measured by lnRR in experimental and observational studies in the focal meta-analysis. The results are model estimates from a meta-analytical model (Supplementary Note 2), presented as mean ± 95% CI (n = 75 for experiments and n = 83 for observations).

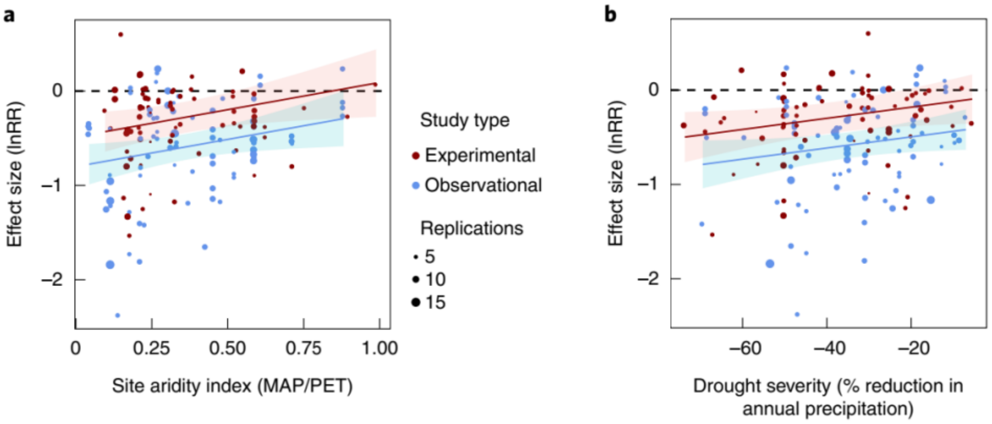


Figure 14. Responses of aboveground biomass to drought in experimental and observational studies as functions of site aridity and drought severity. The lines depict relationships between

lnRR and site aridity index (AI) (a) and drought severity (b) modelled using a meta-analytical model, and the shaded bands show 95% CIs (n = 75 for experiments (red) and n = 83 for observations (blue)).