Final report

on the work and achievements in the K112576 projekt entitled „Ecological effects of extreme drought and precipitation manipulations in grasslands”

György Kröel-Dulay, 30 January, 2018

1. Introduction

In this project we continued previous work in climate change experiments and started work at a unique new experimental facility (ExdRain: Extreme Drought and Rain Manipulation Experiment), which was established through an infrastructure grant form the Hungarian Academy of Sciences in 2013 (before the grant started).

The overall objective of the reserach was to investigate the separate and interactive effects of extreme drought and recurring moderate precipitation changes (moderate drought, strong drought, irrigation) in a field experiment in open sand steppe in central Hungary. Specific objectives were to investigate the immediate and short to medium term effects of extreme drought and recurring moderate precipitation changes on: 1. Vegetation composition and net primary productivity; 2. Fine-scale plant species assembly; 3. Plant quantitative anatomy; 4. Litter decomposition, 5. Nematode diversity, 6. Fungi diversity, 7. Plant root biomass and activity.

Below we first summarise the major achievements of the project, shortly summarise the experimental design, provide a detailed and illustrated report on the work done and results obtained according to the structure of the original proposal (Research topics), and also report on research which was originally not planned, but became the part of the research project.

2. Major achievements

(a) We kept the experiment running (conducted treatments every year, operated micrometeorological sensor network, provided continuous maintenance, ensured site safety);

(b) We conducted field sampling across all years in all research topics planned in the reserach proposal;

(c) We published three papers based on previous data that provide foundations for our ongoing work (Kröel-Dulay et al. 2015, Ónodi et al, 2017a, Ónodi et al 2017b)

(d) We already published two papers form this experiment (Mojzes et al. 2018, Tóth et al. 2017), and a third one has been submittd (Florián et al. submitted)

(e) Participation in international collaborations and research synthesis resulting in several papers (Nature, Methods in Eoclogy and Evolution, Ecosystems, Global Change Biology, Advances in Ecological Research, Science of the Total Environment)

(f) Two PhD degrees were completed that were based on research in the experiment (Tóth Zsolt, Florián Norbert)

(g) New research projects were initiated and got funded linked to the experiments: two PhD topics (Ildikó Orbán, Amira Fatime Abbas) and two postdoc projects (Andrea Mojzes, Gábor Ónodi)

3. Experimental design

In the experiment we manipulate two factors: single extreme drought (in 2014) with two levels (extreme drought vs. control) and long-term (multi-year) precipitation with four levels (severe drought, moderate drought, control, water addition), in a full factorial design and in six replicates (Figure 1 and 2.). We monitor many response variables, and the work was organised according to the various response variables

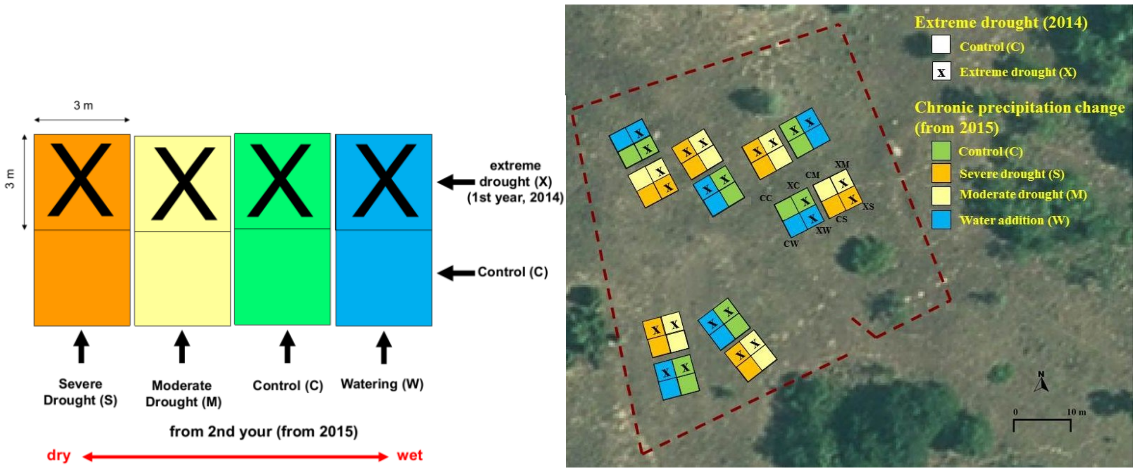


Fig 1. Experimental design, and the layout of the experimental plots in the field

Fig 2. Treatments. Extreme drought treatment in 2014 (left) and watering (right)

4. Work completed and results, according to the original Research Topics

4.1. Research Topic 1. Vegetation composition and net primary productivity

Work completed

We sampled the vegetation in four, 1m * 1m plots in each of 48 study plots, three times a year, from 2013 to 2018. Presence/absence of vascular plants was detected, and their percentage cover were estimated. In each year, the maximum value (form the three sampling dates) were retained for all species and plots. Cover values were converted to biomass based on species-specific calibration curves obtained through cover estimation and subsequent biomass harvest outside the study plots. These data were used for subsequent analysis of vegetation composition and net primary productivity.

As part of the vegetation studies we investigated the intra- and transgenerational plasticity of a subordinate annual grass, Secale sylvestre. This species can gain dominance in response to repeated and more intense droughts, and we hypothesized that its plasticity can facilitate this process.

Results

Extreme drought induced a major reduction in aboveground net primary productivity (ANPP) in 2014 (Fig 3.), and high mortality of the dominant perennial grasses (Kröel-Dulay et al. 2018). Chronic precipitation change from 2015 affected ANPP, with higher ANPP in watered plots and lower ANPP in drought plots (Fig 3). Perennial grasses showed slow recovery in irrigated plots, and no recovery in chronic drought plots (Fig 4). Annual species gained dominance (Fig 4), but these species could only partially offset productivity decline. This altered species composition (Fig 5) thus led to a lower productivity even three years after the extreme drought (Fig 3). We are presently working on a manuscript that summarises these results

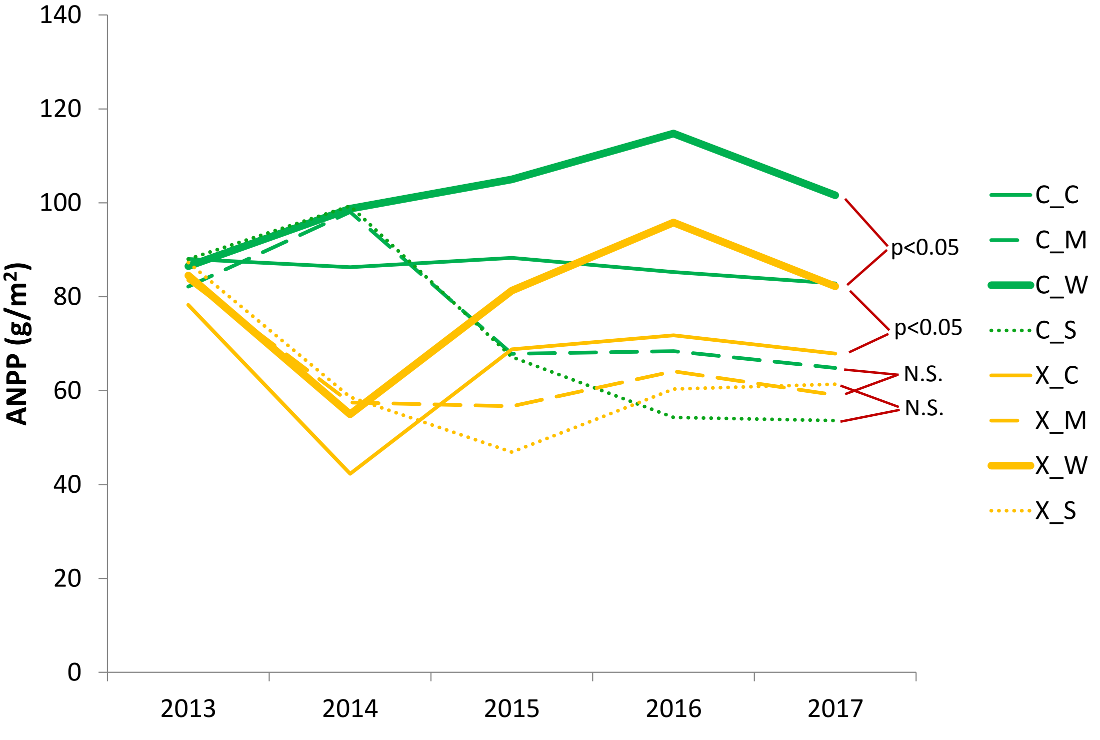


Figure 3 Aboveground NPP in 2013-2017 (1st letter: C: control; X: extreme drought; 2nd letter: C: control; W: watered; M: moderate drought; S: severe drought)

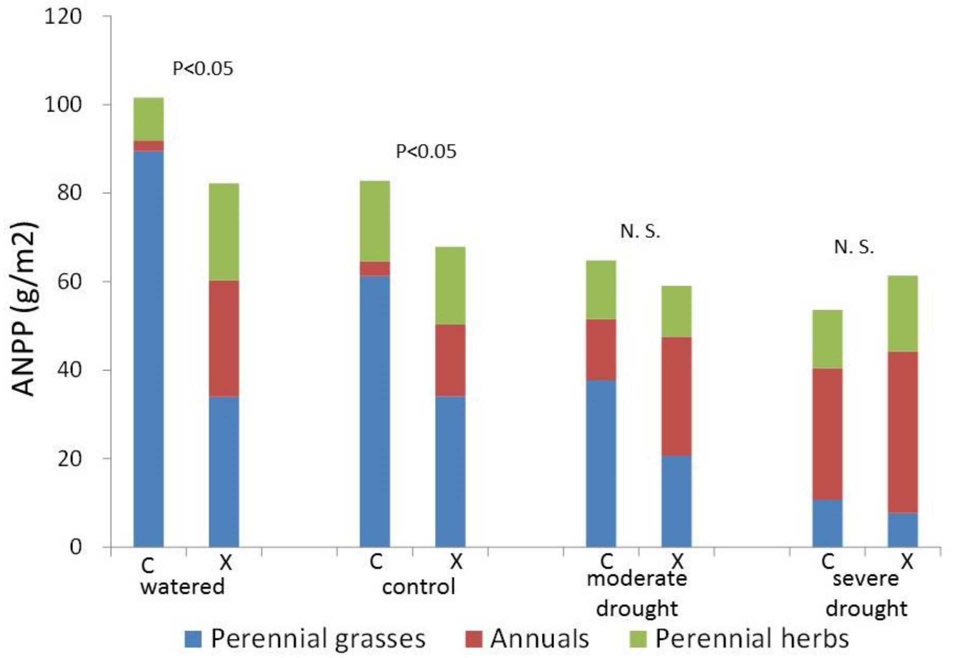


Figure 4. Aboveground NPP in 2017 and its partitioning among major life forms (C: control; X: extreme drought)

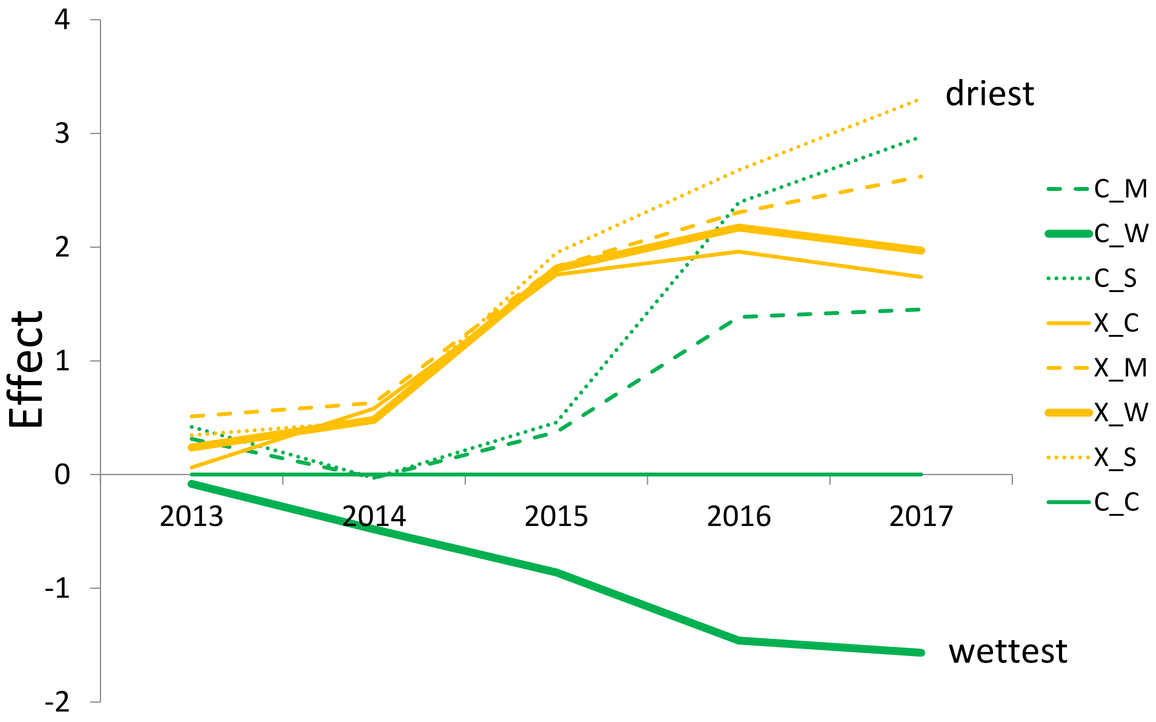


Figure 5. Change in species composition (principal response curve) (1st letter: C: control; X: extreme drought; 2nd letter: C: control; W: watered; M: moderate drought; S: severe drought)

Compared to control plots, S. sylvestre plants grew 38% taller, and produced 32% more seeds in severe drought plots, while plants in watered plots were 17% shorter, and had 22% less seeds (Mojzes et al. 2018). Secale seed mass was greatest in severe drought plots. Plants growing in drought plots had offspring with enhanced juvenile shoot growth compared to the progeny whose mother plants grew in watered plots (Figure 6). These responses are most likely explained by the decreased cover of previously dominant perennial grasses in severe drought plots, which resulted in wetter soil compared to control and watered plots during the peak growth of S. sylvestre.

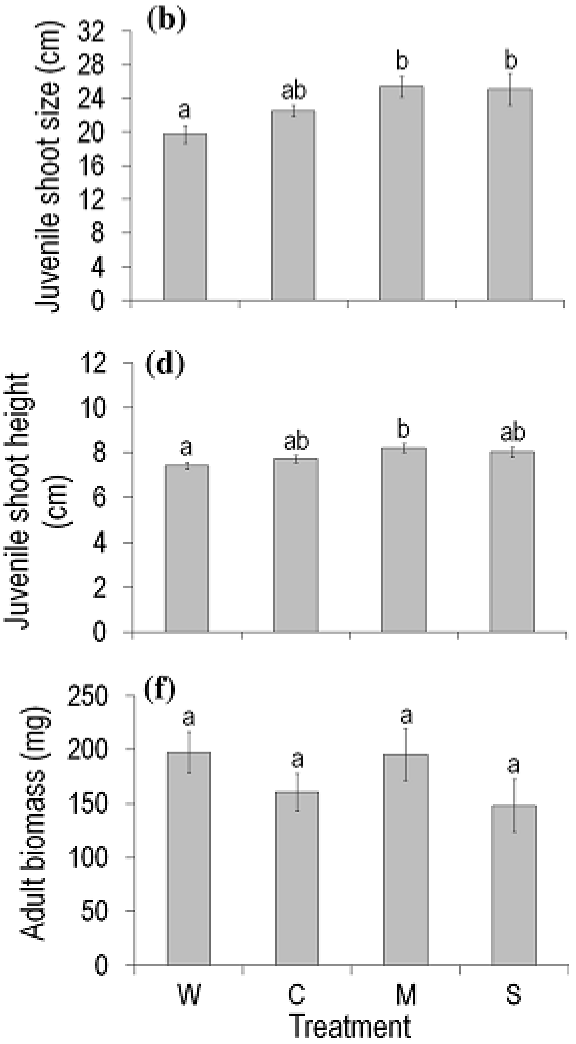


Figure 6. Effects of differences in the maternal environment (field plots; W: watered, C: control, M_ moderate drought, S: severe drought) on Secale sylvestre off-spring generation grown in the same environment (pots in common garden).

4.2. Research Topic 2. Fine-scale plant species assembly

Work completed

We sampled a 50cm*150 cm plot by 5cm*5cm resolution in each plot each year for presence absence of vascular species, moss and litter (Figure 7). From 2015, we sampled twice a year (May and September).

Figure 7. Fine-scale sampling of plant species composition

Results

By comparing temporal patterns of overall spatial associations (associatum) of functional groups within the permanent quadrats, we found that associatum collapsed in 2015 one year after the extreme drought treatment and recovered slowly in subsequent years (Figure 9.). Severe drought in 2015 had similar effects as the extreme drought in 2014 (Figure 8). We also found that the temporal patterns of associatum became synchronized due to the extreme drought event (unpublished results).

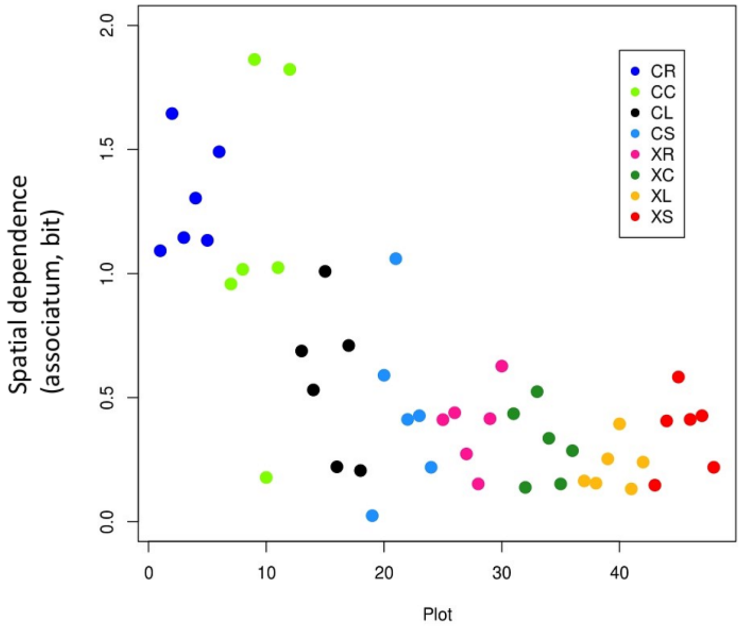


Figure 8. Small scale spacial dependence (associatum) of the eight treatment combinations in 2015, one year after the extreme drought treatment. (coding: first letter is control („c”) of

extreme drought („x”) in 2014, while the second letter indicates rain addition („r”), control („c”), light drought („l”), or severe drought („s”).

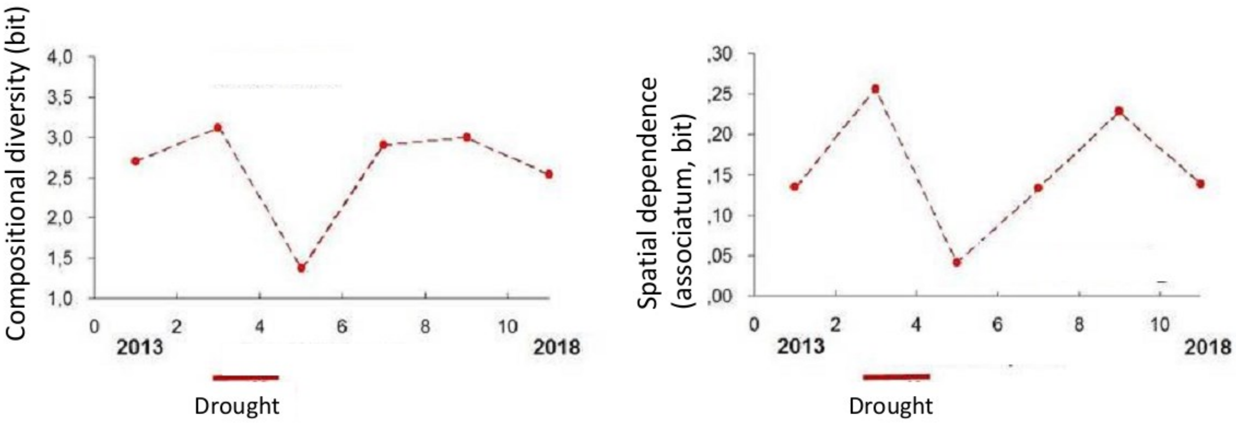


Figure 9. Changes in fine-scale compositional diversity (florula diversity) and spatial dependence (associatum) in the control plots in response to the xtreme droughttreatment.

4.3. Research Topic 3. Plant quantitative anatomy

Work completed

We collected plant samples (leaves) of Festuca vaginata, Stipa borysthenica, Alkanna tinctoria, Artemisia campestris in all years between 2014-2018. In addition, in 2014 we also collected leaf samples of three additional species, Secale sylvestre, Medicago minima, and Fumana procumbens (number of collected leaves are 893 (in 2014), 779 (in 2015), 748 (in 2016), 726 (in 2017, and 714 (in 2018)). So far, we worked on Festuca and Stipa leaves from 2014-2016. We measured 43 anatomical variables on 1563 leaves, resulting in 67209 records.

Results

Based on the data processed so far, both Festuca and Stipa leaves in the drought plots differ from those in the control and the watered plots (Figure 10.). In response to drought, leaves are more closed, have shorter hairs, and a higher proportion of sclerenchymatic tissues. Further analysis are needed to decide on the strength of these differences.

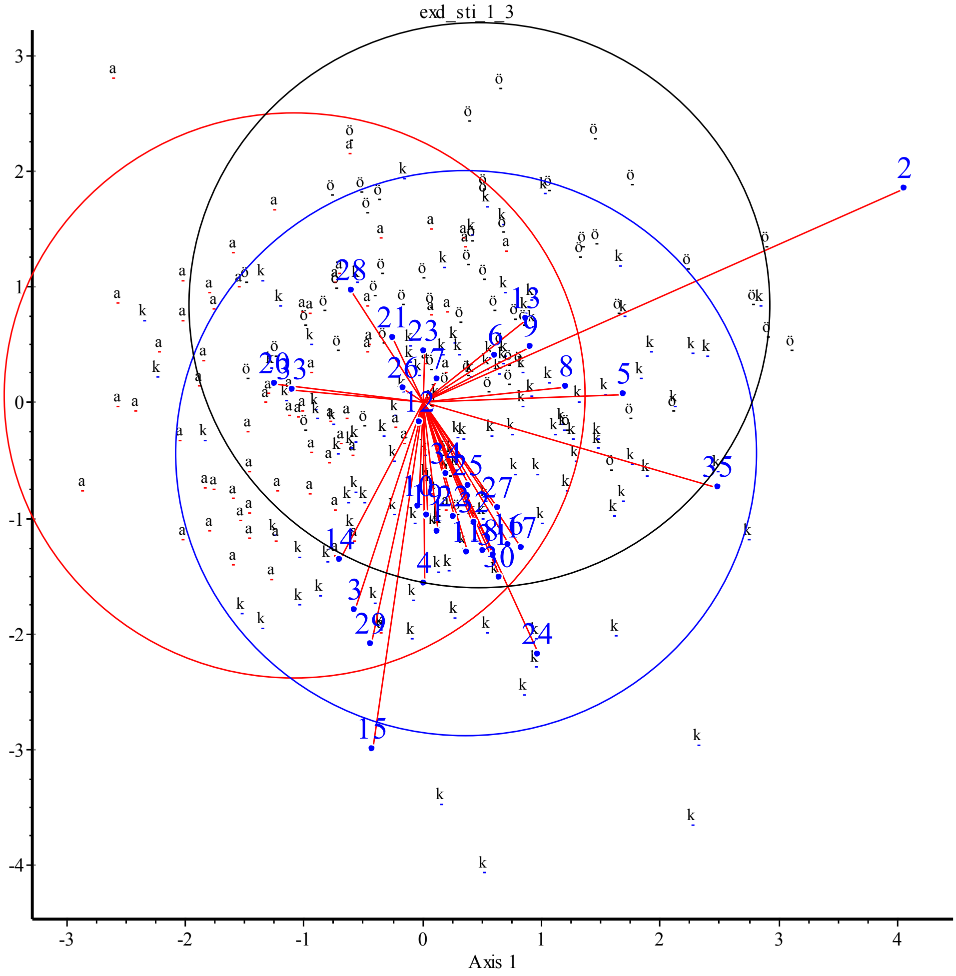


Fig. 10. Principal component analysis of Stipa leaves based on multiple leaf anatomical variables (CV1=64%, CV2=36%). Most important variables are distance between the margins of the closed leaf blade (#2 on the figure) and average length of papillae on the abaxial surface (#35 on the figure)

4.4. Research Topic 4. Litter decomposition

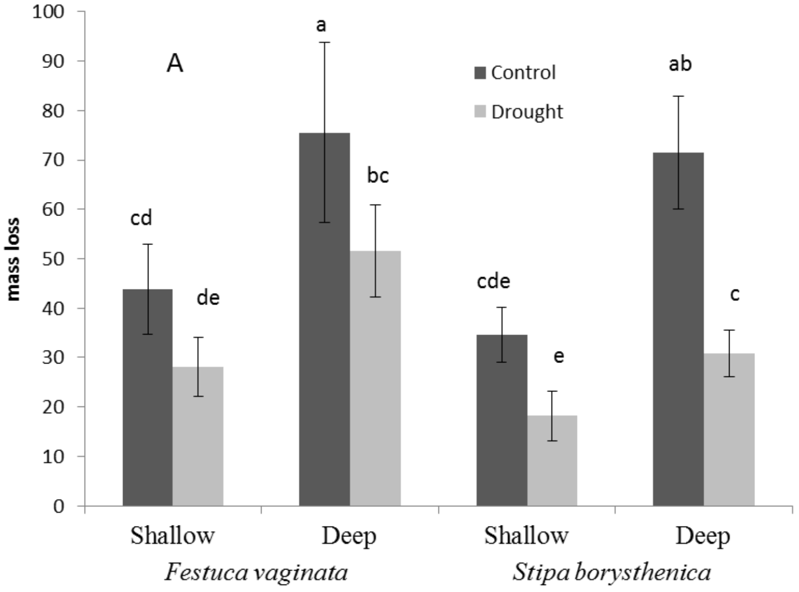
Work completed

We use the minicontainer method to study the decomposition of Festuca and Stipa leaves and roots, in two different soil depths. A series of plastic bars containing the minicontainers were put in each plot (48 plots), and one per plot (and per species) were removed after 2,4,6,and 12 months. We repated this trial in three years, 2014, 2015, and 2016. From 2018, we applied a more simple design: use only leaves, and two dates (6 months and 12 months) and two depths, but skipped roots. Weight loss of litter placed inside the minicontainers (following removal and cleaning) is used as a measure decomposition rate. I addition to the minicontainer method, we also assessed decomposition by using the tea bag method.

Results

After 12 months, an average of 43.5% of the total organic matter was decomposed in the control plots, while only 26.7% in the drought plots (Figure 11). Weight loss was greater for Festuca vaginata (38.6%) than for Stipa borysthenica (30.5%), and for shoots (44.3%) than for roots (24.9%), which is consistent with differences in C:N ratio and lignin content between plant species and plant parts (both being lowest in Festuca leaves and highest in Stipa roots) Weight loss was greater for deep soil (42.8%) than for shallow soil (26.4%), most likely due to soil moisture differences. As the effects of the additional factors (plant species, plant parts, soil depth) were similar in magnitude to direct drought effect, we conclude that for a reliable estimate of decomposition rates in an altered climate, not only direct but also indirect climatic effects must be considered, such as those arising from changing species composition, root-to-shoot ratio, and rooting depth (Seres et al., manuscript). We also found a higher decomposition rate in the control plots with the tea bag method (Fig, 12., Tóth et al. 2017)

Preliminary analysis of decomposition in subsequent years shows that decomposition rate was higher in plots previously exposed to extreme drought compared to control plots. This was most likely related to higher soil moisture content in extreme drought plots as a consequence of dieback of dominant perennial grasses during the drought.



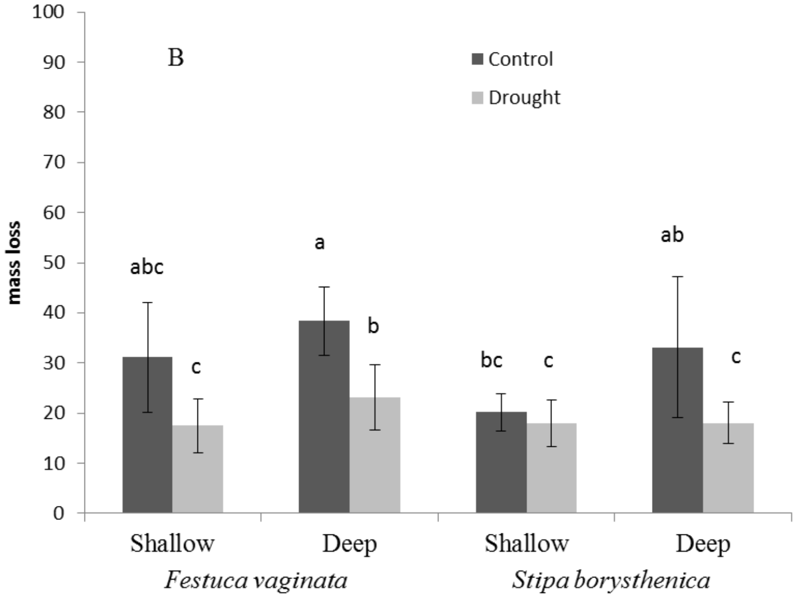


Fig. 11. Mass loss (12 months) of the different plant species leaves (A) and roots (B) in two soil depths (0-2.5 cm, 10-12.5cm) in the soil of control and extreme drought plots in 2014. Columns shown are the averages of six independent replicates ± SD. Different letters mean significant differences at least at the level of $p<0.05$.

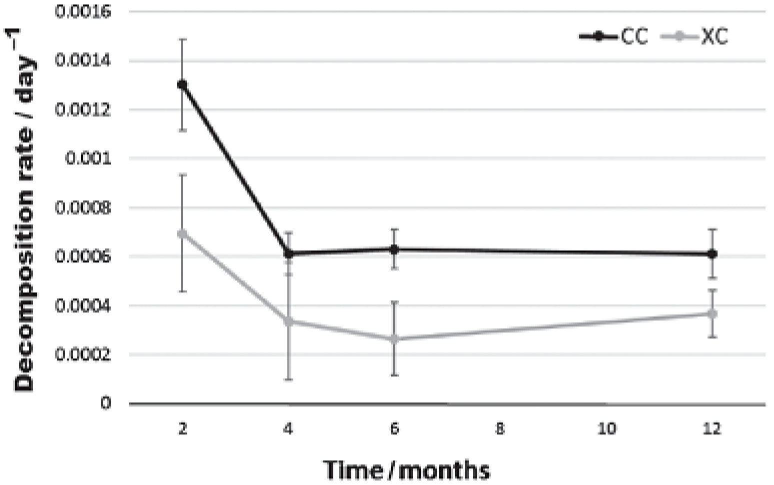


Figure 12. Decomposition rate through time in the control and extreme drought plots as assesses with the tea bag method

4.5. Research Topic 5. Nematode diversity

Work completed

2015: We decided to use only a single soil depth (0-10 cm) in order to decrease work load. However, we decided to continue the work in two microsites, plant and interspace, because previous work has shown that this factor is a major determinant of nematode density and composition. In 2014 and 2015 we sampled nematodes four times a year (March, April, July, September) in all 48 study plots (Figure 13). From 2016, we samples only once per year (September).

Extraction of 2*25 g of soil from each sample was performed with a modified Baermann-funnel technique. After extraction, samples were counted for total number of nematodes and fixed in formaldehyde solution for taxonomic identification.

Figure 13. Collecting soil samples for nematodes and sample processing in the lab

Results

Before the drought treatment started, vegetated microsites had higher nematode density than bare microsites (Szakálas et al 2015). Drought decreased nematode density in the vegetated plots and prevented the increase in nematode density during the year in the bare plots (Figure 14). The drought induced an increase in the densities of Nothacrobeles and Acrobelophis, and a decrease in the densities of Aphelenchoides, Cephalobus, Plectus, Geomonhystera, Tylenchorhynchus and Filenchus. The bacterial-feeding nematodes appeared to be the most sensitive group to drought.

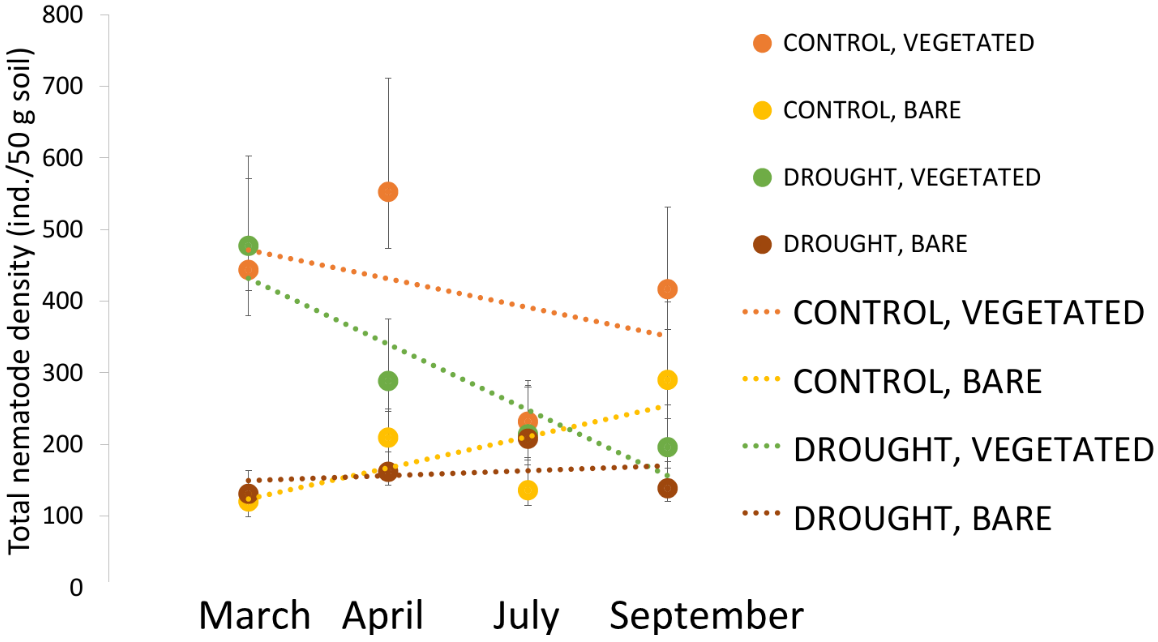


Figure 14. Seasonal change in total nematode density in two microsites (vegetated vs. drought) in the drought and the control plots in 2014. Rain exclusion (drought) was applied between April and September.

4.6. Research Topic 6. Fungi diversity

Work completed

We collected soil samples for molecular diversity from the 48 plots from each year (2014-2018), soil sampling was matched with the flowering time of on of the dominant grassland species, Stipa borysthenica. DNA was extracted was done, and samples ae deep frozen and will be analysed together. Methods develop very fast in this research fields, thus a major emphasis was put on selecting the appropriate method. We finished the analyses of the pilot NGS (Next Generation Sequencing) metabarcoding with ITS1F-ITS4 primers run on Illumina platform. Financial resources in the project did not allow for analysis of the samples; we search for resources to complete the analysis.

Results

The pilot NGS resulted in 6.000-39.000 pruned ITS2 reads passing the pipeline per samples (Fig 15). Their rarefaction curves showed saturation for all the samples, but the 5 g samples revealed higher number of OTUs even if the OTUs of 3 parallel 0.3 g samples were averaged artificially per samples.

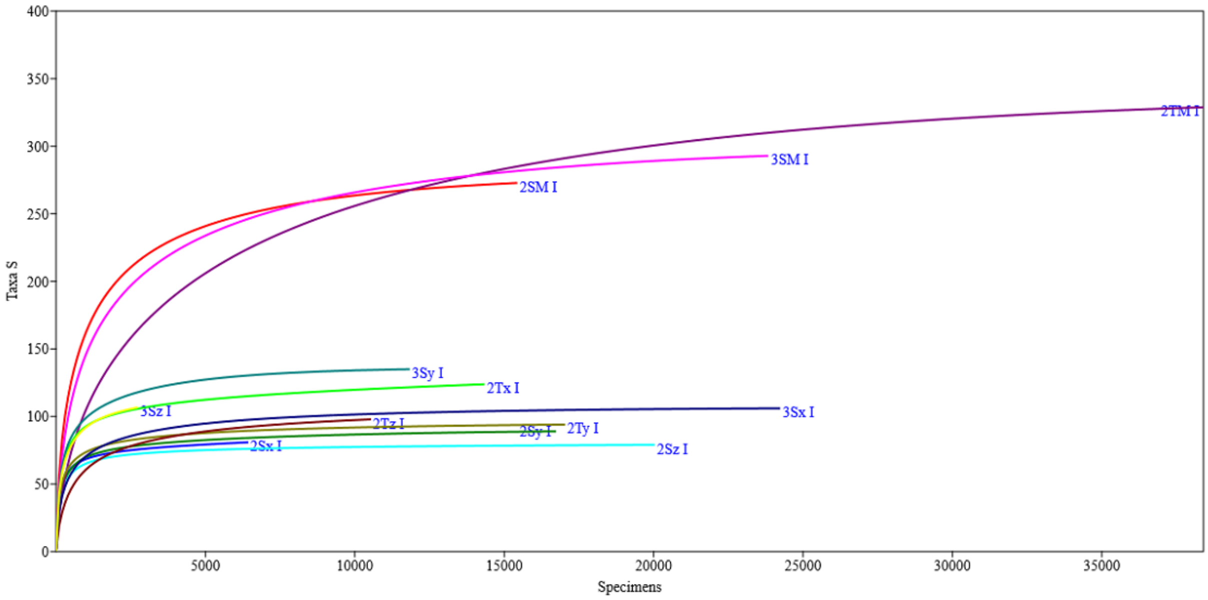


Figure 15. Rarefaction curves of taxa found in the twelve pilot samples.

Most of the sequences were classified to Ascomycota, Basidiomycota represented a less diverse group, whereas 2-6% of the sequences were classified to Glomeromycota (Figure 16). Many sequences of fungi previously isolated from Fülöpháza region (dark septate endophytes, ectomycorrhizal fungi) were also found.

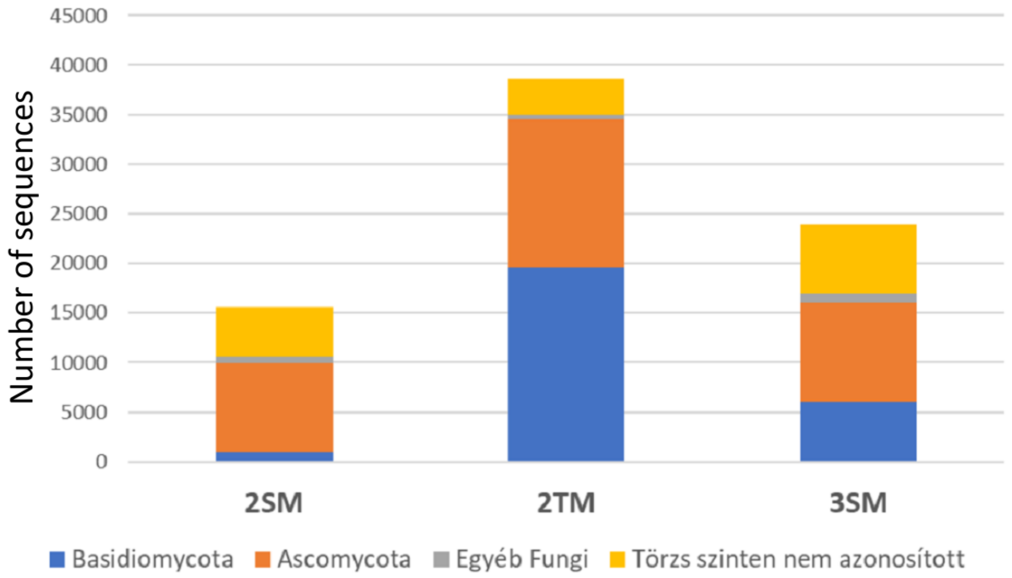


Figure 16. Fungal composition of some pilot samples

4.7. Research Topic 7. Plant root biomass and activity

Work completed

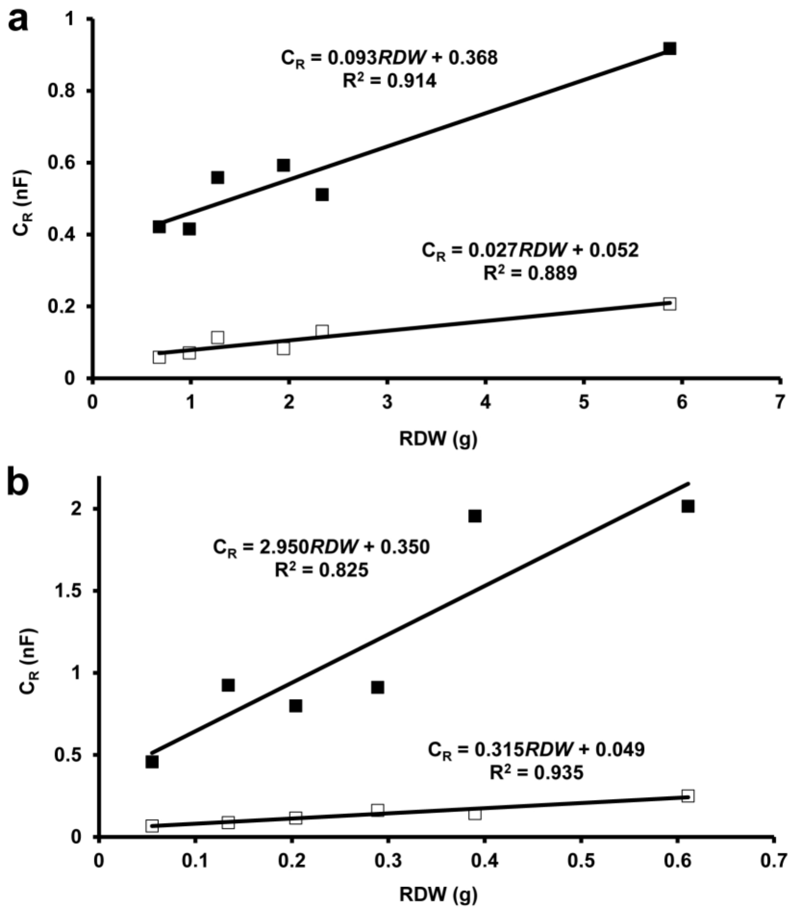
We measured electrical capacitance and impedance on individuals of different sizes for Stipa borysthenica, Conyza canadensis, and Crepis rhoedifolia. We found a strong relationship between plant size (root dry weight) and electrical capacitance (Fig 17). Soil was watered and repeated measurements were taken in order to assess the effects of soil moisture content.

We analysed the electrical capacitance and impedance measurements for Stipa borysthenica and Crepis rhoedifolia in more detail. We found that for each species the relationship between measured capacitance and relative soil moisture content can be characterised by the same parameters, independently from plant size. This means that we can use the same relationship for plants of different size.

In 2016 and 2017, we conducted a field campaign and followed two individuals of both species (Stipa and Crepis) at a monthly basis in the control and the severe drought plots. We measured root capacitance and soil moisture content at about every fourth week in the growth period of each species (in 2017, Stipa: 4 April, 4 may, 31, may, 21 June, 20 July, 23 August, 26 Sept; Crepis: 31, may, 21 June, 20 July, 23 August, 26 Sept).

Results

Our results showed that the Stipa had the maximum root activity during flowering in both 2016 and 2017, which was followed by a minor, second peak in autumn (Figure 18). Root activity was lower in the drought plots than in the control plots, but it recovered fast after the drought ended. Crepis had also the highest root activity during flowering, but it monotonically decreased afterwards. Drought had a negative effect on root activity in Crepis as well. Our results suggest that root capacitance can be used to monitor seasonal changes of root activity, and it shows the effect of droughts (Cseresnyés et al. in preparation).



Figre 17**.** Linear regressions between root electrical capacitance (C_R in nanofarads, nF) and root dry weight (RDW) in *Stipa borysthenica* (**a**) and *Crepis rhoeadifolia* (**b**) plants. Markers □ and ■ represent C_R data recorded in dry ($\theta_{rel} \sim 0.08$) and water-saturated ($\theta_{rel} = 1$) soil, respectively. Each regression is significant at $p < 0.01$ level.

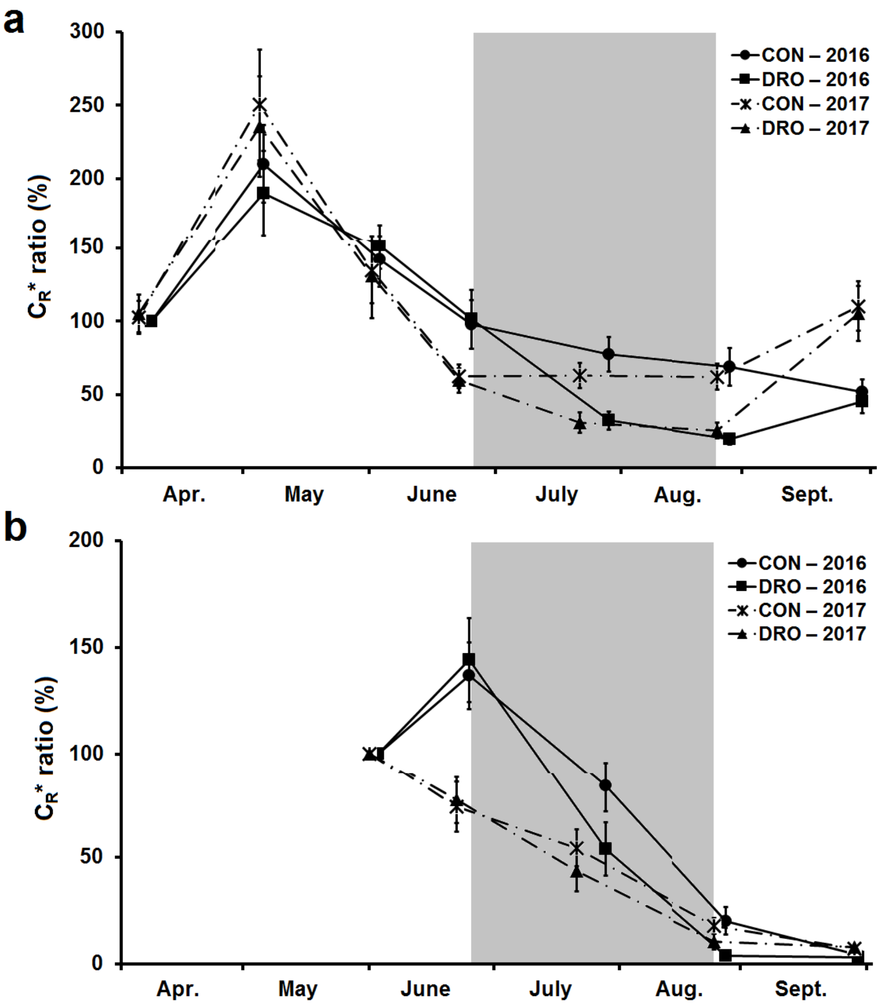


Figure 18: Changes in root activity represented by CR* ratio (mean ± SD) in Stipa borysthenica (a) and Crepis rhoeadifolia (b) during the growing seasons of 2016 and 2017. CR* is the apparent root electrical capacitance. CR* calculated for the first sampling time of 2016 (and of 2017 for C. rhoeadifolia) for each plant specimen was regarded as 100%, from which the following CR* ratios were derived. CON: control; DRO: two-month summer drought treatment (indicated by the shaded area).

5. Achievements originally not planned in the research proposal

5.1. Synthesis of vegetation responses in long-term drought and warming experiments in European shrublands

As part of a previous European collaboration we summarised vegetation responses in long-term climate change experiments (Kröel-Dulay et al. 2015). We showed that vegetation (abundance, species richness and species composition) across seven European shrublands was quite resistant to moderate experimental warming and drought, and responsiveness was associated with the dynamic state of the ecosystem, with recently disturbed sites responding to treatments. These results suggest that successional state influences the sensitivity of ecosystems to climate change, and that ecosystems recovering from disturbances may be sensitive to even modest climatic changes.

5.2. Comparing different non-destructive vegetation sampling methods and assesing their applicability in climate change experiments

Using data from previous long-term monitoring in the same ecosystem (sand grasslands) and experience from these ongoing climate change experiments, we published two papers ont he comparison of different non-destructive vegetation sampling techniques. We showed that visual cover estimation, which is often considered subjective and thus less reliable, performs as well as methods considered more objective (field-based remote sensing or point intercept measurements) in estimating aboveground biomass in grassland (Ónodi et al 2017b). In particular, we showed that the relationship between biomass proxies (cover, NDVI) and actual biomass is dependent on the particular year and the amount of precipitation (Ónodi et al. 2017a). This indicates that using the same relationship across multiple years and/or precipitation treatments may cause a systematic error in biomass estimations.

5.3.. Soil micro-arthropod responses to precipitation manipulations

A group of scientists working on soil arthropods and using a novel method of sampling (Edapholog) have joined the research. They sampled soil arthropods in all plots (48) for two years. We found that extreme drought treatment had negative effect on the catch of all Collembola groups, whereas all mite groups increased in activity density (Florián et al., submitted). In case of severe drought, moderate drought and water addition we did not find considerable changes in the second year (2015), except in case of epedaphic Collembola.

5.4. Participation in cross site synthesis work

Previous and ongoing research projects have provided several possibilities to get involved in various research synthesis on the effects of climate change on terrestrial ecosystems. These include changes in soil carbon content in response to warming (Crowther et al 2018), the response of net primary productivity to drought experiments (Estiarte et al. 2016) the sensitivity of soil respiration to temperature and soil moisture (Lellei-Kovács et al. 2016), Primary production resistance and resilience to extreme droughts (Stuart -Haentjens et al. 2018), and the use of isotopic methods in climate manipulation experiments (Andresen et al. 2018).

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