Project type and number: **NKFI PD_16 120844**

Duration: **2016.12.01–2019.11.30.**

Project title: **Reproductive phenology and transgenerational plasticity of grassland species in response to rainfall manipulations**

Principal investigator: Mojzes Andrea

Final report on the results of the research

1. *Publishing the results of an earlier study that served as one of the bases of this NKFI project*

Aim

To assess the effects of year-round nocturnal warming or late spring rain exclusion on selected plant ecophysiological traits of three abundant species in the Pannonian sand forest-steppe in a plot-scale climate simulation field experiment over six consecutive years. The target species, which represented different plant functional types, were *Festuca vaginata* (C_3 bunchgrass), *Cynodon dactylon* (spreading C_4 grass), and *Populus alba* (shrub-sized root suckers of a deciduous tree).

Activities

Field measurements, and sampling for subsequent laboratory measurements were conducted during 2003-2008. However, synthesizing results and manuscript preparation were completed by early 2017 (i.e. during the first year of this NKFI project).

Results

In general, experimental treatments had smaller effects than weather fluctuations including extremities. Rain exclusion reduced leaf physiological activity or growth, but only during or just after the treatment, and in certain years. For example, for *P. alba*, smaller leaves developed in the drought plots compared to the control and warming plots in June 2005, and also in the dry year 2007 irrespective of treatments (Figure 1). When assessing the effects of treatments and background climatic variation together, in spring, leaf area growth was consistently stimulated by increasing temperature for each species, but decreased with longer rainless periods for *P. alba* and *F. vaginata*. Longer summer droughts reduced leaf gas exchange, particularly for *F. vaginata*.

These results suggest that shallow rooted C_3 bunchgrasses can be the most susceptible to climatic variation, thus their abundance is expected to decline in the Pannonian sand forest-steppe. In contrast, plants having deeper roots and clonal integration will probably be less affected by the projected warming and drying climate, thus they are expected to be more resistant or even increase in abundance.

This study provided an essential basis for the field monitoring part of the NKFI project (section 2).

Publication

Mojzes, A., Kalapos, T., Kovács-Láng, E. (2017) Plant ecophysiological responses to drought, nocturnal warming and variable climate in the Pannonian sand forest-steppe: results of a six-year climate manipulation experiment. *Biologia* 72(12): 1431-1445.

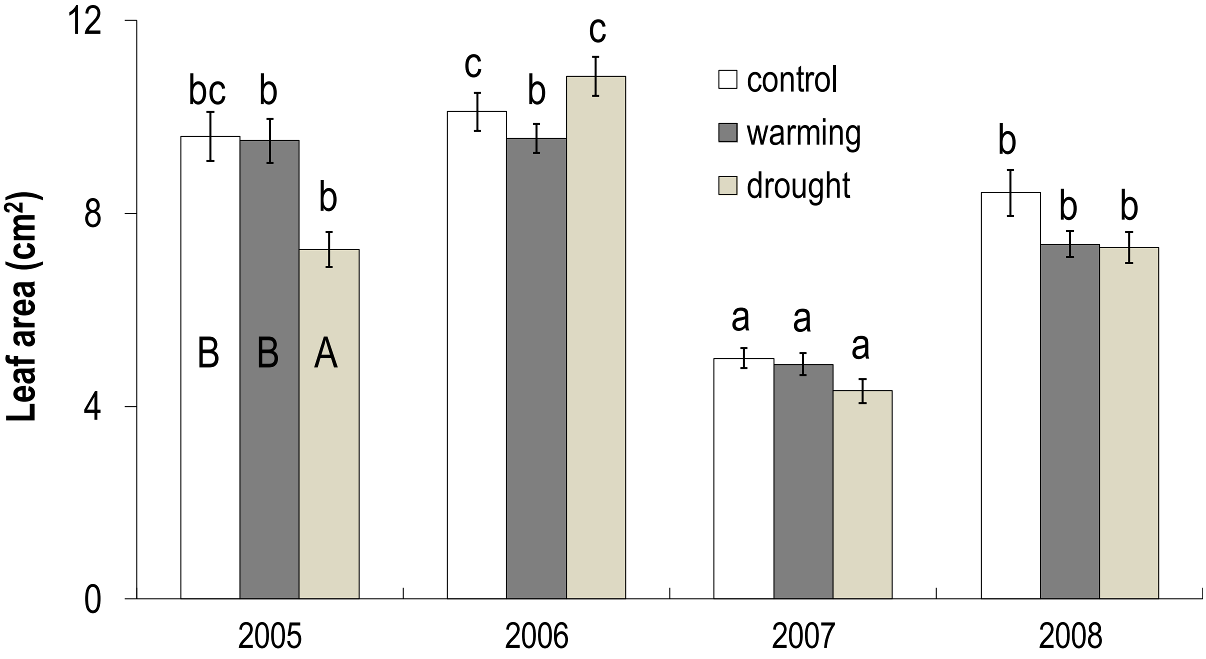

Figure 1. Leaf area of *Populus alba* in early June in years from 2005 to 2008. Different letters indicate significant ($P < 0.05$) differences: lower case letters between years within each treatment, upper case letters between treatments in 2005. Values are treatment means ± SE.

2. *Monitoring reproductive phenology and reproductive performance*

Aim

To assess the impact of recurring precipitation changes (moderate drought, severe drought, watering) on the shoot growth, reproductive phenology and reproductive performance (seed mass and number) of three abundant or characteristic species in open sand steppe in a rainfall manipulation field experiment over three consecutive years (2017-2019). The target species, which represented different plant functional types, were *Stipa borysthenica* (perennial bunchgrass), *Secale sylvestre* (winter annual grass), and *Conyza canadensis* (summer annual forb).

Activities

Field monitoring was carried out for *S. sylvestre* (in 2016 as a pilot study and in 2017) and for *C. canadensis* (in 2016). For *S. borysthenica*, and for *C. canadensis* in 2017, low plant density in the experimental plots precluded collecting field data and enough seeds for the germination and growth experiment (i.e. section 3). For *S. sylvestre*, as field-based data collection was successful for two consecutive years, we decided that additional years of field monitoring (2018 and 2019) would not provide further insight. Instead, in 2018 and 2019, we focused on pot experiments (see section 3).

Results

The results of field monitoring showed that both *S. sylvestre* and *C. canadensis* exhibited phenotypic plasticity in growth and reproduction in response to precipitation manipulations.

For *S. sylvestre*, plants growing in the severe drought plots had both higher maximum vegetative shoot height and greater seed number per ear than those growing in the control and watered plots in both 2016 and 2017 (Figure 2a ,b). Individuals growing in the moderate drought plots had intermediate values. Mean seed mass was greatest in the severe drought plots in 2016 (Figure 2c). For this trait, a similar trend was found in 2017, although there was no significant difference between treatments. These responses are most likely explained by the fact that rain exclusion applied in the previous year decreased the cover of previously dominant perennial grasses in the severe drought plots. This resulted in wetter soil in these plots compared to the control and watered plots in spring, during the peak growth of *S. sylvestre*: volumetric soil water content at 0–30-cm depth in May 2016 was 6.1% in the severe drought plots, while 5.2% and 5.0% in the control and watered plots, respectively.

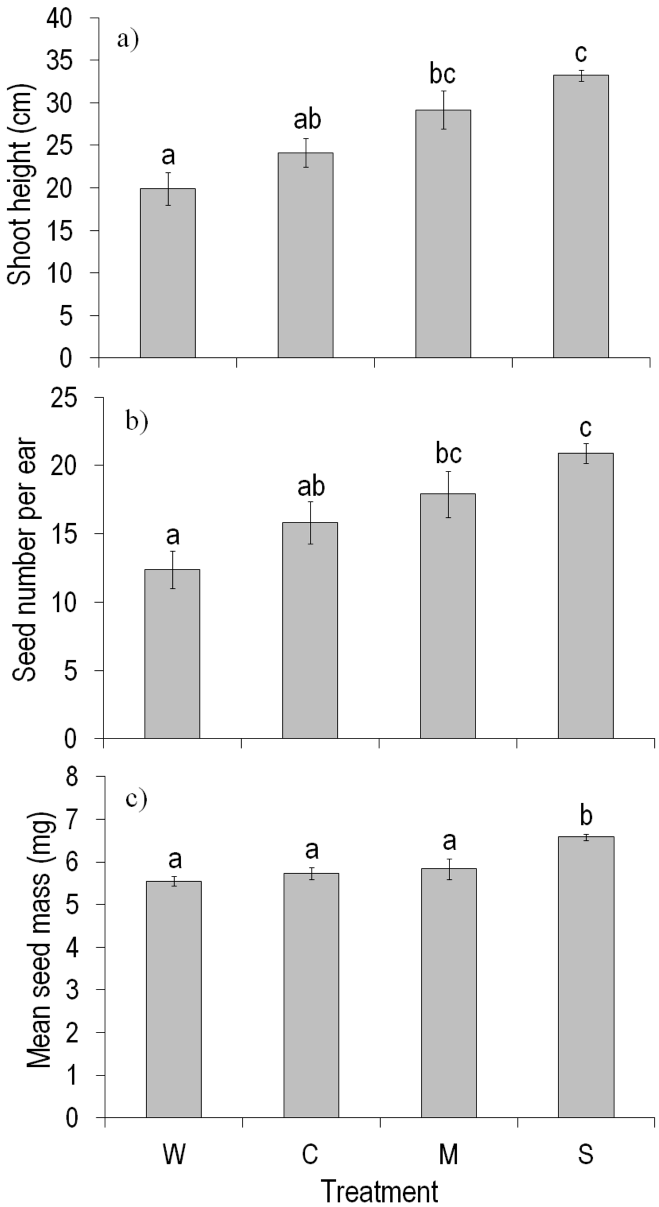

Figure 2. Effects of previous-year (2015) rainfall manipulations on **a**) maximum vegetative shoot height (cm), **b**) seed number per ear and **c**) mean single seed mass (mg) of *Secale sylvestre* growing in the plots of the field experiment in 2016. Values are treatment means ± SE. Treatments are watering (W), control (C), moderate drought (M), severe drought (S). Different letters above the bars indicate significant ($P < 0.05$) differences between treatments.

Overall, *C. canadensis* exhibited greater individual performance in the drought plots than in the control and watered plots with the strongest response to moderate drought: they grew 2.5-times taller than in the control and watered plots, and produced twice and 2.5-times more seeds than in the watered and control plots, respectively (Figure 3). The negative effect of severe drought on reproductive phenology was compensated by a second phase of flowering. This higher performance of *C. canadensis* in the drought plots is most likely due to the decreased abundance of previously dominant perennial grasses resulting from rain exclusions.

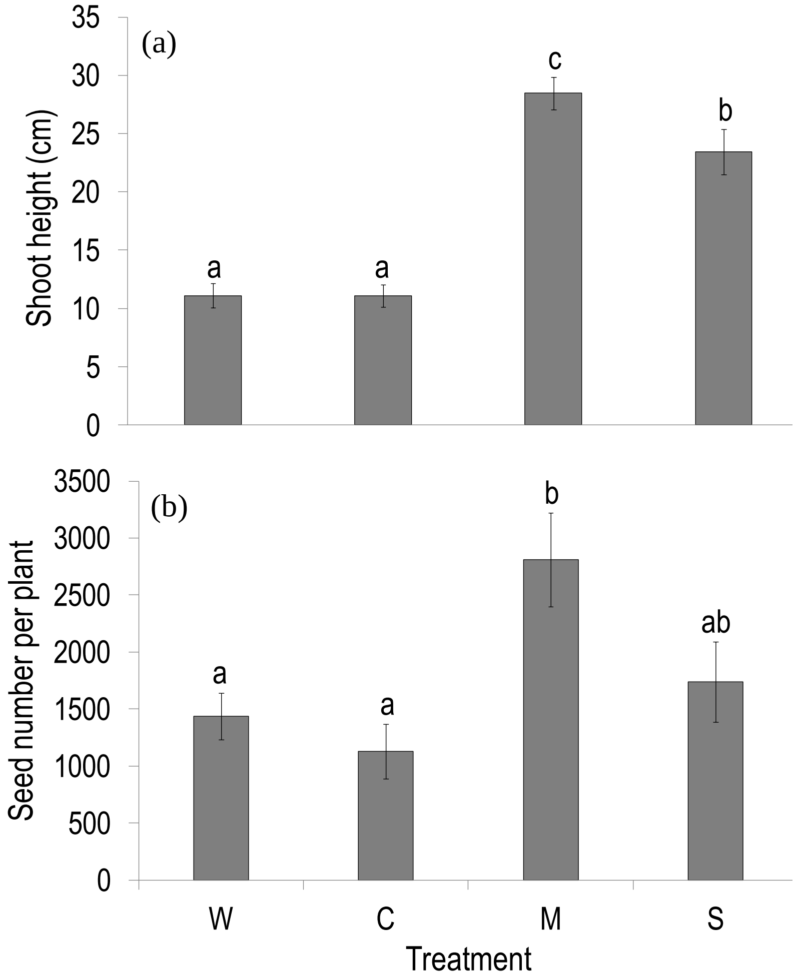

Figure 3. Effects of rainfall manipulations on **a**) the maximum vegetative shoot height (cm), and **b**) seed number per plant of *Conyza canadensis* growing in the plots of the field experiment in 2016. Values are treatment means ± SE. Treatments are watering (W), control (C), moderate drought (M), severe drought (S). Different letters above the bars indicate significant ($P < 0.05$) differences between treatments.

We conclude that the phenotypic plasticity of these two annual species in response to changing environment may help to increase their abundance with recurring droughts that suppress perennial grasses, thus contributing to the shift from perennial grasses to annuals in open sand grasslands.

<u>Publications</u>

For *S. sylvestre*:

Mojzes A., Ónodi G., Lhotsky B., Kalapos T., Csontos P., Kröel-Dulay Gy. (2018) Within-generation and transgenerational plasticity in growth and regeneration of a subordinate annual grass in a rainfall experiment. *Oecologia* 188(4): 1059-1068.

For *C. canadensis*:

Mojzes A., Ónodi G., Lhotsky B., Kalapos T., Kröel-Dulay Gy. (2019) Experimental drought enhances the growth and reproductive performance of an invasive annual weed. *Oecologia* (submitted, under review).

3. *Testing transgenerational plasticity (maternal effect)*

For *S. sylvestre*, three pot experiments were set up at an open-air experimental facility.

a) Experiment 1

Aim

To investigate the effects of different environment resulting from rainfall manipulations applied in 2015 (maternal environment) on the seed germination and growth of offspring (second) generation. Mother plants (first generation) grew in the plots of the field experiment in 2016.

Activities

The experiment was conducted between March and July 2017 with seeds collected from the plots of the field experiment in 2016. Germination data was collected, and certain growth traits (shoot height, leaf number, leaf length) were measured regularly. At the end of July, the oven-dried weight of live shoot biomass was determined. As 89% of plants remained vegetative (most likely due to the lack of exposure to chilling required for flowering) until the end of the experiment, the effects of maternal environment on the reproductive traits of offspring could not be examined (but see Experiment 2).

Results

Precipitation changes in the maternal environment influenced juvenile offspring growth: the three-week-old offspring of mother plants growing in the drought plots achieved greater shoot size (calculated by multiplying the total number of leaves by the length of the longest fully expanded leaf) than the progenies whose mothers grew in the watered plots (Figure 4). This indicates that mother plants experiencing less competitive and thus more favourable environment (i.e. in the severe and moderate drought plots, where the cover of dominant perennial grasses was low), facilitated the early growth of their offspring. Final germination percentage and the shoot biomass of four-month-old plants were not significantly affected by the maternal environment.

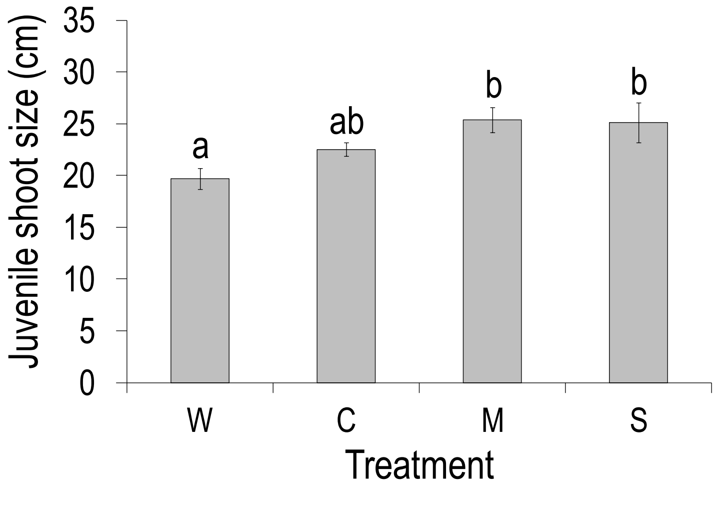

Figure 4. Effects of differences in the maternal environment resulting from rainfall manipulations (applied in 2015) on the juvenile shoot size of the offspring (second) generation of *Secale sylvestre*. Their mother plants (first generation) grew in the plots of the field experiment in 2016. Treatments are watering (W), control (C), moderate drought (M), severe drought (S). Different letters above the bars indicate significant ($P < 0.05$) differences between treatments.

Publication

The results of this experiment were included in the paper published in the journal *Oecologia* in 2018 (see section 2).

b) Experiment 2

Aim

To investigate the effects of different environment resulting from rainfall manipulations applied in 2015 and 2016 (maternal environment) on the growth and reproductive performance of offspring (second) generation under well-watered and reduced watering conditions (offspring environment). Mother plants (first generation) grew in the plots of the field experiment in 2017. The reduced watering treatment in the offspring environment simulated spring drought, which can influence plants directly during their peak growth period.

Activities

The experiment was set up in September 2017 with seeds collected in June of the same year from the plots of the field experiment. Since early April 2018, reduced watering treatment was applied to half of the pots. Drought-treated plants received half (and later one third) of the amount of water given to the adequately watered pots each time. Shoot growth and reproductive phenology were monitored regularly. Seed production per plant and mean seed mass were determined at the end of the plants' life cycle.

Results

Both maternal environment and the reduced watering treatment of offspring had no or only little influence on the maximum vegetative shoot height, the onset of flowering and fruiting, and seed number per plant. However, the mean seed mass of drought treated plants was consistently lower than that of adequately watered plants most likely due to the decreased seed provisioning (i.e. the reduced allocation of nutritive reserves to the developing seeds) of water-stressed plants (Figure 5).

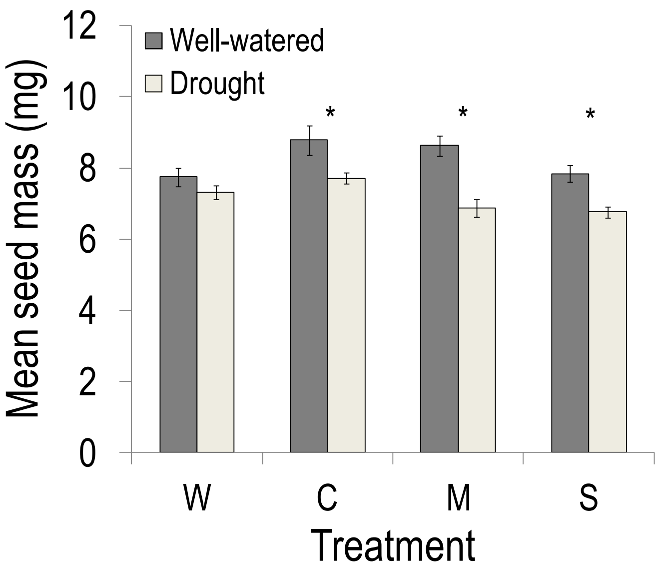

Figure 5. Mean seed mass of the offspring (second) generation of *Secale sylvestre* growing under well-watered and drought conditions (offspring environment) in spring 2018. Their mother plants (first generation) grew in the plots of the field experiment in 2017. Values are treatment means ± SE. Treatments of the field experiment (maternal environment) are watering (W), control (C), moderate drought (M), severe drought (S). Asterisks denote significant ($P < 0.05$) differences between watering conditions within each maternal environment.

Planned publication

The results of this experiment are planned to be included in the same paper as the results of Experiment 3 (see section 3c).

c) Experiment 3

Aim

To test the transgenerational effects of well-watered or reduced watering condition experienced by the second generation in Experiment 2 (maternal environment) on the growth and reproductive performance of offspring (third) generation growing under well-watered and reduced watering conditions (offspring environment).

Activities

The experiment was set up in September 2018 with seeds produced in Experiment 2. We used only the seeds of plants whose mothers grew in the moderate (M) and severe drought (S) plots of the field experiment in 2017 in order to ensure appropriate sample size for each treatment combination: treatments of the field experiment with two levels (M and S), maternal environment with two levels (well-watered and reduced watering conditions in Experiment 2), and offspring environment with two levels (well-watered and reduced watering conditions in Experiment 3). Since early March 2019, reduced watering treatment was applied to half of the pots. Drought-treated plants received one third of the amount of water given to the adequately watered pots each time. Shoot growth and reproductive phenology were monitored regularly. Seed production, mean seed mass, and cumulative shoot biomass (produced since early spring until the final harvest) were determined at the end of the plants' life cycle.

Results

Treatments of the field experiment (M or S) had no significant effect on either of plant traits studied, thus data were merged and analyzed using two factors (maternal and offspring environment). Reduced watering of mother plants had a pronounced influence on offspring performance, irrespective of offspring environment. The offspring of drought-treated mother plants showed reduced early growth compared to the progenies of well-watered mothers: shoot height (measured as stretched length) was lower mostly due to the shorter first fully-expanded leaf (Pearson correlation coefficient between first leaf length and shoot height at the stage of plants having one fully-expanded leaf was 0.91; $P < 0.0001$; Figure 6).

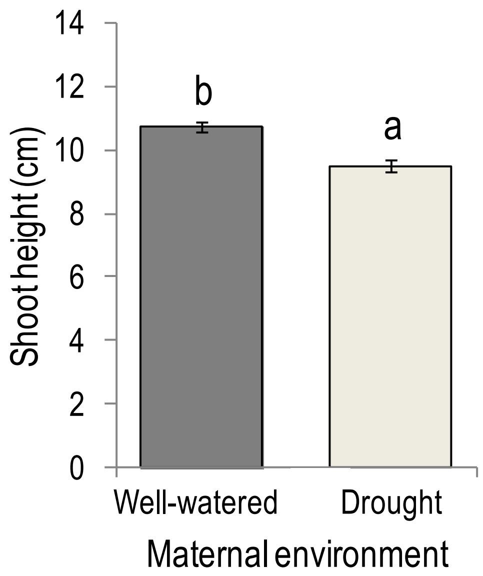

Figure 6. Shoot height of the offspring (third) generation of *Secale sylvestre* at the stage of plants having one fully-expanded leaf in autumn 2018. Their mother plants (second generation) were grown under well-watered and drought conditions (maternal environment) during spring 2018. Values are treatment means ± SE. Different letters above the bars indicate significant ($P < 0.05$) difference between maternal environments.

However, the negative transgenerational effect of reduced watering treatment was reversed one-month later, and remained positive throughout the next spring, which resulted in enhanced cumulative shoot biomass and seed production compared to the offspring of well-watered mother plants (Figure 7).
We conclude that little effects of drought on the growth and reproduction of maternal generation may eventuate in large differences in offspring performance.

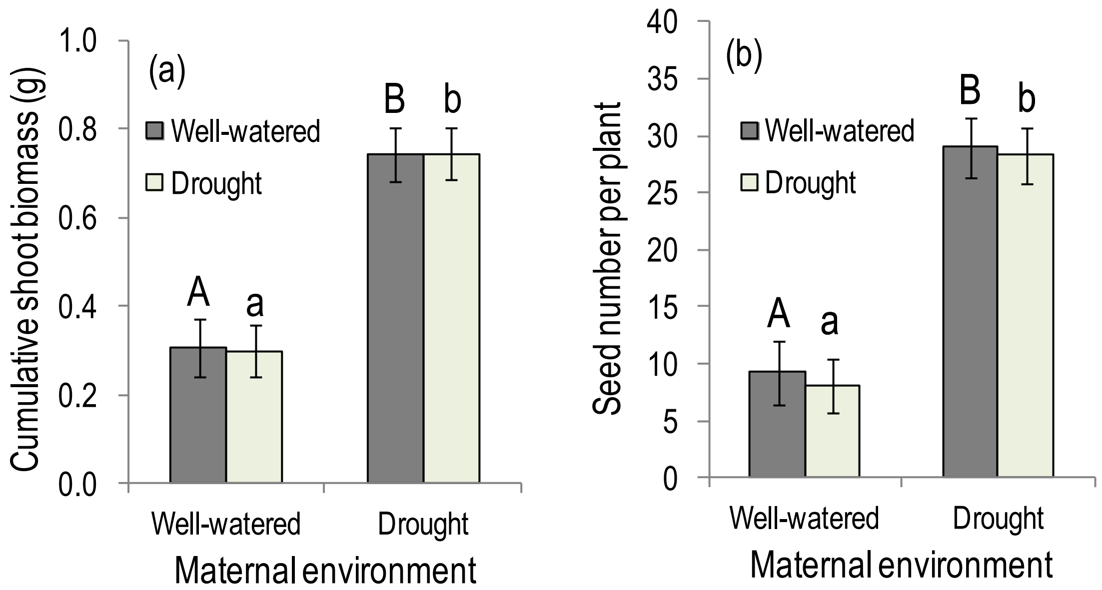

Figure 7. **a**) Cumulative shoot biomass and **b**) seed number per plant of the offspring (third) generation of *Secale sylvestre* adult plants growing under well-watered and drought conditions (offspring environment, denoted by shaded and white bars, respectively) during spring 2019. Their mother plants (second generation) were grown under well-watered and drought conditions (maternal environment, indicated on x axis) during spring 2018. Values are treatment means ± SE. Different letters above the bars indicate significant ($P < 0.05$) differences between maternal environments (upper case letters within well-watered offspring, and lower case letters within drought-treated offspring).

<u>Planned publication</u>
Mojzes A., Kalapos T., Kröel-Dulay Gy. Changes in the effects of drought on growth and reproduction of an annual grass across two generations (preliminary title), planned to submit to a Q1 journal in spring 2020.

4. *Other research topics connected to this NKFI project*

a) Planned systematic review paper on the transgenerational plasticity of plants in climate change field experiments

Workplan included a preparation of a review paper in the topic of studying transgenerational plasticity of plants in field experiments manipulating temperature, precipitation and/or the level of atmospheric CO_2. Literature search and data collection have been started, but only about thirty relevant papers were found by systematic search in the Web of Science database on this topic, despite extending experiment types to common garden and ecotron experiments. Therefore, the plan of the review was abandoned, and instead, I have been participated in preparing another review paper about ecosystem responses to drought (see section 4b).

b) Planned systematic review paper (Kröel-Dulay et al. in prep.) on ecosystem responses to drought

The objective of this review is to compare the productivity responses of ecosystems to natural droughts (detected in observational studies) with the responses to experimental droughts (obtained from rainfall manipulation field experiments). So far, 57 observational and 38 experimental studies have been found (in the Web of Science database, from the cited references of the relevant Web of Science records and previous meta-analyses or reviews on the topic). I have participated in the literature search and collecting data from relevant papers. Data analysis is going on, and the preparation of the manuscript has been started (target journal: *Nature Climate Change* or *Global Change Biology*). First results have been presented in the 11th Hungarian Ecological Congress (see publications). These results indicate that drought experiments underestimate ecosystem responses to drought.

c) Co-authorship in a manuscript about the reintroduction success of sand grassland species at an abandoned field

The objective of this study was to test the usability of seed accessions of the Pannon Seed Bank for reintroduction of ten characteristic sand grassland species in order to restore sand grassland at an abandoned field. Field work was conducted during 2011-2016, but synthesizing results and manuscript preparation were completed by 2019 (i.e. during the third year of this NKFI project). The results of the study showed that reintroduction success was not significantly affected by the short-term (one- or two-year) storage of seeds, but was highly influenced by the year of seeding. These results indicate that the weak seed yield of certain years and the low supply of native seeds in the market can be mitigated by using stored seeds, and a multi-year, scheduled seeding is recommended to eliminate the negative impacts of weather and increase the restoration success.
Publication:
Kövendi-Jakó A., Szitár K., Halassy M., Halász K., Mojzes A., Török K. (2019) Storing seeds for restoration of dry grasslands. *Restoration Ecology* (submitted).

In each publication mentioned in this report including submitted manuscripts, financial support provided by the National Research, Development and Innovation Fund (PD_16 120844) is acknowledged.