

This manuscript is contextually identical with the following published paper:

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.
DOI: 10.1007/s00442-018-4264-6

The original published pdf available in this website:

<https://link.springer.com/article/10.1007/s00442-018-4264-6>

Title page

Within-generation and transgenerational plasticity in growth and regeneration of a subordinate annual grass in a rainfall experiment

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Within-generation and transgenerational plasticity in growth and regeneration of a subordinate annual grass in a rainfall experiment

1 Abstract

2

3 Precipitation changes may induce shifts in plant species or life form dominance in
4 ecosystems, making some previously subordinate species abundant. The plasticity of certain
5 plant functional traits of these expanding subordinate species may be one possible mechanism
6 behind their success.

7 In this study, we tested if the subordinate winter annual grass *Secale sylvestre* shows plasticity
8 in growth and reproduction in response to altered environment associated with field-scale
9 rainfall manipulations (severe drought, moderate drought, watering) in a semiarid grassland,
10 and whether the maternal environment influences offspring germination or growth in a
11 subsequent pot experiment.

12 Compared to control plots, *S. sylvestre* plants grew 38% taller, and produced 32% more seeds
13 in severe drought plots, while plants in watered plots were 17% shorter, and had 22% less
14 seeds. Seed mass was greatest in severe drought plots. Plants growing in drought plots had
15 offspring with enhanced juvenile shoot growth compared to the progeny whose mother plants
16 grew in watered plots. These responses are most likely explained by the decreased cover of
17 previously dominant perennial grasses in severe drought plots, which resulted in wetter soil
18 compared to control and watered plots during the peak growth of *S. sylvestre*.

19 We conclude that the plasticity of this subordinate annual species in response to changing
20 environment may help to gain dominance with recurring droughts that suppress perennial
21 grasses. Our results highlight that exploring both within-generation and transgenerational

22 plasticity of subordinate species may lead to a better prediction of changes in plant species
23 dominance under climate change.

24

25 **Keywords**

26 climate change, maternal environment, plant trait, population interaction, *Secale sylvestre*

27

28 **Introduction**

29 In arid and semiarid grasslands, water availability is a strong determinant of plant
30 diversity, primary production, and community stability (Sala et al. 1988; Bai et al. 2004;
31 Suttle et al. 2007; Seddon et al. 2016). In these ecosystems, altered precipitation regimes can
32 often result in shifts in functional group abundances, species reordering or even replacement
33 of species within a community (Suttle et al. 2007; Smith et al. 2009; Scott et al. 2010; Dudley
34 et al. 2017). In such cases, altered conditions may favour coexisting subordinate or transient
35 species at the expense of previous dominants (Mariotte et al. 2013; Yang et al. 2016). The
36 identification of mechanisms at the level of functional group or individual species underlying
37 these marked vegetation changes can be important to better understand and predict the
38 impacts of climate change.

39 Plant functional traits of subordinate species have received relatively little attention
40 compared to dominant species, despite the evidence that subordinates can also play a
41 substantial role in maintaining ecosystem functions under stress (Walker et al. 1999; Mariotte
42 et al. 2013; Mariotte 2014). Furthermore, the fact that subordinate species are often impacted
43 indirectly by altered climatic conditions via changes in competitive interactions with the
44 dominant species (Kardol et al. 2010; Mariotte et al. 2013, but see Levine et al. 2010) can
45 make their response more difficult to forecast. This highlights the need to improve our
46 understanding of how traits of subordinate species respond to altered climate change drivers,
47 such as precipitation.

48 Phenotypic plasticity is one of the key mechanisms – besides shifts in species distribution
49 and evolutionary adaptation – that can allow plant populations to adjust to climate change
50 (Nicotra et al. 2010; Franks et al. 2014; Parmesan and Hanley 2015). Phenotypic plasticity is
51 defined as the ability of a single genotype to express different phenotypes under different
52 environmental conditions (Franks et al. 2014). Plasticity of various plant traits, such as plant

53 height, leaf size, specific leaf area, and seed size and number is considered to be important in
54 species responses to climate change (Nicotra et al. 2010). However, the plasticity of certain
55 regeneration traits, such as seed germination and seedling growth are highly unknown, despite
56 the critical role of early life history stages in plant population persistence (Walck et al. 2011;
57 Parmesan and Hanley 2015).

58 Plastic response of an individual to environmental conditions can be expressed not only in
59 its own phenotype (within-generation phenotypic plasticity). Maternal environmental effect
60 (or transgenerational phenotypic plasticity) refers to the phenomenon when the ecological
61 environment experienced by the mother plant influences the offspring's phenotype
62 independently of the genetic inheritance of causative alleles (Roach and Wulff 1987; Herman
63 and Sultan 2011). It can be mediated by multiple, often interacting mechanisms, for instance
64 changes in seed provisioning (i.e. the allocation of nutritive reserves to the developing seed),
65 seed hormone content, or epigenetic marks (such as DNA methylation; Herman and Sultan
66 2011). The potential importance of transgenerational plasticity in plant species' responses to
67 global environmental changes is highlighted by an increasing number of studies (e.g.
68 Hovenden et al. 2008; Pias et al. 2010; Schuler and Orrock 2012; Fenesi et al. 2014; Walter et
69 al. 2016). If the progeny environment is reliably predictable from the maternal environment –
70 e.g. for species with short-distance seed dispersal (Galloway and Etterson 2007) – the mother
71 can adjust the phenotype of her offspring to enhance its performance under conditions that it
72 is likely to encounter (Agrawal et al. 1999; Sultan et al. 2009; Herman and Sultan 2011;
73 Fenesi et al. 2014). However, when increased stochasticity in temperature and/or precipitation
74 associated with climate change decrease the reliability of environmental cues,
75 transgenerational effects could reduce offspring performance (Schuler and Orrock 2012).
76 Climate change experiments in natural vegetation have shown that rainfall manipulations in
77 the maternal environment could influence various traits of offspring including seed

78 germination and viability, seedling growth or leaf C:N ratio. However, most of these studies
79 focused on dominant species (Breen and Richards 2008; Pías et al. 2010; Tielbörger and Petru
80 2010; Chamorro et al. 2016; Walter et al. 2016), and little research addressed the responses of
81 other coexisting species (e.g. Li et al. 2011).

82 In semiarid regions, ecosystems on sandy soils can be particularly sensitive to precipitation
83 changes, partly due to the low water-holding capacity of the soil (Yang et al. 2010; Gao et al.
84 2015; Huang et al. 2017). This is also the case in the open perennial sand grassland
85 component of the Pannonian sand forest-steppe in Hungary (Kovács-Láng et al. 2000). For
86 example, extreme droughts in 2000 and 2003 resulted in a marked drop in the cover of the
87 dominant perennial grasses, with a concomitant increase in the abundance of previously
88 subdominant or subordinate annuals in these grasslands in the Danube-Tisza Interfluve,
89 Central Hungary (Kovács-Láng et al. 2006). With a higher probability of drought in summer
90 projected for the country (Bartholy et al. 2014), such annual-dominated patches may persist.
91 The aims of this study were to assess 1) how the altered environment associated with field-
92 scale experimental rainfall manipulations in a perennial sand grassland affect the growth, seed
93 production, and seed mass of the characteristic subordinate winter annual grass *Secale*
94 *sylvestre*, and 2) whether changes in the maternal environment caused by rainfall treatments
95 influence the seed germination and offspring growth of this species in a subsequent pot
96 experiment. We hypothesized that (H₁) plants growing in the experimental plots (mother
97 plants) show plasticity in the studied traits in response to the different environment resulting
98 from rainfall treatments; (H₂) the effect of maternal environment manifests in the offspring
99 generation.

100

101 **Materials and methods**

102

103 *Study site and rainfall manipulation experiment*

104 The study site is located in the Danube-Tisza Interfluve, near the village Fülöpháza
105 (46°52'N, 19°25'E) in the Kiskunság National Park. The climate is moderately warm
106 semiarid temperate with continental and sub-Mediterranean influences. Annual mean
107 temperature is 10.4 °C, and yearly average precipitation is 500-550 mm (1961-1990; Kovács-
108 Láng et al. 2000). Midsummer drought is typical in July and August, and it is amplified by the
109 coarse-textured calcareous sand soil.

110 The species selected for our study, *Secale sylvestre* Host is one of the most frequent winter
111 annual grasses in open sand grasslands of the area. It is a characteristic subordinate
112 component of perennial grasslands, but may become abundant on bare soils as a colonizer
113 during secondary succession after disturbance (Kovács-Láng et al. 2000; Molnár 2003).

114 In 2015, we set up an experiment in an open sand grassland characterised by the
115 dominance of two perennial bunchgrasses, *Festuca vaginata* Waldst. and Kit. ex Willd. and
116 *Stipa borysthena* Klokov ex Prokudin. Experimental units were 3 m × 3 m plots with a 50
117 cm buffer strip along each side inside the boundaries of each plot, thus the effective sampling
118 area was 2 m × 2 m. Plots were laid out in a completely randomized block design with three
119 treatments and a control (ambient rainfall), in six replicates (6 blocks, each block containing
120 one plot of each treatment). Treatments were as follows: severe drought from late June to late
121 August (ca. two months), moderate drought from late July to late August (ca. one month), and
122 watering as one event of ca. 25 mm per month from late May to late August (i.e. 100 mm per
123 year, ca. 20% increase over the long-term annual mean; for exact dates see Table 1). Thus, at
124 the beginning of this study on *S. sylvestre* (April 2016), treatment plots had received one year
125 of rainfall manipulations (in 2015). Treatments were repeated also in 2016 with a similar
126 timing, but *S. sylvestre* plants studied received only the first watering treatment in late May
127 before completing their life cycle in early June.

128 Drought treatments were conducted by excluding rain from the plots using permanent,
129 transparent plastic foils. Watering treatment was applied by using spraying heads at 1 m
130 height, in a 1 m × 1 m grid. Side curtains were used during both treatments to avoid water
131 addition to the plots neighbouring watered plots or prevent rain coming from the side to
132 drought plots.

133 Air temperature at 20 cm height and volumetric soil water content (SWC, %) at 0-30 cm
134 depth (i.e. averaged over the entire soil profile up to 30 cm) were recorded in each plot by
135 installed temperature and moisture sensors (Sensirion SHT75 and Campbell CS616,
136 respectively) connected to a data logger. Precipitation was measured with rain gauges (Davis
137 DS7852) at 30 cm.

138

139 *Background conditions for the studied S. sylvestre plants: precipitation, soil water content*
140 *and plant abundance*

141 Severe and moderate drought treatments excluded 62% and 49% of ambient rainfall,
142 respectively, while watered plots received 50% more rainfall than control plots between 1
143 May and 31 August 2015 (Table 1). During this 4-month period on average, rain exclusions
144 decreased soil water content from 4.0% (control) to 3.2% and 3.4% in severe and moderate
145 drought plots, respectively, whereas watering treatment increased average SWC to 4.3%.
146 Severe drought treatment in summer 2015 decreased the cover of *F. vaginata* and *S.*
147 *borysthena* by ca. 80% by April 2016 compared to April 2015, while in watered plots the
148 cover of these perennial grasses increased by 20% (Table 1). As a result, in April 2016, the
149 abundance of the two dominant perennial grasses in severe drought plots was 83% lower than
150 in control plots, and 87% lower than in watered plots. On the contrary, the cover of *S.*
151 *sylvestre* increased almost fivefold in severe drought plots from April 2015 to April 2016,

152 which led to eightfold and fourfold higher abundance of this grass in these plots than in
153 watered and control plots, respectively, in April 2016.

154

155 *Field sampling and data collection*

156 In April 2016, 10 individuals were selected and marked for repeated measurements within
157 the 4-m² sampling area of each plot. We measured the maximum (vegetative) shoot height
158 (stretched length of the shoot; accuracy 0.5 cm) according to the protocol of Cornelissen et al.
159 (2003). The length of ear without arista was measured in early June, in the ripening phase,
160 when caryopses (referred to as seeds hereafter) had reached their final size, but were not yet
161 loosening (Lancashire et al. 1991). For the few individuals that developed tiller(s), the longest
162 shoot and ear were chosen. Seed number per ear was estimated by using a linear regression
163 equation ($r^2 = 0.98$, $P < 0.0001$) between the length and seed number of ears determined on an
164 additional 30 individuals outside, but close to the experimental plots at the same date. Fully-
165 ripened seeds from 15-20 randomly selected individuals per plot were collected on 9 June
166 2016, when most of the *S. sylvestre* plants had completed their life cycle. Seeds were stored in
167 paper bags at room temperature (ca. 28 °C in summer and 15 °C in winter) until used for the
168 germination experiment. Fifty “apparently viable” seeds per plot (i.e. that appeared to be
169 intact and resisted gentle pressure; Roberts 1981) were weighed individually (accuracy 0.1
170 mg) to determine mean single seed mass.

171

172 *Germination and growth experiment*

173 To examine the effects of maternal environment on offspring germination and growth, a
174 common garden pot experiment was set up in Fót (47°37'N, 19°10'E), ca. 83 km from the
175 field site, on 16 March 2017. In this experiment, 4 half-litre pots were used to represent one
176 experimental field plot. Thus, 96 pots in total (4 treatments, 6 blocks) filled with nutrient-poor

177 sandy soil were placed onto the bench of an outdoor, open-air growth facility. Pots were
178 exposed to natural weather conditions except for excluding precipitation by a transparent
179 plexiglass roof. From each plot of the field experiment, 36 seeds were sown in four pots (9
180 seeds per pot). Final percentage of germination (i.e. coleoptile emerged ≥ 2 mm above the soil
181 surface) was determined after 35 days. Seedlings were thinned to the largest (≥ 5 cm) one per
182 pot, and were grown under well-watered conditions (as the major growth period of this grass
183 (April-May) is usually not water-limited). Pots were rotated weekly on the bench to minimize
184 the micro-environmental differences associated with pot position. Until the end of July, when
185 shoot biomass was harvested, only 11 individuals entered the reproductive phase, and 85
186 plants remained vegetative (most likely due to the lack of exposure to chilling required for
187 flowering; Chouard 1960). Shoot height was measured at two life stages: for 3-week-old
188 plants (juveniles, which had two fully-expanded leaves), and for 4-month-old plants (referred
189 to as adults). In addition, total leaf number and the length of fully-expanded leaves were
190 determined at juvenile stage. Juvenile shoot size was calculated by multiplying the total
191 number of leaves by the length of the longest fully-expanded leaf. This index is frequently
192 used as a non-destructive estimate for biomass, particularly of juvenile plants (e.g. Van
193 Groenendael and Slim 1988; Vergeer and Kunin 2013). It showed strong correlation with
194 juvenile shoot biomass also for *S. sylvestre* (Pearson's $r = 0.90$, $P < 0.0001$), measured on an
195 additional 30 three-week-old plants in a separate experiment. Green (live) biomass was
196 harvested from 4-month-old adult plants (referred to as adult biomass), oven-dried at 60 °C
197 for 48 h and weighed. Reproductive adults and those that died during the experiment (4
198 plants) were excluded from data collection at adult stage. Thus, in the growth experiment, 1-4
199 individuals (pots) corresponded to a single treatment plot of the field experiment.

200

201 *Statistical analysis*

202 For each plant response variable, statistical analyses were done on mean values per plot as
203 the experimental unit ($n = 6$). General Linear Mixed Models with treatment as a fixed effect
204 and block as a random factor were conducted for maximum shoot height, seed number per
205 ear, and mean single seed mass of maternal generation. Data met the assumptions of
206 normality of residuals and homoscedasticity (Quinn and Keough 2002). For post-hoc
207 comparison of means Tukey's HSD tests were used. In order to assess the effect of shoot
208 height on seed number and seed mass after controlling for the effect of treatments, shoot
209 height was also included in the model as a continuous predictor variable, and the partial
210 correlation coefficients (R) were calculated.

211 For monthly average SWC during the growth and reproduction of the studied plants, two-
212 way repeated measures ANOVA was used with treatment as a fixed effect and month as the
213 repeated-measures effect. Subsequently, Tukey's HSD tests were applied between treatments
214 within each month separately. For each analysis, the TIBCO Statistica software (TIBCO
215 Software Inc. 2017) was used, and differences were considered significant at $P < 0.05$.

216

217 **Results**

218 *Maternal generation*

219 During the peak growth period of the maternal generation of *S. sylvestre*, soil water content
220 was higher in severe drought plots than in both watered and control plots in May 2016 (severe
221 drought and watered plots differed also in April with marginal significance: $P = 0.091$; Fig.
222 1).

223 Rainfall manipulations had a significant effect on each plant response variable studied in *S.*
224 *sylvestre* growing in the plots of the field experiment (Table 2). Plants growing in severe
225 drought plots had both higher maximum vegetative shoot height and higher seed number per
226 ear than those growing in control and watered plots (Fig. 2a, b). Consistently, individuals

227 growing in moderate drought plots also showed higher values than those in watered plots.
228 Mean single seed mass was greater in severe drought plots than in control and the other
229 treatment plots (Fig. 2c). Difference between the highest and the lowest treatment means
230 (severe drought and watering, respectively), expressed as percentage of the lowest mean
231 ($[(\text{Max}-\text{Min})/\text{Min}] \times 100, \%$), was 3.7-times higher in seed number (68.9%) than in seed mass
232 (18.6%). However, when controlling for the effect of treatments, both of these components of
233 reproductive success showed a strong positive partial correlation with shoot height ($R = 0.89$,
234 $P < 0.0001$ for seed number; $R = 0.74$, $P = 0.0012$ for seed mass).

235

236 *Offspring generation*

237 Seeds produced in control and rainfall manipulated plots did not differ in final germination
238 percentage (67-80%); only a marginally significant difference ($P = 0.086$) was found between
239 watering and moderate drought treatments; Table 2, Fig. 3a). However, maternal environment
240 had significant effects on the three-week-old offspring (Table 2, Fig. 3b, c, d). Both juvenile
241 shoot size and the length of the first fully-expanded leaf were higher for the offspring whose
242 mother plants grew in severe or moderate drought plots than for the progeny whose mothers
243 developed in watered plots (Fig. 3b, c). Similar differences were found in juvenile shoot
244 height, though severe drought and watering treatments differed with only marginal
245 significance ($P = 0.058$; Fig. 3d). At the time of harvest, neither shoot height nor shoot
246 biomass of the adult progeny varied significantly with the environment of their mothers
247 (Table 2, Fig. 3e, f).

248

249 **Discussion**

250

251 *Plasticity of maternal generation*

252 Shoot growth and seed production of the studied *S. sylvestre* plants provided evidence in
253 favour of H₁, which was that *S. sylvestre* growing in the experimental plots exhibited
254 phenotypic plasticity in the studied traits in response to the different environment caused by
255 rainfall manipulations. The positive relationships of both seed mass and number with shoot
256 height indicate that on average at plot level, plants experiencing better resource availability
257 can allocate more assimilate to both vegetative growth and reproduction. Lower variation in
258 seed mass than in number is consistent with the previous consideration that seed size is often
259 the least plastic component of reproductive yield within a species (Harper et al. 1970).
260 Nevertheless, *S. sylvestre* has a limited seed dispersal capacity, and most seeds fall beneath
261 the mother plant. For such species, density-dependent mortality can be high, e.g. due to
262 intense competition between progeny seedlings, thus larger maternal plants may benefit from
263 producing larger seeds (Venable 1992). The seed number and mass values obtained across
264 treatments in our study were within or close to the wide range reported for these traits in
265 several populations of this species within the Kiskunság region (i.e. 5.3-7.9 g for thousand
266 seed mass, and 12 and 26 as a minimum and maximum number of grains per ear, respectively;
267 Vörösváry et al. 2000).

268 In several other water manipulation experiments in arid and semiarid ecosystems,
269 reduction in the amount of rainfall usually limited plant growth and/or seed production,
270 whereas increased water supply had an opposite effect (e.g. Poulin et al. 2007; Breen and
271 Richards 2008; Gao et al. 2015; Volis et al. 2015). In contrast, *S. sylvestre* in our study,
272 showed enhanced growth and reproductive performance in the experimental plots exposed to
273 2-month drought in the previous year, particularly compared with the individuals growing in
274 plots that received supplemental watering. The most probable explanation for these apparently
275 contradictory results is that *S. sylvestre* was not impacted *directly* by dry conditions (either in
276 2015 or 2016), because this grass usually completes its life cycle in early June, i.e. before the

277 start of severe drought treatment in late June (Table 1). This phenological rhythm is typical
278 for winter annual species in sand grasslands (Kárpáti and Kárpáti 1954). However, this
279 subordinate species might have benefited *indirectly* from rain exclusion, as severe drought
280 treatment in 2015 negatively affected the abundance (and thus the competitive effect) of the
281 two dominant perennial grasses, which did not recover by April 2016 (Table 1). This likely
282 resulted in better resource (particularly water) availability for *S. sylvestre* during its peak
283 growth period in spring 2016. In contrast, the moderate increase in the cover of dominant
284 perennials in response to watering during summer 2015 (Table 1), might have enhanced the
285 suppression of the coexisting annual *S. sylvestre* in spring 2016. This interpretation is
286 supported by the higher soil water content in severe drought plots compared to watered plots
287 in April and particularly in May 2016 (Fig. 1), most likely due to the lower transpirational
288 water loss of the decreased perennial grass cover.

289 Our results also suggest that with recurring severe droughts, the higher abundance of *S.*
290 *sylvestre* in severe drought plots in April 2016 after a single 2-month drought of the previous
291 summer (Table 1) may be further augmented by the enhanced growth and reproductive
292 capacity of this annual grass due to the negative response of the concurrent dominant
293 perennial grasses to drought. Similar to our results, in a California grassland, experimentally
294 extended spring rainfall imposed limited direct effects on winter annual grasses due to their
295 early phenology, but in the subsequent year, these grasses benefited indirectly from the
296 decomposition of the initially expanding N-fixing forbs (Suttle et al. 2007). Our results are
297 also in line with those of Violle et al. (2006), who reported that the experimental removal of
298 standing biomass of the dominant perennial grass (with the retention of litter) enhanced the
299 total final and the seed biomass per plant of two early successional annual species in an old-
300 field.

301 In our study, *S. sylvestre* in watered plots was directly exposed to watering in May both in
302 2015 and 2016, during flowering. However, the competitive effect of the dominant perennial
303 grasses might have overridden the potential direct positive impact of supplemental water on
304 the annual *S. sylvestre*. Similarly, in another water manipulation experiment in a mountain
305 steppe, Liancourt et al. (2013) demonstrated that the negative effects of competition with
306 neighbouring plants (including dominant species) could offset the direct benefit of added
307 water on the above-ground biomass of a characteristic species. However, the net effect of
308 supplemental rainfall may depend on how strongly precipitation change alters competition,
309 and also on the sensitivity of inferior species to the altered competitor abundance (Levine et
310 al. 2010).

311

312 *Plasticity of offspring generation*

313 In agreement with H₂, which was that differences in the maternal environment caused by
314 rainfall manipulations affected the offspring generation of *S. sylvestre*, we found plasticity in
315 the growth of progeny at the juvenile stage. In contrast, seed germination percentage and the
316 adult growth of offspring were not influenced by the environmental conditions of their mother
317 plants. When seed dormancy is imposed by biochemical constraints, drought during seed
318 development usually decreases dormancy and increases germinability (Fenner 1991), which
319 has also been demonstrated in some recent rainfall manipulation experiments with annual
320 species (Karimmojeni et al. 2014; Gao et al. 2015). Nevertheless, some other studies reported
321 similar or higher germination percentage in response to better water conditions in the maternal
322 environment (Poulin et al. 2007; Breen and Richards 2008; Pías et al. 2010; Li et al. 2011).

323 In the juvenile phase, the size of both the first leaf and the whole shoot was greater for the
324 progeny whose mothers grew in drought plots compared with the offspring whose mothers
325 developed in watered plots. This indicates that mother plants experiencing less competitive

326 and thus more favourable (moisture) environment (i.e. in severe and moderate drought plots,
327 where the cover of dominant perennial grasses was low; Table 1) facilitated the early growth
328 of their offspring. Larger plant size in the early phase of the life cycle might provide a great
329 advantage for survival, as mortality rate of young plants is often high (Leishman et al. 2000),
330 and can be size-dependent within a species, especially in resource-limited conditions, such as
331 under water stress (Cook 1980; Parker 1982). Such a positive maternal effect can allow
332 offspring to avoid the initial time lag that is required for the development of the offspring's
333 own plasticity to its actual environment (Agrawal et al. 1999; Herman and Sultan 2011).
334 Numerous prior studies reported that better water availability for the studied species in the
335 maternal environment had positive transgenerational effects on offspring growth in early
336 seedling or juvenile stage, i.e. in the phase that can be critical for establishment (Breen and
337 Richards 2008; Pías et al. 2010; Li et al. 2011; Walter et al. 2016). Larger seedlings usually
338 germinate from larger seeds, and greater seed mass often reflects a higher amount of seed
339 reserves (Leishman et al. 2000). In our experiment, greater mass was detected only for seeds
340 produced in severe drought plots, thus other potential mechanisms than seed provisioning
341 (reviewed by Herman and Sultan 2011) should (also) account for the differences in juvenile
342 growth observed between the progeny whose mother plants grew in severe or moderate
343 drought plots and in watered plots.

344 We found no difference in shoot height and biomass of four-month-old progeny according
345 to the environment of their mothers. These results are consistent with previous studies
346 reporting that the beneficial maternal effects diminished or disappeared in a later stage of
347 offspring's life cycle (Pías et al. 2010; Walter et al. 2016), but contrast with the other studies
348 where positive transgenerational effect was detected in the final fitness of adult progeny or
349 both in an earlier and adult stages (Roach and Wulff 1987; Fenesi et al. 2014). The
350 persistence of positive maternal influence may depend on its underlying mechanism (Herman

351 and Sultan 2011), and also on the environmental conditions experienced by the offspring. For
352 example, the improved seedling vigour of Austrian winter field peas established from large
353 seeds could increase the seed yield compared to the yield of peas planted from small seeds
354 under adverse conditions, but not in environment more favourable for pea growth at
355 Grangeville, Idaho (Murray et al. 1984). Thus, the fact that in our experiment, the progeny of
356 *S. sylvestre* were grown under well-watered conditions might provide one possible
357 explanation why the benefit of enhanced growth of juveniles did not appear in the adult stage.
358 Nevertheless, to our best knowledge, our study provides the first experimental evidence that
359 altered rainfall amounts, this key element of climate change, can trigger transgenerational
360 effects on offspring growth of a subordinate species indirectly via changes in the competitive
361 interactions with the dominant species.

362

363 **Conclusions**

364 Our field experiment showed that a subordinate species in perennial sand grasslands, *S.*
365 *sylvestre* exhibited phenotypic plasticity in shoot growth and seed production when growing
366 in different environments caused by a single year of rainfall manipulations. This plasticity is
367 most likely a response to the altered population interactions in the growth environment
368 resulting from the previous-year precipitation changes, which led to enhanced performance of
369 this species with decreasing amount of rainfall. Moreover, maternal environmental effect
370 found in the early growth of offspring might amplify the immediate response that can be
371 achieved by within-generation plasticity alone (Sultan et al. 2009; Herman and Sultan 2011).
372 Based on these results, we expect that summer drying projected for Hungary in the future
373 (Bartholy et al. 2014) will favour the growth and reproduction of *S. sylvestre*. This better
374 performance may contribute to the increase in abundance of this annual grass, and thus to the
375 shift from perennial grasses to annuals in sand grasslands of the study region. Our study

376 highlights that both within-generation and transgenerational plasticity of subordinate species
377 should be taken into account to better understand and predict shifts in plant species or
378 functional group abundances under climate change.

379

380 **Acknowledgements**

381 This work is a part of the projects no. 120844 and no. 112576, which has been implemented
382 with the support provided by the National Research, Development and Innovation Fund
383 (NRDI Fund) of Hungary, financed under the PD_16 (A.M.) and K (G.K-D.) funding scheme,
384 respectively. This study was also part of the project Sustainable Use of Ecosystem Services
385 (GINOP-2.3.2-15-2016-00019) funded by the NRDI Office (G.K-D. and G.Ó.). This research
386 was also supported by the János Bolyai Research Scholarship of the Hungarian Academy of
387 Sciences (G.K-D.). We are grateful to the Kiskunság National Park for the support to our field
388 work. We thank Péter Ódor for his advice on statistical analyses. We also thank the two
389 anonymous reviewers for their helpful comments on an earlier version of the manuscript.

390

391 **Author contribution statement**

392 G.K-D. designed and established the rainfall manipulation experiment. A.M. and G.K-D.
393 conceived the concept of the research. A.M. conducted fieldwork with the help of B.L. in
394 developing the methodology. G.Ó. collected and processed the micrometeorological and
395 vegetation cover data. A.M., T.K. and P.C. designed, and A.M. performed the pot experiment.
396 A.M. analysed the data, and wrote the manuscript with major inputs from all co-authors.

397

398 **Compliance with ethical standards**

399 **Conflict of interest** The authors declare that they have no conflict of interest.

400 **Ethical approval** This article does not contain any studies with human participants or animals
401 performed by any of the authors.

402

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566 **Table 1** Precipitation (total sum) and daily average volumetric soil water content (SWC, %)
 567 between 1 May and 31 August 2015 (i.e. during the period covering each treatment in the year
 568 preceding our study) and cover (%) of the two dominant perennial grasses *Festuca vaginata*
 569 and *Stipa borysthenica*, and *Secale sylvestre* in the experimental plots in April (i.e. prior to the
 570 current year's treatments). For SWC and cover data, data are means \pm SE (n = 6)

	Treatment			
	Control	Severe drought	Moderate drought	Watering
Treatment period	–	23.06–25.08	22.07–25.08	18.05, 22.06, 21.07, 25.08
(number of days)	(–)	(63)	(34)	(4 distinct)
Precipitation (mm)	196.0	74.2	100.6	294.5
SWC (%) at 0-30 cm	4.0 \pm 0.1	3.2 \pm 0.1	3.4 \pm 0.1	4.3 \pm 0.1
Cover (%) of <i>F. vaginata</i> and <i>S. borysthenica</i> (2015)	10.0 \pm 1.1	9.6 \pm 1.3	9.3 \pm 1.1	12.8 \pm 0.9
Cover (%) of <i>F. vaginata</i> and <i>S. borysthenica</i> (2016)	12.0 \pm 1.5	2.0 \pm 0.5	7.2 \pm 1.1	15.4 \pm 0.8
Cover (%) of <i>S. sylvestre</i> (2015)	0.58 \pm 0.22	0.96 \pm 0.31	0.76 \pm 0.25	0.22 \pm 0.04
Cover (%) of <i>S. sylvestre</i> (2016)	1.1 \pm 0.3	4.5 \pm 1.0	2.7 \pm 0.9	0.55 \pm 0.14

571 **Table 2** Results of General Linear Mixed Models for traits of maternal and offspring
 572 generations of *Secale sylvestre*. Mother plants grew in the field experiment in 2016 and
 573 offspring in a common garden pot experiment. *P*-values of < 0.05 are considered significant.
 574 Subscripts on F-values are degrees of freedom of the numerator (MS Predictor) and
 575 denominator (MS Error), respectively

Plant response variable (predictor)	F _{3; 15}	<i>P</i>
<i>Maternal generation</i>		
Shoot height (cm)	16.34	< 0.0001
Seed number per ear	11.96	0.00029
Mean seed mass (mg)	8.69	0.0014
<i>Offspring generation</i>		
Final germination %	2.81	0.075
Juvenile shoot size (cm)	5.25	0.011
First leaf length (cm)	4.34	0.022
Juvenile shoot height (cm)	5.03	0.013
Adult shoot height (cm)	0.16	0.92
Adult biomass (mg)	1.31	0.31

576 **Figure legends**

577 **Fig. 1** Effects of rainfall manipulations on volumetric soil water content (%) at 0-30 cm depth
578 in the plots of the field experiment during the period of growth and reproduction of the
579 studied *Secale sylvestre* plants (maternal generation). Values are treatment means \pm SE (n = 6)
580 in each month. Treatments are watering (W), control (C), moderate drought (M), severe
581 drought (S). Different letters above the bars denote significant ($P < 0.05$) differences, while
582 N.S. indicates the lack of significant differences between treatments within each month
583 separately. Results of Tukey's HSD tests following two-way repeated measures ANOVA are
584 shown

585

586 **Fig. 2** Effects of previous-year (2015) rainfall manipulations on **a**) maximum vegetative shoot
587 height (cm), **b**) seed number per ear and **c**) mean single seed mass (mg) of *Secale sylvestre*
588 growing in the plots of the field experiment in 2016 (maternal generation). Values are
589 treatment means \pm SE (n = 6). Treatment symbols are defined in the legend of Fig. 1.
590 Different letters above the bars indicate significant ($P < 0.05$) differences between treatments.
591 Results of Tukey's HSD tests following General Linear Mixed Models are shown

592

593 **Fig. 3** Effects of differences in the maternal environment resulting from rainfall manipulations
594 (which were applied in 2015) on *Secale sylvestre* (offspring generation), whose mothers grew
595 in the plots of the field experiment in 2016. Offspring were grown in a subsequent common
596 garden pot experiment. Plant response variables include **a**) final germination percentage (%),
597 **b**) juvenile shoot size calculated by multiplying the total number of leaves by the length of the
598 longest fully-expanded leaf (cm), **c**) length of the first fully-expanded leaf (cm), **d**) juvenile
599 shoot height (cm), **e**) adult shoot height (cm), and **f**) adult live biomass (mg). Values are
600 treatment means \pm SE (n = 6). The treatments of the field experiment are abbreviated as in

601 Fig. 1. The statistical tests applied, and the indication of significant ($P < 0.05$) differences
602 between treatments are the same as described in Fig. 2

Fig. 1

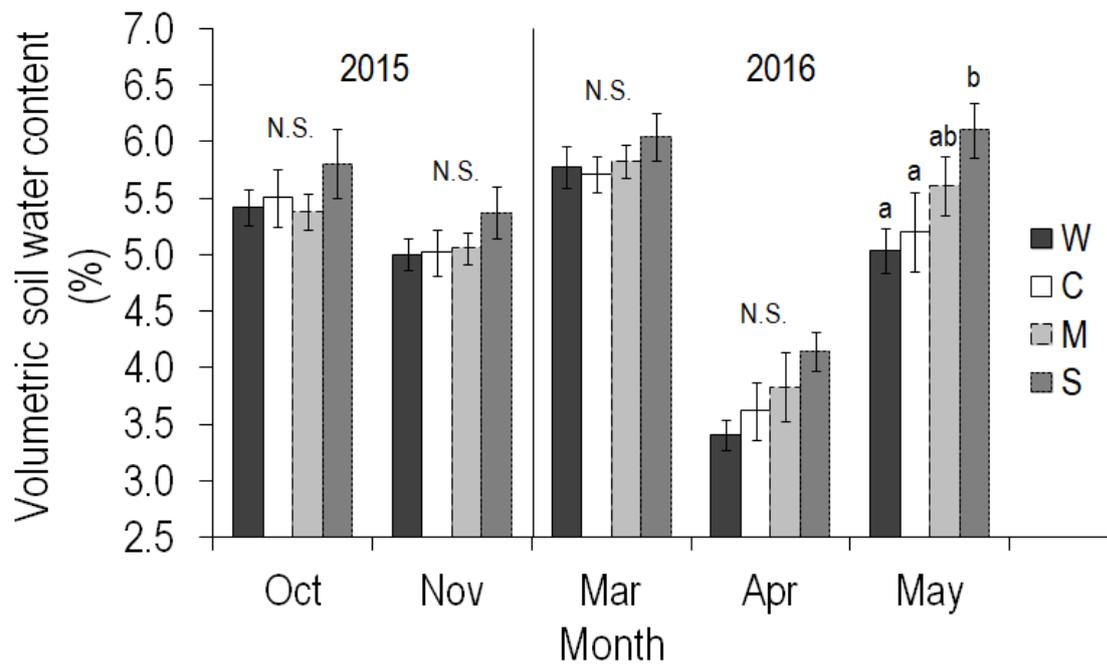


Fig. 2

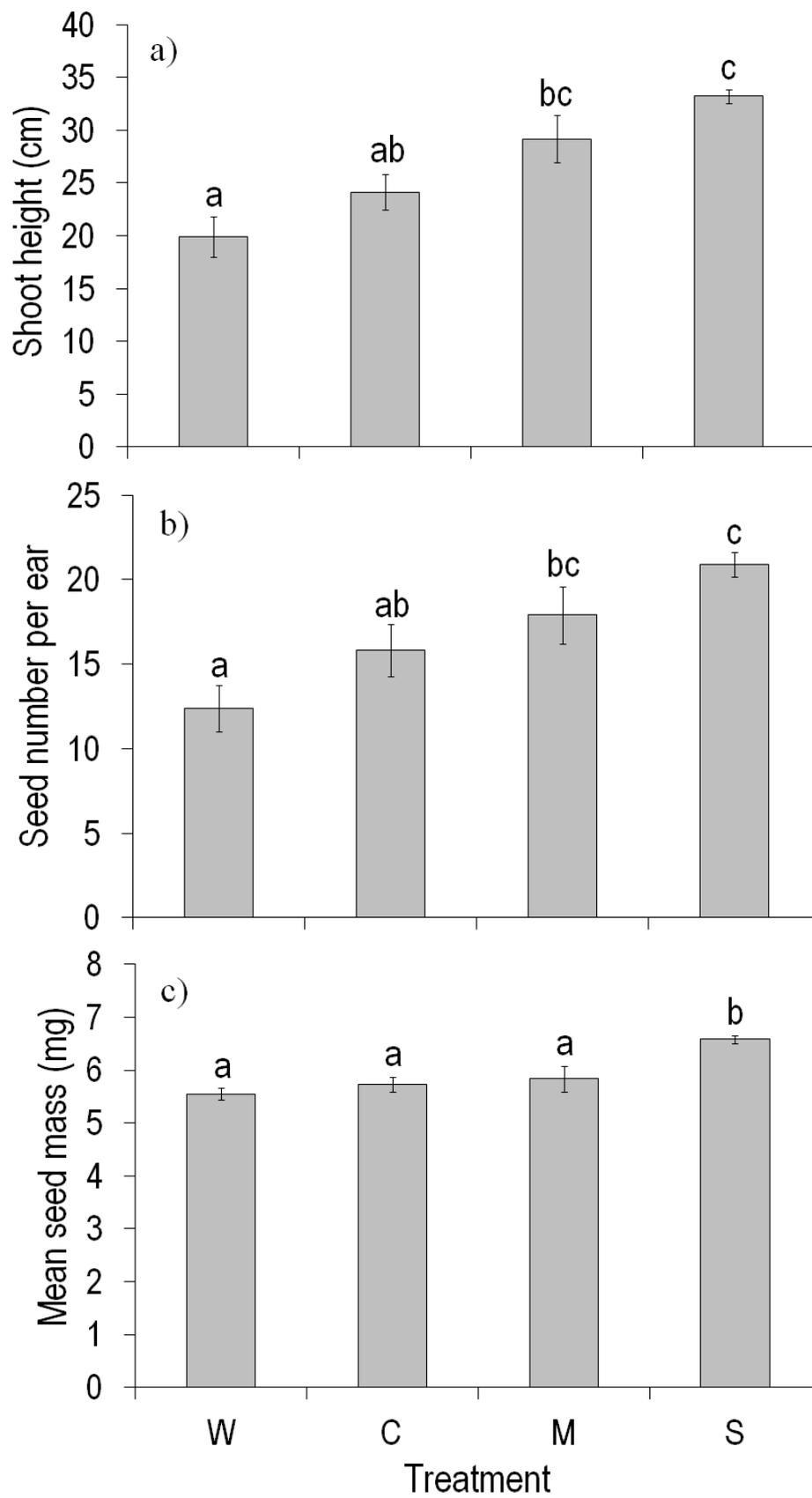


Fig. 3

