

**Effects of global changes in terms of fertilization and drought
on the vegetative growth, pollen and seed quality of closely related
threatened and common plant species of Switzerland**

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handed in by

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1. Abstract

Predicting how species, particularly threatened species, will react to global change is a challenge in ecology. Two factors of global change that strongly influence plant growth, reproduction and survival and also play an important role in biodiversity loss are fertilization and drought. We therefore wanted to test whether fertilization, drought and their interaction influence more strongly threatened than common plant species. We used ten threatened and eight common closely related plant species of Switzerland and we measured plant growth, pollen viability, flowering, seed weight and plant survival. We then tested for the effects of the global change treatments, plant rarity and all possible interactions on these plant traits. We found that drought decreased and fertilization increased plant height of both threatened and common plant species. Further, threatened species flowered less frequently than common plant species in general. Moreover, while none of the treatments nor their interaction affected pollen viability of the five flowering species, drought decreased the seed weight of *Erysimum cheiranthoides*. Our results indicate that global changes affect both threatened and common plant species, meaning that not only already threatened but also common plant species can be in danger. This can potentially increase the already high number of threatened plant species. Further multi-species studies addressing other aspects of global change and their interaction are crucial for the identification of plant species which are particularly sensitive to global changes, and to provide informed advice on their conservation strategies.

2. Introduction

Biodiversity, including plant species richness, is threatened worldwide (Díaz et al., 2006; Tilman et al., 2006; Butchart et al., 2010), and it decreases at a rate that has never been observed before (IPBES, 2019; WWF, 2020; Carrington, 2021). While some species are common, others are threatened, and in order to being able to conserve threatened plant species and slow down biodiversity loss, it is crucial to understand the different causes of plant rarity (Church, 2014).

Many studies have investigated the causes of plant rarity, for example by comparing their niche breadth (Brown 1984, Slatyer et al. 2013), germination rates (Anderson, 1980; Hodgson, 1986; Murray et al., 2002; Vincent, 2017) or pollinator limitation (Karron, 1987; Harper, 1979). However, in a time of global changes when most of the biodiversity is being lost due to human activities (Forester & Machlist, 1996; Rafferty, 2019), studies focusing on the role of different aspects of global change for plant rarity are needed. Global changes are alterations in natural systems (National Research Council, 2000) produced by human activity, causing the loss of biodiversity, environmental goods and services (CAESCG, 2014; Vitousek, 1994). Increased nutrients, as part of land-use intensification, and drought, as a part of climate change, are two important factors of global change influencing plant diversity (Pierik et al., 2011; Tilman, 1992). Understanding how they affect common and threatened plant species will strongly influence the strategies of how to conserve plant species in a changing environment.

Regarding the nutrient enrichment of habitats (eutrophication), it has been shown that plant species richness declines with increasing soil fertility (DiTommaso &

Aarssen, 1989; Rosenzweig & Abramsky, 1993; Gough et al., 2000; Suding et al., 2005). Moreover, threatened plant species are hypothesized to have adaptations suited for resource conservation rather than acquisition (Reich et al., 1999), and are consequently believed to be limited to resource-poor habitats as they cannot compete with competitive (common) species in nutrient-rich habitats (Drury, 1974; Grime, 1977; Murray et al., 2002). Based on these hypotheses, common plant species may be able to use additional nutrients more efficiently, increasing their competitive advantage and potentially out-competing threatened plant species in previously nutrient-poor habitats. Experiments comparing the responses of common and threatened plant species to increased level of nutrients are therefore an important tool for investigating the effects of global change on plant communities and the survival of plant species in future global change scenarios.

Drought is commonly defined as a deficit in supply of water, or low supply of water relative to demand (FAO, 2016). It can alter plant photosynthetic rates (Wang et al., 2018), damage plant tissues and even lead to mortality (Silva et al., 2013). It has been also shown that plant fitness declines in response to increasing drought frequency (Matesanz et al., 2009; Pratt & Mooney, 2013; Anderson J., 2016) and that water stress diminishes seed recruitment and can cause complex phenological changes (Peñuelas et al., 2004). Further, threatened plant species are expected to be less tolerant and particularly vulnerable to climate change (Schwartz et al., 2006). In this way, Vincent et al. (2020) reported that threatened plant species are less able to cope with changes in climate (precipitation) compared to more widespread ones, which might even benefit from these changes.

As different global change factors do not occur isolated, it is also important to consider the interacting effects as well. For drought and fertilization, it is suggested that an increased nutrient supply does not improve plant growth under simultaneous severe drought (Hu & Schmidhalter, 2005). However, little is known about the interaction of drought, fertilization and plant rarity. Therefore, so that plant ecologists are better able to advise on conservation practices, it is important to understand if common plant species may have an even greater competitive advantage over threatened plant species if drought and fertilization are altered in future global change scenarios.

Here, we present a multi-species experiment where we compare the effect of global changes on the fitness of threatened and common plant species. To test this, we grew ten pairs of closely related threatened and common plant species from nine different plant families in Switzerland under drought and fertilization treatments. We measured plant fitness in terms of plant height, flowering, pollen viability (using impedance flow cytometry technology), seed weight and plant survival.

Specifically, we addressed the following four questions: Do drought and fertilization, simulating global changes, affect 1) the growth, 2) the pollen quality and 3) the seed quality of threatened plant species more strongly than of common plant species? 4) Does rarity affect flowering and plant survival?

3. Materials and Methods

3.1. Study species

To test whether threatened and common plant species respond differently to global changes, we selected 20 plant species. Ten species were classified as threatened, since they have a Swiss conservation priority (Bornand et al., 2019) and are considered as near-threatened or threatened by the Swiss Red List of vascular plants (Bornand et al., 2016), and ten plant species were classified as common since they are not priority of conservation in Switzerland. The 20 species belonged to nine different plant families. To control for the phylogenetic relationship, we used closely related species and worked with pairs consisting of one common and one threatened species from the same family or genus.

Seeds from threatened and common plant species were collected in Swiss natural populations (one population per species). For each species, plants originated from several mother plants (seed families). Table 1 provides information on closely related pairs, plant populations and their location.

Table 1. Plant families and plant species selected for the experiment and the location of the populations where the seeds were collected from.

| Pair | Plant family | Threatened species | Location of threatened species | Common species | Location of common species |
|-------------|---------------------|--------------------------------|---------------------------------------|--------------------------------|-----------------------------------|
| 1 | Brassicaceae | <i>Cochlearia pyrenaica</i> | Gantrisch, Bern | <i>Lunaria rediviva</i> | Bönigen, Bern |
| 2 | Rosaceae | <i>Potentilla multifida</i> | Gornergrat, Zermatt, Wallis | <i>Potentilla argentea</i> | Herbriggen, Zermatt, Wallis |
| 3 | Papaveraceae | <i>Papaver occidentale</i> | Zweisimmen 1 | <i>Papaver rhoeas</i> | Bern Insel, Bern |
| 4 | Scrophulariaceae | <i>Scrophularia auriculata</i> | Choulex, Genf | <i>Scrophularia nodosa</i> | Magnedens, Freiburg |
| 5 | Lamiaceae | <i>Prunella laciniata</i> | Le Landeron, Neuenburg | <i>Prunella grandiflora</i> | La Mayette, Nods, Bern |
| 6 | Plantaginaceae | <i>Veronica austriaca</i> | Les Jordans / Les Baulles, Neuenburg | <i>Veronica urticifolia</i> | Holzflue, Bern |
| 7 | Campanulaceae | <i>Campanula cervicaria</i> | Forst 1, Bern | <i>Campanula rotundifolia</i> | Le Lieu, Vaud |
| 8 | Caryophyllaceae | <i>Silene viscaria</i> | Ried-Mörel, Wallis | <i>Silene vulgaris</i> | Nods, Bern |
| 9 | Brassicaceae | <i>Erysimum ochroleucum</i> | Chasseral 2, Jura | <i>Erysimum cheiranthoides</i> | Holligen, Bern |
| 10 | Hypericaceae | <i>Hypericum richerii</i> | Combe des Ambourneux, Jura | <i>Hypericum perforatum</i> | Güterbahnhof, Bern |

3.2. Seed preparation

In summer 2020, we sowed seeds of our 20 study species in small pots (6x7x7 cm) filled with seedling substrate (Klasmann-Deilmann GmbH). Then, we stratified the seeds for two months in the dark at 4 °C and kept the soil in the pots moist. After stratification, we allowed the seeds to germinate in the research greenhouse of the Institute of the Plant Sciences of the University of Bern in Ostermundigen, Switzerland (46°57'59.6"N 7°29'13.0"E). During germination, water was provided via ebb and flow system, the average light was 20.9 klx (minimum: 0 klx, maximum: 73.1 klx) and the average temperature was 21.3 °C (minimum: 14.3 °C, maximum: 25.5 °C).

After germination, we pricked out the seedlings individually in pots (11x11x12 cm) filled with a 1:9 ratio of sand:plant substrate (Selmaterra, mixed soil containing 20% compost, 25% agricultural field soil, 25% wood fiber and 30% peat; pH = 6.8-7.2; fertilizer=1.8 mS). All plant species were kept in the same greenhouse conditions to control for bias before the application of treatments. Due to low germination of *Campanula rotundifolia* and Oidium infestation of *Papaver rhoeas*, we were only able to carry out our experiment with 18 species in total, having two threatened species (*Campanula cervicaria* and *Papaver occidentale*) without a closely related common species.

3.3. Treatments

We used a full factorial design, including a drought treatment with three levels (none (**N**), medium (**M**), severe (**S**)), a fertilization treatment with two levels (fertilized (**F**), not fertilized (**NF**)) and all possible combinations. This resulted in six treatments in total: Control (**N x NF**); **N x F**; **M x NF**; **M x F**; **S x NF**; and **S x F**, which were applied during 139 days (from September 2020 until January 2021). During the experiment, the average light was 11.6 klx (minimum: 0 klx, maximum: 100.9 klx) and the average temperature was 17 °C (minimum: 10.6 °C, maximum: 25.2 °C).

For the drought treatment, watering was automated with the ebb and flow system. Three ebb and flow benches were used, each representing one treatment level. Plants in the first bench were watered every 48 hours (**N**), plants in the second bench were watered every 96 hours (**M**), and plants in the third bench were watered every 192 hours during the first month and then every 144 hours (**S**). The watering regime for the severe drought was modified to avoid high plant mortality rates because as plants grew, their water demand increased.

For the fertilization treatment we used Wuxal®, a commonly used soluble NPK fertilizer. First, we prepared a mix with a concentration of 2 mL Wuxal/L water. Then, we applied 20 mL of the mix to each plant once a week during the first month of the experiment, on a day without irrigation to avoid leaching. Afterwards, in order to enhance the effect of the fertilizer, we applied 30 mL of the mix to each plant once every week. In total, we applied fertilizer 19 times during the entire experiment.

3.4. Experimental design

The treatments were arranged in a split plot design with drought as the main plot and fertilization as the sub-plot. Fertilization treatments were replicated four times per ebb and flow bench, resulting in a total of 24 sub-plots (Figure 1).

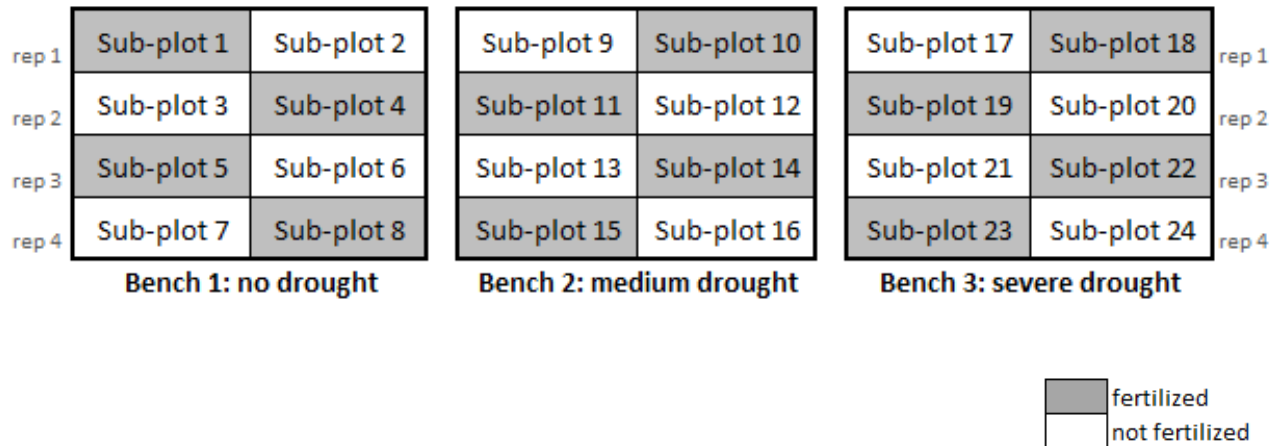


Figure 1. Experiment design (split plot), where each main plot has one drought level and the sub-plot has one of the two fertilization levels. The two levels of the fertilization treatment were applied within each main plot and repeated four times. This resulted in 24 sub-plots.

For nine plant species (*Cochlearia pyrenaica*, *Veronica austriaca*, *Campanula cervicaria*, *Erysimum ochroleucum*, *Hypericum richerii*, *Lunaria rediviva*, *Prunella grandiflora*, *Veronica urticifolia*, *Erysimum cheiranthoides*), we included 24 plants per species in the experiment, i.e., one plant for each subplot. For *Prunella laciniata*, we had low germination and could only include 16 plants. We distributed those plants equally in the “none” and “severe” drought treatments.

Moreover, we included additional replications of seed families for species with sufficient high germination rates. Depending on the germination rates of the seed families, we had two different cases. 1) For two species (*Potentilla multifida* and *Scrophularia auriculata*), we included two plants originating from different seed families per sub-plot, which resulted in 48 plants per species in total. 2) For another six species (*Papaver occidentale*, *Silene viscaria*, *Potentilla argentea*, *Scrophularia nodosa*, *Silene vulgaris*, *Hypericum perforatum*), we included three plants in four sub-plots per ebb and flow bench. From those three plants, two plants were from the same seed family and one plant was from another seed family. This case also resulted in 48 plants per species in total.

Overall, we included a total of 616 plants in our experiment. Individuals within each sub-plot were randomly distributed. Information about the number of plants per species can also be found in the Supplementary information: Table S1.

3.5. Measurements

We measured plant height as an indicator for plant growth, from soil level to the highest point of the plant. We measured it before starting the treatments and at the end of the experiment to obtain initial and final plant height measurements.

During our experiment, only five study species flowered (*Erysimum cheiranthoides*, *Papaver occidentale*, *Scrophularia auriculata*, *Scrophularia nodosa*, *Silene vulgaris*). We placed organza bags from the beginning of the reproductive stage of every plant until the end of the experiment. This allowed us to avoid pollen contamination and uncontrolled cross-pollinations between plants from different treatments. Regarding pollination, *Papaver occidentale* and *Silene vulgaris* needed hand pollination while *Erysimum cheiranthoides*, *Scrophularia auriculata* and *Scrophularia nodosa* did not (self-pollination).

Pollen viability, considered as an important parameter of pollen quality (Dafni & Firmage, 2000), was measured for the five species that flowered. When the flowers were fully open, we checked if pollen had been released using a magnifying glass. If so, we cut the flower and placed it into a tube. For small flowers (*Erysimum cheiranthoides*, *Scrophularia auriculata*, *Scrophularia nodosa*), we used 1.5 mL Eppendorf tubes. For big flowers (*Papaver occidentale*, *Silene vulgaris*), we used 15 mL centrifuge tubes. We took pollen samples once per week and assessed pollen viability with impedance flow cytometry technology, using the AmphaZ32 instrument and Amphasoft 2.0 software at the Amphasys laboratory (Technopark Lucerne, Root D4, Switzerland). This technology consists of a flow cytometer equipped with a

microfluidic chip that measures changes in the electrical impedance of a fluidic medium when suspended pollen cells pass through an applied electric field. A specific set of consumables, sample preparation routines and instrument configurations were required for different pollen types, as they differ morphologically and biophysically. The critical elements are summarized in Table 2. Once in the laboratory, we classified all the samples per species and then worked with one sample at a time. First, we removed the stamens with tweezers and mixed them with their specific measurement buffer, by shaking the suspended stamens in an Eppendorf tube. After that, we filtered the pollen mix with a specific filter, in order to obtain a cleaner sample and avoid clogging of the microfluidic chip. Subsequently, the sample is diluted with more measurement buffer and then attached at the sample holder of the AmphaZ32 instrument. Then, we attached the microfluidic chip (that is also species-specific) in the instrument, and run the sample. Pollen viability was calculated by the Amphasoft directly as percentage. We then converted the percentages to decimals.

Table 2. Details on the preparation of pollen samples using Amphasys technology for the five species that flowered in our experiment. Information on the type of chip, type of buffer, volume (mL) of buffer, type of filter, number of flowers used per individual and pollen extraction method is given.

| Species name | Chip Type | Buffer | Buffer (mL) | Filter | Flowers | Pollen extraction |
|--------------------------------|------------|--------|-------------|--------|---------|-------------------|
| <i>Erysimum cheiranthoides</i> | D (120 µm) | AF7 | 2 | 100 µm | 3 | shaking |
| <i>Papaver occidentale</i> | D (120 µm) | AF6 | 3 | 100 µm | 1/4 | shaking |
| <i>Scrophularia auriculata</i> | D (120 µm) | AF6 | 2 | 100 µm | 3 | shaking |
| <i>Scrophularia nodosa</i> | D (120 µm) | AF6 | 2 | 100 µm | 3 | shaking |
| <i>Silene vulgaris</i> | E (250 µm) | AF6 | 3 | 150 µm | 3 | pestle + shaking |

We further recorded seed weight, an indicator for seed quality (Deivasigamani & Swaminathan, 2011; Afshari et al., 2011), by counting the number of seeds per fruit and weighing them. We then calculated the weight of hundred seeds in order to have a standardized metric among all species. During the experiment, we also recorded flowering and survival, giving us the percentage of flowering plants and survival of threatened and common plant species.

3.6. Statistical analysis

To test whether the effect of drought and fertilizer on final plant height, pollen viability and seed weight differ between threatened and common plant species, we used linear mixed-effect models (lmer) in the R program version 4.0.4; R Development Core Team 2021. We used the following packages: devtools, dplyr, effects, ggplot2, lme4, lmerTest and remef (Wickham, Hester & Chang, 2020; Wickham et al., 2020; Fox & Weisberg, 2019; Wickham, 2016; Bates et al., 2015; Kuznetsova et al., 2017; Hohenstein & Kliegl, 2021). We log-transformed plant height (cm) and seed weight (mg), and used the arcsine square root function for pollen viability to meet lmer requirements on residual distributions.

For the plant height model, the explanatory variables were rarity (threatened and common), fertilization (fertilized, not fertilized), drought (none, medium, severe) and all possible two-way and three-way interactions. The random terms were plant family, plant species, seed family (these three nested as plant family/plant species/seed family) and sub-plot number. Because initial plant height affects the potential final height, it was added in the model as a covariate.

As only five species produced flowers, we ran independent models for each species, with pollen viability and seed weight as response variables. The explanatory variables were fertilization (fertilized, not fertilized), drought (none, medium, severe) and their interaction. The random terms were seed family and sub-plot number. The exception was *Erysimum cheiranthoides*, which had one observation per sub-plot, thus we didn't include sub-plot number as random term. Additionally, we also calculated

models including all five species together for both pollen viability and seed weight (Supplementary information: Table S8, Table S14). For these models, we used plant rarity, drought, fertilization and all possible two- and three-way interactions as explanatory variables and used plant species, seed family (nested as plant species/seed family) and sub-plot number as random terms.

For all the models described above, we proceeded to do a model reduction by using a backward stepwise procedure. We ran the models and removed the least significant term calculated with the Satterthwaite's method of approximation until obtaining the simplest model possible, i.e., the model containing only significant terms included in significant interactions (Kuznetsova et al., 2017). In order to avoid Type I errors, we then corrected all *P*-values from all models with the Hochberg correction for multiple tests.

To test whether flowering and survival rate differed between threatened and common plant species overall, we used a Chi-squared test on the categorical flowering data ("vegetative", "flowering") and a Fischer test on the categorical survival data ("alive" or "dead"), including all the plants of our experiment. We used a Fischer test because the number of dead plants in our experiment was five, and this test is the most suitable for small frequencies.

4. Results

4.1. Plant height

Overall, we found that common plant species were higher than threatened plant species, regardless of the global change treatments.

When taking all species together, the medium drought treatment reduced plant height by 10.6%, while the severe drought treatment strongly decreased plant height by 26.3% when compared with no drought stress ($P < 0.001$, Supplementary information: Table S2; Figure 2, left). The reduction of plant height with medium or severe drought did not depend on the rarity of the plant species nor did it depend on whether we fertilized the plants or not.

When taking all species together, fertilization increased plant height by 17.8% compared with plants that were not fertilized ($P < 0.001$, Supplementary information: Table S2; Figure 2, right). The increase in plant height with fertilization did not depend on the rarity of the plant species nor on the drought treatment.

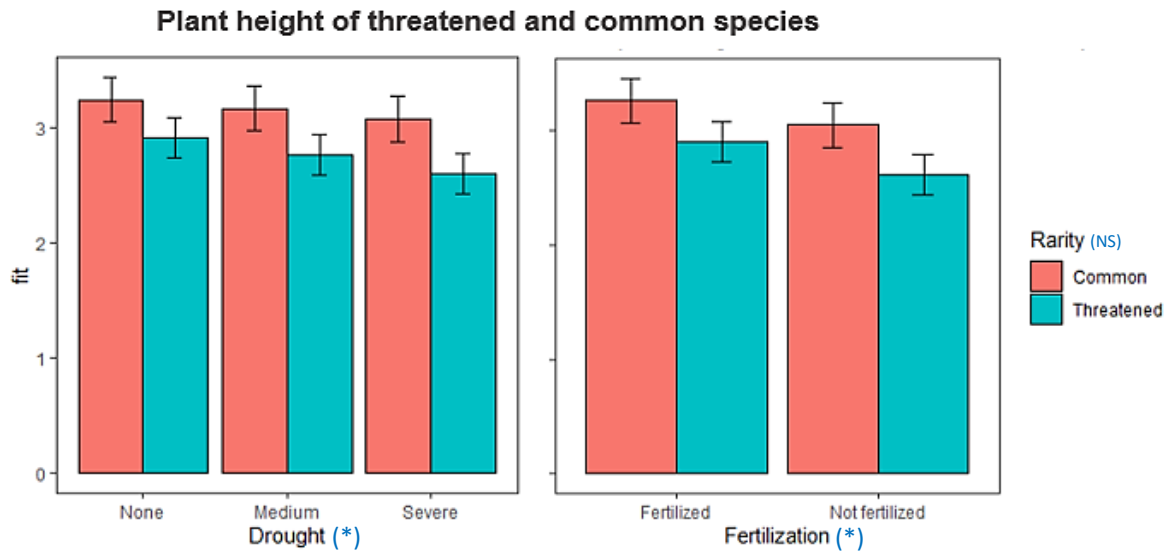


Figure 2. Plant height of common (pink) and threatened (blue) plant species, under the effects of drought with three levels: none, medium and severe (left), and under the effects of fertilization with two levels: fertilized and not fertilized (right). It was found that drought and fertilization, each independently, influenced the plant height of our study species. Shown are model fitted estimates. Error bars indicate confidence intervals (obtained from the effect package in R).

4.2. Pollen viability

Neither drought, fertilization nor their interaction influenced the pollen viability of any of the five species. Instead, we found that the response of pollen viability to the global change treatments drought and fertilization was very species-specific (Figure 3-4).

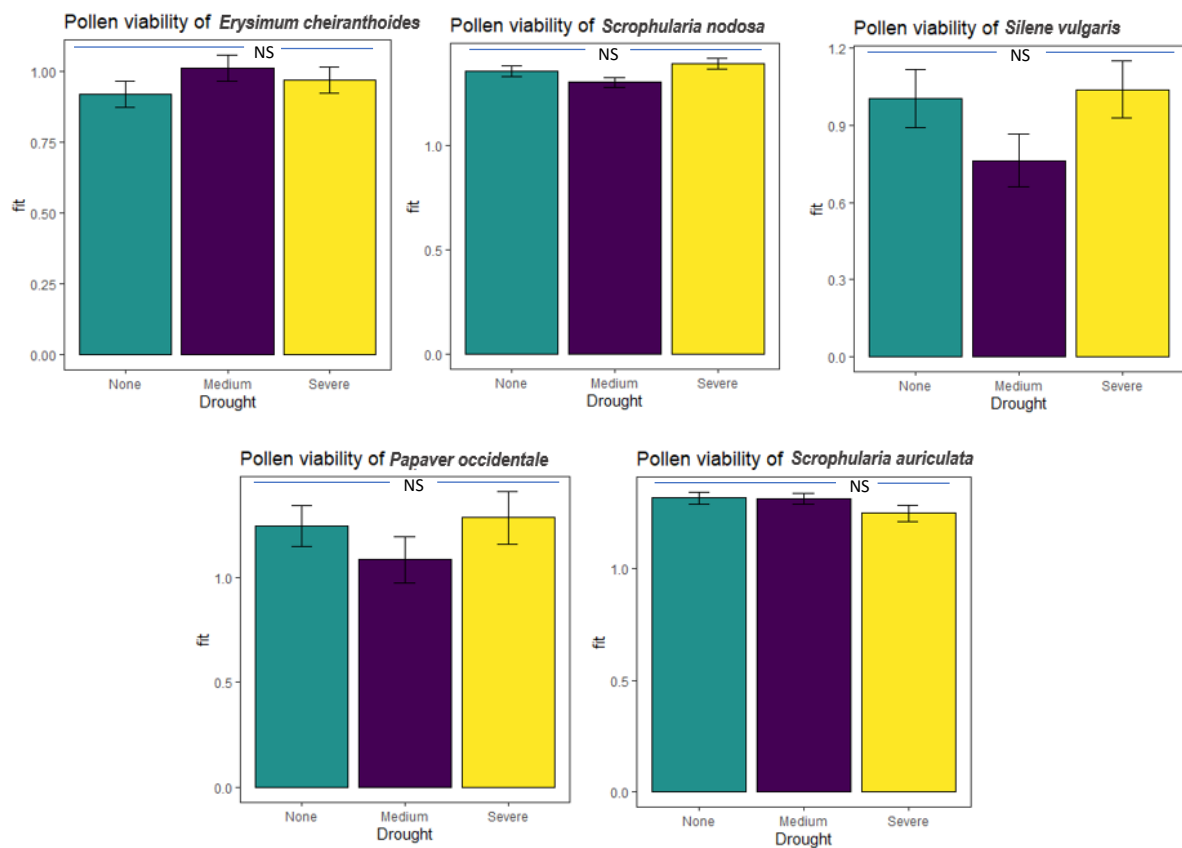


Figure 3. Pollen viability of three common (top of figure) and two threatened (bottom of figure) plant species, under the effects of drought with three levels: none (blue), medium (purple) and severe (yellow). No effects of drought on the pollen viability of the five species were found. Shown are model fitted estimates. Error bars indicate confidence intervals (obtained from the effect package in R).

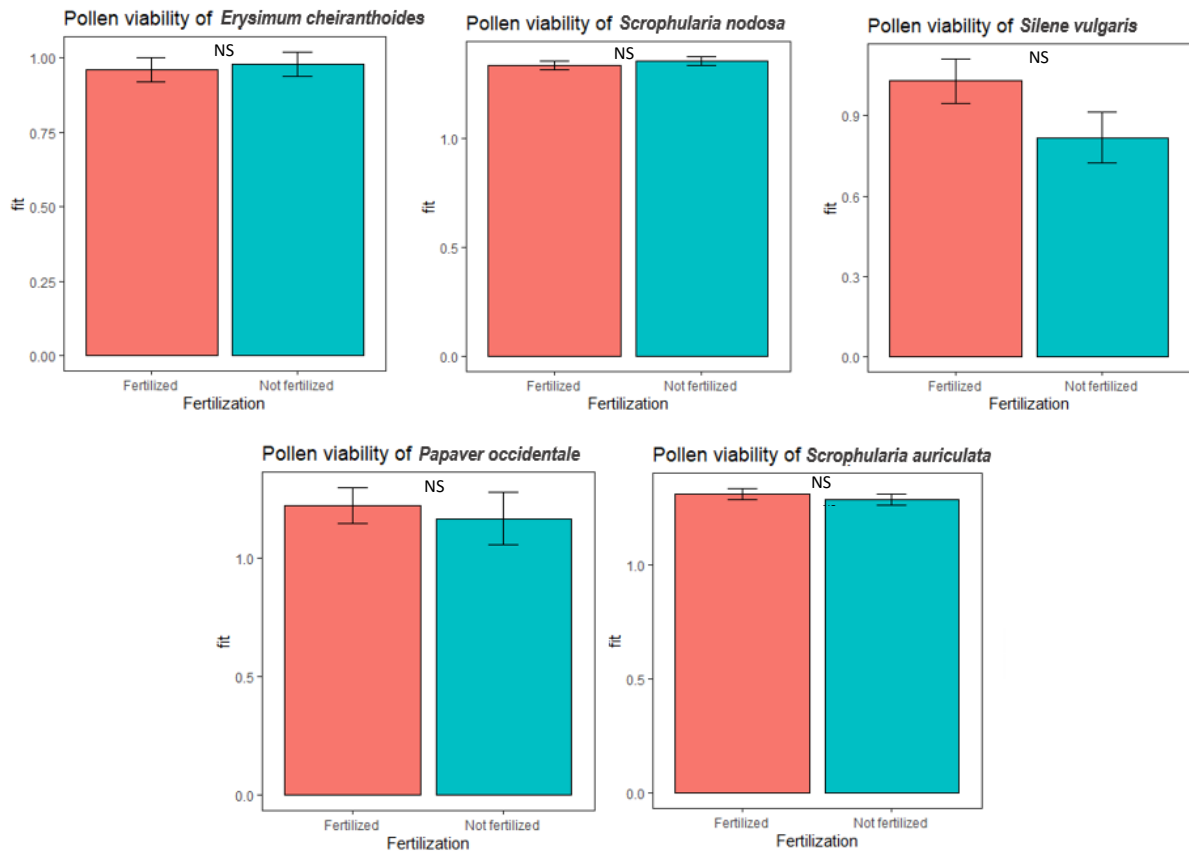


Figure 4. Pollen viability of three common (top of figure) and two threatened (bottom of figure) plant species, under the effects of fertilization with two levels: fertilized (pink) and not fertilized (blue). No effects of fertilization on the pollen viability of the five species were found. Shown are model fitted estimates. Error bars indicate confidence intervals (obtained from the effect package in R).

When analyzing the five species together, neither the treatments fertilization and drought nor their interaction influenced pollen viability (Supplementary information: Table S8).

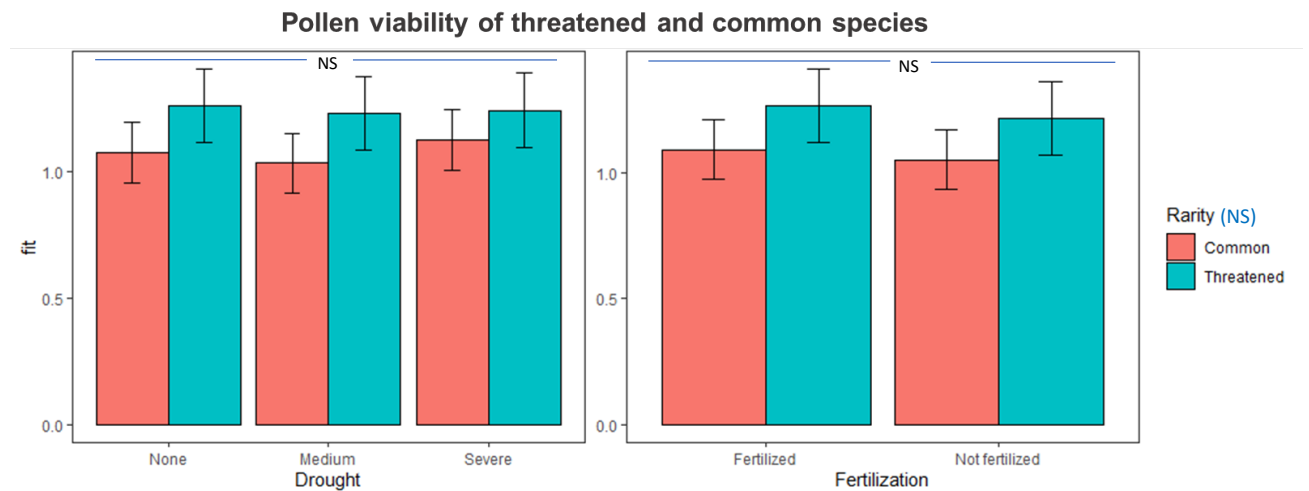


Figure 5. Pollen viability of common (pink) and threatened (blue) plant species, under the effects of drought with three levels: none, medium and severe (left), and under the effects of fertilization with two levels: fertilized and not fertilized (right). No effects of drought, fertilization nor of their interaction were found. Shown are model fitted estimates. Error bars indicate confidence intervals (obtained from the effect package in R).

4.3. Seed weight

After analyzing each species separately, we found that the global change treatment drought affected the seed weight of *Erysimum cheiranthoides* (Supplementary information: Table S9). However, drought, fertilization and their interaction did not influence the seed weight of the other four species (Supplementary information: Table S10-13).

Erysimum cheiranthoides had the heaviest seeds for the plants growing under no drought ($P=0.0006244$, Supplementary information: Table S9). Its seed weight then gradually decreased as the severity of the drought increased (Figure 6, left). Fertilization did not influence the seed weight of this species (Figure 6, right).

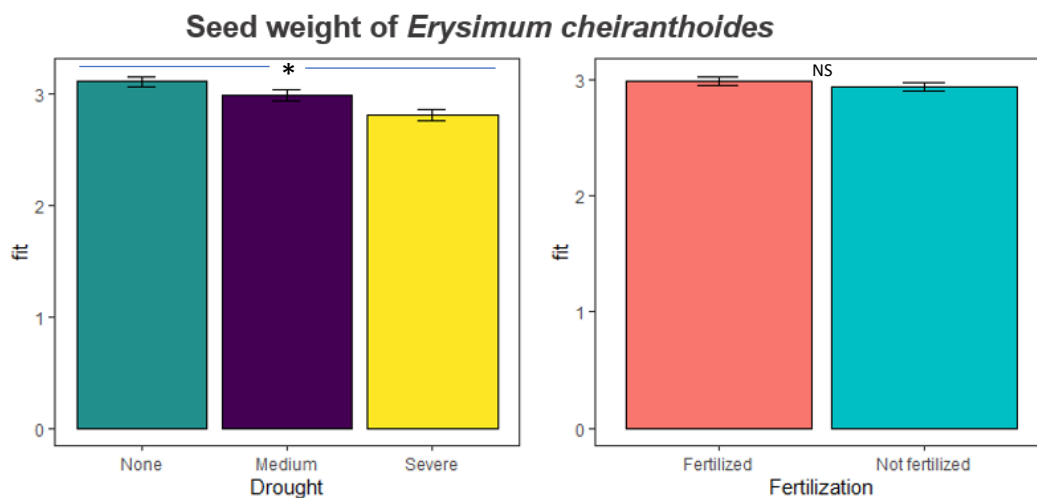


Figure 6. Seed weight of *Erysimum cheiranthoides* under the effects of drought with three levels: none (blue), medium (purple) and severe (yellow), shown on the left; and under the effects of fertilization with two levels: fertilized (pink) and not fertilized (blue), shown on the right. Only the drought treatment influenced the seed weight of *Erysimum cheiranthoides*. Shown are model fitted estimates. Error bars indicate confidence intervals (obtained from the effect package in R).

When analyzing the five species together, we did not find any effects of drought, fertilization or their interaction on the seed weight (Supplementary information: Table S14).

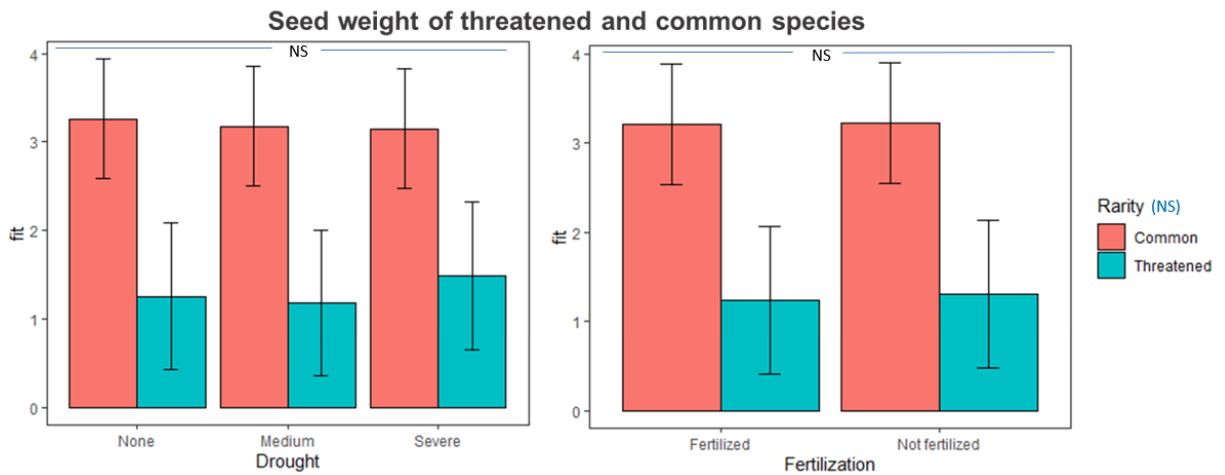


Figure 7. Seed weight of common (pink) and threatened (blue) plant species, under the effects of drought with three levels: none, medium and severe (left), and under the effects of fertilization with two levels: fertilized and not fertilized (right). No effects of drought, fertilization nor of their interaction were found. Shown are model fitted estimates. Error bars indicate confidence intervals (obtained from the effect package in R).

4.4. Flowering and survival

We found that flowering differed between threatened and common plant species, and that threatened species flowered less often than common species. While only 16% of threatened plants flowered in our experiment, 29% of common plants flowered. When analyzing survival, we did not find that plant survival differed between threatened and common plant species.

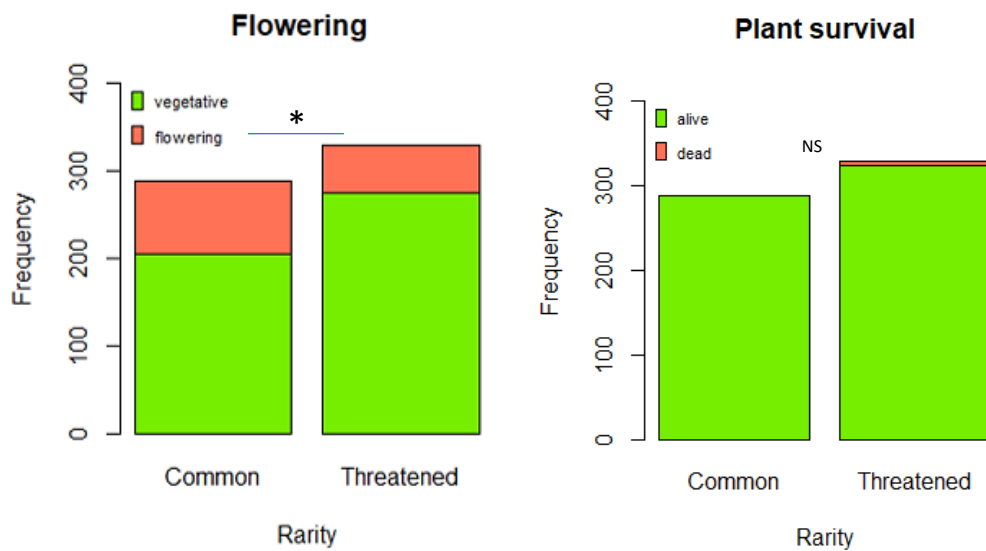


Figure 8. Flowering and survival of common and threatened plant species. Effects of rarity were found only for flowering (left), where pink is the fraction of plants that flowered and green is the fraction of plants that did not flower. No effects of rarity were found for plant survival (right), where pink is the fraction of plants that died during our experiment, and green is the fraction of plants that stayed alive. Shown are frequencies from a Chi-squared test (flowering) and Fischer test (survival).

5. Discussion

We found that drought and fertilization, both important aspects of global change, affected the plant growth of all studied plant species, independently of their rarity. While drought decreased plant height, fertilization increased it. For the five species that flowered, we found that none of the treatments nor their interaction affected pollen viability, and that drought decreased the seed weight of *Erysimum cheiranthoides*. We further found that threatened plant species flowered less often than common plant species.

Plant growth is influenced by many abiotic and biotic factors, amongst which water availability and fertilization play an important role. Several studies have reported that fertilization increases plant growth (Hu & Schmidhalter, 2005; King et al., 2006; Santachiara et al., 2017; Wu et al., 2019) and biomass (Liu & Greaver, 2010; Liu et al., 2014) while drought tends to decrease it (Yuyan et al., 2007; Riaz et al., 2010; Hamayun et al., 2010). This is in agreement with the effects that we found in our study. However, while these effects haven been shown to vary in function of plant rarity, we did not find such a change for threatened and common plant species. Regarding drought, Vincent et al. (2020) reported that above-ground biomass of threatened plant species was hardly affected by differences in precipitation while common species showed plasticity and produced more biomass in drier and wetter conditions. Regarding fertilization, Dawson et al. (2012) showed that common plant species were better than threatened plant species in taking advantage of increased nutrient availability, while Kempel et al. (2020) reported that common and threatened plant species did not differ in their response to fertilization. Furthermore, we did not find

interactions between drought and fertilization, which is in contrast to the findings from Smika et al. (1965) and Hu et al. (2005). Both studies have reported that fertilizer did not increase yield without sufficient water. Furthermore, plant phenology differed between the 18 plant species during our experiment, and as suggested by Dawson et al. (2012), we should therefore be careful with comparisons between groups of species, as the experimental period may not have been long enough to establish the maximum biomass attainable for all species. We thus suggest that future studies should include observations on a longer period of time, so more species can achieve their maximum potential growth. Taking into account that common plant species grew higher in general, we cannot exclude that global change will not shift competition patterns and therefore endanger already threatened plant species even more.

Pollen viability is an important indicator of tolerance to abiotic stresses (Junyi et al., 2019) and has been shown to be influenced by fertilization and water stress in a general and species-specific manner. While some species' pollen viability is very sensitive to water stress, the pollen viability of others does not depend on water availability in reproductive tissues (Saini, 1997; Barnabas et al., 2008). Similarly, for some species pollen viability is higher under nutrient addition while for others it is higher under low nutrient conditions (Tak-Cheung Lau & Stephenson, 1993; Atasay et al., 2013; Pers-Kamczyc et al., 2020), whereas for others pollen viability is not influenced by changes in nutrient availability at all (Anderson, 1980; Murray et al., 2002). Additionally, Banks (1980) reported that threatened plant species had generally lower pollen viability than common plant species. In our study, we did not find effects of drought, fertilization or plant rarity on pollen viability in any of the study species. Even though we could analyze pollen viability for only five species, we can conclude

that drought and fertilization have no effect on the pollen viability in our study. The use of pollen viability in the studies on the effects of global changes can help us to predict species dispersal, fitness and survival of the next plant generation (Impe et al., 2020). Thus, we suggest to include pollen viability in future projects to study in more depth whether viability varies in function of plant rarity. Moreover, pollen analyses with this new type of technology (impedance flow cytometry) are a fast tool, especially when compared to seed quality or seed germination. We also recommend to include other pollen features, i.e., pollen grain size and the number of pollen grains per anther, since they also have been shown to be drought-sensitive (Yamburov et al., 2014), and together they could give a more complete picture about pollen quality in regards to global change.

Next to pollen viability, we also looked at the flowering percentage. Lavergne et al. (2004) reported that common plant species produce more flowers than threatened plant species. Similarly, we found that common plants flowered more often than threatened plants. Threatened plant species may flower less frequently because they have a smaller population size (Gaston et al., 2000), a narrow fundamental niche (Brown, 1984) and a lower colonization ability than their common congeners (Fiedler, 1987; Byers & Meagher, 1997). This disadvantage may limit their opportunities to colonize new sites and to increase their local population (Silvertown et al., 1993). However, it is also possible that even though threatened plants flower less often, they may produce fruits with a higher number of seeds and a higher ratio of viable seeds than common plant species (Bürli et al., in prep.).

Several studies have shown that seed weight was increased by fertilization (Asare & Scarisbrick, 1995; Breen & Richards, 2008; Zareie et al., 2011; Olama et al., 2014), and decreased by drought (Mogensen, 1992; Samarah et al., 2009; Alqudah et al., 2011). Nevertheless, we found that even though *Erysimum cheiranthoides* is a common species, its seed quality was highly susceptible to water stress. Drought alters the plant nutrient uptake (Nieves-Cordones et al., 2019) and developing seeds cannot accumulate enough starch in the endosperm (Sehgal et al., 2018), resulting in a reduced grain weight and seed size (Nicolas et al., 1985; Samarah et al., 2004). In addition, the indicator values for moisture (H) according to Landolt et al. (2010) indicate that *Silene vulgaris* lives in a moist habitat (humidity factor of 2+), whereas *Erysimum cheiranthoides* lives in a wet habitat (humidity factor of 3+). This can explain why *Erysimum cheiranthoides* was sensitive to water stress, whereas the seed weight of *Silene vulgaris* remained very stable among the severity of the treatment (no effects of drought). Regarding *Scrophularia nodosa* and *Scrophularia auriculata*, which grow in very moist and flooded habitats respectively, they did not produce enough seed samples in the medium and severe drought treatments due to fruit abortion. This observation suggests that these two species are likely to suffer from drought. Regarding *Papaver occidentale*, which grows in medium wet habitats, more time would have been needed to collect more seed weight data. Hence, assumptions on these species should be taken carefully. Regarding plant rarity and seed quality, there are comparative studies on seed production, seed size, type and length that show controversial results (Murray et al., 2002). For instance, Vincent et al (2017) reported that threatened species tended to have lower seed mass than common species, but this difference was marginally significant. Moreover, finding a negative impact of drought on the seed quality of *Erysimum cheiranthoides* shows that also common plant

species will most likely suffer from global change. Further, with only two threatened species we cannot conclude that fertilization or drought do not have an influence on the seed weight of threatened plant species in general. In order to make a prediction on how global change will affect threatened and common plant species, future studies should increase the study time to enable more species to flower and, therefore, to analyze seed quality on more plant species.

To conclude, our study shows that both common and threatened plant species increased their growth with the application of fertilizers. This challenges the hypothesis that common species may profit more than threatened plant species from eutrophication, achieving an even greater competitive advantage. Moreover, the fact that all species showed decreased plant height with drought and that seed quality of *Erysimum cheiranthoides*, a common species, was found to be negatively affected by it, raises the concern not only threatened but also common species will suffer from global change. This may increase the already high number of threatened plant species in the future. Lastly, it is important to consider in future studies other important global change drivers besides drought and fertilization (e.g., land-use intensification, extreme temperatures, carbon cycle, pollution), especially in regards to plant rarity, and considering the effects on their own and in interaction. Studies of longer duration will additionally allow the inclusion of important parameters besides plant height, such as pollen and seed quality. This will improve our ability to predict how global changes affect common and threatened plant species, and thus will improve our decision making in applied plant conservation.

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7. Supplementary information

Table S1. Information on the plant species, the number of seed families per sub-plot, the number of plants per species per sub-plot and the total number of plants per species used in the experiment is given.

| Threatened species | Nb seed family/subplot | Number of plants/subplot | Total plants |
|---------------------------------|------------------------|--------------------------|--------------|
| <i>Cochlearia pyrenaica</i> | 1 | 1 | 24 |
| <i>Potentilla multifida</i> | 2 | 2 | 48 |
| <i>Papaver occidentale</i> | 1 or 2 | 1 or 3 | 48 |
| <i>Scrophularia auriculata</i> | 1 or 2 | 2 | 48 |
| <i>Prunella laciniata</i> (***) | 1 | 1 | 16 |
| <i>Veronica austriaca</i> | 1 | 1 | 24 |
| <i>Campanula cervicaria</i> | 1 | 1 | 24 |
| <i>Silene viscaria</i> | 1 or 2 | 1 or 3 | 48 |
| <i>Erysimum ochroleucum</i> | 1 | 1 | 24 |
| <i>Hypericum richerii</i> | 1 | 1 | 24 |

| Common species | Nb seed family/subplot | Number of plants/subplot | Total plants |
|--------------------------------|------------------------|--------------------------|--------------|
| <i>Lunaria rediviva</i> | 1 | 1 | 24 |
| <i>Potentilla argentea</i> | 1 or 2 | 1 or 3 | 48 |
| <i>Scrophularia nodosa</i> | 1 or 2 | 1 or 3 | 48 |
| <i>Prunella grandiflora</i> | 1 | 1 | 24 |
| <i>Veronica urticifolia</i> | 1 | 1 | 24 |
| <i>Silene vulgaris</i> | 1 or 2 | 1 or 3 | 48 |
| <i>Erysimum cheiranthoides</i> | 1 | 1 | 24 |
| <i>Hypericum perforatum</i> | 1 or 2 | 1 or 3 | 48 |

| | |
|---------------------------------------|------------|
| Total plants in the experiment | 616 |
|---------------------------------------|------------|

*** *Prunella laciniata*: 16 plants from one single seed family were included in the experiment

Table S2. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on plant height of all the plant species of the experiment after the model reduction (i.e., after non-significant terms have been dropped). Performance analysis was conducted on 616 observations, 18 plant species and nine plant families. For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and *P* value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding *P* value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and *P* value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding *P* value respectively. The Corrected *P* value refers to the *P* value after Hochberg correction for multiple tests. Significant *P* values are highlighted in boldface type. * *P* < 0.05; ** *P* < 0.01; and *** *P* < 0.001.

| Plant height – all plant species | | | | | | | |
|----------------------------------|----------|----------|----|---------|---------|--------------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | <i>P</i> value | Corrected <i>P</i> value |
| Height at installation | 3.411 | 3.411 | 1 | 597.45 | 32.585 | < 0.001 *** | < 0.001 *** |
| Drought | 5.574 | 2.787 | 2 | 18.75 | 26.625 | < 0.001 *** | < 0.001 *** |
| Fertilization | 8.866 | 8.866 | 1 | 18.35 | 84.694 | < 0.001 *** | < 0.001 *** |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | <i>P</i> value | Corrected <i>P</i> value |
| Family/Species/Seed family | 0.012 | 0.109 | 1 | -251.74 | 9.991 | 0.002 ** | 0.091 |
| Number of subplot | 0.000 | 0.021 | 1 | -246.80 | 0.099 | 0.753 | 1.000 |
| Family/Species | 0.275 | 0.525 | 1 | -287.33 | 81.176 | < 0.001 *** | < 0.001 *** |
| Family | 0.029 | 0.171 | 1 | -246.78 | 0.069 | 0.793 | 1.000 |
| Residual | 0.105 | 0.324 | | | | | |

Table S3. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the pollen viability of *Erysimum cheiranthoides* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and *P* value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding *P* value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and *P* value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding *P* value respectively. The Corrected *P* value refers to the *P* value after Hochberg correction for multiple tests. Significant *P* values are highlighted in boldface type. * *P* < 0.05; ** *P* < 0.01; and *** *P* < 0.001.

| Pollen viability of <i>Erysimum cheiranthoides</i> | | | | | | | |
|---|-----------------|-----------------|-----------|---------------|----------------|----------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | P value | Corrected P value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | P value | Corrected P value |
| Seed family | 0.004 | 0.064 | 1 | 8.6153 | 3.06 | 0.080 | 1.000 |
| Residual | 0.008 | 0.092 | | | | | |

Table S4. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the pollen viability of *Papaver occidentale* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and P value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding P value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and P value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding P value respectively. The Corrected P value refers to the P value after Hochberg correction for multiple tests. Significant P values are highlighted in boldface type. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

| Pollen viability of <i>Papaver occidentale</i> | | | | | | | |
|---|-----------------|-----------------|-----------|---------------|----------------|----------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | P value | Corrected P value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | P value | Corrected P value |
| Number of subplot | 0.032 | 0.179 | 1 | -1.995 | 2.284 | 0.131 | 1.000 |
| Seed family | 0.000 | 0.000 | 1 | -0.853 | 0.000 | 1.000 | 1.000 |
| Residual | 0.018 | 0.133 | | | | | |

Table S5. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the pollen viability of *Scrophularia auriculata* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and P value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding P value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and P value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding P value respectively. The Corrected P value refers to the P value after Hochberg correction for multiple tests. Significant P values are highlighted in boldface type. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

| Pollen viability of <i>Scrophularia auriculata</i> | | | | | | | |
|---|-----------------|-----------------|-----------|---------------|----------------|----------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | P value | Corrected P value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | P value | Corrected P value |
| Number of subplot | 0.000 | 0.000 | 1 | 20.09 | 0 | 1.000 | 1.000 |
| Seed family | 0.000 | 0.000 | 1 | 20.09 | 0 | 1.000 | 1.000 |
| Residual | 0.010 | 0.100 | | | | | |

Table S6. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the pollen viability of *Scrophularia nodosa* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and P value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding P value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and P value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding P value respectively. The Corrected P value refers to the P value after Hochberg correction for multiple tests. Significant P values are highlighted in boldface type. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

| Pollen viability of <i>Scrophularia nodosa</i> | | | | | | | |
|---|-----------------|-----------------|-----------|---------------|----------------|----------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | P value | Corrected P value |
| Drought | 0.053 | 0.027 | 2 | 39 | 3.207 | 0.051 | 1.000 |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | P value | Corrected P value |
| Number of subplot | 0.000 | 0.000 | 1 | 33.601 | 0 | 1.000 | 1.000 |
| Seed family | 0.000 | 0.000 | 1 | 33.601 | 0 | 1.000 | 1.000 |
| Residual | 0.008 | 0.091 | | | | | |

Table S7. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on pollen viability of *Silene vulgaris* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and *P* value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding *P* value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and *P* value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding *P* value respectively. The Corrected *P* value refers to the *P* value after Hochberg correction for multiple tests. Significant *P* values are highlighted in boldface type. * *P* < 0.05; ** *P* < 0.01; and *** *P* < 0.001.

| Pollen viability of <i>Silene vulgaris</i> | | | | | | | |
|---|----------|----------|----|--------|---------|----------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | <i>P</i> value | Corrected <i>P</i> value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | <i>P</i> value | Corrected <i>P</i> value |
| Number of subplot | 0.060 | 0.245 | 1 | -4.685 | 4.191 | 0.041 * | 1.000 |
| Seed family | 0.000 | 0.000 | 1 | -2.589 | 0.000 | 1.000 | 1.000 |
| Residual | 0.001 | 0.034 | | | | | |

Table S8. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the pollen viability of the five species that flowered during our experiment, after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and *P* value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding *P* value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and *P* value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding *P* value respectively. The Corrected *P* value refers to the *P* value after Hochberg correction for multiple tests. Significant *P* values are highlighted in boldface type. * *P* < 0.05; ** *P* < 0.01; and *** *P* < 0.001.

| Pollen viability – five flowering species | | | | | | | |
|---|----------|----------|----|--------|---------|-----------------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | <i>P</i> value | Corrected <i>P</i> value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | <i>P</i> value | Corrected <i>P</i> value |
| Number of subplot | 0.000 | 0.000 | 1 | 50.746 | 0.000 | 1 | 1.000 |
| Species/Seed family | 0.000 | 0.000 | 1 | 50.746 | 0.000 | 1 | 1.000 |
| Species | 0.038 | 0.195 | 1 | 39.595 | 22.302 | < 0.001 *** | < 0.001 *** |
| Residual | 0.023 | 0.152 | | | | | |

Table S9. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the seed weight of *Erysimum cheiranthoides* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and P value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding P value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and P value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding P value respectively. The Corrected P value refers to the P value after Hochberg correction for multiple tests. Significant P values are highlighted in boldface type. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

| Seed weight – <i>Erysimum cheiranthoides</i> | | | | | | | |
|---|-----------------|-----------------|-----------|---------------|----------------|-----------------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | P value | Corrected P value |
| Drought | 0.368 | 0.184 | 2 | 21 | 10.702 | < 0.001 *** | 0.037 * |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | P value | Corrected P value |
| Seed family | 0.000 | 0.000 | 1 | 9.209 | 0.000 | 1.000 | 1.000 |
| Residual | 0.017 | 0.131 | | | | | |

Table S10. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the seed weight of *Papaver occidentale* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and *P* value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding *P* value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and *P* value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding *P* value respectively. The Corrected *P* value refers to the *P* value after Hochberg correction for multiple tests. Significant *P* values are highlighted in boldface type. * *P* < 0.05; ** *P* < 0.01; and *** *P* < 0.001.

| Seed weight – <i>Papaver occidentale</i> | | | | | | | |
|---|-----------------|-----------------|-----------|---------------|----------------|-----------------------|---------------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | <i>P</i> value | Corrected <i>P</i> value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | <i>P</i> value | Corrected <i>P</i> value |
| Number of subplot | 0.003 | 0.052 | 1 | -19.955 | 0.009 | 0.926 | 1.000 |
| Seed family | 0.000 | 0.000 | 1 | -19.955 | 0.000 | 1.000 | 1.000 |
| Residual | 0.189 | 0.434 | | | | | |

Table S11. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the seed weight of *Scrophularia auriculata* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and P value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding P value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and P value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding P value respectively. The Corrected P value refers to the P value after Hochberg correction for multiple tests. Significant P values are highlighted in boldface type. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

| Seed weight – <i>Scrophularia auriculata</i> | | | | | | | |
|---|-----------------|-----------------|-----------|---------------|----------------|----------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | P value | Corrected P value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | P value | Corrected P value |
| Number of subplot | 0.019 | 0.138 | 1 | -11.279 | 3.507 | 0.061 | 1.00 |
| Seed family | 0.007 | 0.083 | 1 | -11.017 | 2.983 | 0.084 | 1.00 |
| Residual | 0.054 | 0.232 | | | | | |

Table S12. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the seed weight of *Scrophularia nodosa* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and *P* value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding *P* value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and *P* value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding *P* value respectively. The Corrected *P* value refers to the *P* value after Hochberg correction for multiple tests. Significant *P* values are highlighted in boldface type. * *P* < 0.05; ** *P* < 0.01; and *** *P* < 0.001.

| Seed weight – <i>Scrophularia nodosa</i> | | | | | | | |
|---|----------|----------|----|--------|---------|-----------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | <i>P</i> value | Corrected <i>P</i> value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | <i>P</i> value | Corrected <i>P</i> value |
| Number of subplot | 0.027 | 0.164 | 1 | 8.230 | 8.005 | 0.005 ** | 0.261 |
| Seed family | 0.007 | 0.084 | 1 | 11.504 | 1.458 | 0.227 | 1.000 |
| Residual | 0.020 | 0.140 | | | | | |

Table S13. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the seed weight of *Silene vulgaris* after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and *P* value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding *P* value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and *P* value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding *P* value respectively. The Corrected *P* value refers to the *P* value after Hochberg correction for multiple tests. Significant *P* values are highlighted in boldface type. * *P* < 0.05; ** *P* < 0.01; and *** *P* < 0.001.

| Seed weight – <i>Silene vulgaris</i> | | | | | | | |
|---|----------|----------|----|--------|---------|-----------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | <i>P</i> value | Corrected <i>P</i> value |
| As nothing was significant, no fixed terms were left in the reduced model | | | | | | | |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | <i>P</i> value | Corrected <i>P</i> value |
| Number of subplot | 0.005 | 0.069 | 1 | 0.978 | 0.796 | 0.372 | 1.000 |
| Seed family | 0.151 | 0.389 | 1 | -3.422 | 9.597 | 0.002 ** | 0.111 |
| Residual | 0.030 | 0.174 | | | | | |

Table S14. Results of the linear mixed effect model testing for the effect of global change treatments drought and fertilization on the seed weight of the five species that flowered during our experiment, after the model reduction (i.e., after non-significant terms have been dropped). For the fixed effects, Sum Sq, Mean Sq, DF, DenDF, F value and *P* value refer to the sum of squares, the mean sum of squares, the degrees of freedom, the denominator degrees of freedom, the F-statistic value and the corresponding *P* value using the Satterthwaite's method respectively. For the random effects, Std. Dev, DF, logLik, LRT and *P* value refer to the standard deviation, the degrees of freedom, the log-likelihood value, the log-likelihood ratio test statistic and the corresponding *P* value respectively. The Corrected *P* value refers to the *P* value after Hochberg correction for multiple tests. Significant *P* values are highlighted in boldface type. * *P* < 0.05; ** *P* < 0.01; and *** *P* < 0.001.

| Seed weight – five flowering species | | | | | | | |
|--------------------------------------|----------|----------|----|---------|---------|-----------------------|--------------------------|
| Fixed effects | Sum Sq | Mean Sq | DF | DenDF | F value | <i>P</i> value | Corrected <i>P</i> value |
| Drought | 0.307 | 0.154 | 2 | 21.874 | 2.531 | 0.103 | 1.000 |
| Fertilization | 0.193 | 0.193 | 1 | 46.981 | 3.179 | 0.081 | 1.000 |
| Rarity | 0.186 | 0.186 | 1 | 3.008 | 3.066 | 0.178 | 1.000 |
| Drought:Rarity | 0.451 | 0.226 | 2 | 218.345 | 3.716 | 0.026 * | 1.000 |
| Drought:Fertilization | 0.332 | 0.166 | 2 | 26.101 | 2.737 | 0.083 | 1.000 |
| Fertilization:Rarity | 0.260 | 0.260 | 1 | 221.614 | 4.280 | 0.040 * | 1.000 |
| Drought:Fertilization:Rarity | 0.524 | 0.262 | 2 | 219.382 | 4.314 | 0.015 * | 0.799 |
| Random effects | Variance | Std. Dev | DF | logLik | LRT | <i>P</i> value | Corrected <i>P</i> value |
| Number of subplot | 0.005 | 0.070 | 1 | -42.138 | 2.802 | 0.094 | 1.000 |
| Species/Seed family | 0.005 | 0.073 | 1 | -42.499 | 3.524 | 0.061 | 1.000 |
| Species | 1.355 | 1.164 | 1 | -63.294 | 45.114 | < 0.001 *** | < 0.001 *** |
| Residual | 0.061 | 0.246 | | | | | |

8. References

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