

Contrasting effects of precipitation and fertilization on seed viability and production of *Stipa krylovii* in Mongolia

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Abstract

In drylands, primary production is predominantly limited by water availability; however, there is evidence for co-limitation by nutrients. We tested whether improved water and nutrient availability facilitate reproduction of dominant steppe species, and studied the effects of increased moisture and fertilization on seed production of the steppe grass *Stipa krylovii*.

Effects of water availability on seed production and seed viability were investigated in a large-scale study on three sites with decreasing precipitation in Mongolia, over three to five consecutive years. In dry southern Mongolia, we additionally conducted an *in situ* irrigation and fertilization experiment to clarify the role of environmentally induced effects on seed production.

Seed viability of *S. krylovii* was negatively correlated with annual precipitation over five years at the driest study site. The relation between annual precipitation and seed viability in the large-scale study was not as clear, however, in the two moister regions there was a trend of lower seed viability. Experimental irrigation also significantly decreased seed viability and seed mass. Seed production per hectare was not affected by irrigation, while fertilization resulted in a more than fivefold increase in both seed weight and number of viable seeds. The underlying mechanisms for these unexpected results were not investigated. However, a switch from cleistogamous pollination under dry conditions to less effective cross-pollination in moist years may be an explanation. Our data indicate that plant reproduction may show complex and unexpected reactions, and that nutrient limitation must be considered in global change scenarios even for dry regions.

Zusammenfassung

In Trockengebieten ist die Primärproduktion vor allem durch Wasserverfügbarkeit limitiert, es gibt jedoch auch Hinweise auf eine Ko-Limitierung durch Nährstoffe. Wir haben getestet, ob sich Wasser- und Nährstoffverfügbarkeit auf die Reproduktion eines dominanten Steppengrases auswirken. Dazu untersuchten wir die Auswirkung unterschiedlicher Bewässerung und Düngung auf die Samenproduktion und -qualität der Federgrasart *Stipa krylovii*. Den Effekt der Wasserverfügbarkeit testeten wir in einem großräumigen Vergleich abnehmender Niederschläge in der Mongolei; die Messungen liefen über drei bis fünf aufeinanderfolgende Jahre. Darüber hinaus führten wir in der südlichen Mongolei, im trockensten der drei Untersuchungsgebiete, ein *in situ* Experiment mit Beregnung und Düngung durch, um die Rolle von umweltinduzierten Effekten auf die Samenproduktion zu analysieren.

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Die Samenlebensfähigkeit im trockensten Untersuchungsgebiet korrelierte signifikant negativ mit dem jährlichen Niederschlag. Das Verhältnis zwischen jährlichem Niederschlag und Samenlebensfähigkeit in der großräumigen Studie verhielt sich weniger eindeutig; die Lebensfähigkeit in den feuchteren Regionen war jedoch tendenziell niedriger. Experimentelle Bewässerung reduzierte sowohl die Samenlebensfähigkeit als auch das Samengewicht. Die Gesamtproduktion von Samen pro Hektar wurde durch die Bewässerung nicht beeinflusst; Düngung hingegen führte zu einer mehr als fünffachen Zunahme von Gesamt-Samengewicht und Anzahl lebensfähiger Samen.

Die Ursachen für diese unerwarteten Ergebnisse wurden von uns nicht untersucht, jedoch könnte ein Wechsel von kleistogamer Bestäubung bei trockenen Bedingungen zu weniger effektiver Fremdbestäubung in feuchteren Jahren unsere Ergebnisse erklären. Unsere Daten zeigen, dass die Reproduktion von Pflanzen komplexe und unerwartete Reaktionen auf den Klimawandel zeigen könnte, und dass Nährstoffmangel in entsprechenden Zukunftsszenarien selbst für sehr trockene Gebiete einbezogen werden sollte.

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Introduction

In arid environments water is regarded as the driving force limiting plant productivity and performance (Noy-Meir 1973). Water availability is thus the main topic of discussions on potential climate change effects on the vast drylands of Central Asia (Christensen, Coughenour, Ellis, & Zuo 2004; Zheng, Xie, Robert, Jiang, & Shimizu 2006). Recent climate change scenarios predict an increase in precipitation for the Gobi and the deserts of north-western China (Gao, Zhao, & Giorgi 2002; Christensen et al. 2007). Following more favourable water conditions, nutrient availability may become increasingly important (Hooper and Johnson 1999; LeBauer and Treseder 2008). Nitrogen depositions are also predicted to increase in Central Asia (Galloway et al. 2004), however, experimental tests on water and/or nutrient limitation on plant growth are scarcely available for the region.

Precipitation is known to have pronounced effects on plant vigour, including reproductive parameters (Wang and Gao 2004; Wesche, Ronnenberg, Retzer, & Miede 2010). Considerable differences in seed numbers and/or seed quality have been reported between habitats occupied by a given species (Renison, Hensen, & Cingolani 2004; Loha, Tigabu, Teketay, Lundkvist, & Fries 2006), and also between years (Houle and Filion 1993). Some populations, especially in harsh environments, produce few viable seeds and almost exclusively depend on prolonged clonal growth for long-term persistence (Liang, Michalk, & Millar 2002; Honnay and Bossyut 2005; Wesche, Ronnenberg, & Hensen 2005). These differences in reproductive strategies can either be genetically determined (e.g. Bischoff, Vonlanthen, Steinger, & Müller-Schärer 2006) or they are the result of phenotypic plasticity as a response to different maternal environments. Nutrients are also known to affect reproductive activity in plants (especially P-limitation, Marschner 1995), corroborated by evidence for co-limitation of nutrient and water availability affecting seed number and quality in the Californian desert shrub *Sarcobatus vermiculatus* (Breen and Richards 2008). Recent evidence also indicates that nutrient limitation may constrain plant

performance in arid northern hemisphere grasslands, including the arid steppes of southern Mongolia (Slemnev, Sanjid, Khongor, & Tsooj 2004; Harpole, Potts, & Suding 2007; Wesche, Nadrowski, & Retzer 2007).

The genus *Stipa* is widespread and dominant in large parts of the zonal grasslands of the northern hemisphere (Lavrenko and Karamysheva 1993). Our study species *S. krylovii* is widespread and important as a fodder plant in Central Asia, where it is typical of the dry southern Mongolian mountain steppes and semi-deserts (Wesche, Miede, & Miede 2005).

Over the course of 3–5 years, we investigated the main indicators of seed production and seed quality of *S. krylovii* in three study sites of decreasing precipitation in Mongolia. Additionally, a factorial field experiment combining the factors water and nutrients was conducted at our driest study site.

The following hypotheses were tested: (1) Water availability positively affects seed production, seed mass and seed viability of *S. krylovii*. (2) Measures of seed production are thus higher in moister regions and increase in moist years in a given region (3) Fertilization enhances seed-production, mass and viability.

To our knowledge, this is the first time that reproductive traits of one of the most important grass species of the Central Asian steppes have been studied comparing several sites and years in a large-scale study.

Materials and methods

Study sites

The main study site for seed (*sensu stricto*: caryopses) collection and field experiments was situated at an altitude of 2300 m asl. (N 43°36.76'; E 103°46.36') in the Dund Saykhan mountain range in Gobi Gurvan Saykhan National Park, southern Mongolia (Fig. 1). This region receives an estimated 180 mm mean annual precipitation (Hijmans, Susan, Cameron, Parra, & Jones 2005, Table 1). The two other

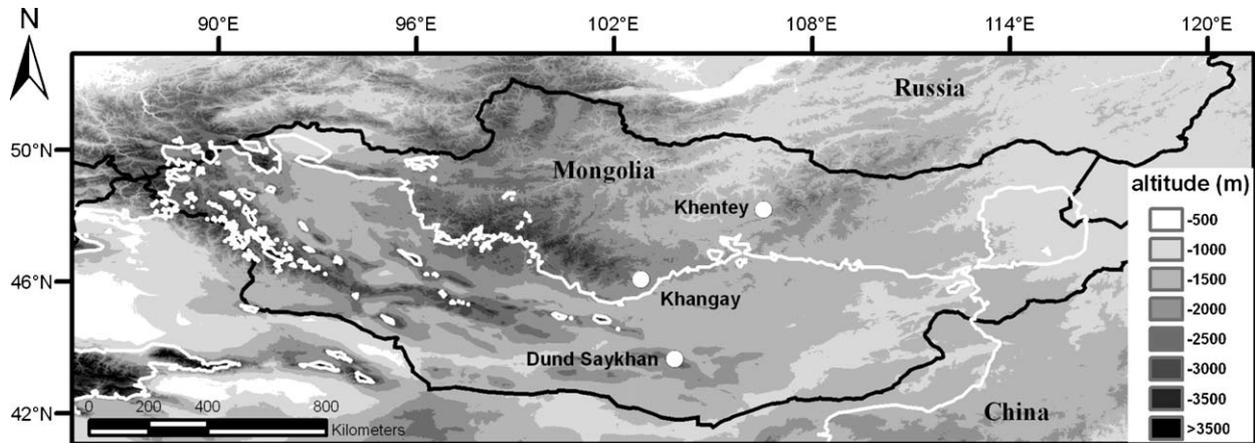


Fig. 1. Topographical map of Mongolia showing the three sampling sites (Khentey, Khangay and Dund Saykhan) of the large-scale study, and the 200 mm isohet (white line) that divides the country in the dry south and south-west, and the moister north.

sites (Fig. 1) were chosen with respect to the presence of *S. krylovii*-dominated vegetation and geographical position. The “Khangay sampling site” was located at the eastern edge of the Khangay mountains, at 1700 m asl. (N 45°55.42'; E 102°46.37'), receiving about 255 mm mean annual precipitation (Table 1). The “Khentey sampling site” is situated in the westernmost outposts of the Khentey mountains at an altitude of 1500 m asl. (N 48°15.43'; E 106°33.23'). Here, the *Stipa*-steppes are restricted to south-facing extrazonal slopes. We assume that – in line with Yuan, Zhou, Wang, Han, and Wang (2007) – even though mean annual precipita-

tion (270 mm) is only slightly higher than at the Khangay site, the water stress experienced by the plants is less severe than at the Khangay site where field capacity (water mass/saturated weight fraction) of the soil is lower (Table 1), and the amount of plant available water decreases much faster after rainfall. Summer temperatures are similar in the three study regions (Table 1).

Vegetation is very sparse in the Dund Saykhan and Khangay sites (Table 1); and *Stipa krylovii* contributes 8% and 6%, respectively, of the total cover. The proportion of annuals is comparably high at the Khangay site; the perennial

Table 1. Key-characteristics of climate, vegetation and soil in the three study regions (mean climate values, extrapolated data, Hijmans et al. 2005); summer: April–September, winter: October–March). For vegetation, we present species richness, total cover, the relative share of *S. krylovii* of vegetation cover, and list three other abundant species, their relative cover contribution and life form (H – hemicryptophyte; G – geophyte; T – therophyte).

| | Dund Saykhan | Khangay | Khentey |
|---|---|---|---|
| Mean precipitation (mm) | | | |
| Annual total | 180 | 255 | 270 |
| Summer total | 160 | 220 | 250 |
| Mean temperature (°C) | | | |
| Annual mean | 0.9 | 1.6 | –2 |
| Winter mean | –9.9 | –8.9 | –17.7 |
| Summer mean | 10.9 | 10.9 | 10.6 |
| Vegetation | | | |
| Vegetation zone | Desert steppe | Desert steppe | Meadow steppe |
| Mean α diversity per m ² | 11 | 10 | 15 |
| Total vegetation cover (%) | 10 | 15 | 50 |
| Cover <i>S. krylovii</i> as a fraction of total cover | 8% | 6% | 5% |
| Three most abundant species (life form; share of cover) | <i>Agropyron cristatum</i> (H; 17%) <i>Allium polyrrhizum</i> (G; 43%) <i>Stipa gobica</i> (H; 13%) | <i>Salsola pestifera</i> (T; 34%) <i>Carex stenophylla</i> (H; 6%) <i>Chenopodium acuminatum</i> (T; 19%) | <i>Carex stenophylla</i> (H; 10%) <i>Leymus chinensis</i> (H; 10%) <i>Potentilla acaulis</i> (H; 25%) |
| Soil | | | |
| Soil type | Degraded chestnut soil | Degraded chestnut soil | Degraded chernozem |
| Field capacity (%) | 12–25 | 14–20 | >40 |

Carex stenophylla was also abundant, indicating that grazing pressure is high. Vegetation at the Khentey site is more luxuriant but *S. krylovii* has a similar relative contribution.

Study species

Stipa krylovii Roshev. (Poaceae, section – *Leiostipa*), a perennial tussock grass, is widespread in Mongolia, China, Kazakhstan and Russia (Gubanov 1996; Wu and Raven 2006). It is characteristic of the grass steppes of the central region and dry mountain steppes of southern Mongolia, and inhabits a wide macroclimatic range. Like most species of the area, its phenology is highly dependent on rainfall (Yuan et al. 2007). In an average year, flowers are wind-pollinated in July and seeds mature in August with southern populations starting their reproductive cycle earlier than the northern populations. *Stipa* spp. are known to utilise facultative cleistogamy as a flowering strategy, which appears to be strongly related to water availability (Brown 1952; Ponomarev 1961). This is also the case for *S. krylovii*; in dry conditions, flowers never open and pollination is exclusively cleistogamous. Levels of seed dormancy vary between almost 80% in Khentey and just over 40% in Dund Saykhan (as a fraction of seeds viable after 20 days, Ronnenberg, Wesche, & Hensen 2008). Establishment is extremely rare in southern Mongolian feather grasses. However, in a genetical study on clonal diversity (Ronnenberg et al. unpublished), we found no evidence for extensive clonal growth. Previous studies also showed that biomass production is strongly nutrient limited while effects of irrigation are minor (Ronnenberg and Wesche 2010).

Methods

All plants came from extensive stands (>100 ha). Given that the density is >10 tussocks/m², population size was at least 10⁶ and possibly much larger. Plants were selected haphazardly; we made sure, however, not to take several samples from the same plants within a year. Plants (especially in the south) flower irregularly over the years, and we could not resample the same individuals every year. Inflorescences were collected at the peak of maturity over five consecutive years in the Dund Saykhan range (2002–2006) and over three years (2004–2006) in the Khentey and Khangay range. In 2006, seeds from the Khangay site were not completely mature; therefore, we only took a reduced data-set for that year and region (see below). Apart from years 2002 and 2003 we always counted the number of seeds per inflorescence ($n=20$) and conducted a TTC-viability test (Tetrazolium Chloride Test, see Baskin and Baskin 2001) because this is a more direct test of viability than a germination trial. The number of replicates in the viability tests differed between years: In 2002 and 2003 we only had 3 replicates, however, in the years 2004–2006, we tested five replicates (each 50 seeds per replicate). For logistical reasons, seed mass was

based on a small sample ($n=20$ seeds, without awn) and thus has to be treated with care.

Since *Stipa* seeds mature from the top to the bottom of the inflorescence with the lower ones often failing to mature at all, we only took the upper seeds that were loosely attached to the glumes as an indication of maturity. Moreover, we took care to sample seeds that had no apparent external damage. The TTC-test was performed under lab conditions one (in 2005) to three months (in 2004) after seed collection. In two years (2004 and 2005), the TTC-test was also conducted in the field directly after seed maturation. Results from the same seed batch were compared in a lab test to rule out damage through transport or storage (Mann–Whitney *U*-tests between field and lab results $p>0.6$).

For the irrigation × fertilization study in Dund Saykhan, we used a randomised block design with ten blocks (replicates) including the following treatments: FI – fertilized and irrigated, F – fertilized, I – irrigated, C – control. The plot-size was 1 m² and small cages were installed to prevent grazing by the abundant livestock herds and pikas (*Ochotona pallasi*, the most common small mammal in the region). Cages were set up in August 2004 in the unaltered natural vegetation, and fertilization and irrigation treatments were applied over two years (2005 and 2006) following the same procedure: During summer, plots were irrigated every two days with 5 mm water, adding up to a total surplus of 100 mm, which corresponds to the difference between the Dund Saykhan and the Khentey site. The daily dosage was based on own precipitation records in July and August 2004, when 75% of daily rainfall was below 6 mm (median 2.3 mm). We applied water-soluble NPK-fertilizer (FLORY 2, PLANTA Germany) with 8.5% Nitrate-N; 6.5% Ammonia-N; 5% P₂O₅; 25% K₂O; 2% MgO, and the micro elements B, Cu, Fe, Mn, Mo, and Zn. We added fertilizer equivalent to 20 gN/m² (200 kgN/ha) in two applications, which proved to have effects in a preliminary study (see Ronnenberg and Wesche 2010; for further details).

Seeds were collected in 2006. Whenever possible, we collected seeds and inflorescences from 10 different *Stipa* plants in the plot. However, the number of flowering tussocks per plot varied widely and in a few cases all seeds were taken from the same plant. If available, numbers of seeds of 20 inflorescences were counted and 20 seeds were weighed and their viability was tested. Based on these figures we estimated seed mass and maximum number of viable seeds per hectare. Numbers of viable seeds are therefore overestimated because we did not exclude the morphologically damaged seeds from these calculations.

Effectiveness of the irrigation treatment was assessed by checking soil moisture with a Theta probe (Delta-T instruments) 24 h after irrigation. On irrigated plots soil moisture was on average 12% (volume fraction), which was significantly higher than on non-irrigated plots (10% volume fraction, $p<0.001$, paired *t*-Test).

All measurements were related to monthly rain data from the nearest governmental weather stations, namely Dalanzadgad (near Dund Saykhan), Arvaykheer (in Khangay

region) and Ulan Bator (near Khentey station). Data thus represent only rough approximations for precipitation and water availability at our study sites because stations were at distances of 30–70 km. Annual rainfall at these stations was below the extrapolated data of the model by Hijmans et al. (2005), because all of these stations are situated at lower altitudes than our study sites.

Statistical analyses

Percent values were arcsine square-root transformed; quantitative data was rank (all variables except for seed viability in the fertilization \times irrigation study) or log₁₀ transformed (seed mass and number of inflorescences in the large-scale study), depending on their statistical distribution, to meet assumptions of ANOVA with respect to normality and homogeneity of variances. Seed viability at the Dund Saykhan site over five years (fixed factor) was analyzed by means of a one-way ANOVA with a subsequent Scheffé-*post hoc* test, as the number of replicates differed between years (3–5 replicates with 50 seeds each). For the five year study at the Dund Saykhan site, the relation between seed viability and annual precipitation of the current and previous year was analyzed with Pearson correlations. In the model for the large-scale study, we calculated a repeated measure ANOVA followed by a Tukey *post hoc* test, with sampling years representing the repeated measures (fixed factor). Note, that the factor year cannot be seen as a simple temporary variable as we could not sample seeds from the same plants every year. Thus, the year effect might be confounded by genotypic differences or spatial heterogeneity in e.g. soils, though samples came from the same sites and are assumed to be representative of the same population. Tests for seed mass and viability were based on two years (2004–2005) only, because data for 2006 were missing for the Khangay region. In the ANOVA-model for the fertilization and irrigation experiment, the factors fertilization and irrigation (both fixed) were crossed and block was integrated as an unreplicated random factor. All statistics were calculated with the software SPSS 12.0G (SPSSInc. 2003).

Results

In the Dund Saykhan, mean seed viability of *S. krylovii* varied between more than 90% (2002) and about 50% (2003; Fig. 2A). The ANOVA revealed clear differences between years ($p=0.004$): The driest year, 2002 (60 mm), differed strongly from the wettest years, 2003 (140 mm) and 2004 (110 mm, station Dalanzadgad; Scheffé tests $p=0.008$ and 0.027 , respectively). Seed viability was thus negatively correlated with annual precipitation over the five years tested ($r=-0.97$; $p=0.007$; Fig. 2B), whereas the previous year's precipitation had no influence ($r=0.03$; $p=0.96$).

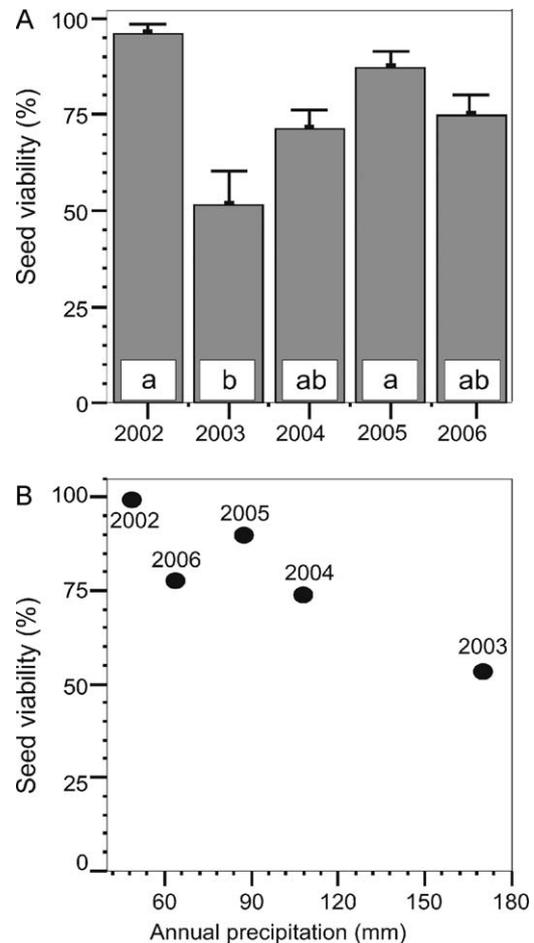


Fig. 2. (A) Seed viability (%) in the dry Dund Saykhan site over five years (2002–2006, mean + standard error). Different letters indicate significant differences at the significance level ($p < 0.05$). (B) Correlation between seed viability (%) and the annual precipitation of the nearest weather station at the same year ($r = -0.97$, $p = 0.007$).

Inflorescences of *S. krylovii* in the northern regions produced on average more seeds than those in the Dund Saykhan (Fig. 3A, repeated measures ANOVA, site $p < 0.001$, Table 2). However, there was no difference between years and no significant interaction between years and regions ($p > 0.5$). Seed mass also differed between regions ($p < 0.001$, Fig. 3B) according to the Tukey *post hoc* test showing significant differences between all three regions. Year did not show a significant effect ($p = 0.17$), while the interaction between regions and year did ($p < 0.001$). Seed mass was higher in 2005 than in 2004 for Dund Saykhan and Khangay, whereas in Khentey year had the opposite effect.

Seed viability was on average highest in the Dund Saykhan and lower in the two northern regions. A repeated measures ANOVA on seed viability data over the first two years showed significant effects of both study region and year ($p = 0.001$ and $p < 0.001$, respectively, Table 2), while their interaction was not significant ($p > 0.8$). The Tukey *post hoc* test revealed that viability was significantly higher in the Dund Saykhan

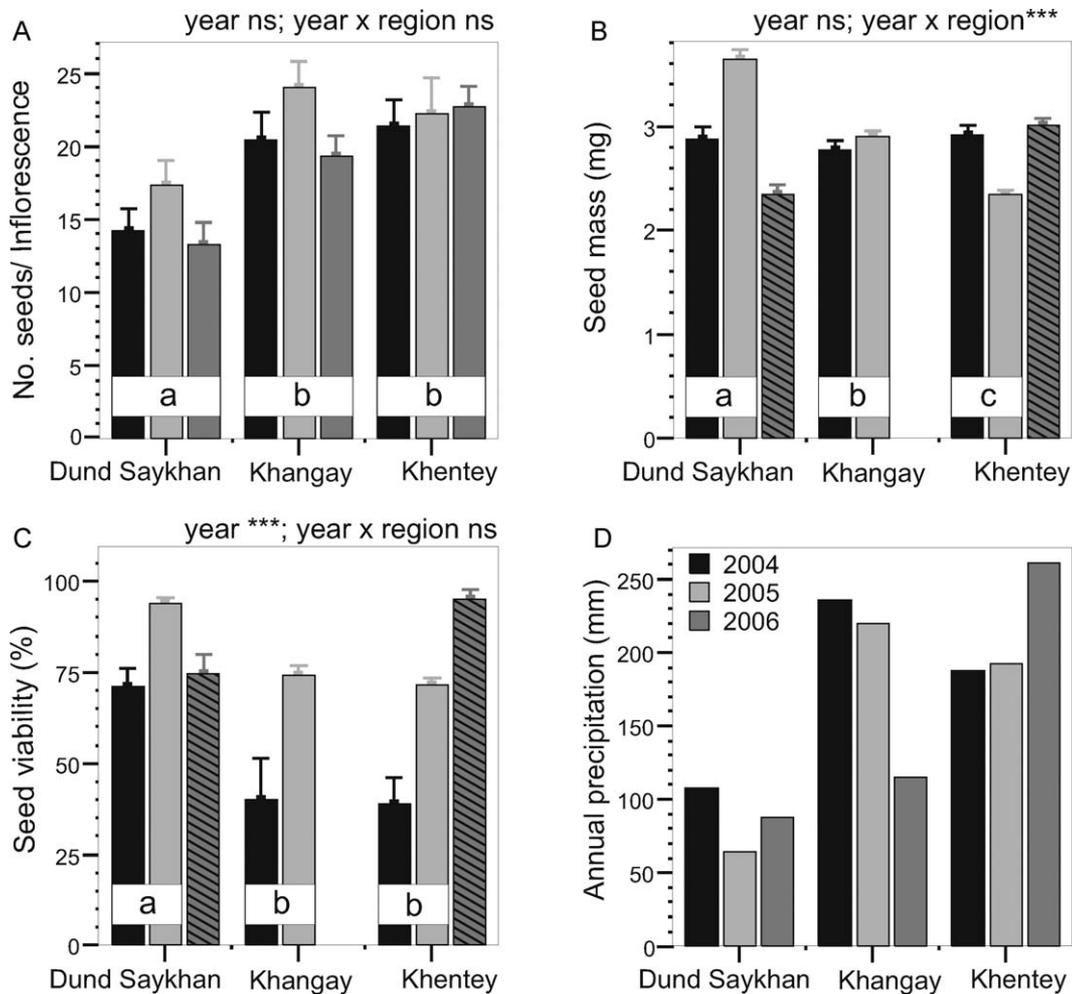


Fig. 3. Large-scale study covering three years (2004–2006) and three regions (Dund Saykhan, Khangay and Khentey). Means (+SE) of (A) number of seeds per inflorescence, (B) seed mass (mg), (C) seed viability (%), and (D) annual precipitation of the closest weather stations, i.e. Dalanzadgad (Dund Saykhan); Arvaykheer (Khangay) and Ulan Bator (Khentey). Data for seed mass and viability from the Khangay are incomplete as seeds were not mature in 2006. Different letters indicate significant differences between regions ($p < 0.05$). Significant differences between years are shown above the figures (ns: not significant, *** $p < 0.001$). Hatched treatments were not integrated in the statistical model.

compared to the other two regions. Data for 2006 were not tested due to lack of suitable seeds from Khangay; in that year, however, more seeds from the Khentey region were viable than from Dund Saykhan (Fig. 3C), where 2006 was an average year (Fig. 2A).

Experimental irrigation significantly increased the number of seeds per inflorescence ($p = 0.013$, Fig. 4A). We found, however, a trend of decreasing mean seed mass with additional water supply ($p = 0.058$, Appendix A: Table 1a), which was more apparent in the non-fertilized than in the fertilized plots (interaction $p = 0.045$; Fig. 4B, Appendix A1b). Concomitantly, mean seed viability was lower on irrigated plots ($p < 0.001$; Fig. 4C, Appendix A1c). Fertilization increased the number of inflorescences by a factor of almost three ($p < 0.001$, Fig. 4D, Appendix A1d), while the effect of irrigation was small. Accordingly, total seed mass per ha and the maximum number of viable seeds were strongly affected by

fertilization ($p < 0.001$), and not by irrigation ($p > 0.3$, Fig. 4E and F; Appendix A1e,f).

Discussion

The results failed to support most of our hypotheses concerning the effect of water availability. The large-scale study and the experiment showed that improved water availability indeed increased seed production per inflorescence, whereas there were no consistent effects on seed mass. The number of inflorescences was also not affected in the irrigation experiment. In contrast to ecological theory (e.g. Verdú and García-Fayos 1998; Renison et al. 2004), seed viability was even negatively affected by increasing precipitation and by irrigation.

Table 2. Repeated measures ANOVA showing the effects of sampling region and sampling year on (a) number of seeds, (b) seed mass and (c) seed viability of *Stipa krylovii* inflorescences (see Fig. 3). The factor region represents three sites of a large scale study differing in annual rainfall.

| Treatment | df | MSS | F-values | p-values |
|--|----|--------|----------|----------|
| (a) Number of seeds per inflorescence (log 10 transf.) | | | | |
| Region | 2 | 459.01 | 12.95 | <0.001 |
| Error | 27 | 35.45 | | |
| Year (3 years) | 2 | 68.878 | 2.332 | 0.107 |
| Region × year | 4 | 19.944 | 0.675 | 0.612 |
| Error | 54 | 29.54 | | |
| (b) Seed mass (log 10 transf.) | | | | |
| Region | 2 | 2.02 | 31.14 | <0.001 |
| Error | 27 | | | |
| Year (2 years) | 1 | 0.17 | 1.99 | 0.17 |
| Region × year | 2 | 2.26 | 26.66 | <0.001 |
| Error | 27 | 0.85 | | |
| (c) Seed viability (arcsin transf.) | | | | |
| Region | 2 | 0.379 | 6.868 | 0.002 |
| Error | 9 | 0.026 | | |
| Year (2 years) | 1 | 0.622 | 33.73 | <0.001 |
| Region × year | 2 | 0.00 | 0.02 | 0.985 |
| Error | 9 | 0.018 | | |

Nutrient addition (especially P) is known to enhance seed-set (Marschner 1995), and positive effects of nutrient addition on biomass productivity were now also demonstrated for different areas in the Gobi desert (e.g. Bai et al. 2009; Niu et al. 2009; Ronnenberg and Wesche 2010). The effect of fertilization on the reproductive output of *S. krylovii* was stronger than expected. Nutrient addition increased the maximum number of viable seeds per ha more than twentyfold (treatments F versus C); the corresponding factor was >12 on the plots receiving additional water (FI versus I). In contrast, the number of viable seeds on irrigated plots was only 1.5 times higher than on the controls (I versus C, see Fig. 4).

Results from the field experiment, consecutive surveys at the driest study site (Dund Saykhan) and the large-scale study, consistently indicated that seed viability was negatively correlated with increased moisture availability. This was apparently related to trends of decreasing seed mass as observed in the large-scale study and in the irrigation × fertilization experiment.

Keeley (1977) also found precipitation, in this case the previous year's, to be positively correlated to seed production in two shrub species. In our study, current-year precipitation was clearly more important for seed production and seed quality than previous-year precipitation. This coincides with Oosterheld, Loreti, Semmartin, and Sala (2001), who reported that current-year precipitation explained a higher percentage of the inter-annual variation in above-ground net primary production of a North American short-grass prairie than previous-year precipitation. In contrast to short-lived species, perennials are often buffered against short-term fluctuations through storage organs or by exploiting large soil

volumes with extensive clonal structures. Our study species has none of these structures and tussocks have a limited volume which may explain that there was no effect of previous year, and only immediate effects of current year precipitation.

The apparent negative correlation between seed viability and annual rainfall in the Dund Saykhan (Fig. 2B) was surprising given that we did not account for precipitation seasonality, and the respective weather station was not located nearby. Moreover, seeds were not collected from the same plants every year, thus genotype, spatial (e.g. edaphic conditions) and temporal heterogeneity (temperature, periods of snow cover) may have interfered with the potential precipitation effects. Still, in light of the experimental data this significant 'year' effect is probably best explained by water availability in different years.

Results in the large-scale study were not as clear. In the Khentey region, for example, seed viability was highest in the most humid year, 2006, and lowest in the driest year, 2004. This indicates that not only the macroclimate determines plant performance but also several other factors, including genetic aspects. Wind pollination, however, reduces genetic differentiation among populations (Nybom and Bartish 2000), which was supported by an unpublished molecular marker analysis, in which we found <10% of variation being partitioned among populations.

In the caged areas of the experiment, seed mass and percentages of viable seeds were higher and numbers of seeds per inflorescence were lower than in seeds collected over a larger area but in the same population (Fig. 3 vs. Fig. 4). It seems that exclusion of large herbivores over three years also had an impact on *S. krylovii* tussocks.

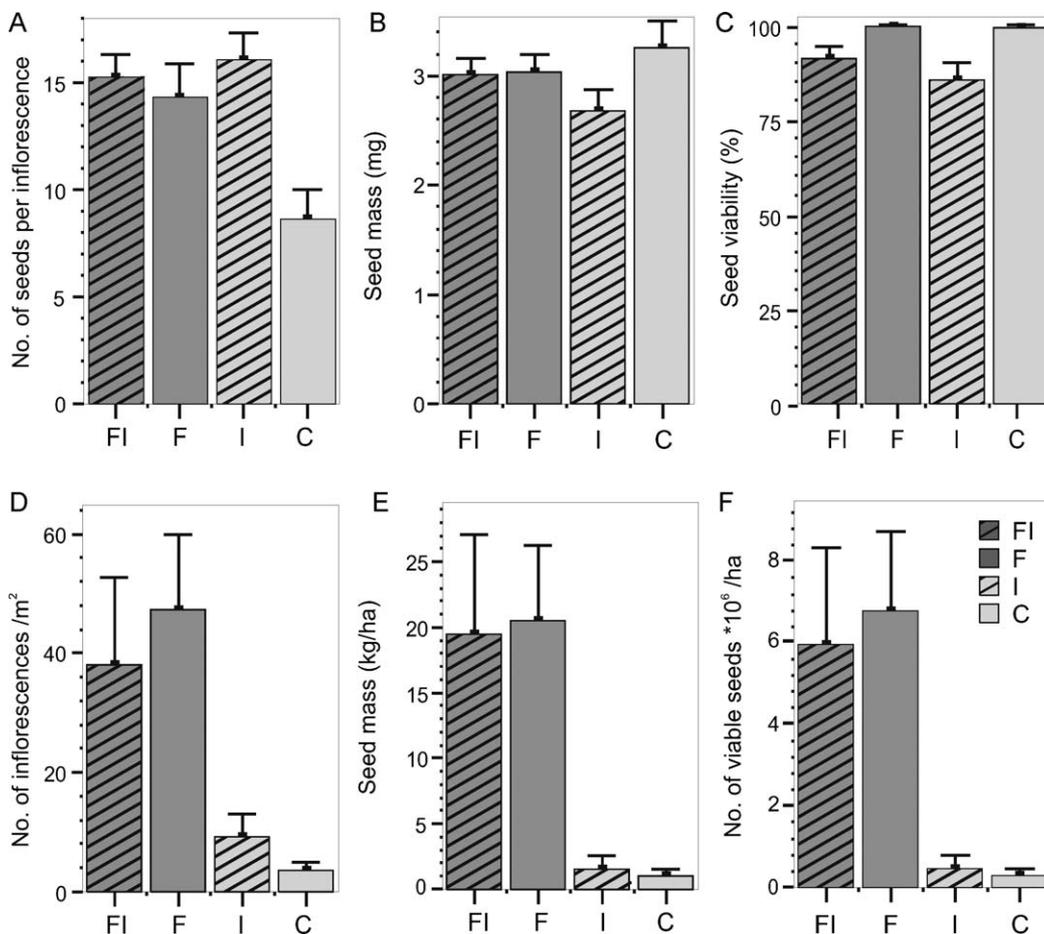


Fig. 4. Irrigation \times fertilization study. Means (\pm SE) of (A) number of seeds per inflorescence, (B) seed mass (mg), (C) seed viability (%), (D) number of inflorescences m^{-2} (E) Seed mass per hectare ($kg\ ha^{-1}$), (F) maximum number of viable seeds ($10^3\ ha^{-1}$). FI – fertilized and irrigated, F – fertilized, I – irrigated, C – control. See online Appendix A: Table 1 for ANOVA-table showing the statistical effects.

The negative correlation between seed number and seed mass in our field experiment reflects a common trade-off (Moles, Falster, Leishman, & Westoby 2004; Moles and Westoby 2006). In our large-scale comparison, the effect of experimental irrigation on seed mass was only marginally significant, while the effect on seed viability was highly significant. As we did not find any indication for the infestation of pathogens at higher water availability, one possible explanation for higher seed viability under drier site conditions can be drawn from differences in the pollination type. In the moister regions, a high fraction of florets opened for pollination; while in the Dund Saykhan most flowers were cleistogamous. In years with lower precipitation, such as 2002 or 2005, when seed viability was highest, flowers of *S. krylovii* there did not even emerge from leaf sheaths. In moist years like 2003, a higher proportion of flowers opened allowing outcrossing (own field observations). The ratio between cleistogamous and chasmogamous flowers in *Stipa* spp. indeed tends to decrease with increasing moisture availability (Brown 1952; Ponomarev 1961). In studies on *Stipa leucotricha*, percent germination of seeds originating from cleistogamous florets was higher compared to chasmog-

amous florets (Call and Spoonts 1989). This may hold true for *S. krylovii* and this effect may be due to a limited effectiveness of chasmogamous pollination under dry conditions. Mechanisms of higher effectivity of cleistogamous pollination are clearly not fully understood, but there are examples of other wind pollinated species in dry environments that support this idea. *Juniperus oxycedrus* shows low seed viability caused by low pollen vigour or pollen viability (Arista, Ortiz, & Talavera 2001). The pollen triggers the formation of the seed but dies before fusing with the ovary, resulting in the abortion of the seed. Pollen viability of *S. krylovii* is indeed short under ambient temperature and light conditions (pollen grains lost over 80% viability within 30 min) and despite large numbers of pollen grains per flower, the pollen rain was found to be very low in the Dund Saykhan (Ronnenberg et al. unpublished). Hence, in years with low numbers of inflorescences per area, cross-pollination might just be too risky, rendering selfing as the safer option.

Fertilization appeared to have no effect on the percentage of viable *S. krylovii* seeds. However, the effect on the number of inflorescences per area and thus the overall production of viable seeds per area was very pronounced. The lack of irriga-

tion effects on the number of inflorescences was remarkable because data over several years revealed a strong correlation between total annual precipitation and number of inflorescences (Wesche et al. 2010). We suspect that only strong rain events of more than 5 mm/day activate the production of reproductive organs in *S. krylovii*, which is supported by observational evidence found in 2006, where hardly any flowers were produced until exceptionally high rainfalls of 36 mm and 20 mm/day in late July. This resulted in mass flowering and germination (Ronnenberg et al. 2008) highlighting the importance of the magnitude and timing of single rain events.

Conclusions

The surprisingly strong evidence for nutrient constraints on reproduction even under the dry conditions of the Dund Saykhan raises concerns because steppes have been subject to nutrient translocation and depletion for several centuries (Stumpp, Wesche, Retzer, & Miede 2005; Wesche et al. 2007). Rangeland biomass productivity in Central Asian steppes is also greatly enhanced by nutrient addition (Bai et al. 2009; Ronnenberg and Wesche 2010). Atmospheric nitrogen depositions will increase even in Central Asia (Galloway et al. 2004; Kinugasa, Shinoda, & Tsunekawa 2008), but the relatively low amounts are unlikely to compensate for the losses. Nutrient constraints add to the strong reduction in flower numbers due to direct grazing effects (Wesche et al. 2010), which imposes further constraints on the already rare sexual regeneration in drylands (Ronnenberg et al. 2008).

The second implication is related to climate change models that predict more precipitation for our study region (Christensen et al. 2007) but differ with respect to the timing and intensity of rains. The latter may be more important than changes in the overall mean precipitation (Chen, Lin, Huang, & Jenerette 2009), and our data point in the same direction. However, further research on the effect of different rain pulses on *S. krylovii* would be necessary to prove the effect of higher water availability. Studies on the consequences of changing water availability in drylands generally focus on biomass production rather than on reproduction of plant species which is of similar importance and may respond differently. Our study shows that changes may affect reproduction and eventually even population sizes, implying that nutrient availability and its effect on plant recruitment should be considered even in dry environments.

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Appendix A. Supplementary Material

The online version of this article contains additional supplementary data. Please visit doi:10.1016/j.baae.2010.12.002.

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