

Article

Seed Germination in Alpine Meadow Steppe Plants from Central Tibet in Response to Experimental Warming

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Abstract: Clarifying the effects of climate warming on seed germination is critical for predicting plant community assembly and species renewal, especially in alpine grassland ecosystems where warming is occurring faster than in other biomes globally. We collected matured seeds of 19 common species from a typical alpine meadow steppe community in Central Tibet. Seeds were germinated in three incubators with manipulated day-night temperatures to impose three treatments: (1) theoretically optimal values of 25/15 °C, (2) temperatures observed in the field (control), and (3) a warming of 3 °C above the observed temperatures. We calculated seed germination percentage (SGP) and mean germination time (MGT) per species at different treatments. Our results showed that SGPs of *Stipa capillacea*, *Kobresia macrantha*, *Potentilla saundersiana*, *Saussurea tibetica*, *Pedicularis kansuensis*, and *Androsace graminifolia* were higher under the warming treatment than under control. Among them, the MGTs of *S. capillacea*, *K. macrantha*, and *And. graminifolia* were significantly shortened, while the MGT of *Pe. kansuensis* was significantly lengthened by warming of 3 °C. Significant decreases in MGT induced by warming were only observed for *Festuca coelestis* and *Anaphalis xylorhiza*. Additionally, the treatment with theoretically optimal temperatures restrained germination of *Stipa purpurea*, *S. capillacea*, *F. coelestis*, and *Sa. tibetica* seeds but promoted germination of *K. macrantha*, *Astragalus strictus*, *P. saundersiana*, *Potentilla bifurca*, *Pe. kansuensis*, *Swertia tetraptera*, *Pleurospermum hedinii*, and *And. Graminifolia* seeds, when compared with the control and warming treatments. Therefore, the response of seed germination to warming differs among alpine species, implying that future warming could result in significant changes in community assembly of alpine grasslands on the Tibetan Plateau.

Keywords: alpine grasslands; climate warming; incubator; germination time; seed germination percentage; Tibetan Plateau

1. Introduction

Seed germination plays a vital role in the propagation and renewal of plant communities [1], which represents a predictable ecosystem function in response to changes in biotic and abiotic factors [2,3].

Generally, seed germination characteristics differ among species. Seeds of some species can germinate immediately after leaving the parent plant, while for other species seed germination is always delayed to avoid hostile environmental conditions to increase chances of successful growth, development, and survival [4,5]. At the community level, variations in seed germination characteristics influence species co-existence and distributions among microenvironments that are heterogeneous in terms of resource availability over time or across space [6,7].

In alpine grassland ecosystems, it is generally believed that low temperatures and short growing seasons result in high seed mortality and low seedling survival [8,9]. This indicates that seed germination contributes little to vegetation renewal and seedling establishment in an alpine ecosystem [10] because the process of alpine vegetation renewal is mainly achieved through clonal propagation [11]. Studies on seedling renewal have also revealed that seedlings germinated from soil seed banks account for a more substantial proportion of all seedlings and exhibited great variety among ecosystems [12,13]. Therefore, sexual propagation might be vital in the renewal and establishment of alpine plants.

Global air temperatures are predicted to increase by 1.8–4.0 °C by 2100 under expected CO₂ emission scenarios [14]. Alpine vegetation on the Qinghai-Tibetan Plateau is vulnerable to global warming [15], partly because the warming speed on this plateau was twice that of the global average over the past few decades [16,17]. Under climate warming, alpine grasslands are likely to degrade as permafrost reduction and glacial melting increases [18]. Moreover, changes in soil temperature, moisture, and nutrient content caused by warming will consequently threaten biodiversity conservation and sustainable management of alpine pastures on the Qinghai-Tibetan Plateau [19].

Changes in temperature can influence seed germination time in plants [20,21] and can thereby determine the establishment and survival of seedlings in alpine ecosystems [3,22]. Increasing temperatures can accelerate the metabolic responses of seeds during germination. Extreme low or high temperatures will alter cell membrane permeability and restrain catalytic activities [5,23]. For example, the germination percentage of arctic dwarf shrub seeds was found to increase with warming within a specific temperature range [24]. Therefore, clarifying the effects of temperature on seed germination is critical for predicting changes in plant distributions and community assembly and renewal, which will also be beneficial for sustainable management and conservation of natural ecosystems in the context of climate change.

In this study, we collected matured seeds of 19 common plant species from a typical alpine meadow steppe community and investigated the seed germination percentage (SGP) and mean germination time (MGT) under different incubation temperatures in the Central Tibet. Specifically, we aimed to test the hypothesis that climate warming enhances seed germination percentage and shortens mean germination time for alpine grassland species on the Tibetan Plateau. We also wanted to determine whether there are differences among alpine grassland plants in seed germination responses to experimental warming.

2. Materials and Methods

2.1. Study Area and Seed Collection

Seeds were collected near to the Damxung Grassland Station located in the southern foothills of Nyenchen Tanglha (91°05' E, 30°51' N, 4333 m above sea level), an area characterized by a plateau semi-arid monsoon climate. The non-growing season is cold and long (from October to April of the following year), and the growing season is warm and short (from May to September, Figure 1) [25]. The mean annual temperature is 1.3 °C and mean annual precipitation is 476.8 mm [26], approximately 80% of which falls in the growing season from June to August (Figure 1). The soil type is alpine meadow soil with a depth of 50–70 cm and relatively high gravel content [27].

The natural vegetation grazed by yaks close to the Damxung Grassland Station belongs to a *Kobresia-Stipa* co-dominant community (Table 1). The most dominant species are *Kobresia pygmaea* C. B. Clarke, *K. macrantha* Bockl., *Stipa purpurea* Griseb., and *Stipa capillacea* Keng [25]. Forb species,

such as *Anaphalis xylorhiza* Sch.Bip. ex Hook. f., *Leontopodium nanum* Hand.-Mazz., *Artemisia wellbyi* Hemsl. et Pears. ex Deasy, *Astragalus strictus* Grah. Ex Bend., and *Potentilla bifurca* Linn. are also common in this study area [25]. We collected seeds from the middle of August to the end of September 2017, when the seeds of most plants were mature [28]. In total, we collected seeds from 19 common species—belonging to 16 genera and 10 families—in the study area (Table 1). At least 1500 seeds were collected per species. The collected seeds were pre-processed (i.e., glumes and pinnae were removed), air dried in paper bags, and then stored for more than 6 months in the lab of the Damxung Grassland Station until germination experiments were carried out. The temperature conditions in the lab (the average temperature is about -2°C and the temperature ranges from -11 to 12°C) are similar to the local natural conditions, and it can meet the needs of most seeds for lower temperature conditions in winter in the alpine region.

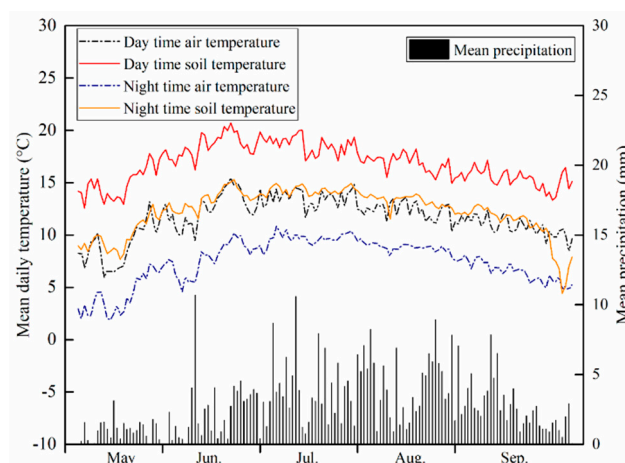


Figure 1. Daily temperature and precipitation during the plant growing seasons recorded from May to September 2007–2011 at the Damxung Grassland Station. Solid lines represent soil temperatures at 5 cm depth and dash-dotted lines represent air temperature 2 m above the soil surface. Histograms represent the mean daily precipitation during the five plant growing seasons of 2007–2011.

Table 1. Identification of all species used in the seed germination experiments.

Species	Family	Functional Type	Abbreviations ‡
<i>Stipa purpurea</i> Griseb.	Gramineae	Grass	<i>S. purpurea</i>
<i>Stipa capillacea</i> Keng	Gramineae	Grass	<i>S. capillacea</i>
<i>Festuca coelestis</i> Krecz. et Bobr.	Gramineae	Grass	<i>F. coelestis</i>
<i>Kobresia pygmaea</i> C. B. Clarke †	Cyperaceae	Sedge	<i>K. pygmaea</i>
<i>Kobresia macrantha</i> Bocklr.	Cyperaceae	Sedge	<i>K. macrantha</i>
<i>Oxytropis stracheyana</i> Benth. ex Baker	Leguminosae	Legume	<i>O. stracheyana</i>
<i>Astragalus strictus</i> Grah. Ex Bend.	Leguminosae	Legume	<i>A. strictus</i>
<i>Potentilla saundersiana</i> Royle	Rosaceae	Forb	<i>P. saundersiana</i>
<i>Potentilla bifurca</i> Linn.	Rosaceae	Forb	<i>P. bifurca</i>
<i>Saussurea tibetica</i> C. Winkl.	Compositae	Forb	<i>Sa. tibetica</i>
<i>Leontopodium nanum</i> Hand.-Mazz.	Compositae	Forb	<i>L. nanum</i>
<i>Anaphalis xylorhiza</i> Sch.-Bip. ex Hook. f.	Compositae	Forb	<i>Ana. xylorhiza</i>
<i>Artemisia wellbyi</i> Hemsl. et Pears. ex Deasy	Compositae	Forb	<i>Art. wellbyi</i>
<i>Pedicularis kansuensis</i> Maxim.	Scrophulariaceae	Forb	<i>Pe. kansuensis</i>
<i>Silene waltonii</i> Williams	Caryophyllaceae	Forb	<i>Si. waltonii</i>
<i>Arenaria pulvinata</i> Edgew.	Caryophyllaceae	Forb	<i>Are. pulvinata</i>
<i>Swertia tetraptera</i> Maxim.	Gentianaceae	Forb	<i>Sw. tetraptera</i>
<i>Pleurospermum hedinii</i> Diels	Umbelliferae	Forb	<i>Pl. hedinii</i>
<i>Androsace graminifolia</i> C. E. C. Fisch.	Primulaceae	Forb	<i>And. graminifolia</i>

† Seed germination percentage was too low (less than 5%) to be included in statistical analyses. ‡ Abbreviations were used where species were referred to in both figures and text.

2.2. Germination Experiment Design

Seed germination is mainly controlled by the plant growing season temperature (GST), and increasing temperatures may advantage or disadvantage the seed germination of alpine plant species [29]. In previous studies of seed germination, experimental temperatures were mainly controlled to be theoretically optimal (e.g., 20/10 °C or 25/15 °C for day and night) [3,30–32]. However, neither natural temperature variations nor the effect of climate warming has been well considered previously.

To fill these knowledge gaps, we set up a seed germination experiment with three treatments under different manipulated day/night temperatures (see Table 2 for details): (1) theoretically optimal temperatures of 25/15 °C (optimal), (2) real-world temperatures (control) deduced from observations in the field, and (3) warming of +3 °C from the observed temperatures (warming). Four replicates were performed per treatment. The day/night temperatures in the control treatment were manipulated to follow the records of the mean soil temperature at the 5 cm depth (Figure 1) taken from the Damxung Grassland Station with a ten-day time resolution during the plant growing seasons from May 1st to September 30th, 2007–2011. The air temperature on the Qinghai-Tibetan Plateau is predicted to increase by approximately 3 °C by the end of the 21st century [33]. Therefore, our modelling of this rise based on the actual observed temperatures could provide information on possible effects on seed germination under predictions of future warming. In addition to the three different temperature treatments, the light within the incubators was also switched on/off every 12 h to simulate photoperiod.

Table 2. Experiment design and manipulated temperatures in seed incubators over the experiment period.

Phase	Dates †	Incubation Temperature (°C)		
		Control	Warming (+3 °C)	Optimum
1	1st May–10th May	14.9/9.2	17.9/12.2	25/15
2	11th May–20th May	15.1/9.3	18.1/12.3	25/15
3	21st May–30th May	17.7/12.0	20.7/15.0	25/15
4	31st May–9th June	18.6/12.5	21.6/15.5	25/15
5	10th June–19th June	20.2/14.3	23.2/17.3	25/15
6	20th June–29th June	19.6/13.8	22.6/16.8	25/15
7	30th June–9th July	19.7/14.4	22.7/17.4	25/15
8	10th July–19th July	18.8/14.2	21.8/17.2	25/15
9	20th July–29th July	18.3/14.1	21.3/17.1	25/15
10	30th July–8th August	17.6/13.5	20.6/16.5	25/15
11	9th August–18th August	17.7/13.7	20.7/16.7	25/15
12	19th August–28th August	17.4/13.5	20.4/16.5	25/15
13	29th August–7th September	17.4/13.4	20.4/16.4	25/15
14	8th September–17th September	17.2/12.7	20.2/15.7	25/15
15	18th September–27th September	15.9/11.6	18.9/14.6	25/15

† On the last day of each phase, the number of seeds germinated was recorded, and the germinated seeds and seedlings were removed from incubators.

2.3. Seed Germination Observation

For each temperature treatment, 100 seeds per species were incubated and replicated four times. Prior to the germination experiment, seeds were washed 3–5 times with distilled water and then placed in Petri dishes (90 mm diameter) with two layers of filter paper. The seed germination status was checked and counted every 10 days, with radicle emergence from the seed coat recorded as germination [34]. During the germination period, the filter papers were periodically replenished with distilled water to make sure that water was available throughout the experiment. Seeds that had already germinated were removed from the Petri dishes after each 10-day check.

2.4. Calculations of Germination Percentage and Time

The seed germination percentage (SGP) was defined as the proportion of germinated seeds out of the total number of seeds per replicate under each treatment expressed in percent. After 150 days (the length of the plant growing season at the Damxung Grassland Station), we calculated SGP and mean germination time (MGT) for each species (Equation (1)), and the latter was used to determine the germination speed of alpine grassland plants under different temperature treatments.

$$MGT = \sum_1^i n_i t_i / N, \quad (1)$$

where n_i is the number of seeds that germinated within consecutive intervals of time, t_i is the time between the beginning of the test and the end of a given interval of measurement, and N is the total number of seeds that germinated (Milbau et al. 2009).

2.5. Statistical Analysis

The data of *K. pygmaea* were excluded because its SGP was too low—less than 5% in the statistical analyses. One-way analysis of variance (ANOVA) with the least significant difference (LSD, $p < 0.05$) was performed to examine the differences in SGP and MGT between different temperature treatments in SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). The normal distribution and variance equality required by the one-way ANOVA was tested by Shapiro–Wilk and Levene’s test, respectively. If the test failed, we applied the nonparametric tests to examine the differences in SGP and MGT under multiple warming treatments.

3. Results

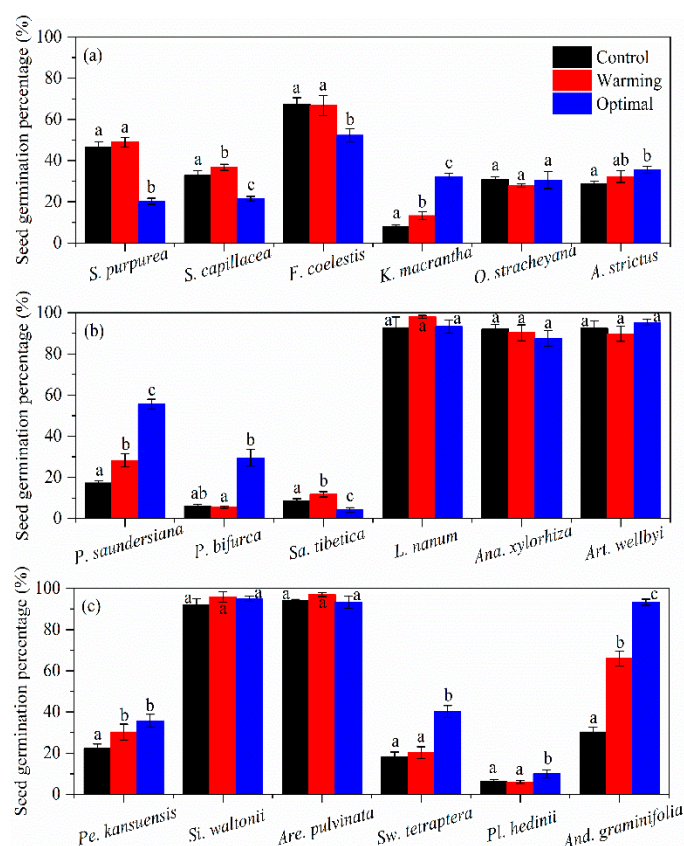
3.1. Seed Germination Percentage

Basing on the test of normal distribution and variance equality, we applied the one-way ANOVA and nonparametric tests to analyze the differences of SPG under different temperature treatments (Table 3). The SGPs of *Festuca coelestis* Krecz. et Bobr., *L. nanum*, *Ana. xylorhiza*, *Art. wellbyi*, *Silene waltonii* Williams, and *Arenaria pulvinata* Edgew. were over 60%. The only significant differences in SGP between the temperature treatments occurred in *F. coelestis*, where SGP was approximately 15% lower under the optimal treatment than under the control or warming treatments (Figure 2).

The SGPs of *S. purpurea*, *S. capillacea*, *Oxytropis stracheyana* Benth. ex Baker, *A. strictus*, *Pedicularis kansuensis* Maxim., and *Androsace graminifolia* C.E.C. Fisch. were between 20% and 60% (Figure 2). No significant differences in SGP between the control and warming treatments were found for *S. purpurea*, *S. capillacea*, *O. stracheyana*, or *A. strictus*. Compared to the control treatment, the warming and optimal treatments significantly enhanced the SGPs of *Pe. kansuensi* by 8% and 14%, respectively. However, no significant difference in the SGP of *Pe. kansuensi* was observed between warming and optimal treatments. In addition, the SGPs of *And. graminifolia* significantly increased with increasing temperatures. The SGP of *S. capillacea* was approximately 4% higher under the warming treatment compared to the control treatment and 11% lower than under the optimal treatment. The SGP of *S. purpurea* under the optimal treatment was approximately 26% lower than that under both the control and warming treatments.

Table 3. The hypothetical test (p values) of normal distribution (Shapiro-Wilk; SW) and variance equality (Levene's test) in one-way ANOVA under the comparisons of SGP.

	Levene's Test	Control_SW	Warming_SW	Optimal_SW
<i>S. purpurea</i>	0.587	0.240	0.062	0.850
<i>S. capillacea</i>	0.059	0.123	0.850	0.972
<i>F. coelestis</i>	0.123	0.262	0.233	0.976
<i>K. macrantha</i>	0.149	0.683	0.798	0.850
<i>O. stracheyana</i>	0.081	0.850	0.272	0.662
<i>A. strictus</i>	0.457	0.224	0.556	0.272
<i>P. saundersiana</i>	0.241	0.406	0.584	0.962
<i>P. bifurca</i>	0.024	0.024	0.024	0.998
<i>Si. waltonii</i>	0.312	0.024	0.952	0.161
<i>Are. pulvinata</i>	0.002	0.683	0.024	0.513
<i>Sw. tetraptera</i>	0.814	0.734	0.513	0.900
<i>Pl. hedinii</i>	0.274	0.272	0.683	0.577
<i>And. graminifolia</i>	0.314	0.650	0.995	0.850
<i>Pe. kansuensis</i>	0.184	0.911	0.332	0.584
<i>Sa. tibetica</i>	0.674	0.972	0.224	0.406
<i>L. nanum</i>	0.024	0.467	0.683	0.584
<i>Ana. xylorhiza</i>	0.197	0.972	0.332	0.798
<i>Art. wellbyi</i>	0.449	0.899	0.650	0.850

**Figure 2.** Comparisons of seed germination percentages (SGPs, %) between different temperature treatments at the species level. The three treatments were: (1) control (black bars), with real-world temperatures as observed at the Damxung Grassland Station where the seeds were collected; (2) warming (red bars), an increase of 3 °C from the observed temperatures; and (3) optimal, with the theoretically optimal temperatures of 25/15 °C for day/night time. Different letters denote significant differences (LSD, $p < 0.05$). Details of species name abbreviations can be found in Table 1 and the temperature manipulations during the experimental period in Table 2.

The SGPs of *K. macrantha*, *Potentilla saundersiana* Royle, *P. bifurca*, *Saussurea tibetica* C. Winkl., *Swertia tetraptera* Maxim., and *Pleurospermum hedinii* Diels were less than 20%, with no significant differences between control and warming treatments observed except for that of *Sa. tibetica* (Figure 2). The SGPs of *Sw. tetraptera*, and *Pl. hedinii* were approximately 22% and 4% higher under the optimal treatment than under the control treatment, respectively. Additionally, the SGP of *P. bifurca* was approximately 24% higher under the optimal treatment than under the warming treatment. Compared with that under the control treatment, the SGP of *Sa. tibetica* was improved by approximately 3% under the warming treatment and was reduced by approximately 4% under the optimal treatment. Furthermore, the SGPs of *K. macrantha* and *P. saundersiana* were significantly different between the three treatments and increased along with temperature (from the control to the warming and then the optimal treatments). The decreasing SGPs under the optimal treatment compared to both the control and warming treatments observed in *S. purpurea*, *S. capillacea*, and *F. coelestis* suggests that an increase of more than 3 °C would likely result in more significant negative impacts on alpine steppe communities that are dominated by these plants (Figure 2).

3.2. Mean Germination Time

We examined the normal distribution and variance equality before using the ANOVA test, and then employed the one-way ANOVA and nonparametric tests to analyze the differences of MGT under different temperature treatments (Table 4). The MGTs of *K. macrantha*, *O. stracheyana*, *A. strictus*, *P. bifurca*, and *Pl. hedinii* were all longer than 45 days (Figure 3). The MGT of *K. macrantha* under the warming treatment was approximately six and ten days shorter than that under the control and optimal treatments, respectively. However, there was no significant difference in the MGT of *K. macrantha* between the warming and optimal treatments. The MGT of *Pl. hedinii* under the optimal treatment was approximately ten days longer than those under the control and warming treatments. In contrast, the MGT of *A. strictus* under the optimal treatment was approximately six days shorter relative to the other two treatments.

Table 4. The hypothetical test (*p* values) of normal distribution (Shapiro-Wilk; SW) and variance equality (Levene's test) in one-way ANOVA under the comparisons of mean germination time (MGT).

	Levene's test	Control_SW	Warming_SW	Optimal_SW
<i>S. purpurea</i>	0.787	0.972	0.216	0.103
<i>S. capillacea</i>	0.056	0.272	0.504	0.515
<i>F. coelestis</i>	0.403	0.848	0.187	0.343
<i>K. macrantha</i>	0.201	0.599	0.503	0.030
<i>O. stracheyana</i>	0.843	0.442	0.432	0.420
<i>A. strictus</i>	0.599	0.599	0.609	0.201
<i>P. saundersiana</i>	0.102	0.790	1.000	0.001
<i>P. bifurca</i>	0.074	0.011	0.223	0.318
<i>Si. waltonii</i>	0.013	0.272	0.001	—
<i>Are. pulvinata</i>	0.075	0.272	0.683	—
<i>Sw. tetraptera</i>	0.341	0.079	0.414	0.086
<i>Pl. hedinii</i>	0.738	0.598	0.437	0.431
<i>And. graminifolia</i>	0.380	0.217	0.392	0.610
<i>Pe. kansuensis</i>	0.613	0.605	0.948	0.798
<i>Sa. tibetica</i>	0.040	0.125	0.118	—
<i>L. nanum</i>	0.029	0.272	0.001	—
<i>Ana. xylorhiza</i>	0.248	0.850	0.798	0.272
<i>Art. wellbyi</i>	0.033	0.001	0.972	—

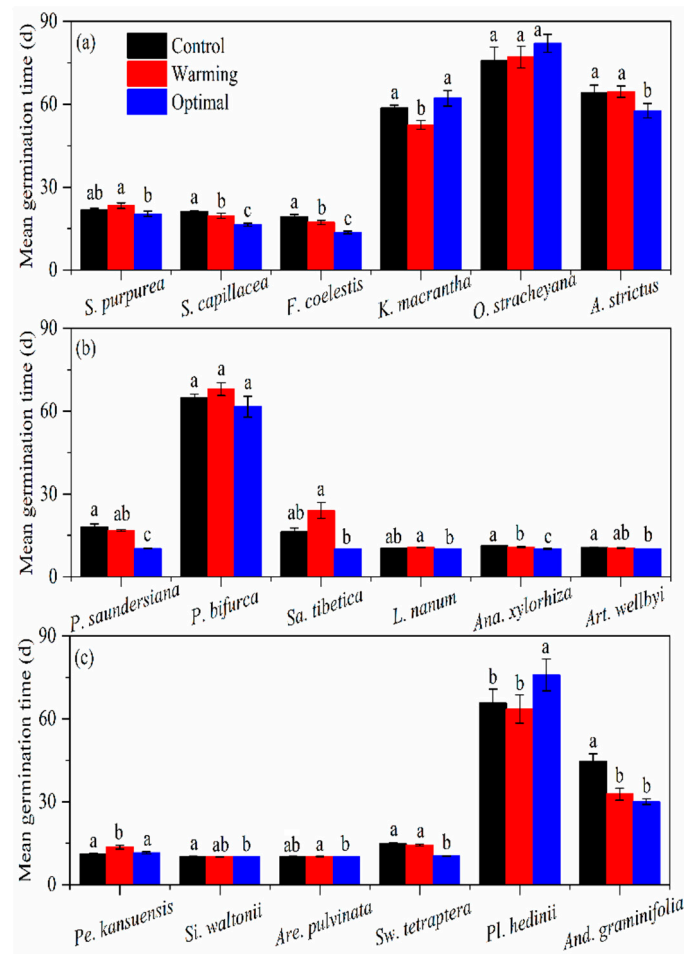


Figure 3. Comparisons of mean germination time (MGT, day) between different treatments at the species level. The three treatments were: (1) control (black bars), with real-world temperatures as observed at the Damxung Grassland Station where the seeds were collected; (2) warming (red bars), an increase of 3 °C from the observed temperatures; and (3) optimal, with the theoretically optimal temperatures of 25/15 °C for day/night time. Different letters denote significant differences (LSD, $p < 0.05$). Details of species name abbreviations can be found in Table 1 and the temperature manipulations during the experimental period in Table 2.

The MGTs of *S. purpurea*, *S. capillacea*, *F. coelestis*, *P. saundersiana*, *Sa. tibetica*, and *And. graminifolia* ranged from 15 to 45 days (Figure 3). The MGTs of *And. graminifolia* under the warming and optimal treatments were approximately 12 and 15 days shorter than that under the control, respectively. In addition, with the rising temperatures from the control to warming and then optimal treatments, the MGTs of *S. capillacea*, *F. coelestis*, and *And. graminifolia* decreased.

The MGTs of *L. nanum*, *Ana. xylorhiza*, *Art. wellbyi*, *Pe. kansuensis*, *Si. waltonii*, *Are. pulvinata*, and *Sw. tetraptera* were less than 15 days (Figure 3). The MGT of *Pe. kansuensis* under the warming treatment was 2.65 and 2.05 days longer than under the control and optimal treatments, respectively. The MGTs of *Art. Wellbyi* and *Si. waltonii* were approximately one day shorter under the optimal treatment than those observed under the control treatment; for *Sw. tetraptera* the MGT was approximately five days shorter under the optimal treatment. The MGT of *Ana. xylorhiza* decreased generally with increasing temperatures.

3.3. Germination Phenological Patterns

At the level of plant functional types, we found that the phenological germination patterns (i.e., the trend of accumulated germination percentage) were different among forbs, grasses, sedges, and

legumes (Figure 4). Approximately 50% of grass species' seeds germinated within 20 days under both the control and warming treatments at the beginning of the experiment, which was approximately 20% higher than that under the optimal treatment at the same time (Figure 4a).

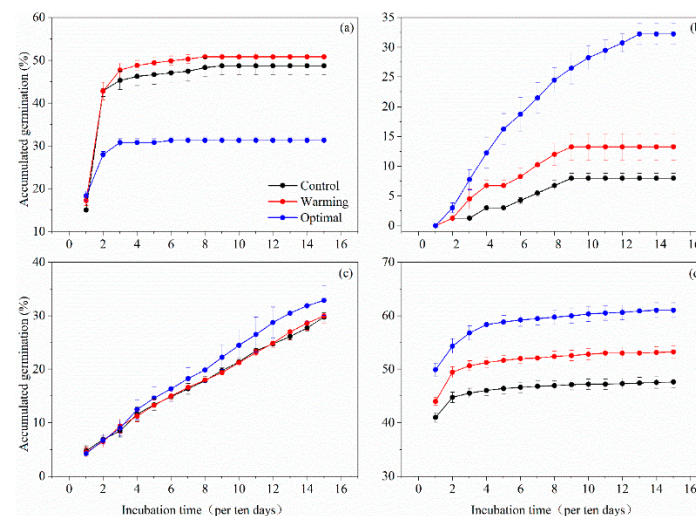


Figure 4. Accumulated seed germination percentage over time at the level of plant functional groups. The panels (a), (b), (c), and (d), represent grasses, sedges, legumes, and forbs, respectively.

The seed germination of sedge species was slow, taking approximately 100 days for 8% and 12% under the control and warming treatments, respectively (Figure 4b). Under optimal temperatures, the seeds of sedges germinated much faster than that under both the control and warming treatments (Figure 3b). However, the final SGP of sedges at the end of the experiment was still lower than in the other three groups (Figures 2–4).

The seeds of legumes germinated slowly and did not show significant differences between the three treatments (Figure 4c). However, their SGPs were comparable to those of grasses and sedges under the optimal treatment at the end of the experiments. Most of the forbs' seeds germinated within the first forty days (Figure 4d), with their SGPs at the end of the experiment being 61% under optimal, 53% under warming, and 48% under control treatments, respectively.

4. Discussion

In the current study, we wanted to examine possible differences in seed germination among alpine grassland plants in response to experimental warming. Furthermore, we aimed to test the hypothesis that climate warming enhances SGP and shortens MGT for alpine grassland species on the Tibetan Plateau. We found that different incubation temperatures significantly changed seed germination time and/or germination percentage at both species and functional group levels. In particular, under the warming scenario of +3 °C from real-world temperatures (observed in the field), the seeds of six species exhibited higher germination percentages than when under current temperature conditions. The MGTs were shortened for three of these six species and prolonged for the other one species in our experiments modelling climate warming. Compared to the control treatment, the average germination times of only two species were shortened when under a climate warming scenario.

4.1. Effects of Warming on Seed Germination of Alpine Grassland Plants

Lower temperatures and shorter growing seasons are the main limiting factors in the survival of alpine plant seedlings [35–37]. Slight changes in these conditions at the beginning and/or end of seed germination significantly influence seedling renewal. A recent study found that the seed germination of species adapted to lower temperatures generally requires higher accumulated temperatures (daily mean over zero or five degrees Celsius) [38]. For instance, plants in the Arctic and Alps regions require higher

temperatures to avoid early germination risks caused by climate extremes—e.g., frosts [39]. However, other studies have demonstrated that species adapted to higher elevations can germinate successfully at lower temperatures [32], and the minimum temperature threshold for their seed germination is much lower than that for species adapted to warm environments [38,40]. In this study, we found that most species were able to germinate under the current temperature conditions of the study area in early May, except for *K. pygmaea*, which had a low SGP (less than 5%). As the dominant species, *K. pygmaea* may have already evolved to reproduce asexually as an adaptation to the cold environment in Central Tibet. Our results also suggest that temperatures of 14.9/9.2 °C (sustained for 10 days) are sufficiently high for seed germination in alpine grassland plants found in Central Tibet [41] because over 50% of the species we tested germinated early on in the experiment (within the first 40 days, Figure 4). The subsequent rainy season (late June to late July) also favored seedling establishment in these species [10,42] because of the increasing temperatures (Figure 4) and greater availability of moisture in soils (Figure 1).

The seed germination of alpine meadow steppe plants in Central Tibet is sensitive to temperature changes. In particular, under the warming scenario of +3 °C to real-world temperatures observed in the field, the SGP and/or MGT of some species notably changed; these changes may affect the regeneration and establishment of seedlings [43]. Six species showed significant increases in their SGPs. As temperatures increased, the number of species that showed significant changes in SGP increased to eleven, indicating that under a warming scenario, the SGP of certain species is dependent on the magnitude of temperature increase [34]. A previous experiment on dormancy breaking and germination in dwarf shrub seeds from arctic, alpine, and boreal sites showed that incubation temperature was positively correlated with SGP [24]. However, the SGPs in this study were not entirely consistent with the conclusions drawn by Graae and Ejrnaes [24]. Specifically, among all of the tested species, we only found that *K. macrantha*, *And. graminifolia*, and *P. saundersiana* exhibited increases in germination percentage with increasing temperatures under the three different treatments. In contrast, the SGP of *Sa. tibetica* and *S. capillacea* decreased with increasing temperatures across the three temperature treatments.

Milbau et al. (2009) studied the influence of warmer summer temperatures on seed germination in subarctic species and found that a moderate temperature increase of 2.5 °C decreased the MGTs of more than 80% of the species they tested. In our study, the MGTs of one third of the tested species were significantly influenced under conditions of simulated temperature increase during the growing season, with four species exhibiting a significant decrease in MGT and two species exhibiting a significant increase. The shorter MGT in certain species can favor the rapid occupation and utilization of resources and continuous expansion of their competitive advantage [5,43,44]. In addition, delayed germination also signifies that the species may be subjected to exclusion and expulsion in a highly competitive environment, which may lead to the loss of a favorable ecological niche [45,46].

4.2. Effects of Species Identity on Seed Germination in Alpine Grassland Plants

Unsuitable germination times will cause seedlings to miss the optimum growing period and significantly reduce the fitness of the species. Many studies have confirmed that when the habitat or distribution of a species changes, its germination strategy will also change, and can even show completely different germination characteristics [47,48]. At the community level, variations in germination strategy among species promotes better use of environmental resources in time and space, which is conducive to the coexistence of species [49].

Previous studies have shown that seed germination in *Stipa* is somewhat dependent on alternating temperatures [31,50]. In this study, the germination percentage of *S. purpurea* under the warming and control treatments was higher than when under the optimal treatment; this further supports the hypothesis that variable temperature conditions promote seed germination in *S. purpurea*, likely due to the fact that the seed coat expands, shrinks, and ruptures under the changeable conditions to allow gas exchange and nutrient transformation within seeds [5,23]. This may also explain why

higher germination percentages were observed for *S. capillacea* and *F. coelestis* under the warming, rather than optimal, treatment. For *O. stracheyana*, neither the SGP or MGT showed responses to the control, warming, and optimal treatments. It may be possible that germination was mainly limited by the species dormancy characteristics rather than temperature [51,52]. Moreover, during the experimental process, mildew development was found in the seeds of *Sa. tibetica* despite daily sterilization, which could be a critical factor in germination percentage reduction in the species.

Some plants are capable of both sexual and asexual reproduction, with the preferred mode of reproduction depending on environmental conditions and genotypes [53]. Estimations of the relative importance of sexual and vegetative reproduction in *Leymus chinensis* (Chinese wildrye) grassland in Inner Mongolia showed that when the population density was low, reproduction was primarily sexual to quickly allow occupation of the optimal habitats [54]. As a result, the plants overcame the limited dispersal distances possible under vegetative propagation [55]. In our study, although the germination percentage of *P. bifurca* was not sensitive to the warming treatment, it was significantly enhanced with the optimum treatment when compared with the warming treatment, which indicates that temperature conditions determine the selection of propagation methods by the species to a certain extent [56,57]. Specifically, *P. bifurca* has a tendency to select asexual propagation under low temperature stress conditions, while sexual propagation is favored under high temperature conditions. The combination of both propagation methods ensures the maintenance of the competitive advantage of *P. bifurca* in the varied environments of alpine regions.

In general, studies on germination and emergence of primed grass seeds under field and simulated field temperature regimes have shown that emergence rates in the field are lower than in the laboratory (Hardegree and Van Vactor, 2000). Climate warming is likely to cause a series of changes in biotic or abiotic factors in the natural environment; however, the success or failure of seedling establishment depends not only on harsh living conditions such as cold, drought, and soil depletion, but also on the fierce competition with other surrounding plants. Therefore, there are some limitations to the prediction of seed germination and seedling regeneration using simulated field temperature experiments. Further research on seed germination should consider a series of biotic and abiotic factors, including temperature, to provide a more comprehensive prediction of seed germination response and new patterns of seedling regeneration in a climate warming scenario.

4.3. Implications

The characteristics of seed germination play a key role in determining whether plants can colonize new habitats and achieve species coexistence at the community level. Therefore, elucidating the relationship between temperature and seed germination can provide valuable data for the prediction of future species distributions and changes in community composition and structure in alpine meadow plants in Central Tibet. In addition, germination time and germination percentage may have effects on the renewal and restoration of degraded alpine grassland in Central Tibet, especially in some severely damaged grassland ecosystems. The response of seed germination to temperature is often an essential reference for the selection of suitable local herbage and the artificial addition of seeds.

5. Conclusions

When compared to the control treatment, the number of species we examined that showed a significant change in germination percentage as the temperature increased rose from six in the warming treatment to eleven in the control treatment. Therefore, we deduce that the response of germination percentage to climate warming in certain species is dependent on the magnitude of temperature increase. Specifically, under a 3 °C increase scenario, *S. capillacea*, *K. macrantha*, *P. saundersiana*, *Sa. tibetica*, *Pe. kansuensis*, and *And. graminifolia* exhibited higher germination percentages than in the present climate; among these six species, the mean germination times were shortened for *S. capillacea*, *K. macrantha*, and *And. graminifolia* in a climate warming scenario. Additionally, compared to the control treatment, *F. coelestis* and *Ana. xylorhiza* also exhibited shorter mean germination times in a climate warming

scenario. Therefore, we predict that the sexual propagation abilities of these eight species will be substantially enhanced by a warmer climate. In particular, the renewal of these eight species by clonal propagation coupled with higher germination percentages and/or faster germination times will favor the rapid occupation of space and acquisition of resources in harsh living conditions in alpine meadows.

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