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ASSOCIATE EDITOR'S COMMENTS TO THE AUTHORS

Associate Editor

Comments to the Author:

Dear Authors,

Thank you for submitting the revised version of your manuscript. An additional independent reviewer has evaluated your work and found the study both impressive and timely. However, the reviewer also noted some technical and language issues and has recommended certain improvements to enhance clarity. We encourage you to address these points for further refinement of the manuscript.

Dear Editor and Associate Editor,

We are very pleased to know that the review we provided has been positively received by the Associate Editor and Reviewer. We have considered all comments and our and incorporated them into a revised version. Please find below our point by point responses (shown in blue) to the comment made by the reviewers. We hope that you find this revised manuscript acceptable for publication in Functional Ecology. All authors contributed to and approved this revised submission.

Yours sincerely,

The autors.

REVIEWERS' COMMENTS TO THE AUTHORS

Reviewer: 1

Comments to the Corresponding autor

This is an interesting work and I enjoyed reading the manuscript. Clearly, understanding germination is critical, and efforts should shift from just temperature assessment in the face of climate change. In this regard, this study focusing on water stress or limitation is a welcome addition to the literature. The authors worked with physiological germination models and attempted to make some ecological sense from the results. This is an interesting approach, an area that has been overlooked in the field of ecology since the dawn of time. As with some preliminary attempts before, the authors could not completely achieve this, but it is certainly a good start.

We were very pleased to see that Reviewer 1 had a positive view of our research and valued the novelty of the question and the approach we used. We deeply appreciate the additional time dedicated to improving this manuscript.

The use of R packaging for data analysis and Bradford's hydro-time model in several populations of the seeds makes this manuscript more interesting. I am on the side favoring publication of the idea, but I have some comments, mainly for writing, which should be incorporated to improve the readability. Thus, the science is OK, but the presentation needs intense tweaking. Especially the newly added section or those in the 'track changes' have plenty of grammar errors.

We have reviewed all the sections to correct the grammar error the reviewer refers to.

Please see below, where I cannot ascertain what the authors attempted to say.

Please see below our responses to your comments.

Introduction  
Generally, well written, but in places, the authors did not complete the idea.  
L91- Do you have evidence for this happening late in other ecosystems? I also think you should replace 'advanced' with 'early'. Perhaps "... arid conditions show early flowering, germination, and seedling emergence."

Thanks for pointing this out. Yes, in other alpine regions (without significant water limitation) the germination pattern is to delay germination after snow-melting and once temperature rise above certain threshold (e.g., , Fernández-Pascual et 2021, New Phytologist) we added explanation in L 87-90. We rephrased the sentence as suggested by the reviewer in L95.

L94-98: How and why are early germination or seedling emergence advantageous?

We have expanded the reasoning behind this idea in L92-94. According to literature, early germination benefit individuals to access first the available resources (Verdú and Traveset, 2005) and provide them with a longer growing season (Donohue et al., 2010), which in turn can potentially increase the amount time for the correct development of a root system with more chances to survive summer drought (see L 90-92).

L108- I think it is important for the readers to know from which ecosystem you have studied the species and set the scene for your methods and results. It appears that you have talked about all the ecosystems and possible climatic change effects but lack a sense of belonging to the study species. You must also explain the choice of study species.

We agree with the reviewer comment and have introduced the requirements we followed to selecting the study species in L 110-112. To be able to study intraspecific variation at subpopulation level it is important to select a locally abundant species present along an environmental gradient in the study area (Albert et al 2011). Additionally, previous knowledge about optimal germination conditions was also needed to correctly assess that responses observed were because of water stress levels (L 125-126).

Materials and methods

L126- I always struggle with studies using months and seasons together, which would confuse readers who know less about the study area. For instance, you say flowering onset occurs in early June and ripe seeds are dispersed during August, and suddenly talk about germination occurring mainly during late summer/early autumn. I suggest sticking to seasons with month patterns, both described properly.

We agree with the reviewer point and used both seasons and patterns consistently in L 135-137.

L144- Did you bury the datalogger at the surface or at some centimeters below ground? How did you ensure the loggers stayed at this position for three years?

We clarified the datalogger buried at 5 cm deep (L 151). We checked data loggers at least every 6 months to retrieve data and make a backup and from previous experiments we know that dataloggers do not move, partly also because of their size. We added clarification in L 155-156.

L174- This March to November do not have summer?

Yes, it includes summer period, however the mean soil temperature we report in the text refers to annual average. We added clarification in L 183.

L218- How long is "until the start of the germination experiments"?

Germination experiments started at different time points depending on the storage treatment applied. For fresh seeds, germination experiment started 10 days after collection and for after ripened seeds it started 45 days after collection. We added clarification in L 229-230.

L211- How did you judge that the seeds had reached maturity for collection? You also need to explain why you stored the seeds for 3 months in silica gel to determine the dry mass. Did you also measure the fresh mass?

Thanks for pointing this out. We agree that more details are needed. Mature seeds are indicated with the opening of the capsule, turning the capsule upside down seeds are easily retrieved. We added clarification in L 223. Seed mass measurement is very dependant of seeds relative humidity, thus to ensure comparability between seed lots we decided to follow the international seed bank protocol who measured the dry seed mass weight (reference). We did not measure fresh see mass. We added justification in L 233-234.

L231- Above, you talk about after-ripening. Which treatment can release dormancy? Has the previous germination experiment been published or is it a preliminary study? Not sure who conducted this and how rapid is "rapid"?

For non-deep physiological dormancy can be relieved by after ripening at room conditions following Baskin et al 2006 (Seed Science Research). We added clarification in L 239-240. The previous germination experiment has just been accepted for publication in Journal of Ecology, we added reference in L 242 but are still waiting for doi details to include it in the reference list. Here rapidly refer to less than two weeks, we have added clarification in L 242-245.

L257- Petri dish (and elsewhere, e.g., L 316) – "Petri dish" is the correct format as it is named after Julius Richard Petri, a German bacteriologist. Was it not open? Or opened? This sentence needs to be improved. It is not easy to understand. How few is "few"?  
I still do not understand clearly. Above, you mentioned seeds were collected at the time of natural dispersal (August 7-8th, 2023), and then you incubated the seeds at summer temperature? It looks like the seeds are dispersed at the end of summer. However, I am not sure about this, as you mentioned both months and seasons. What is the datalogger measuring for summer temperature (soil temperatures, as the ambient temperature may be low, but soil temperatures may be high)?

We appreciate the reviewer comment and have changed the terms to “Petri dish” throughout the manuscript. We have rewritten the sentence to improve clarification to: “each Petri dish was only open for 30 seconds a day” in L 272. Seeds were collected during summer, although specific dates change every year. 2023 was a specially warm year thus seeds were ripe already at the beggining of August. Seeds were incubated with constant 20ºC which corresponded with the mean temperatures recorded by end of summer (last weeks of August and first weeks of September) in our study area, added clarification in L 273-276, data can be checked in Figure 3A or looking at the raw data files (“temperature\_ibuttons.csv” or “wp\_villa\_2020\_2024.csv”). Datalogger is measuring soil temperatures as specified in L 152.

L268- Why did they not germinate? Were they dormant? I guess water potential may also induce dormancy, but I'm not sure if you tested this.

Some seeds did not germinate because they were empty or with fungus, although a small percentage looked viable. Some of the seeds indeed seemed to be dormant as the germination results for fresh seeds suggested, however, for the after ripened seeds germination reach close to 95% in average, indicating that after ripening was a successful method for dormancy alleviation see details in L 353-357. Still we cannot completely discard the idea that some seeds might in fact have a higher level of dormancy that was not alleviated, we added clarification in L 285-286. We did not test if water potential induced dormancy, we explained the lower levels of germination under water stress due to the increasing difficulty of the seeds to absorb sufficient water to trigger germination.

Results  
Please include effect sizes or confidence intervals alongside p-values when reporting results.

Thanks for spotting this. We have added parameter estimate and confidence intervals in L 364.

L339- This is a discussion point.

We appreciate the comment, but we think that the sentence appropriately describes the germination results thus we would like to keep it in results, but we let the decision in hands of the Associate Editor.

L341- It might be interesting to also note the germination speed between control and after-ripened seeds. I think this would also contribute to the success of seedling establishment, especially in the context of climate change.

We agree that with the reviewer’s comment that germination speed might be of importance in the climate change context. We have added information about the calculation, results and importance of germination speed in L306-308, L 365-367 and L 401-402.

In the results section, it is always good practice to use accurate germination percentages. Perhaps you can write "almost 100%" or "below 50%" in the discussion.

We agree with the comment of the reviewer however, we are using the average values across all our subpopulations to highlight the general patterns across all subpopulations. We have added clarification in L 356-357.

L342- Did you see if the seeds dried during after-ripening? In my experience, this happens a lot and affects the water potential experiments.

During after ripening, all seed lots were kept at room conditions with 22ºC and 35% of relative humidity (L 228-229) and we did not observed dried seeds. Affects the water potential how??

L361- "We firstly checked"? But what are the potential effects of seed mass on? Seed death?  
In one page of results, the authors did not explain the results of several other experiments they included in the methods. For example, the climatic data were completely excluded. If they are not useful, then why write about the experimental process in the method section?

We appreciate reviewer detailed comments. Seed mass is a known trait that can modulate germination responses and is sometimes used as a proxy of maternal effects. Despite this, the literature reports contradictory effects of seed mass when used as an explanatory factor to germination responses under drought, in some cases are small seeds the ones germinating better while in others are larger seeds. Potential effects and literature from the topic are explained in L 347-353.

Climatic data was used to calculate each subpopulations growing degree days, a bioclimatic index that highly correlated with the amount of drought measured in the soil and thus experienced by the seeds L 205-213. Additionally, the other climatic data was reported as a descriptions of the subpopulations environmental conditions L 214-219. Climatic data is also used in the discussion to interpret the plausible explanations for the results obtained L 442-445.

Discussion  
I am really surprised the authors say "our results" in the discussion many times but never mention which specific results.

We partially agree with the reviewer comment, in most cases those specific results are specified at the beginning of each paragraph nevertheless, we have clarified to which results we refer to at every point. See L 406-407, L 432, L 435, L 446-447, L 460-461, L494-495.

L371- I am not sure if you provided enough evidence to make this the "take-home" message, especially given that only one constant temperature was used for germination.

Thank you for noticing this. We feel confident about our take-home message despite the study limitations. Nevertheless, we have rephrased the sentence to diminish the strength of the statement in L 393-395.

L380- Unless you explain that fully matured seeds were collected, I am also skeptical about the dormancy claim. This is crucial as you discuss the implications extensively later, e.g., L384.

We collected what we consider fully matured seeds, based on the exclusive collections of open capsules (added specification in L 223).

L399- I still do not understand what was tested and where you showed the results.

Seed mass effects are tested following the process described in L 380-388, with detailed descriptions of what was tested and the models results written in the text.

L397- What about water stress during seed maturation? Would this affect the size and dormancy status of the seeds in different populations?

Thanks for your comment. In agreement with this comment, water stress can affect seeds during maturation decreasing the level of dormancy as well as seed size and seed production. We have incorporated such ideas in L 510-512.

L428-494: The text can be more concise.

Following reviewer comment changes have been made throughout the text to improve concision.

Fig 4- Did you see some populations germinating more and others showing less germination percentage? I don't know if you mixed the seeds from different populations. Your legend says "average," but lines 236-239 are not clear. Please avoid using abbreviations in the figures, e.g., WP. I understand it is water potential, but for someone skimming through the paper, it may be hard to understand.

We appreciate the reviewers comment; yes there was some differences between subpopulations that can be checked in the supplementary figure SF1. We did not mixed seeds between different subpopulations, but only for the figure representation, we showed the average germination across subpopulations. We have clarified Fig 4 caption in L 790-791 and changed abbreviations to Water potential in the figure 4.