Answer to reviewers

25-Sep-2024  
Microclimatic variation regulates seed germination phenology in alpine plant communities. JEcol-2024-0743

Our responses to the comments provided by the editor and both reviewers are shown in blue

Your manuscript has now been assessed by reviewers and by James Dalling, the Associate Editor handling your manuscript. We require you to make some revisions, before we can make a final decision for your paper.  
As you can see, both referees and Associate Editor feel this is an interesting manuscript with the potential to make a valuable contribution to the area. I have considered your manuscript in light of their assessment and agree that it is potentially suitable for the journal. However, several substantial points need to be addressed.  
Please spend some time on the Abstract and highlighting the novelty of the work in this section of the paper. Well-written Abstracts make a potential reader more likely to read the paper and therefore to understand your important work.

Dear Editor,

Thank you for your positive evaluation of our manuscript. The comments and suggestions of the associate editor and reviewers have helped us to identify key points that needed revision to improve the impact our research can achieve. We have considered all comments and we have incorporated them into a revised version. Please find below our point-by-point responses to the comments made. We hope that you find this revised manuscript acceptable for publication in Journal of Ecology. All authors contributed to and approved this revised submission.

Yours sincerely,

The authors  
  
Associate Editor's comments:  
  
Dear Authors.

Thanks for submitting your manuscript to Journal of Ecology. I’m pleased to report that the two reviewers were positively impressed with this work. I agree that this has the potential to be an important contribution. I’ve added my comments below – which mostly concern clarification of experiment details, presentation of your figures, and language use. I do think though that you could make the abstract of your paper more impactful. You make the case for why your work is important in the discussion (line 319). This does not come across clearly in the abstract where changes in germination need to be put into the context of the associated temperature change. I also thought that you might think about the terminology that you use to describe the plant community (‘system’) and the climate regime (‘incubator’). Someone skimming through the paper would not understand what a system\*incubator interaction would mean. Where clarity is lacking (e.g., line 232) you could add some explanation, or change the language use entirely.

We were very pleased to see that the Associate Editor 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.

We agree that the abstract can be substantially improve to highlight the novelty and impact of the study, see modifications across the whole abstract.

We agree with the reviewer that the manuscript will benefit from more clear terms, therefore we have changed both terms, “system” to “community” and “incubator” to “climate regime” throughout the manuscript.

Please see below our response to your comments.  
  
  
Minor Comments  
Abstract  
line 15: Unclear what is meant by ‘anticipate germination 52 days on average’. Please rephrase this. ‘Anticipar’ doesn’t really translate as anticipate in English in this context.

We agree with the comment, we changed the term to “advance germination“ in line 28.   
  
Introduction  
Line 31: ‘competitive edge in the use of limited resources versus individuals germinating later’

Thank you for this observation. We made the change (line 83).  
  
Line 63: I think this needs to revised somewhat. The previous 10 lines describe alpine and mediterranean high elevation germination syndromes in some detail – which seems a bit incongruous with the statement that little is known.

Thank you for this comment, we wanted to clarify that although the general syndromes have been described for alpine areas, germination strategies are known to vary in response to local conditions and it is not known how phenology of alpine species varies in response to microclimatic variation caused by the complex topography. We agree with the comment and have removed the statement.

Line 66: bedrock type  
Changed as requested (line 119).

Line 69: use heterogeneity for ‘roughness’  
Changed as requested (line 122).

Line 74: ‘fellfield’ needs to be defined first time it is used – this term may not be familiar to readers

We agree with the Editor comment and have added clarification in lines 127-130.   
  
Methods  
  
Line 120 Convert precipitation data to mm?

We have converted precipitation to mm in line 178 and line 183, as well as in Figure 1b.

Line 164 “Each incubator was configured in Aralab climatic chambers”. Not sure what this means. Isn’t a climatic chamber the same as an incubator? Are these chambers placed within the incubator?

We clarified this point, each incubator is indeed one climatic chamber, we have rewritten the sentence in line 225 “Each condition was configured in one Aralab climatic chamber … using Fitolog 9000 software” and changed the term “incubator” in lines 227 and 230.

Line 184 and 223: loupe = stereoscope

Changed as requested (line 245 and 284).  
  
Line 195: contemplate = concern

Changed as requested (line 256).  
  
Line 204: exact = predicted

Changed as requested (line 265).  
  
Line 210: to represent

Thank you for this observation. We made the change (line 269).  
  
Line 219: This is quite deep – is this a realistic burial depth for this experiment?

We based our depth on that used by Schwienbacher et al 2010 (Flora, doi:10.1016/j.flora.2008.10.007) with alpine species. Other studies buried their seeds even deeper (7-10 cm) (Moravková et al., 2022, <https://doi.org/10.1038/s41598-022-12884-0>). Please note that the upper cm of soil are relatively unstable in the alpine environment and can experience movement from year to year as a consequence of, for example, snow weight. In our experience, this can cause the bags to surface and move away from the experimental plot, if buried closer to the surface.

Additionally, germination was recorded in all species in the bags. Thus, we assume that depth did not interfere with germination, or if it did the effect was the same for all species, because all bags were buried at the same depth.

Line 232: this might confuse readers a bit – they might wonder why incubator is not a random factor. However, there are only two incubators so you should say here what incubator represents (ie fellfield vs snowbed) – see also line 250 – a reader skipping to the results would not follow what incubators signifies here. I wonder if it is just simpler to replace incubator with something else throughout?

We agree with the Editor, and we have added clarification in line 293 and line 297. To facilitate understanding throughout the manuscript we have changed the term “incubator” per “climate regime”.

Line 250: “Indistinctively of the system”. Meaning unclear. Independently of..? What does ‘system’ refer to here?

Thank you for the comment, here were referring to both communities (Mediterranean and Temperate). Following the Editor’s suggestion we have change the term “system” to “community” in line 298 and throughout the paper to improve clarity.

Line 266: ‘anticipate’ is not the correct verb here. Do you mean advance their germination?

Yes, we have changed the terminology (line 329), thank you for the comment.

Line 319 – write as 2-3 degrees C. I think this is the striking result that you need to emphasize better in the abstract with line 321-3 comment in the abstract synthesis.

We agree with the Editor comment and have added this in the Abstract lines 17-20.

Line 361 and 375: postponed = delayed germination

Changed as requested (line 429 and 447).

Line 378: snowbed conditions  
Changed as requested (line 450).

Fig. 2 b and c. I wonder if there is a better way to represent these germination curves. At present they do not convey much information as the individual species cannot be identified. If there is a pattern that the authors want to illustrate with these graphs then perhaps it is worth rethinking how to display it. Otherwise I don’t think Fig. 2b and 2c add anything to the paper.

Thank you for the comment, we wanted to represent the notable variability of germination strategies within each community. REMOVE them or add a legend with all species names?

Fig. 3 legend for Fig. 3a needs to be extended. Its unclear what is being plotted here. What is the x axis, what does the y axis – going from 0-1% represent? The shift is relative to what?

We agree with the Editor’s comments, we have added clarification in lines 679-683, as well as we have updated figure 3a.

Line 611: Phylogenetic not phylogenetical

Changed as requested (line 683).

Fig. 4 line 617: trait not traits. Line 623 condition not conditions.

Changed as requested (line 688-697).

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Reviewers’ comments:

Reviewer: 1

COMMENTS FOR THE AUTHOR

This is a very welcome paper investigating the germination phenology of a considerable number of alpine species by using a novel approach that implements simulated seasonal temperature cycles resulting from microclimate data collected in the wild. The experimental design is coupled with a backup from field observations, thereby providing a complete picture of the germination phenology of the target species.

The novelty is exactly in the title of the study, i.e. an accurate simulation of the real microclimatic conditions that seeds experience in the wild. To the best of my knowledge this has never been investigated so far and it is very important as it allows to identify germination phonologies that haven’t been described yet. Indeed, by applying an accurate simulated cycle of temperatures that seeds experience in the wild, this paper highlights for the first time that alpine species spread the germination in different time periods (seasons) after seed dispersal and not predominantly in spring (as previously thought). This is clearly a bet-hedging strategy that buffer the risk of recruitment failure due to the unpredictable environmental conditions of the alpine life-zone. This study further highlights that this strategy is not the same across alpine plants but vary depending on both the macroclimate (i.e. alpine-temperate vs. alpine Mediterranean regions) and the microenvironment conditions (i.e. fellfield vs. snowbed). The analysis of traits describing the germination phenology is novel and comprehensive, including metrics able to synthetize a great amount of information in a simple, yet informative way.  
  
Overall I think that this paper, including its experimental and data analysis approach might be of inspiration also for other ecological systems. I have just a few minor comments to consider.

We were very pleased to see that Reviewer 1 understood the importance and the novelty of the question and the new methodology we applied to study germination phenology. Please see below our response to your comments.

Line 97-100. This hypothesis might make sense, but it is not sufficiently justified. Why do you expect such responses? For a not expert in the specific field (ie seed germination of alpine species) might not be obvious.

We hypothesized that fellfield conditions will result in higher total germination, due to longer growing season, earlier germination, due to higher temperatures, and lower or no germination during winter, due to below 0 temperatures. While the snowbed conditions will show lower total germination, due to shorter growing season, delayed germination, due to lower temperatures, and germination under winter (snow-covered) conditions, due to temperature not dropping below 0 degrees Celsius. We have added justification in lines 153-157..

Line 150. Please specific if you have taken an average of the temp recorded from 2008-2019.

Yes, We used temperature data series collected in the sampling sites of the temperate community (Picos de Europa National Park) from 2008 to 2019 to calculate and average minimum and maximum temperature per day which we used as a reference for identifying regional alpine microclimates. We have added clarification in lines 209-210.

Line 163. It is not clear to me how long, during the course of a day (24h), it took the temperature to shift from Tmax to Tmin? e.g. °C x hour.

The duration of the temperature shift depended on the range between Tmax and T min and the hours of light (changing every month). We have added a supplementary table with the detailed climatic chambers programs, see supplementary Table S1

Line 167. Please specify the temperature below 0 that you have used

Below zero temperatures ranged between -0.5 to -2 ºC, we have added clarification in line 228-229 and see details in supplementary Table S1.

Lines 219-220. Why did you choose not to monitor autumn germination in field experiments? This seems not consistent with the lab simulation.

We agree with the reviewer comment nevertheless, the year we sowed the seeds in the field, temperatures starting dropping already in mid-October with snow covering our sites and impeding us to check germination in the field. ~~Temperatures experienced were below the average we used in the lab and that is the reason why we decided to, instead, have two retrieval times in early spring and late autumn.~~

Lines 335-358. OK. But please consider that that your experiments were conducted in the lab, which may not resemble exactly all conditions experienced by seeds in nature: lab may facilitate seed germination compared to what happen in the wild, where seeds may not germinate because of buried too deep, under darkness (if light sensitive) or do not receive enough water. Supporting this view, fled experiments conducted here show lower germination. I would add a few words about this.

We agree with the reviewer comment and have added sentences in lines 382-384 and 419-421.

Line 359. “germination before winter” It surprised me the significantly higher germination occurred for fellfield species in autumn, as these places are supposed to be exposed to more frequent frost event. How would you explain this pattern?

Thank you for this insightful comment, yes fellfield areas are exposed to more frequent frost events but also to higher temperatures (compared to snowbed areas) and thus promote advanced germination if water is available. We need to keep in mind that our experiment provided a continuous supply of moisture for germination, as discussed in lines 236-238 In the field, water might not be available in early autumn because of a lack of rainfall, and this might prevent germination until temperatures have dropped under a value not suitable for germination.

Line 364. “future climate scenario”. What about autumn warming? This is expected to increase seed germination, especially in weakly dormant seeds.

We agree with the reviewer and have added a sentence to include this idea in the discussion, lines 436-438.

Lines 416-418. I could not find where this conclusion come from by looking at the results. Which species will anticipate the germination, and which will be affected by a lack of clod stratification? Moreover, it is not clear which is the “climatic trend” mentioned.

Thank you for the comment, we clarified that the climatic trend is referring to the warming of alpine areas (line 489). The conclusion comes from the fellfield regime showing advanced germination compared to snowbed regime. Germination responses are species-specific but all species except for *Cerastium ramosissimum, Helianthemum urrielense* and *Spergula morisonii* showed advanced germination responses in the warmer microclimate (fellfield), additionally *Dethawia splendens, Luzula caespitosa, Phyteuma hemispahericum* and *Phyteuma orbiculare* showed higher germination in snowbed regime, we added clarification in line 490-492. Species specific germination curves can be checked at supplementary Figure S2.

Figure 5. please indicate which species are from Mediterranean and which from temperate alpine biomes.

We have added the information as requested in figure 5.

Figure 3. I think that part a) needs to be better described as it might not be obvious its interpretation for everyone: what x and y axes represent?

We agree with the reviewer’s comments, X- axis represent the area between fellfield and snowbed germination curves, y-axis represent the density of the values. We have added clarification in lines 680-632 as well as we have updated the figure axis.

Table 1. Under “ecological significance” at the second row, I would just say: “species able to germinate under the snow like conditions (zero degrees and darkness)”. Then I would add: “maximizes ability of taking advantage of water available after snowmelt for rapid seedling recruitment”.  
I was also wondering whether it makes sense to add a column about “drawbacks” beside each ecological strategy, as it might help the reader understanding these different trade-offs. For example, autumn/winter germination will expose seedling to the long-lasting winter season and to high risk of frost (especially in fellfield); this latter may apply also for early spring germination while summer germination will expose seedling to the risk of drought.

Thank you for your suggestion we have added the column potential drawbacks to Table 1.

Reviewer: 2

COMMENTS FOR THE AUTHOR

This paper explores the important and understudied question of how microclimate variation affects germination phenology in temperate and Mediterranean alpine systems. The study investigated fresh seeds of an impressive number of species (54) from temperate and Mediterranean alpine communities. The experimental approach was strong with both in situ and ex situ components. In the lab, a continuous seasonal experiment mimicked 2 contrasting microclimate regimes, fellfield and snowbed, in growth chambers using soil temperature data collected in the field over many years. In situ, seed burial experiments were conducted on a subset of species (six dominant species per study system), buried in fellfield and snowbed conditions within each site. The study is thoughtful, thorough and well written. I agree that results suggest microclimate influences germination phenology in these alpine species and that this is new information for alpine ecosystems. The paper is very well written and was a pleasure to read – congratulations to the authors.

We appreciate the Reviewer 2 positive opinion about our research. We appreciate all the comments and suggestions, which have been incorporated in the manuscript and discussed in the comments down below.

Line 251 – 254. Please check that these sentences accurate describe what is seen in Fig 2a.

We have revised and clarified the sentence in lines 312-314.

Line 258 – suggest you highlight that this ‘change’ is a ‘delay’ – to aid interpretation of the next para.

Changed as requested (line 321).

Line 267 – check grammar.

Thank you for this suggestion, we have rewritten the sentence (line 330-331).

Lines 269 and 270 – add the family in parentheses for each species

We added the information (line 333-334).

Line 308 – For Armeria duriaei, fig 5 seems to show fellfield germination in spring..?

Yes, indeed very few germination for Armeria duriaei happened in spring. We have added clarification in line 371.

Line 312 – Add ‘Results of’ before ‘our experiments’

Changed as requested (line 375).

Line 313. Better to say ‘in these alpine species’ than suggest all alpine species everywhere.

We agree with the reviewers comment, we have included the suggestion in line 376.

Line 337-338. Better to refer to autumn and spring rather than early season and late season, I think.

We agree with the reviewer point, nevertheless we did not want to confuse the reader because here late season refers to the second autumn. We made the change in lines 403-404.

Line 341 – is the word ‘contrastingly’ correct here?

The reviewer is correct, “Constrastingly” is wrong here, we have removed this connector in line 407.

Line 343 – what do you mean by ‘and successfully break seed dormany’ ? Unclear.

Thank you for this comment; we wanted to say that snow like conditions break the dormancy and species are able to successfully germinate. We have rewritten the sentence to clarify in line 408-409.

Line 347 – Are you saying that higher temp requirement for germination prevents autumn germination without the need for dormancy?  This point could be cleaner.

We agree with the reviewer comment and have added clarification in lines 424-425.

Line 353 – should this say ‘interspecific’ and not ‘intraspecific’?

Both actually (line 422), we are referring to the differences between species but also to the plasticity observed in some species between fellfield and snowbed germination curves, see for example *Iberis carnosa* in supplementary Figure S2.

Line 378- lower total germination – assuming high seed viability, no?

Yes, we have added the clarification in line 451.

Line 395 – change ‘factor’ to ‘effect’

Changed as requested in line 468.

Figure 1c and 1d suggest that experimental temperatures mimicked soil temperature data extremely well.

Yes, we took the daily average soil temperature records for 2008-2019, and transformed into weekly programs, you can check the detailed climatic chamber programs in supplementary table T1.

Line 601 – change ‘target’ species to ‘study’ species

Changed as requested in line 672.

Line 604 – ‘Density plots within each system represents the calculated area between individual species cumulative germination curves’ – I don’t understand this.

Sorry this was a mistake from the caption, it was referring to figure 3, we have removed it.

Line 607 – delete ‘Show’ before ‘cumulative’

Changed as requested line 677.

Fig 3a – add/improve axis titles

Thank you for the suggestion, done

Line 610 – change ‘as’ to ‘in’

Thank you for this observation, we have rewritten this part of the caption.

612 – change ‘species’ to ‘species’’ (i.e. germination shift belonging to each species)

Changed as requested in line 680.

619 – do you mean to say ‘confidence intervals’?

Credible intervals are the Bayesian analog of confidence intervals in frequentist statistics, and the correct term for the MCMCglmms that we used (p.e. Lee, P.M. 1997, Bayesian Statistics: An introduction)

Line 629 – change ‘each removal time’ to ‘each retrieval’ time’ and be consistent throughout

Changed as requested line 699.

Fig 5 – add key to explain stats depicted.

We have modified figure 5.

Fig 5. You use ‘early/late season’ here, and ‘spring/autumn’ in the text. Better to use seasonal names, I think.

Changed as requested figure 5.