Palma de Mallorca, 6th December 2022

Evaluation report

To whom it may concern:

The thesis entitled "Ecology of alpine plants at their uppermost limit in the Himalayas" proposed by the PhD student Mgr. Zuzana Chlumská is suitable to be defend it in order to obtain the PhD degree by the candidate. The results and conclusions are fully covered by the scientific methodology employed. The thesis reads well, being an excellent document about the latest data about high mountain plant ecology. Additionally, it shows novel findings with relevant significance about plants living at the roof of the world and the effects of climatic change in Ladakh mountains (Himalayas). Altogether, the thesis quality is really high as it can be also confirmed by the papers published thanks to the development of this work.

Major questions in relationship with the chapters and results showed in this thesis:

-1-You mention in the page 4 the different abiotic factors that CC is altering: rising temperatures, precipitations, extreme climatic events... permafrost thawing... and page 6 the abiotic factors that drive plant communities at high mountain ecosystems.
Moreover, your thesis is focused to plant´s ecophysiology regarding the NSC (Chapter III and IV directly, but also indirectly the other chapters I and II), that mostly will depend on the photosynthetical capacity and final carbon allocation.

I am curious that most of the literature in the topic do not refer to the increasing CO2 parcial pressure as an extra factor to consider driving plant dynamics (my search was not exhaustive at all, so I could be partially mistake in my impression). CO2 concentration is one of the most well-described and certain factor driven by CC, that strongly affects the whole plant physiology (net photosynthesis, water use efficiency... etc) and thus its fertilizer effect should be taking in mind describing the dynamics of the uppermost plant´s communities in Himalaya?

If so, how the rising CO2 could somehow counteract or show synergies with the other abiotic factors driving plants dynamics at high mountain ecosystems?

-2-Chapter II your results nicely illustrate the limits of the vascular plants. The snowfall event you mention additionally open more information to this point,

could you give us more information about that event? How long was? For how long was frozen the plants? the soil?

How much change this event the previous established limits about soil temperature... etc? Thus, those plants that survive there for 3 years, you think that they collapse why? 1) They do not fulfill the positive carbon balance at the end of the summer season, 2) I do not think that there plants were not acclimated to freezing temperatures (all nights get temperatures below 0ºC) (accumulate sugars, anti-freezing proteins, changes in lipid membrane composition... , thus they should tolerate snow, isnt it? Or do you think they suffer important damages leaded to generalized oxidative stress and thus finally lead to point 1?

Also, it becomes quite curious to me that the literature related to the subject of the vascular limits do not mention the essential role of respiration, a process that is highly sensitive to the temperature. Beyond ATP production also synthetizes the main building blocks for growth and stress tolerance response, of course, non-vascular plants reach further abiotic limits than vascular, and they do respirate, but still it is curious that is a point not too much explored in the high altitude flora?

My point here is that I am curious why in the literature and here in your thesis do not mention the effect of the temperature in one essential physiological process: the respiration. Respiration is highly affected by temperature, and meanwhile plants can live without photosynthesis for really long time, respiration should be sustained even at really really low rates... Thus, if you characterize it that extreme and unfrequent event, how it breaks the previous limits of the vascular plants in terms of soil temperature, or the total positive carbon balance in the season?

-3-Chapter III. I think here what you should demonstrate is that one of the extraction methods is equivalent in the extraction yield than the typical one after freezing the samples. This way you just need to compare each of them respect to the control "freezing" samples, isnt it?
If so, why do not use simple comparison between two treatments as T-students test? Multiple comparisons by Tukey post-hocs do not provide you much more relevant information in this sense, what is the meaning if one of the new treatments is different to other? I think your objective is compared each of them, independently, against the conventional method (freezing), avoiding multiple-comparisons test, you are increasing your freedom degrees and wining statistical power comparing one vs other, isnt it?

In figure 2 the violin plots show the presence of "outliers" of the 95 and 5% of the distribution of the data. In this kind of comparison studies, should be worthy to perform some previous outlier analysis to ensure that if differences

are not found between treatments are not due to "weird-outlier" data (that could be artifactual) out of the main data distribution of each treatment?

-4-Chapter IV-

This chapter opens new challenging scientific questions. Your data demonstrate that at the limit´s of life for vascular plants they are not limited by carbon acquisition, thus it will be expected that the goal of the final annual net carbon positive balance are fulfilled by the end of the growing season. Sugars have several roles as basic carbon storage driving plant metabolism to specific roles regarding low temperature, freezing, drought tolerances ... So, then if photo-assimilates are covered... which one/s are the ecophysiological reasons that limits plant´s life at the highest altitude that you studied?

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