

Response RC1: Alain Vanderpoorten

Dear Alain Vanderpoorten,

We would like to thank you very much for your detailed and constructive feedback on our manuscript *Temperature-driven vapor pressure deficit structures forest bryophyte communities across the landscapes*. We are grateful for your positive assessment of our manuscript and for appreciating the novelty and timing of our work. We will do our best to incorporate your helpful suggestions to make our manuscript clearer and more interesting for the readers.

In the following response, your reviewer's comments are written in standard black font, and our responses are written in blue.

Sincerely, on behalf of our author team,

Anna Růžicková

I found this paper most original and useful as it addresses the very timely question of microclimates on diversity patterns, with a special emphasis on one key parameter: VPD. To give the paper the impact that it deserves and emphasize the relevance of VPD as an important, ecologically meaningful variable, I have two suggestions to make: (i) define VPD and explain, in the Introduction, what its ecological relevance as compared to other microclimatic variables such as T or RH alone (in other words, why would it be important to integrate VPD in ecological studies) and (ii) show that VPD is indeed a better predictor of species richness and composition than T or RH alone. This could be easily done by re-running the analyses, using T and RH as predictors, and showing that the use of VPD results in a higher percent variance of bryophyte richness and composition community explained.

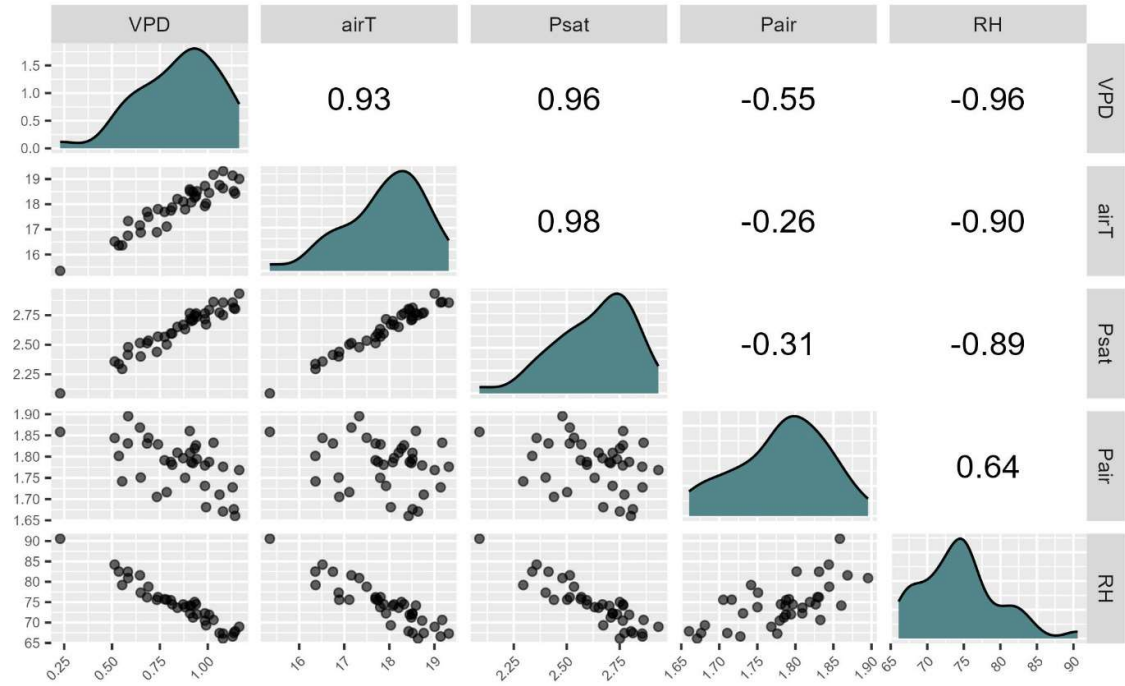
Regarding your first suggestion (i), we agree, and we will add a definition of VPD to the Introduction, as well as an explanation of its ecological relevance compared to other microclimatic variables. Our original aim was to make the Introduction as concise as possible and to focus directly on the VPD. But we agree with the reviewer that it is useful to emphasize and explain in more depth the reasons why it is important to integrate VPD into ecological studies instead of, e.g. the temperature itself or relative humidity.

Regarding the second suggestion (ii) - explanatory effects of VPD compared to T and RH. These three variables are closely correlated in our study area (see correlation matrix below). Therefore, we cannot statistically separate their effects. We agree that this is important information, which was not fully presented in the previous paper version. Therefore, we will add this information to the revised paper. In the paper, we focused directly on VPD, because it is the most physiologically relevant variable (as we discuss on lines 239-255 and 270-287), and because it integrates both temperature and air humidity in a theoretically and physically preferable way (Anderson 1936). However, we acknowledge that it is difficult to separate the effects of these variables with observation data collected on landscape scale and we will expand the discussion of this topic in the revision.

Because of the very tight correlation between VPD, T and RH in our study (see correlation matrix below), using these variables in analyses resulted in similar proportion of explained variability (see table below). Nevertheless, VPD consistently explained more variability in species composition than either T or RH. For species richness, VPD explained more variability than RH, but slightly less than T. However, for reasons given above (e.g. physiological relevance), we prefer to focus on VPD and clearly acknowledge that the VPD is closely related to the temperature within our study region. Of course, it would be interesting to separate effects of T and VPD, but this would require more extensive dataset collected across larger area or preferably experimental approach (as we already discuss on lines 203-209).

Nevertheless, while thinking about this comment from the reviewer, we realized that we should also explore not only the effects of the maximum VPD, but also average VPD. We initially decided to use the maximum VPD, because maximum temperature was the most important microclimatic variable affecting species composition of vascular plants (Macek et al., 2019) as well as bryophytes (Man et al., 2022). However, thanks to the reviewer's second comment (ii), we have renewed the discussion in our author team, and we have decided to reanalyze our data with the average VPD. The main motivation for this change was that the averages better capture the long-term characteristics of site microclimatic conditions, which are likely more important for bryophytes than short-term extremes captured by the maximum VPD (which can however be more relevant for vascular plants as they lack bryophytes unique ability to tolerate desiccation).

Using the average VPD instead of maximum VPD resulted in substantial increase in the explained variation, both in bryophyte community composition as well as species richness (see table below). Therefore, we decided to focus on mean VPD instead of maximum VPD in our revised paper. This change increases the proportion of explained variability in bryophyte species composition and species richness, but did not affect other conclusions about the importance of temperature as the main driver of VPD variability across the landscape.



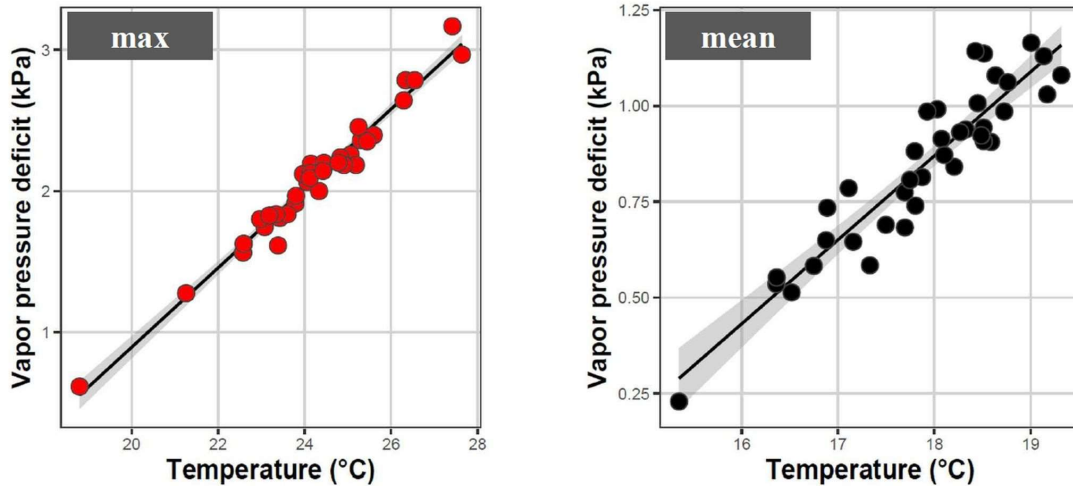
Correlation matrix of used microclimatic variables (VPD – the average daily mean VPD; airT – the average daily mean T ; Psat – the average daily mean P_{sat} ; Pair – the average daily mean P_{air} ; RH – the average daily mean RH).

	Species composition (db-RDA)		Species richness (GAM)
	Sørensen	Simpson	Number of species
VPD max	10.95 % **	13.52 % **	31.2 % ***
VPD mean	16.09 % ***	17.15 % ***	32.8 % ***
T _{air} mean	15.36 ***	13.67 **	36.7 % ***
RH mean	15.09 ***	16.09 ***	27.2 % ***

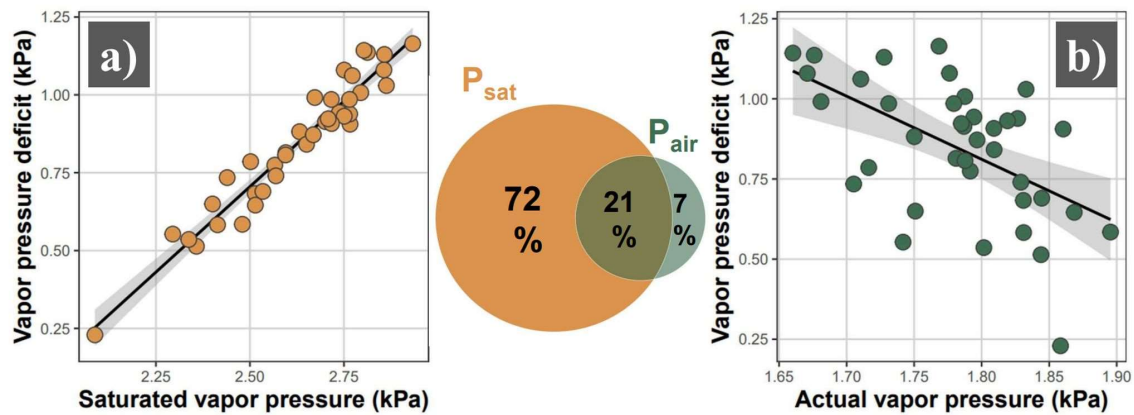
Table with results of db-RDA and GAM models

One of the first results being discussed is the tight relationship between VPD and Tmax-it does not seem to me that this results directly stems out the analyses presented? Could this relationship be actually evidenced based on the data generated?

Yes, based on our data, we have evidenced a close relationship between VPD and T. This result stems from the analyses presented (Results part 3.1 VPD variability, second paragraph). However, it is true that we do not directly report the relationship between VPD and T, but instead the relationship between VPD and saturation vapor pressure (P_{sat}), which is solely a function of temperature (as we stated on line 43 and 109), so the relationship between VPD and P_{sat} also illustrates the relationship between VPD and T. We agree that the result presented in this way, without re-emphasizing the relationship between P_{sat} and T, can be a little bit unclear for the reader not familiar with VPD calculation. Therefore, we will explain this more clearly and add to the Appendix a plot directly showing the relationship between VPD and T (see below). To elucidate the results even further, we will show in the revised Figure 3 results of variation partitioning based on a multiple linear regression model with the average daily mean VPD as the response variable and the average daily mean P_{sat} and P_{air} as the predictors (see updated Figure 3 below).



On the left scatterplot the average daily maximum VPD ~ the average daily maximum T; on the right scatterplot the average daily mean VPD ~ the average daily mean T



Updated Figure 3 shows the relationships between VPD and saturated (a) and actual (b) vapor pressure. Inset Venn diagram shows that the landscape-scale variation in VPD is dominantly controlled by saturated vapor pressure (based on the result from variation partitioning).

In the meantime, if VPD is tightly correlated with T_{max} , does this not slightly undermine the premises of the study, that is, the potential benefit of an integrative variable such as VPD in ecological studies as compared to a ‘simple’ variable like T_{max} ?

While we found here that in the temperate forest landscape, VPD is dominantly driven by saturated vapor pressure (respectively T), and the contribution of actual vapor pressure is minor, the situation at larger spatial scales or near water bodies may be different. Under these conditions, the role of actual vapor pressure may be more prominent, and T alone may no longer be a good proxy of VPD. Therefore, we cannot recommend the use of a “simple” variable such as T instead of VPD. Generally, VPD is more physiologically relevant than T (as we discuss within the manuscript) and even if we found that the T is closely correlated to VPD in our study area, this correlation can change under the different conditions elsewhere. Therefore, we would still recommend measuring VPD directly, but also acknowledge that on landscape and finer scales, gradients in VPD and temperature can be very closely correlated and therefore difficult to distinguish (as we discuss on lines 270-287).

Since the second main result discussed is that it is possible to estimate VPD from local T measurements with HR measured as nearby weather stations, I suggest moving the content of this appendix into the result section of the main text. Would that mean that one can efficiently characterize microclimates using temperature sensors only, which are much cheaper than sensors combining T +HR?

We considered including the estimation of VPD from local T measurements with air humidity measured at a nearby weather station in the main results, but we decided not to do it because this is more of an application of our results rather than a hypothesis we have had at the beginning of our research. For this reason, we would prefer to leave this section in the Appendix. However, we are prepared to move these results into the main text, if the editor prefers to do so.

The answer to the second part of your question, whether that would mean that one can efficiently characterize microclimates using temperature sensors, is partly discussed in our response to your previous question about using simple variables like T instead of VPD. Our approach to the VPD estimation can indeed be useful in cases where air humidity measurements are not available, but this should be done carefully and should be more widely tested across different biotopes and spatial scales. Moreover, as we stated in the Appendix (lines 317-320), this approach does not provide a reliable estimate of

absolute local atmospheric VPD, but rather the relative position on the VPD gradient. So, we would not recommend characterizing microclimate using temperature sensors only.

I was a bit surprised by the relatively limited contribution of VPD (about 11%) to variation in species composition among plots, whereas the introduction rightly emphasizes that in poikilohydric organisms like bryophytes, one could expect VPD to be a prime factor driving community composition. Looking at Fig1, one would think that communities at the top of a cliff would be very different from those in buffered conditions. I wonder whether this could be due to the fact that, as Fig1 suggests, this is a very rugged terrain, and that there is hence a huge (intra-plot) micro-habitat variation that is actually more important than (inter-plot) microclimatic variation. More information on the sampling sites would be welcome to understand the spatial heterogeneity potentially present in the sampling plots.

Compositional variation explained by a multivariate analysis depends on a dataset internal heterogeneity and therefore it is not comparable among datasets (Økland, 1999). Especially in ecological studies covering different vegetation types with limited overlap of the species composition between plots (as in our case), the percentage of the compositional variation explained by one variable is typically in similar range (Økland, 1999). To put the percentage of the explained variation further into the perspective, the best theoretically possible variable can explain max. of 30 % (Sørensen dissimilarity), respectively 54 % (Simpson dissimilarity) of compositional variation in our dataset (for methodological explanation see e.g. Macek et al. 2019). We decided not to complicate the paper with the reporting of this maximum explainable compositional variability which will require additional description of the methods used. However, we are ready to add this information into the revised paper if the Editor think it will be useful for better understanding of our results.

Moreover, motivated by the reviewer comment about the relevance of maximum VPD, we recalculated our results with average VPD instead of maximum VPD, which resulted in the substantial increase in the compositional variation explained by our analyses (db-RDA with average VPD: 16 % explained (Sørensen dissimilarity), 17 % explained (Simpson dissimilarity)). We are therefore confident that these results support our conclusion that atmospheric VPD is an important driver of bryophyte species composition across the studied landscape.

It would help the reader if it was reminded in the Result section based on which analysis each result was obtained. For example, the variation partitioning and db-RDA are not mentioned in the Result section, and mentioning them would help the reader making a link between the M&Ms and results. For example, I am not sure which analysis was implemented to reach the result described L179-180 ('ecological relevance of VPD as compared to HR alone').

In the results, we will clearly state which results are based on which analysis. Specifically, the results presented on lines 179-180 were obtained by the *envfit* function from the *vegan* R package with 999 random permutations (as described on lines 141-143).

In the discussion, it would be interesting to add a section explaining what could be the factors accounting for the spatial variation of VPD reported, and why Pair exhibits such a comparatively narrow range of variation. At present the discussion falls a bit short—especially since the entire §4.2 (from L239 onwards) actually belongs to the Introduction (why bryophytes would be sensitive indicators of VPD variation), and not to the Discussion as it does not help interpreting the results presented.

Thank you for this suggestion. We agree that section 4.2 in the Discussion belongs more to the Introduction. Therefore, we will move this section to the Introduction. Regarding the factors accounting for the spatial variation of VPD, we already discussed them in section 4.1 of the Discussion (lines 223-230). However, in the revised paper, we will expand this discussion even further.

References

Anderson, D. B.: Relative humidity or vapor pressure deficit, *Ecology*, 17(2), 277–282, <https://doi.org/10.2307/1931468>, 1936.

Macek, M., Kopecký, M., and Wild, J.: Maximum air temperature controlled by landscape topography affects plant species composition in temperate forests, *Landscape Ecol.*, 34, 2541–2556, <https://doi.org/10.1007/s10980-019-00903-x>, 2019.

Man, M., Wild, J., Macek, M., and Kopecký, M.: Can high-resolution topography and forest canopy structure substitute microclimate measurements? Bryophytes say no., *Sci. Total Environ.*, 821, 153377, <https://doi.org/10.1016/j.scitotenv.2022.153377>, 2022.

Økland, R.H.: On the variation explained by ordination and constrained ordination axes, *J. Veg. Sci.*, 10, 131–136, <https://doi.org/10.2307/3237168>, 1999.