Review PhD-Thesis Eliska Vicherova by Dr. Annemieke Kooijman

The candidate Eliska Vicherova wrote a very nice thesis on the ecology of peatland bryophytes, a subject very dear to me, and I would like to congratulate her with this result.

The candidate worked on the shift in dominance from brownmosses to *Sphagnum* species, associated with the transition from mineral-rich to poor fens. Mineral-rich fens belong to the most species-rich habitats within the European Union, but are highly threatened in many countries, among which the Netherlands. The bryophyte community forms an important part of these alkaline mires, and the transition from brownmoss to *Sphagnum* species has been studied in the past, including by myself. However, the candidate focussed on topics overlooked or unresolved, such as calcium toxicity and tolerance of fen mosses, and communication between moss species.

The thesis is well written, and not only contains a nice introduction and general conclusion, but also four scientific papers, which mostly have already been published or accepted. The first paper consists of a series of cultivation experiments with 15 fen mosses, which together showed that calcium toxicity in *Sphagnum* spp. is an important mechanism to protect rich fens against invading *Sphagnum* species, as long as Ca levels and pH values remain high. The candidate tested this in the field in the second paper, in a four-year transplantation experiment in 8 different fens. This experiment showed that high Ca was indeed toxic for *Sphagnum* species, but that they could survive on hummocks with low Ca levels, especially in more humid climates. In the third paper, the candidate again showed that high Ca levels were not a problem for brownmosses, but may lead to high and toxic intracellular Ca concentrations in *Sphagnum* species, presumably because they have problems to get it out again. The fourth paper is also very nice, and shows that mosses can communicate with each other through volatile organic compounds, and brownmosses may be warned in the presence of *Sphagnum* species to grow a bit higher, to reduce the risk of being overgrown.

The thesis is a consistent piece of work, and I really enjoyed reading it. But I have been invited as opponent in this thesis defence, so I have some questions and points for discussion. The first questions are about methods and interpretation of the results in particular chapters, and the last about how the new knowledge acquired in the thesis might help us in the protection and restoration of rich fens.

Question 1: In the introduction (page 2), the candidate gives an overview of ombrotrophic and minerotrophic peatland habitats, with Ca levels below 5 mg/L in bogs and poor fens, and above 30 mg/L in rich and extremely rich fens. However, the intermediate fen, with Ca levels between 5-30 mg/L between 5.5-7, is not mentioned, nor treated in the cultivation experiments of chapter 2 and 4. In my experience, this is the stage in which calcicole and calcifuge species co-exist and the shift from brownmoss to *Sphagnum* dominance is a real threat. Why was the intermediate fen stage left out in the experiments?

Question 2: In chapter 2, the plants were mainly grown submerged (page 29-31). However, all brownmoss and *Sphagnum* species used in the experiments usually grow above the water table in the field, and in the experiments indeed grew better when allowed to do so. How representative are the culture experiments then for the actual field situation? I ask this especially since species that do grow submerged, such as Scorpidium scorpioides and Sphagnum cuspidatum, were used in chapter 4.

Kooyman

Question 3: In chapter 2, in the results (page 38), it is stated that *Sphagna* were limited by high Ca and high pH, while brownmosses were not, which points to calcium toxicity in calcifuge species. However, it was also mentioned that bicarbonate plays a specific role. Without bicarbonate, *Sphagna* were able to grow in Ca-rich water, and extend their fundamental niche. Other studies (Koks et al. 2019) claim that bicarbonate is even more important than Ca. If bicarbonate is really more important than Ca, would that change the ideas about the importance of calcium toxicity to keep *Sphagnum* species away from rich fens?

Question 4: I really like the field experiment in chapter 3, and figure 3 clearly suggests that *Sphagnum* species showed higher survival in humid than in drier fens (page 124). But was this the case in all humid fen sites? In fig S3 (page 148), *Sphagnum* expansion was much higher in Zlata buka and Reka than in Liptovska Tepicka 1 and 2, and the latter did not differ from the drier sites. Also, is higher survival in the humid sites really the effect of climate, or do differences in parent material also play a role? The humid sites are also sites with more acidic parent material and approximately 2 and 1.7 times lower values for lower and upper Ca levels than in the drier sites, and Sphagnum already occurring in two of them.

Question 5: How can the new knowledge acquired in this thesis be used to preserve rich fens in the Czech Republic and Slovakia?

Question 6: As mentioned in the thesis, *Hamatocaulis vernicosus* is an EU-habitat directive species, and thus very important in rich fen conservation. In Sweden and the Netherlands, we found it mainly in rich fens with Fe-rich groundwater (Mettrop et al. 2018). The experiment in chapter 2 with different levels of Fe showed that *H. vernicosus* is indeed tolerant to high Fe (page 86). However, in the Netherlands groundwater levels have dropped, and in many places do not reach the surface any longer. What should we do? Let the water levels drop, and assume that there is enough Fe in the soil to maintain favourable habitat conditions for the species, or increase the water level with Fe-poor surface water?

Question 7: In the Netherlands, *Sphagnum* species (such as *S. flexuosum/fallax and S. palustre*) are now dominating many former rich fens. We are trying to restore rich fens by flooding them from time to time with mineral-rich surface water with Ca concentrations of 60 mg/L. Would this be enough to kill the *Sphagna* in a country like the Netherlands? A problem is that our surface water comes from agricultural polders and is often polluted with N, P and K. Can we restore rich fens with this nutrient-rich water? We can also hydrologically isolate the fens, and keep out the polluted water. However, this may lead to dilution with rain water, and reduced Ca concentrations in the surface water of 25-30 mg/L. What should we do?

Question 8: In restoration of former rich fens, some Dutch nature managers apply liming with solid calcium carbonate, to restore buffer capacity of the peat soil, and we just started a project to evaluate this method. Could this be a good idea to get rid of the *Sphagna*?

Koks, A.H.W., Van Dijk, G., Smolders, A.J.P., Lamers, L.P.M. and Fritz, C. (2019). The effects of alkalinity and cations on the vitality of *Sphagnum palustre* L. Mires and Peat 24, Article 25, 1–14.

Mettrop, I.S., Neijmeijer, T., Cusell, C., Lamers, L.P.M., Hedenäs, L. and Kooijman, A.M. (2018). Calcium and iron as key drivers on brown moss composition through differential effects on phosphorus availability. Journal of Bryology 40, 350-357.