Effect of phosphorus and moisture conditions on the growth and chlorophyll fluorescence of *Sphagnum magellanicum*

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Summary

We studied the effect of P and water level (WL) on the growth of *Sphagnum magellanicum*. In the laboratory experiment the addition of P suppressed the linear growth but enhanced the lateral growth of *S. magellanicum*. The field WL affected the growth of *Sphagnum* in the laboratory. In the field experiment the effect of P upon the growth and chlorophyll (Chl) fluorescence of *Sphagnum* was expressed through the interaction with microsite peculiarities. Neither P nor N was among the primary limiting factors for *Sphagnum* growth. Instead, certain adaptations of *Sphagnum* to habitat conditions turned out to be important.

Key index words: F_v/F_m, Growth, Phosphorus, *Sphagnum magellanicum*, Water level

Introduction

Phosphorus and nitrogen are considered to be limiting elements for *Sphagnum* growth. As the nutrient supply to an ombrotrophic bog is exclusively through precipitation, the *Sphagnum* mosses are able to compensate the insufficiency of nutrients by their high cation-exchange and water-holding capacity. As *Sphagnum* species lack inward specialization to regulate the water movement actively, the transport of water and nutrients to the capitulum depends on the diameter of the capillary spaces. The capillary network is formed by pendent branches which hang down against the stem making possible the vertical transport to the capitulum, in other words, evapotranspiration caused by the water movement (Hayward and Clymo, 1982). The higher on hummocks *Sphagnum* grows, the tighter should *Sphagnum* plants be packed, so that capillary spaces would be smaller. It has been shown that *S. magellanicum* is not heavily dependent on water level (WL) depth and its tolerance in desiccation situations may be explained by its morphological plasticity (Grosvernier *et al.*, 1997). Additionally, *S. magellanicum* improves the capillary structure and water transport by building a tight canopy in very dry situations (Li *et al.*, 1992).

The growth rates of *Sphagnum* are species-specific and depend on the position on the hollow-hummock gradient. Hollow species (*S. fallax*, *S. cuspidatum*) usually outproduce hummock species (*S. magellanicum*, *S. papillosum*) (Pedersen, 1975). The latter are considered to be more tolerant to their growth habitat, which enables them to form their own habitat – hummock. The differences in growth are considered to be related to the chlorophyll content in plants. The species that grow in hollows more loosely can absorb light with the larger plant surface (Clymo, 1970). Further, hollow species also lack metabolically expensive desiccation tolerance mechanisms, but the ability to maintain positive turgor and photosynthesis down to low water contents may optimize their growth rates (Rydin *et al.*, 2006).

Chlorophyll (Chl) fluorescence in bryophytes is measured predominantly to study desiccation tolerance (Deltoro *et al.*, 1998). It also gives information about the responses to other environmental stressors and about changes in photosynthetic efficiency through time (Maxwell, Johnson, 2000). Photosynthetic activity in mosses decreases as the water content declines below the optimum for assimilation. Being constantly water saturated, desiccation tolerance mechanisms are not developed in *Sphagnum* species as effectively as they may be in other mosses (Deltoro *et al.*, 1998).

Materials and Methods

In 2006 two experiments with *Sphagnum magellanicum* were carried out, in laboratory and under field conditions for 12 and 18 weeks, respectively. Samples for laboratory experiment, collected from Kõrgemäe bog in North Estonia (58°57'51"; 25°08'41"), were categorized into two groups based on the original water level (WL) of the microsite at sampling: high (8–20 cm) and low (22–42 cm) WL. The main variable in the laboratory experiment was the N:P ratio. Modified Rudolph's nutrient solution (Rudolph and Voigt, 1986) at 4 levels was added every second day. The N:P ratios were 8, 15 and 30, plus control without P. The field experiment was carried out in an open hollow-hummock complex on the intact part of Rabivere bog (59°05'44"; 24°45'25") in North Estonia. The experiment

lasted from June to the end of October. Solutions with different concentrations of NaH_2PO_4 (0.5 g NaH_2PO_4 l^{-1}), plus control with only distilled water, were sprayed on *S. magellanicum* at two-week intervals. NaH_2PO_4 was added to 400 ml distilled water as 0.25, 0.50 and 1.00 kg P ha^{-1} a^{-1}. Each solution level was marked on every 4 replicates. The water level was measured 7 times during the experiment using perforated tubes. For growth measurements marked wires were used in the field and ca 10 *Sphagnum* plants at every treatment level on six replicates were painted in the laboratory experiment.

Chl fluorescence was measured at the start, in the middle and at the end of the experiment with portable Handy PEA fluorometer (Hansatech Instruments Ltd.). Dark-adaptation time of 7 min was used before every measurement at light intensities of 500–3000 µmol m^{-2} s^{-1}. The stress situation in *S. magellanicum* was measured by the parameter F_v/F_m. Here F_m indicates the fluorescence level when the plastoquinone electron acceptor pool is transiently fully reduced and $F_v = (F_m-F_0)$, where F_0 is the fluorescence level when the plastoquinone electron acceptor pool is fully oxidized. For dark-adaptation measurements leaf clips were used. The parameter F_v/F_m is independent of the leaf area and measurements do not require the whole of the illuminated area of the clip to be filled with the sample (Handy PEA Help). We analysed 3 samples for every treatment level on every replicate.

In laboratory analyses the topmost 3 cm long apical parts of *Sphagnum* plants were used. The samples were divided into two sections: the photosynthetically active topmost part, capitulum (1 cm) and the following 2-cm-long stem section. The dry weight (d.w.) was found (48 h, 70°C). The contents of N and P (% in dry matter, wet ash method) were determined in the Estonian University of Life Sciences. The analyses were performed using the program STATISTICA 4.0.

Results

The average increment of *Sphagnum* in the laboratory experiment was 1.4 mm and stem production was 0.20 g dm^{-2} d.w. The linear growth of *Sphagnum* was negatively correlated with the content of N and P in the capitulum (R=–0.34, R=–0.35). The pre-experiment N:P ratio was possibly close to the optimum for *S. magellanicum* as the N:P ratio correlated well with the P content at the end of the experiment and the increase in the P content suppressed the growth. The content of both P and N in the *Sphagnum* capitulum increased during the experiment (table 1). The values of F_v/F_m were low at the beginning of the experiment but increased near the optimal level at the end of the experiment. The original WL (when samples were collected) was included as one more parameter into the statistical analysis. ANOVA showed that the original WL was an important factor affecting the *Sphagnum* increment, production and Chl fluorescence (table 2). The lower was the original WL, the higher was the linear growth and production of *Sphagnum* in the experiment (R=0.44 and R=0.55, respectively).

In the field experiment the average increment and production were 1.7 mm and 0.29 g dm^{-2} d.w., respectively. During the experiment the N:P ratio, dry weight of the capitulum and density changed notably. The N:P ratio correlated negatively with the capitulum dry weight (R=-0.44). P treatment had no direct effect on the linear growth of *Sphagnum* (R=-0.08) and F_v/F_m (R=0.20). ANOVA showed that *Sphagnum* increment and Chl fluorescence responded to the characteristics of the microsite and average WL (table 3).

P affected the growth and Chl fluorescence of *Sphagnum* not as a single factor but through the interaction with microsite peculiarities. It was especially well expressed on microsite number 3 where the growth rate of *Sphagnum* was lowest (Fig. 1).

Table 1. Parameters of *S. magellanicum* before and after the laboratory experiment

Variables	Pre-experiment	After experiment
Density (ind.dm^{-2})	188 ±61	82 ±20
Dry weight of capitulum (mg)	12.4 ±5.8	26.5 ±7.7
N (% d.w.) of capitula	0.716 ±0.106	1.004 ±0.227
P (% d.w.) of capitula	0.092 ±0.024	0.163 ±0.048
N:P of capitula	8.2 ±2.0	6.5 ±1.7
F_v/F_m	0.181 ±0.086	0.682 ±0.031

Table 2. One-way ANOVA F and P statistics (n=42) for the effect of the pre-experiment water table in the bog on *S. magellanicum* growth and Chl fluorescence, F_v/F_m before (A) and after (B) the experiment

Variable	F	p
Average increment (mm)	9.39	0.0039
Production (g dm^{-2})	18.00	0.0001
Highest yield of PSII of F_v/F_m (A)	0.45	0.5077
Highest yield of PSII of F_v/F_m (B)	5.97	0.0191

Table 3. ANOVA F and P statistics (n=24) for effects of the microsite and WL (p<0.05)

Variable	F	p
Average length increment (mm)	5.81	0.0019
Production (g dm^{-2})	8.17	0.0003
Initial dry weight of cap (mg)	4.03	0.0107
Final dry weight of cap (mg)	1.95	0.1298
Highest yield of PSII of F_v/F_m, July	5.79	0.0019
Highest yield of PSII of F_v/F_m, September	4.46	0.0069
Highest yield of PSII of F_v/F_m, October	11.56	0.0000

Discussion

The average increment and production were quite low in both laboratory and field experiments as these lasted only 12 and 18 weeks, respectively. Still, the density of *Sphagnum* decreased and the dry mass of the capitulum increased significantly. Therefore, it may be concluded that the limitation of P, which was expressed as the increase in P content, did not influence the linear growth of *Sphagnum* but promoted significantly (through the decrease in the N:P ratio) the lateral growth in drought conditions.

The N:P ratio in plant material is a good indicator for primary element limitation. An N:P ratio over 16 indicates P limitation and an N:P ratio less than 14 indicates that N is a limiting nutrient (Verhoeven *et al.*, 1996). In our laboratory experiment this ratio was less than 10 and, consequently, should indicate a limiting effect of N for *Sphagnum*. Therefore, the addition of P should have, and did have in our experiment a suppressing effect upon the linear vertical growth of the *Sphagnum*. However, the increase in the capitulum dry mass correlated negatively with the N:P ratio. This indicates that the higher concentration of P in the topmost part of *Sphagnum* suppressed the linear growth but at the same time stimulated the lateral growth of *S. magellanicum*.

The value of F_v/F_m for the vascular plants approaches under optimum conditions the level of about 0.83; a lower value indicates the inhibition of PSII (Maxwell and Johnson, 2000). As a rule, bryophytes show lower values (Smith, 2002). At the beginning of the laboratory experiment very low values indicate a very intense stress. Stress factors might be related to low temperature and light intensity at the acclimation period of 4 weeks before the experiment. As the samples used in the laboratory experiment were categorized into two groups based on the original WL of the microsite during sampling, we used this parameter as an additional factor in statistical analysis. At the end of the experiment F_v/F_m correlated positively with the original WL: the lower the WL was at the microsite, the higher was the Chl fluorescence of *Sphagnum*. Consequently, neither P nor N was among primary limiting factors for *Sphagnum* growth at the end of the experiment. Instead, adaptation of *Sphagnum* to certain specific habitat conditions, masked by other stress factors, became the primary one. The original WL was an important factor affecting the linear growth of *Sphagnum*, production of its stems and F_v/F_m at the end of the experiment. However, the WL in laboratory conditions was constantly about 5 cm below capitula. Therefore, as the Chl fluorescence of

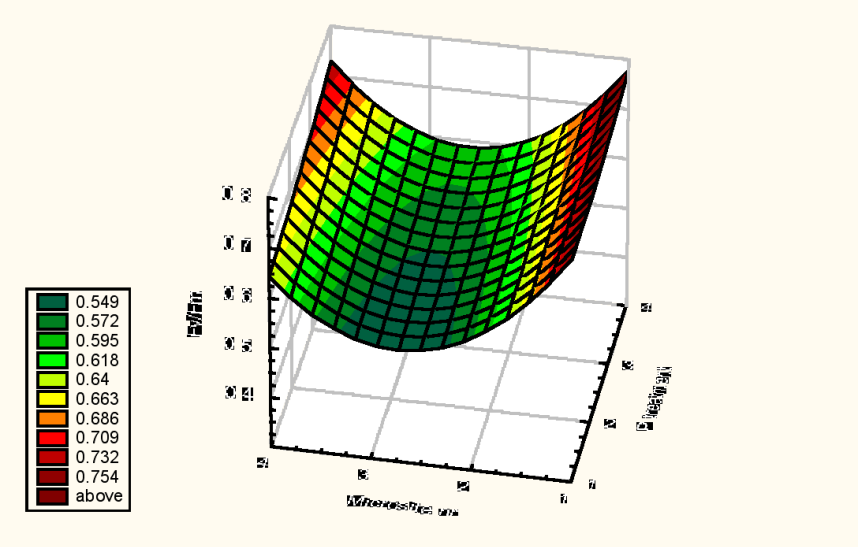

Figure 1. The effect of P treatment and the microsite on the Chl fluorescence (F_v/F_m) of *S. magellanicum* (October 2006).

Sphagnum rose to a near-optimum level at the end of the experiment, the WL should have been one of the limiting factors in the field conditions. It means that the moss samples collected from the microsites with a lower WL benefited more from the constantly higher WL than the samples gathered from microsites with a high WL.

In the field the Chl fluorescence changed only little during the course of the experiment and was not significantly correlated with the concentration of added solutions or the P content in the capitulum. However, the moss increment and Chl fluorescence values differed largely between the sites. The greatest differences were noted in the values of microsite number 3. One-way ANOVA showed the importance of the interaction of the microsite and WL in the increment, production and Chl fluorescence of *S. magellanicum*. As the microsite peculiarities became an important factor also in the P treatment, it may be concluded that the effect of the treatment may be strongly influenced by the visually undistinguishable characteristics of the microsite. In our experiments the interaction between the microsite and WL became the main factor affecting the growth and Chl fluorescence of *S. magellanicum*.

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References

Clymo, R.S. (1970). The growth of *Sphagnum*: methods of measurement. *Journal of Ecology* **58**, 1, 13-49.

Deltoro, V.I., Calatayud, A., Gimeno, C., Barreno, E. (1998). Water relations, chlorophyll fluorescence, and membrane permeability during desiccation in bryophytes from xeric, mesic, and hydric environments. *Canadian Journal of Botany* **76**, 1923-1929.

Grosvernier, P., Matthey, Y., Buttler, A. (1997). Growth potential of three *Sphagnum* species in relation to water table level and peat properties with implications for their restoration in cut-over bogs. *Journal of Applied Ecology* **34**, 471-483.

Handy PEA Help Documentation. (14th May 2002). Version 1.30.

Hayward, P.M., Clymo, R.S. (1982). Profiles of water content and pore size in *Sphagnum* and peat, and their relation to peat bog ecology. *Proceedings of the Royal Society of London Series B* **215**, 299-325.

Li, Y., Glime, J.M., Liao, C. (1992). Responses of two interacting *Sphagnum* species to water level. *Journal of Bryology* **17**, 59-70.

Maxwell, K., Johnson, G.N. (2000). Chlorophyll fluorescence- a practical guide. *Journal of Experimental Botany* **51**, 345, 659-668.

Pedersen, A. (1975). Growth measurements of five *Sphagnum* species in south Norway. *Norwegian Journal of Botany* **22**: 277-284.

Rudolph, H.,Voigt, J.U. (1986). Effects of NH_4-N and NO_3-N on growth and metabolism of *Sphagnum magellanicum*. *Physiologia Plantarum* **66**, 339-343.

Rydin, H, Gunnarsson, U., Sundberg, S. (2006). The role of *Sphagnum* in peatland development and persistence. In: Vitt, DH., Wieder, RK., eds. *Boreal peatland ecosystems*. Ecological Studies, Vol. **188**. Berlin: Springer-Verlag, 49-65.

Smith, E.C. (2002). *In vivo* analysis of rapid chlorophyll flurescence inducyion effects: aspects relating to the study of bryophytes. *Journal of Bryology* **24**, 17-23.

Verhoeven, J.T.A., Koerselman, W., Meuleman, A.F.M. (1996). Nitrogen- or phosphorous- limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. *TREE* **11**, 12, 494-497.