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Potential for fast chlorophyll *a* fluorescence measurement in bryophyte ecophysiology

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Abstract. The aim of the study was to analyse if the measurement of fast fluorescence induction kinetics in bryophyte samples in field conditions could be used for characterizing the photochemistry of photosynthesis in bryophytes. Bryophyte samples were collected in five different habitats of the boreo-nemoral zone growing on various substrates. Twenty-four species were epigeic, six epilithic, ten epiphytic, three epixylic, and six semi-aquatic or aquatic. Extremely high variation was found for fluorescence parameters between bryophyte samples. Performance Index showed the highest variability, reaching 160% in the case of epiphytic bryophytes. There were statistically significant differences for mean values of F_v/F_m, RC/ABS, and F_v/F_0 between epigeic and epiphytic bryophyte samples as well as between epiphytic and semi-aquatic & aquatic samples. For Performance Index, a significant difference was observed only between epiphytic and epigeic bryophytes. It was concluded that bryophytes display a low intensity of the photochemistry of photosynthesis even in relatively wet habitats. In general, measurement of fast fluorescence induction kinetics in field conditions could be a rapid and efficient tool to obtain quantitative data useful for ecophysiological studies.

Key words: boreo-nemoral zone, bryophyte species, chlorophyll *a* fluorescence, habitats, photochemistry of photosynthesis, photosystem II, substrates.

INTRODUCTION

Local distribution of species is considered to be affected by the degree of their physiological tolerance to particular range of environmental conditions (Cleavitt, 2002). Bryophytes represent a group of non-vascular green plants whose species richness considerably increases in cool and wet environments. In contrast to vascular plants relying on a specialized system of interorgan water transfer, bryophytes are mostly poikilohydric plants with water content dependence on environmental conditions (Glime, 2007). In addition, their tissue water holding capacity is poor with almost no regulation by internal structures. These ectohydric mosses use external surface water transport by capillary action, and dry plants have a high rehydration rate reaching recovery within minutes when water becomes available. Most of the water in ectohydric mosses is extracellular and, therefore,

does not affect the water status of cells (Proctor, 2000). In contrast, endohydric mosses possess an internal water conducting system hydrome, which resembles xylem. Endohydric mosses occur on loose substrate, e.g. mineral soil or decaying wood but never on stones or tree bark like epiphytes. However, most mosses could be myxohydric, relying on both external and internal mechanisms of water conduction, though to a various degree (Glime, 2007).

In respect to physiological adaptation to environmental conditions, bryophytes are regarded as typical shade-adapted plants with a high desiccation tolerance. However, desiccation tolerance is reported to be lower in bryophyte species from mesic habitats in comparison to xeric ones (Robinson et al., 2000). A species' desiccation tolerance could have an impact on its growth and distribution. Intensive growth needs prolonged periods of metabolic activity, which is only supported in the hydrated state. Therefore, only bryophyte species with a relatively high desiccation tolerance could have high rates of productivity (Bates, 1997). Even one 24 h desiccation–rehydration cycle on a weekly basis within seven weeks significantly decreases the relative growth rate of bryophytes in controlled conditions (Bates, 1997).

Bryophyte diversity in boreo-nemoral forests is potentially high. While habitats in the boreo-nemoral zone are generally described as rich in moisture (Diekmann, 1994), bryophytes within the same habitat growing on various substrates could exhibit significant variations in water availability due to microenvironmental differences. It appears that even in relatively moist habitats bryophytes are exposed to significant fluctuations of moisture on a weekly or even a daily basis. Consequently, depending on the substrate (tree, decaying wood, stones, soil) as well as adaptation to hold water in tissues, bryophyte species have to persist a considerable period of time in a relatively dry state. As a result, the growth rate and colonization potential between and within bryophyte species can be highly variable, possibly affecting species distribution.

Measurement of chlorophyll *a* fluorescence has been used in bryophyte studies mostly for desiccation tolerance analysis (Deltoro et al., 1998; Maseyk et al., 1999; Proctor & Smirnoff, 2000; Robinson et al., 2000; Cleavitt, 2002; Proctor, 2003; Proctor et al., 2007; Hájek & Beckett, 2008; Hooijmaijers, 2008; Lüttge et al., 2008; Cruz de Carvalho et al., 2011), including photoprotection during rehydration (Beckett et al., 2005). Recently chlorophyll fluorescence analysis has been used to study photosynthetic response to variability of light and moisture (Cui et al., 2009; Hájek et al., 2009), nitrogen deposition (Granath et al., 2009), growth rate (Laine et al., 2011), as well as salinity tolerance (Garbary et al., 2008; Bates et al., 2009). So far, fluorescence measurements have not been performed in natural habitats with several bryophyte species differing in putative adaptation strategies. In addition, mostly a method of pulse-amplitude modulated analysis has been used. Measuring fluorescence emission by modulated fluorescence by means of the saturation pulse method allows quenching analysis of absorbed light energy (Misra et al., 2012). As an alternative, a continuous chlorophyll *a* fluorescence measurement method allows for a rapid on-site screening of a relatively large number of samples. The method uses fast fluorescence induction kinetics to

derive a number of parameters characterizing energy fluxes as well as the overall photochemical performance of photosystem II (PSII) in the measured samples (Strasser et al., 2004). Recently the method has been used in a number of ecophysiological studies including rare and protected plant species in natural habitats where the use of nondestructive and noninvasive instrumental methods has a great advantage (Thach et al., 2007; Jung et al., 2009; Samsone et al., 2009; Zubek et al., 2009; Albert et al., 2011; Andersone et al., 2011).

The aim of the present study was to measure fast fluorescence induction kinetics in a large number of bryophyte samples growing on various substrates in field conditions. It was hypothesized that analysis of the photochemistry of photosynthesis can be used to obtain quantitative traits for ecological studies of bryophytes from the boreo-nemoral zone.

MATERIALS AND METHODS

Bryophyte samples were collected at the end of April before the bud burst of deciduous trees. Five sites (20 m × 50 m) on different habitats (H 1, deciduous forest on slopes of a river with *Tilia cordata*, *Acer platanoides*, and *Ulmus laevis*; H 2, ravine with a stream with *Alnus incana* and *Ulmus laevis*; H 3, mixed wet forest with *Picea abies*, *Quercus robur*, *Betula pubescens*, and *Alnus glutinosa*; H 4, pine forest with *Pinus sylvestris*; H 5, peat bog with *Pinus sylvestris*) located not more than 20 km apart in the central part of Latvia near Riga were searched for the presence of bryophyte species and all unique species for the respective habitat were collected. No precipitation was observed in sampling sites for at least 10 days. Several samples of the same species within the respective habitat were taken if they exhibited an apparently different moisture content. In total, 76 samples including 46 bryophyte species were collected (Table 1). Two species were found on different substrates: *Dicranum scoparium* on decaying wood and

Table 1. Occurrence of bryophyte species analysed in the present study in different habitats: H 1, deciduous forest on slopes of a river with *Tilia cordata*, *Acer platanoides*, and *Ulmus laevis*; H 2, ravine with a stream with *Alnus incana* and *Ulmus laevis*; H 3, mixed wet forest with *Picea abies*, *Quercus robur*, *Betula pubescens*, and *Alnus glutinosa*; H 4, pine forest with *Pinus sylvestris*; H 5, peat bog with *Pinus sylvestris*

Species	Substrate type	H 1	H 2	H 3	H 4	H 5
Sphagnum cuspidatum Ehrh. ex Hoffm.	Aquatic					+
Sphagnum riparium Ångström	Aquatic					+
Atrichum undulatum (Schleich. ex Brid.) Hartm.	Epigeic			+		
Aulacomnium palustre (Hedw.) Schwaegr.	Epigeic					+
Brachythecium glareosum (Bruch ex Spruce) Schimp.	Epigeic	+				
Ceratodon purpureus (Hedw.) Brid.	Epigeic				+	

Continued overleaf

Table 1. *Continued*

Species	Substrate type	H 1	H 2	H 3	H 4	H 5
Climacium dendroides (Hedw.) F. Weber & D. Mohr	Epigeic			+		
Conocephalum conicum (L.) Lindb.	Epigeic		+			
Dicranum polysetum Sw.	Epigeic				+	
Dicranum scoparium Hedw.	Epigeic				+	
Encalypta sreptocarpa Hedw.	Epigeic	+				
Eurhynchium angustirete (Broth.) T. J. Kop.	Epigeic			+		
Fissidens adianthoides Hedw.	Epigeic			+		
Funaria hygrometrica Hedw.	Epigeic		+			
Hylocomium splendens (Hedw.) B., S. et G.	Epigeic			+		
Oxyrrhynchium hians (Hedw.) Loeske	Epigeic	+				
Plagiomnium affine (Blandow ex Funck) T. J. Kop.	Epigeic		+			
Plagiomnium cuspidatum (Hedw.) T. J. Kop.	Epigeic			+		
Plagiomnium undulatum (Hedw.) T. J. Kop.	Epigeic		+			
Pleurozium schreberi (Brid.) Mitt.	Epigeic				+	
Pohlia nutans (Hedw.) Lindb.	Epigeic	+	+			
Polytrichum juniperinum Hedw.	Epigeic				+	+
Rhytidiadelphus triquetrus (Hedw.) Warnst.	Epigeic				+	
Sphagnum capillifolium (Ehrh.) Hedw.	Epigeic					+
Thuidium delicatulum (Hedw.) Schimp.	Epigeic	+				
Thuidium philibertii Limpr.	Epigeic				+	
Amblystegium fluviatile (Hedw.) Schimp.	Epilithic		+			
Anomodon longifolius (Schleich. ex Brid.) Hartm.	Epilithic		+			
Brachythecium rivulare Schimp.	Epilithic		+			
Cratoneuron filicinum (Hedw.) Spruce	Epilithic		+			
Hypnum cupressiforme Hedw.	Epilithic		+			
Schistidium apocarpum (Hedw.) Bruch & Schimp.	Epilithic		+			
Brachythecium rutabulum (Hedw.) B., S. et G.	Epiphytic		+			
Homalia trichomanoides (Hedw.) Schimp.	Epiphytic	+				
Hypnum cupressiforme Hedw.	Epiphytic	+	+	+	+	
Jubula complanata (L.) Corda	Epiphytic	+				
Leucodon sciuroides (Hedw.) Schwägr.	Epiphytic	+				
Neckera pennata Hedw.	Epiphytic	+				
Orthotrichum affine Schrad. ex Brid.	Epiphytic	+				
Pylaisiella polyantha (Hedw.) Grout	Epiphytic	+				
Chiloscyphus profundus (Nees) J. J. Engel & R. M. Schust.	Epixylic			+		
Dicranum scoparium Hedw.	Epixylic			+		
Tetraphis pellucida Hedw.	Epixylic			+		
Calliergon cordifolium (Hedw.) Kindb.	Semi-aquatic			+		
Sphagnum angustifolium (Warnst.) C. E. O. Jensen.	Semi-aquatic					+
Sphagnum magellanicum Brid.	Semi-aquatic					+
Sphagnum rubellum Wilson	Semi-aquatic					+

soil and *Hypnum cupressiforme* on trees and stones. Twenty-four species were epigeic, six species were epilithic, ten epiphytic, three epixylic, and six semi-aquatic or aquatic. Most of the species were specific for a given habitat as they were found only in the particular site. Only *Hypnum cupressiforme* were found in four habitats, and *Pohlia nutans* and *Polytrichum juniperinum* in two habitats. Bryophyte nomenclature follows http://www.theplantlist.org.

Immediately after collection the samples were used for measurement of chlorophyll *a* fluorescence. Chlorophyll *a* fluorescence measurements were performed with a continuous fluorometer Plant Efficiency Analyser (Handy PEA; Hansatech Instruments, King's Lynn, UK). The material used for analysis included several shoot apices or parts of cushions, which were placed in standard leaf clips so that the clip area was filled and darkened for not less than 20 min. For every sample, three to five subsamples were independently measured. Fast fluorescence kinetics was measured for 1 s after illumination of the sample with saturating light pulse (3500 μmol m^{-2} s^{-1}). The data were analysed by PEA Plus software (Hansatech Instruments, King's Lynn, UK). Maximum quantum efficiency of PSII F_v/F_m was calculated as the ratio of variable fluorescence (F_v) to maximum fluorescence value (F_m). Indication of overall PSII activity F_v/F_0 was measured as the ratio of F_v to minimal fluorescence level (F_0). The ratio RC/ABS characterizes the number of active PSII reaction centres in respect to the quantity of light absorbed (Clark et al., 2000). Performance Index was measured as a combination of three independent parameters: total number of active reaction centres per absorption, yield of primary photochemistry, and efficiency with which a trapped exciton can move an electron into the electron transport chain (Appenroth et al., 2001). It is supposed that the general efficiency of the photochemistry of photosynthesis for a given plant species in particular conditions can be characterized by an overall energy conservation efficiency of the absorbed light energy in PSII (Thach et al., 2007) as indicated by Performance Index (Misra et al., 2012).

Three freshly collected apparently dry samples of the epigeic bryophyte *Fissidens adianthoides* were used for the measurement of fluorescence parameters in a native state. As the samples had an extremely low level of fluorescence indices, they were chosen for analysis of recovery of fluorescence parameters after rehydration. Samples were immersed in distilled water and left in closed plastic containers in a dim light for 30 min. Then chlorophyll fluorescence was repeatedly measured after darkening with leaf clips for 20 min.

Data were presented as the means ± SE. Differences between the means were tested by the Tukey–Kramer test, $\alpha = 0.05$. The coefficient of variation was calculated as the standard deviation divided by the mean (%).

RESULTS

Analysis of the dependence of fluorescence parameters on substrate type in samples of bryophyte species revealed extremely high variation (Table 2). The lowest variation was observed for maximum quantum efficiency of PSII (F_v/F_m)

Table 2. Fluorescence parameters in bryophyte groups on different substrates. For F_v/F_m, F_v/F_0, and RC/ABS the difference between the means was significant between epigeic and epihytic as well as epiphytic and semi-aquatic & aquatic bryophytes (Tukey–Kramer test, $\alpha = 0.05$). For Performance Index, the difference was significant only between epiphytic and epigeic bryophytes

	Epigeic	Epilithic	Epiphytic	Epixylic	Semi-aquatic & aquatic
Number of species	24	6	10	3	6
Number of samples	40	7	20	3	6
F_v/F_m range	0.051–0.851	0.073–0.766	0.019–0.768	0.521–0.685	0.685–0.807
mean ± SE	0.590 ± 0.041	0.517 ± 0.120	0.299 ± 0.068	0.590 ± 0.049	0.730 ± 0.018
coefficient of variation, %	45	62	101	14	6
F_v/F_0 range	0.033–5.73	0.082–3.340	0.019–3.330	1.100–2.250	2.370–4.180
mean ± SE	2.388 ± 0.260	1.882 ± 0.559	0.887 ± 0.261	1.553 ± 0.353	2.888 ± 0.279
coefficient of variation, %	70	79	132	39	24
RC/ABS range	0–0.866	0–0.496	0–0.458	0.217–0.384	0.326–0.515
mean ± SE	0.375 ± 0.035	0.290 ± 0.070	0.161 ± 0.037	0.287 ± 0.050	0.426 ± 0.026
coefficient of variation, %	59	64	103	30	15
Performance Index range	0–2.298	0–0.481	0–0.531	0.054–0.249	0.176–0.658
mean ± SE	0.513 ± 0.102	0.243 ± 0.080	0.090 ± 0.032	0.130 ± 0.060	0.371 ± 0.068
coefficient of variation, %	128	88	160	80	45
Mean coefficient of variation, %	76	73	124	41	23

followed by RC/ABS and F_v/F_0. Performance Index showed the highest variability reaching 160% in the case of epiphytic bryophytes. Besides, epiphytic bryophytes were the most variable group in respect to all parameters, with an average coefficient of variation 124%. Epigeic and epilithic bryophytes had similar mean variation coefficients (76% and 73%, respectively) in spite of the different number of analysed samples (40 and 7, respectively). Epixylic bryophytes were characterized by average mean variability (41%), while the semi-aquatic & aquatic group was the least variable (23%). Also, minimum levels of all measured fluorescence parameters were the highest for epixylic and semi-aquatic & aquatic bryophyte samples, but maximum levels were the highest for epigeic and semi-aquatic & aquatic bryophytes.

In spite of large variation, there were statistically significant differences ($\alpha = 0.05$) for mean values of F_v/F_m, RC/ABS, and F_v/F_0 between epigeic and epiphytic bryophyte samples as well as between epiphytic and semi-aquatic/aquatic samples (Table 2). For Performance Index, a significant difference was evident only between epiphytic and epigeic bryophytes.

A dry sample of the epigeic bryophyte *Fissidens adianthoides* showed extreme recovery of all fluorescence parameters after 50 min of rehydration (Table 3). Thus, F_v/F_0 of rehydrated tissues reached nearly the maximum level of the respective parameter characteristic of epiphytic bryophyte species in a freshly collected state (Table 2).

In order to find out some functional meaning of fluorescence parameters in bryophyte tissues, maximum quantum efficiency of PSII (F_v/F_m) was plotted against photochemical efficiency of PSII (F_v/F_0), density of active reaction centres (RC/ABS), and Performance Index of the respective bryophyte samples (Fig. 1). There was a clear exponential relationship between F_v/F_m and F_v/F_0 ($R = 0.99694$; Fig. 1a), indicating an internal relation between the minimal and the maximal fluorescence level of PSII in bryophyte tissues. A more complex dependence between the parameters was seen in the two other cases. Thus, RC/ABS increased linearly with the increase of F_v/F_m up to 0.7, followed by a

Table 3. Fluorescence analysis of freshly collected epigeic *Fissidens adianthoides* in comparison with the same samples hydrated for 30 min in a dim light followed by 20 min of darkness. Data are means ± SE from 3 samples with 3 independent measurements for each sample. For all fluorescence parameters, differences between freshly collected and rehydrated samples were statistically significant (Tukey–Kramer test, $\alpha = 0.05$)

	F_v/F_m (units)	F_v/F_0 (units)	RC/ABS (units)	Performance Index (units)
Freshly collected sample	0.084 ± 0.027	0.09 ± 0.03	0.072 ± 0.017	0.002 ± 0.001
After 50 min of hydration	0.736 ± 0.007	2.80 ± 0.10	0.337 ± 0.025	0.139 ± 0.031

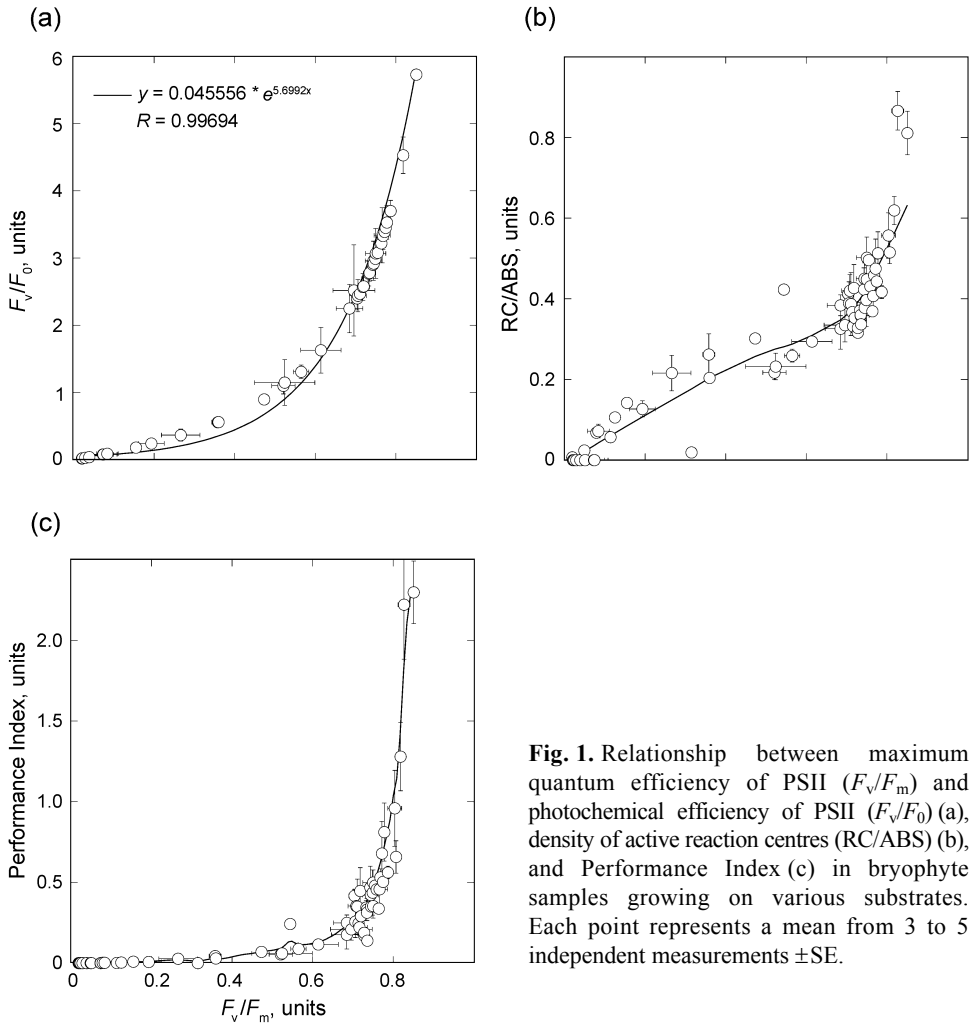

Fig. 1. Relationship between maximum quantum efficiency of PSII (F_v/F_m) and photochemical efficiency of PSII (F_v/F_0) (a), density of active reaction centres (RC/ABS) (b), and Performance Index (c) in bryophyte samples growing on various substrates. Each point represents a mean from 3 to 5 independent measurements ±SE.

steeper rise at higher F_v/F_m values (Fig. 1b). The relationship between F_v/F_m and Performance Index had a triphasic nature (Fig. 1c). No photochemical performance of PSII was seen below F_v/F_m 0.3, then a slow increase of the performance followed up to 0.7. After that Performance Index sharply rose with the further increase of F_v/F_m.

DISCUSSION

In the present study, the use of chlorophyll *a* fluorescence measurements in field conditions in different boreo-nemoral habitats allowed us to capture differences in the photochemistry of photosynthesis for bryophyte species growing on different substrates.

Very often only maximum quantum efficiency of PSII (F_v/F_m) has been used to characterize the photochemical performance of PSII in bryophyte tissues (Robinson et al., 2000; Proctor, 2003; Cui et al., 2009; Granath et al., 2009; Laine et al., 2011). However, the present study showed that other fluorescence parameters might be also useful in characterizing various aspects of photochemistry in bryophytes in field conditions, as there was no general linear relationship between them and F_v/F_m.

Fluorescence parameters derived from fast induction kinetics are based on the theory of energy flow in chloroplast thylakoid membranes (Strasser et al., 2004). Besides F_v/F_m, F_v/F_0 also characterizes the maximum quantum yield of PSII accounting for simultaneous variations in F_m and F_0. In other words, the parameter characterizes PSII performance due to trapping probability and can be used as an indication of overall PSII activity (Lichtenthaler et al., 2005). As F_v/F_m reflects only negative effects on PSII due to downregulation or damage to PSII complexes while F_v/F_0 can reflect the intensity of positive changes, F_v/F_0 showed more variability than F_v/F_m. This can be seen also from the exponential relationship between F_v/F_m and F_v/F_0 (Fig. 1a). Performance Index combines three independent values by quantifying different steps of photochemistry, i.e., the total number of active reaction centres per absorption, yield of primary photochemistry, and efficiency with which a trapped exciton can move an electron into the electron transport chain (Appenroth et al., 2001). Performance Index is believed to be more sensitive to changes in environmental conditions in comparison to other fluorescence parameters and to show correlation with plant physiological performance (Thach et al., 2007). The sensitivity of Performance Index as well as its complex nature were evident also in the present study as indicated by extremely high variability (Table 2).

The ratio RC/ABS is one of the components of Performance Index, characterizing the density of active reaction centres on a chlorophyll basis (Clark et al., 2000). In our study RC/ABS also was highly variable in bryophyte tissues in natural conditions (Table 2). Functional dependence of overall maximum quantum efficiency on density of active reaction centres in PSII was seen in the biphasic relationship between RC/ABS and F_v/F_m (Fig. 1b). Three distinct phases of changes in RC/ABS were evident with decreasing F_v/F_m: linear above 0.7, curvilinear between 0.7 and 0.4, and linearly decreasing down to zero below 0.4.

In higher plants, F_v/F_m correlates with the number of functional PSII complexes; therefore, the decrease of F_v/F_m below 0.83 is suggested to indicate photoinhibition of photosynthesis due to the damage to reaction centres of PSII by suboptimal environmental factors (Öquist et al., 1992). One may wonder what the functional meaning of the decrease in F_v/F_m in desiccated bryophyte tissues can be. Extremely fast reversibility of F_v/F_m during rehydration without a participation of protein synthesis in darkness (Proctor et al., 2007) indicates against physical damage of PSII protein complexes.

In a study with freezing and thawing stress in Antarctic moss it was concluded that the reversible reduction in F_v/F_m during freezing indicates conformational changes in pigment–protein complexes due to desiccation (Lovelock et al., 1995).

A similar mechanism leading to inability to perceive light quanta might be suggested in naturally desiccated bryophytes, representing an adaptive protection mechanism against endogenous oxidative stress in conditions of permanent water shortage. Consequently, the decrease in photochemical parameters is related to downregulation of photosynthesis for the sake of photoprotection. In *Sphagnum* mosses, complete turgor loss is accompanied by a significant decrease in photosynthetic activity while F_v/F_m remains unchanged down to the tissue water content of 0.65 g g^{-1} (Hájek & Beckett, 2008). Most probably, the relatively high F_v/F_m in a dry state is a result of a decrease of basal fluorescence F_0, serving as a photoprotective heat dissipation mechanism in poikilohydric photosynthetic organisms (Heber et al., 2006). On the contrary, a sustained increase in F_0 might be associated with photoinhibitory damage (Cleland et al., 1986). In addition, a relatively low level of maximum F_v/F_m ratios for mosses (about 0.72) even under favourable conditions was suggested to indicate the continuous presence of zeaxanthin and anteraxanthin participating in non-photochemical thermal quenching of light energy in PSII (Lovelock et al., 1995).

The relationship between F_v/F_m and other parameters found in the present study clearly revealed three functionally different stages in the photochemistry of photosynthesis in bryophyte tissues in natural conditions. No efficient photochemical reactions occurred below F_v/F_m 0.3 in spite of the linearly increasing proportion of active reaction centres as indicated by the changes in RC/ABS. In a range of F_v/F_m between 0.3 and 0.7 a slow renovation of photochemical efficiency was evident, reflecting positive conformational changes in electron transfer complexes. A further increase of photochemical performance above F_v/F_m 0.7 most likely was associated with the further improvement of photochemical efficiency due to optimal environmental conditions.

It is evident that the variation of the relative moisture in bryophyte samples caused a variation of F_v/F_m below 0.7. The decrease of F_v/F_m during the artificial desiccation of bryophyte samples has been described in several studies with a number of bryophyte species used (Bates, 1997; Deltoro et al., 1998; Proctor, 2003; Proctor et al., 2007; Hájek & Beckett, 2008; Lüttge et al., 2008). However, no study so far has linked changes in F_v/F_m in natural conditions with the different hydration status of bryophyte tissues. The variability of F_v/F_m on different substrates possibly reflected different water content of tissues due to 10 days without precipitation because of differences of both water retention capacity of substrate and water holding capacity of bryophyte tissues. Epiphytic bryophytes had the lowest mean values of all fluorescence parameters (Table 2). Based on the analysis of F_v/F_m, epihytic bryophytes appeared to be most stressed (F_v/F_m 0.299), while epigeic, epilithic, and epixylic species were only moderately stressed (F_v/F_m from 0.517 to 0.590), and the semi-aquatic & aquatic group showed only a minor stress (F_v/F_m 0.730). The high stress situation of epiphytic bryophytes can be explained by the fact that being located on vertical or near-vertical tree trunks exposed to air current, they are most vulnerable to desiccation. The largest variability in fluorescence parameters within this group of bryophytes most likely

reflects differences in the water retention capacity of individual species (Glime, 2007) or particular microenvironmental conditions. The low variability and relatively high level of fluorescence parameters of epixylic bryophytes growing on decaying wood are most likely associated with a relatively large mass of substrate providing enough moisture for bryophytes for a prolonged period of time. Similarly, the location of semi-aquatic and aquatic bryophytes in a direct contact with a water source probably excluded the water-shortage-dependent component of variability in their fluorescence parameters.

Intensity of photosynthetically active radiation at the site of sampling is another factor directly or indirectly affecting the photochemistry of photosynthesis (Marschall & Proctor, 2004). However, it was not measured in the present study. As sampling was performed relatively early in the season before bud burst of deciduous trees, differences in photosynthetically active radiation between sampling microsites were not large.

In conclusion, by means of chlorophyll fluorescence measurement with a large number of bryophyte samples growing on various substrates it was shown that even in relatively wet habitats bryophytes can display a low intensity of the photochemistry of photosynthesis, which is possibly due to the low tissue water content. The need for easily measurable quantitative traits in bryophyte studies in order to model ecosystem processes was postulated recently (Cornelissen et al., 2007). In this respect, the measurement of fast fluorescence induction kinetics in field conditions provides a rapid and efficient tool to obtain such data. As it has been suggested that for bryophyte species improved water absorption and holding characteristics are most likely mechanisms of increased growth (Rixen & Mulder, 2005), it would be interesting to link the performance of PSII with the water holding capacity of bryophyte species in natural conditions at different levels of water availability to demonstrate putative adaptive mechanisms of the photochemistry of photosynthesis against desiccation.

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