RELATIONSHIP BETWEEN FUNCTIONAL TRAITS, FUNCTIONAL TYPES, AND HABITAT IN BOREONEMORAL BRYOPHYTES

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The aim of the present study was to determine a relationship between physiological traits and functional types of bryophytes from five boreonemoral habitats with a particular emphasis on discriminative ability of these traits. Sampling of 25 species was performed four times during one season. Water content, chlorophyll a fluorescence and photosynthetic pigment concentration were measured in field and water-equilibrated samples. Principal component analysis indicated the existence of an inverse relationship between concentration of pigments and water content. Linear discriminant analysis showed that relatively high mean predicted posterior probabilities of correct classification of functional types by physiological traits were found for water conducting system, followed by substrate and habitat, but it was highly variable and type-specific for life form and relatively less variable for life strategy. Field water content had the highest average discriminative importance among physiological traits, followed by chlorophyll fluorescence indices field F_v/F_m , equilibrated PI, equilibrated RC/ABS, and field RC/ABS. Photosynthetic pigment concentrations had relatively less average importance for classification of functional types.

Key words: chlorophyll, chlorophyll a fluorescence, life form, life strategy, pigments, substrate, water conducting system.

INTRODUCTION

In addition to classification by taxa, different functional types of plants have been described. For example, functional classifications have been used to predict changes in ecosystems due to global climate change (Lavorel *et al.*, 2007). While no single and comprehensive system has been established for vascular plants, both life forms and life strategies have emerged as useful concepts in bryophyte ecology. Relationships between bryophyte life form and habitat have been frequently described since the early studies in bryophyte ecology (Gimingham and Birse, 1957). Life forms are often associated with adaptations to moisture availability and light intensity (Bates, 1998). It is generally accepted that short turfs and cushions are typical for sunny, dry and xeric habitats, while bryophytes forming mats, wefts, tails and fans dominate humid and shady habitats (Bates, 1998; Kürschner, 2004). Recently, life forms have been used to explain a 100-year-long succession of bryophytes in forest habitat, which was associated with increase of available substrates as well as habitat shading (Strazdiņa

et al., 2013). In particular, these changes led to disappearance of compact cushions and short turfs, while smooth and rough mats increased in richness. Evidently, life forms are a reflection of adaptation to the environment.

In addition to life forms, bryophyte classification by life strategies is based mostly on reproduction and life span characteristics (During, 1979; Kürschner, 2004). Recently, distribution of mountain stream bryophytes with different life forms and life strategies in relation to water velocity, light incidence and hydrological zone has been described (Vieira *et al.*, 2012). It is reasonable to suggest that both systems of functional classification of bryophytes, life forms and life strategies, are associated with specific morphophysiological adaptations to environmental conditions. However, physiological traits have not been frequently used for ecological classification. The only physiological trait used so far for functional classification was based on the water-conducting system: ectohydric (transporting water externally) and endohydric (having internal transport system (Glime, 2007b)). However, a considerable number of species

can exhibit both types of water conduction, in reality being myxohydric. In the context of the present paper, “functional types” are groupings of bryophyte species according to habitat, substrate, water conducting system, life form and life strategy. Functional classification systems of bryophytes are rather descriptive, without much experimentally based evidence. Therefore, it can be asked if groups of species with particular “functional type” represent reflections of different physiological adaptations?

Photosynthesis-related traits are widely used in ecophysiology of vascular plants to characterise their physiological state and responses to the environment (Albert *et al.*, 2011). Among them, measurement of chlorophyll *a* fluorescence is a non-destructive analytical method to estimate photochemical activity of plants (Baker, 2008). As photosystem II (PS II) is highly sensitive to both physiological state of a plant as well as changes in the environment, chlorophyll *a* fluorescence measurements have been used in a multitude of studies (Murchie and Lawson, 2013). For bryophytes, this type of analysis has been used mostly in dehydration/rehydration experiments as an indication of the state of photosynthetic apparatus (Proctor, 2003; Beckett *et al.*, 2005; Cruz de Carvalho *et al.*, 2011). Recently, it has been shown that the fast chlorophyll *a* fluorescence method can provide quantitative estimates of the photochemical activity

of bryophytes with potential use in ecophysiological studies (Liepiņa and Ievinsh, 2013). In addition, photosynthetic pigment concentration has been used as an indicator of photosynthetic activity in desiccation experiments (Martin and Warner, 1984) as well as in ecophysiology (Lopez and Carballeira, 1989), e.g., for characterisation of the relationship with light availability (Marschall and Proctor, 2004).

The aim of the present study was to determine whether or not water content and photosynthesis-related traits could be used as indicators for functional classification of bryophyte species. Water content-related as well as photosynthesis-related traits, namely, photosynthetic pigment concentration and characteristics of PS II photochemical activity, were measured for 25 bryophyte species growing on different substrates from relatively similar boreonemoral habitats during a vegetation season. It was hypothesised that it is possible to define functional groups of bryophytes based on these physiological traits.

MATERIALS AND METHODS

Habitats and species. The study was conducted in five boreonemoral habitats located along a 35-km transect in the W–E direction in the central part of Latvia (Table 1). Habitat A was represented by a deciduous forest with *Acer pla-*

Table 1. Functional classification of studied bryophyte species

Habitat	No	Species	Substrate	Water conducting system	Life form	Life strategy
A	1	<i>Brachythecium rutabulum</i>	epiphytic	ectohydric	rough mat	pioneer
A	2	<i>Homalia trichomanoides</i>	epiphytic	ectohydric	smooth mat	stress-tolerant perennial
A	3	<i>Leucodon sciuroides</i>	epiphytic	ectohydric	tail	long-lived shuttle
A	4	<i>Ortotrichum affine</i>	epiphytic	endohydric	small cushion	colonist
A	5	<i>Plagiomnium undulatum</i>	epigeic	endohydric	dendroid	competitive perennial
B	6	<i>Hypnum cupressiforme</i>	epiphytic	ectohydric	smooth mat	stress-tolerant perennial
B	7	<i>Hypnum cupressiforme</i>	epilytic	ectohydric	smooth mat	stress-tolerant perennial
B	8	<i>Anomodon longifolius</i>	epilytic	ectohydric	tail	perennial stayer
B	9	<i>Cratoneuron filicinum</i>	epilytic	ectohydric	weft	perennial stayer
B	10	<i>Conocephalum conicum</i>	epigeic	endohydric	thalloid-mat	long-lived shuttle
C	11	<i>Climacium dendroides</i>	epigeic	endohydric	dendroid	competitive perennial
C	12	<i>Tetraphis pellucida</i>	epixylic	endohydric	short turf	pioneer
C	13	<i>Atrichum undulatum</i>	epigeic	endohydric	tall turf	short lived shuttle
C	14	<i>Calliergonella cuspidata</i>	semi-aquatic	ectohydric	weft	competitive perennial
C	15	<i>Lophocolea heterophylla</i>	epixylic	ectohydric	thread-like mat	pioneer
D	16	<i>Rhytidiadelphus triquetrus</i>	epigeic	endohydric	weft	competitive perennial
D	17	<i>Ptilium crista-castrensis</i>	epigeic	ectohydric	weft	perennial stayer
D	18	<i>Hylocomium splendens</i>	epigeic	ectohydric	weft	competitive perennial
D	19	<i>Dicranum polysetum</i>	epigeic	endohydric	tall turf	competitive perennial
D	20	<i>Pleurozium schreberi</i>	epigeic	ectohydric	weft	competitive perennial
E	21	<i>Sphagnum russowii</i>	epigeic	ectohydric	sphagnoid	long-lived shuttle
E	22	<i>Sphagnum riparium</i>	aquatic	ectohydric	sphagnoid	long-lived shuttle
E	23	<i>Sphagnum magellanicum</i>	semi-aquatic	ectohydric	sphagnoid	long-lived shuttle
E	24	<i>Sphagnum cuspidatum</i>	aquatic	ectohydric	sphagnoid	long-lived shuttle
E	25	<i>Sphagnum capillifolium</i>	epigeic	ectohydric	sphagnoid	long-lived shuttle

Habitats: A, deciduous forest, 56°48'8"N, 24°51'48"E; B, ravine with stream, 56°47'54"N, 24°52'0"E; C, mixed wet forest, 56°49'6"N, 24°50'18"E; D, conifer forest, 56°53'4"N, 24°26'32"E; E, peat bog, 56°52'39"N, 24°21'24"E

tanoides, *Quercus robur*, *Ulmus laevis*, and *Tilia cordata* on slopes of a river; habitat B was sparsely forested ravine with a stream and *Ulmus laevis* and *Alnus incana* on slopes; habitat C was a mixed wet forest with *Picea abies*, *Quercus robur*, *Alnus glutinosa*, and *Betula pubescens*; habitat D was conifer forest with *Pinus sylvestris* and *Picea abies*; habitat E was in the edges of a peat bog with *Pinus sylvestris*. In total, 25 of the common species growing on different substrates were chosen for the study (Table 1). Samples of each species were collected on a specific substrate except for *Hypnum cupressiforme*, which was found both on tree trunks and boulders and was designated as both epiphytic (species No. 6) and epilithic (No. 7). Bryophyte nomenclature follows the Plant List (Anonymous, 2013). Bryophyte life forms and life strategies were classified according to During (1979; 1992). Water conducting system was characterised according to Glime and references included in this source (Glime, 2007b).

Sampling. Bryophytes were sampled four times during summer (June 17, July 22, August 19, September 23) in order to eliminate any season-specific effects. Each sample consisted of about 20 g of bryophyte shoots taken from at least three different clumps, the samples were placed in sealed polyethylene bags and transported to the laboratory within 4 h.

Water content analysis. To measure dry mass, bryophyte samples were weighed before and after drying in an oven at 70 °C for 72 h. Field water content in samples was calculated as a fresh mass minus dry mass divided by dry mass and expressed as a percentage. For other water content traits, samples (about 5 g) were weighed, immersed in distilled water for 1 min, then taken out and allowed water to dry for 15 s, then weighed again to determine fully hydrated water content. Samples were blotted with a four-folded paper towel for 15 s and weighed again to determine blotted tissue water content, which generally represents full-turgor water content (Proctor *et al.*, 1998). The blotted samples were placed on a wet four-folded paper towel in a closed plastic container for 12 h in low light and then weighed to determine equilibrated water content. All estimates of water content were recalculated according to dry mass to determine relative water content. All water content measurements were performed in triplicate from the same sample.

Analysis of chlorophyll *a* fluorescence. Chlorophyll *a* fluorescence was measured both in field and water-equilibrated samples in triplicate. Separate shoots from different clumps of the same sample were used. Field samples were analysed no later than 4 h after collection, while water-equilibrated samples were analysed after equilibration for 12 h in low light as described above. For each sample, five subsamples consisting of shoot apices were individually placed in standard leaf clips and left in the dark in sealed polyethylene bags for at least 20 min. Fast fluorescence kinetics was measured with a continuous fluorometer Plant Efficiency Analyzer (Handy PEA; Hansatech Instruments, King's Lynn, UK). The results were analysed by PEA Plus software (Handy PEA; Hansatech Instruments,

King's Lynn, UK). Maximum quantum efficiency of PS II F_v/F_m , overall PS II activity (F_v/F_0), number of active PS II reaction centres with respect to absorbed light (RC/ABS), and overall energy conservation efficiency of the absorbed energy in PS II (Performance Index, PI) were used as estimates of PS II efficiency.

Measurement of photosynthetic pigments. Samples (about 5 g) for pigment analysis were taken randomly from larger samples for each species and each month (Table 1), dried in low light at room temperature for one week and then stored in closed polyethylene bags at room temperature until analysis. These conditions led only to minimum changes in photosynthetic pigment concentration comparable to the variation between subsamples within the sample. Three subsamples (10 mg each) consisting of shoot apices were prepared from each sample. The tissues were ground with mortar and pestle in the presence of quartz sand and CaCO_3 crystals, extracted with 10 ml 96% ethanol and centrifuged at 2000 g for 5 min. Optical density of supernatants was measured at 440.5, 649 and 665 nm in triplicate. Pigment concentration was calculated according to the equations presented in published research (Wettstein, 1957; Wintermans and De Mots, 1965).

Data analysis. As the number of samples (bryophyte species) per functional group was small, principal component analysis (PCA) was performed first with 18 physiological traits for dimensionality reduction using the R 3.4.1 software (Anonymous, 2017). After PCA, functional group mean values of principal component 1 (PC1) and PC2 scores for functional types were compared using the Tukey–Kramer test. Physiological characteristics had four time points per species, each with three replicates.

To test for ability of 18 physiological traits (scaled variables) to discriminate functional types linear discriminant analysis (LDA) as implemented in package MASS (Venables and Ripley, 2002) of software R 3.4.1. (Anonymous, 2017) was used. Separate analyses were performed for functional types: (a) habitat with five groups, (b) substrate with five groups, (c) water conducting system with three groups, (d) life form with eleven groups, and (e) life strategy with seven groups. LDA was performed using leave-one-out cross-validation option to calculate mean posterior probability for each functional type group to be correctly predicted to known group.

RESULTS

Principal components analysis. Principal components analysis revealed the existence of an inverse relationship between concentration of photosynthetic pigments and water content traits (Fig. 1). Chlorophyll *a* fluorescence traits F_v/F_m and F_v/F_0 in field bryophyte samples were most closely related to water content. Other fluorescence traits measured in field tissues, and particularly in water-equilibrated tissues, were not related to water content traits. To identify possible clustering by functional types, mean

Fig. 1. Principal component analysis of 18 physiological traits. Right plot shows values of the first and second principal components for each species (numbers in circles correspond to species number from Table 1). The first two axes account for 39.02% and 26.39% of the total variation. The numbers represent species numbers according to Table 1.

scores on PCA1 and PCA2 for bryophytes grouped by various functional types were plotted (Fig. 2). Significant differences between the species groups are given in Table 2. In

Fig. 2. Principal component analysis. Grouping of bryophyte species in principal component analysis according to functional types. (A) Habitat. 1: deciduous forest; 2: ravine with stream; 3: mixed wet forest; 4: conifer forest; 5: peat bog. (B) Substrate. 1: epiphytic; 2: epigeic; 3: epilytic; 4: epixylic; 5: aquatic, semi-aquatic. (C) Water conducting system. 1: endohydric; 2: ectohydric non-*Sphagnum*; 3: ectohydric *Sphagnum*. (D) Life form. 1: rough mat; 2: smooth mat; 3: thalloid-mat; 4: thread-like mat; 5: tail; 6: small cushion; 7: dendroid; 8: short turf; 9: tall turf; 10: weft; 11: sphagnoid. (E) Life strategy. 1: pioneer; 2: stress-tolerant perennial; 3: long-lived shuttle; 4: colonist; 5: short-lived shuttle; 6: competitive perennial; 7: perennial stayer. Mean scores ± SE are shown. Significant differences between the species groups for the particular type are indicated in Table 2.

most habitats, except mixed wet forest, species had relatively variable fluorescence traits together with a somewhat fixed species-specific ratio between photosynthetic pigment concentration and water content (Fig. 2A). In mixed wet forest, the presence of two species with contrasting fluorescence traits, *Atrichum undulatum* and *Lophocolea heterophylla*, resulted in large variability for mean PC2 score. Along PC1, peat bog species significantly differed from other habitats (Table 2), and conifer forest species differed from species in deciduous forest and ravine habitats. Along PC2, peat bog species significantly differed from those growing in ravine and mixed wet forest habitats.

Species on different growth substrates showed some distinctive features with respect to water content/photosynthetic pigment concentrations (Fig. 2B). Epiphytic species formed a relatively discrete group based on a narrow pigment/water content ratio and relatively similar fluorescence traits, and the lowest photochemical activity among species. Epigeic, epilithic and epixylic species clustered together with no significant differences between them, while aquatic and semi-aquatic species formed a distinct group that significantly differed from the other species in both PC1 and PC2 scores (except epiphytic species; Table 2).

Both ectohydric non-*Sphagnum* and endohydric species were similar in both photosynthesis- and water-related traits (Fig. 2C). However, ectohydric non-*Sphagnum* species had a low level of photochemical efficiency together with moderate pigment concentration and water content, while endohydric species had a relatively high level of both pigments and photochemical activity. Only endohydric species had both relatively high pigment concentration and relatively low water content together with high photochemical activity of PS II. Endohydric species significantly differed from ectohydric non-*Sphagnum* species (PC1 scores) and both endohydric and ectohydric non-*Sphagnum* species from ectohydric *Sphagnum* species (PC2 scores; Table 2).

Grouping of bryophyte species according to their life forms distinguished several clusters (Fig. 2D). Sphagnoid species (group 11) located far from the other groups, had high water

Table 2. Significant differences in scores on first two axes between functional types of bryophyte species by principal coordinates analysis (Fig. 1)

Group		PC1	PC2
Habitat			
1	Deciduous forest	c	abc
2	Ravine with stream	c	bc
3	Mixed wet forest	bc	c
4	Conifer forest	b	ab
5	Peat bog	a	a
Substrate			
1	Epiphytic	c	ab
2	Epigeic	b	b
3	Epilithic	bc	b
4	Epixylic	bc	b
5	Aquatic, semi-aquatic	a	a
Water conducting system			
1	Endohydric	b	a
2	Ectohydric, <i>Sphagnum</i>	b	b
3	Ectohydric, non- <i>Sphagnum</i>	a	a
Life form			
1	Rough mat	bc	abc
2	Smooth mat	c	ab
3	Thalloid-mat	bc	abc
4	Thread-like mat	c	abc
5	Tail	c	abc
6	Small cushion	bc	abc
7	Dendroid	b	abc
8	Short turf	bc	abc
9	Tall turf	bc	ac
10	Weft	bc	a
11	Sphagnoid	a	a
Life strategy			
1	Pioneer	bc	a
2	Stress-tolerant perennial	bc	a
3	Long-lived shuttle	a	a
4	Colonist	bc	a
5	Short lived shuttle	bc	b
6	Competitive perennial	bc	a
7	Perennial stayer	ab	a

Functional types indicated with the same letter within the particular group are not significantly different ($p > 0.01$)

content together with low photosynthetic pigment concentration and variable intensity of fluorescence traits, and significantly differed from mean PC1 scores of other groups (Table 2). Another well-differentiating cluster was dendroid species (group 7). Species in the opposite corner of the ordination had low water content and high pigment concentration together with high values of fluorescence intensity traits. These species included tail (group 5) and small cushion (group 6) species, with short turf (group 8) and tall turf (group 9) species located closely. A cluster composed of rough mat (group 1), smooth mat (group 2), thalloid-mat (group 3) and thread-like mat (group 4) species had similar water content/pigment concentration ratio together with

relatively low fluorescence intensity traits. Weft species (group 10) had relatively variable traits.

Ordination according to the life strategies (Fig. 2E) identified *Atrichum undulatum* (group 5), the only species with the short-lived shuttle strategy, in the lower left corner, and long-lived shuttle species in the far right upper corner. A distinct cluster with low water content/pigment ratio and low fluorescence intensity traits consisted of stress-tolerant perennial (group 2), colonist (group 4) and fugitive (group 8) species. Another cluster with more intermediate trait values consisted of pioneer (group 1), competitive perennial (group 6) and perennial stayer (group 7) species. Significant differences in scores were evident only between long-lived shuttle and the other species besides perennial stayer species (PC1 scores) as well as between short lived shuttle and all other species (PC2 scores).

Linear discriminant analysis. First and second linear discriminant accounted for 88.8 and 5.6% of total variation of different habitats (Fig. 3A). The most important physiological parameter for the first linear discriminant was field water content (2.615) and for the second it was field RC/ABS (1.878) and field F_v/F_m (−1.394; Table 3). Only peat bog species had a higher field water content clearly separated by the first linear discriminant. Conifer forest species separated from other habitats by the second linear discriminant. Cross-validation showed that mean predicted posterior probabilities correctly classifying objects (species and species groups) according to physiological traits were 100% for peat bog species group, 58.2% for conifer forest group, 43.7% for deciduous forest group, 35.9% for ravine with stream group and 31.4% for mixed wet forest group.

LDA of substrate functional types showed that first linear discriminant explained 54.2 and second 20.6% of variation (Fig. 3B). Equilibrated PI (1.949), field water content (1.451) and blotted water content (−1.392) were the most important parameters for the first linear discriminant, with field RC/ABS being the most important for the second (−2.339; Table 3). Both epigeic and aquatic and semi-aquatic species clearly separated from each other as well as from the rest of substrate groups according to the first discriminant. Epiphytic, epilithic and epixylic species separated according to the second discriminant. Highest mean posterior probability of correct classification was for epigeic group (71.0%) and for aquatic, semi-aquatic group (67.3%). Mean probability was 65.2% for epiphytic group, 52.5% for epilithic group and 49.9% for epixylic group.

First and second linear discriminant accounted for 92.3 and 7.7% of variation of water conducting system functional types (Fig. 3C). Field water content (2.478) was the most important parameter for the first linear discriminant, but equilibrated RC/ABS (2.225) and equilibrated PI (−2.091) were the most important for the second linear discriminant (Table 3). While ectohydric *Sphagnum* species with high field water content separated from the other two groups according to the first discriminant, endohydric and ectohydric non-*Sphagnum* species separated according to the second

Fig. 3. Results of linear discriminant analysis showing coefficients of linear discriminants (higher absolute coefficients value shows higher importance of particular trait for discriminating functional types) of 18 physiological traits by functional type. Symbols show mean and standard error of the first and second linear discriminants for each function group. (A) Habitat. (B) Substrate. (C) Water conducting system. (D) Life form. (E) Life strategy.

discriminant. A 100% mean posterior probability of correct classification was for the ectohydric *Sphagnum* group, and it reached 76.7% for the endohydric group and 56.6% for the ectohydric non-*Sphagnum* group.

Life form function types LDA first and second linear discriminant accounted for 64.7 and 13.2% of variation (Fig. 3D). The most important physiological parameter for the first linear discriminant was field water content (−2.146), and for the second discriminant it was chlorophyll *a+b* concentration (1.970), but the number of other physiological parameters (chlorophyll *b* concentration, field RC/ABS, chlorophyll *a* concentration, field F_v/F_m , equilibrated PI) also had relatively high impact (Table 3). Tail, tall turf, rough mat, smooth mat, thalloid-mat and thread-like mat species showed no separation according to the first discriminant, but differences in photosynthetic pigment concentrations allowed separation according to the second discriminant. Classification of life forms according to physiological traits was clearly life form-specific, as 100% mean posterior probability of correct classification was for the sphagnoid group but for the weft group there were no correct classification cases and only 1.6% for the rough mat group, but 17.0% for the tall turf group and 18.7% for the

thalloid-mat group. For other groups mean posterior probabilities were between 35.5 and 76.5%.

First and second linear discriminant accounted for 45.3% and 20.9% of variation of life strategy functional types (Fig. 3E). Field F_v/F_m (−2.786) and field RC/ABS (2.030) were the most important parameters for the first linear discriminant, and field PI (−1.818) was the most important for the second (Table 3). Long-lived shuttle and colonist species separated from the rest of life strategy groups according to the first discriminant. Colonist, and short-lived shuttle species separated from each other as well as from the rest of life strategy groups according to the second discriminant. However, a group consisting of pioneer, stress-tolerant perennial, perennial stayer and competitive perennial species showed almost no separation according to either discriminant. Mean posterior probabilities of correct classifications were 28.3% for pioneer group, 43.5% for stress-tolerant perennial group, 66.7% for long-lived shuttle group, 73.7% for colonist group, 25.5% for short-lived shuttle group, 51.7% for competitive perennial group, and 25.2% for perennial stayer group.

Comparison between the mean absolute values of coefficients for different physiological parameters revealed that

Table 3. Results of linear discriminant analysis showing coefficients of linear discriminants (higher absolute coefficients value shows higher importance of particular trait for discriminating functional types) of 18 physiological traits by functional type

Trait	Habitat		Substrate		Water conducting system		Life form		Life strategy		Mean absolute values
	LD1	LD2	LD1	LD2	LD1	LD2	LD1	LD2	LD1	LD2	
Water content, field	2.615	0.561	1.451	-0.235	2.478	-0.963	-2.146	0.682	1.562	-0.232	1.29
Water content, fully hydrated	0.724	-0.127	0.924	0.147	0.438	0.180	-1.126	-0.106	-0.296	-0.527	0.46
Water content, blotted	-0.779	-0.129	-1.392	0.679	-0.543	-0.636	1.016	-0.182	0.257	-0.119	0.57
Water content, equilibrated	0.776	0.113	0.657	-0.352	0.742	0.896	-0.787	0.041	0.256	0.448	0.51
F_v/F_m , field	-0.393	-1.394	0.638	-1.368	-1.332	-0.153	0.793	1.447	-2.786	-1.207	1.15
F_v/F_m , equilibrated	0.528	-0.105	0.832	0.524	0.446	-0.062	-0.151	-0.436	0.390	0.916	0.44
RC/ABS, field	0.723	1.878	-0.199	0.664	1.295	-0.301	-1.141	-1.485	2.030	0.923	1.06
RC/ABS, equilibrated	-0.919	0.4691	-1.236	-2.339	-0.489	2.225	-0.149	-1.166	-1.997	0.070	1.11
F_v/F_0 , field	-1.088	0.308	-0.191	1.420	-0.288	0.181	0.758	-0.368	1.531	1.160	0.73
F_v/F_0 , equilibrated	-0.593	-0.518	-1.026	0.252	-0.512	0.100	0.202	0.549	-0.334	-1.026	0.51
$(1-V_j)/V_j$, field	-0.285	0.268	0.078	-0.380	-0.370	-0.556	0.247	-0.502	0.316	0.836	0.39
$(1-V_j)/V_j$, equilibrated	-0.336	-0.009	-1.035	-0.048	-0.103	0.102	0.220	-0.423	0.494	0.969	0.37
PI, field	1.103	-1.051	0.138	-0.776	0.440	0.036	-0.661	1.145	-1.609	-1.818	0.88
PI, equilibrated	0.946	-0.205	1.949	1.711	0.357	-2.091	0.182	1.341	1.408	-1.016	1.12
Chl <i>a</i>	-1.156	0.096	0.142	-0.710	-0.845	1.090	0.844	1.455	-0.241	-0.456	0.70
Chl <i>b</i>	0.890	-0.030	-0.165	-0.273	0.752	0.347	-1.110	-1.541	-0.103	0.676	0.59
Chl <i>a+b</i>	-0.110	1.131	0.901	0.614	0.011	-0.845	0.309	1.970	0.889	-0.591	0.74
Carotenoids	0.343	-0.345	-0.445	0.818	0.062	-1.474	0.361	-1.247	-0.619	-0.225	0.60

field water content had the highest average discriminative importance (1.29), followed by field F_v/F_m (1.15), equilibrated PI (1.12), equilibrated RC/ABS (1.11), and field RC/ABS (1.06; Table 2). Photosynthetic pigment concentrations had relatively less average importance.

DISCUSSION

From a practical point of view, it is usually assumed that functional groups of bryophytes differentially respond to climate changes and biogeographic effects (Baldwin and Bradfield, 2007). For example, it is often stressed that bryophyte life forms are adaptations to low water availability, particular light intensity, and physical characteristics of a substrate (Bates, 1998).

The present study focused on several quantitatively assessed traits related to tissue water content and photosynthesis (concentration of chlorophylls and carotenoids, as well as chlorophyll *a* fluorescence) as possible indicators for functional classification purposes. It was hypothesised that these characteristics could be used to discriminate between different groups of species (functional types) within different systems of functional classification. The study showed that the examined traits differed in their ability to predict functional types.

As expected, water-content traits differed between species groups differing in water conducting system characteristics. Field water content had the highest average discriminative importance for classification of different functional types,

especially for habitat, water conducting system and life form, followed by life strategy and substrate (Table 2).

While field tissue water content (hydration state) is directly dependent on water availability in the microenvironment as well as physical characteristics of a substrate (Glime, 2007a), the ability to accumulate water seems to be a species-specific characteristic, at least, to some extent. Both *Sphagnum* and non-*Sphagnum* aquatic and semi-aquatic species had statistically significant higher field and fully hydrated tissue water content in comparison to other species. It appears that physical capacity to hold water is related to specific morphological features of bryophyte shoots. The presence of large hyaline cells within a layer of leaf cells in *Sphagnum* species is an adaptation for holding a relatively high water content extracellularly (Andrus, 1986). Even within closely related *Sphagnum* species, some may have better ability to hold water, e.g., *Sphagnum magellanicum*, a hummock species, in comparison to other species (Li *et al.*, 1992). Other structural adaptations at the leaf morphology level (concave, multi-layered and overlapping leaves, specialised structures) can provide higher ability to absorb (Proctor, 1979). It should be noted also that extremely high relative water content in bryophyte tissues (above 1000%) can lead to decrease in CO₂ fixation rate, as noted for *Sphagnum* species (Silvola and Aaltonen, 1984). The value over 1000% was exceeded also in the present study in *Sphagnum* species in field state (with an average value for all *Sphagnum* species 1156 ± 66).

In a broader context, photosynthesis-related characteristics, including chlorophyll *a* fluorescence, are widely used in

ecophysiological studies of vascular plants both in relation to plant productivity and physiological vitality (Zarco-Tejada *et al.*, 2002). However, in bryophytes, fluorescence analysis has been used mostly for water availability studies (e.g., Proctor, 2003; Beckett *et al.*, 2005; Cruz de Carvalho *et al.*, 2011) and only relatively few studies have dealt with light effects (Murray *et al.*, 1993; Ren *et al.*, 2010; Proctor and Smirnov, 2011). Relatively high photochemical activity of PS II is usually associated with a well-hydrated state of bryophyte tissues and vice versa (Davey, 1997). Surprisingly, in the present study, intensity of photochemical processes was only to a relatively low extent related to field water content. It is possible that, besides water status of tissues, other environmental factors contribute to changes in photochemical activity of PS II in bryophytes in field conditions, as fluorescence traits in water-equilibrated tissues showed no relationship with pigment concentration and climate variables. It is well known that light intensity directly positively affects both photochemical activity and rate of CO₂ fixation, but many bryophyte species are adapted to low-light conditions (Rincón, 1993). Consequently, low but stable intensity of photochemical activity and photosynthesis is expected in shaded conditions for most bryophyte species, while sudden increase in light availability for well-watered bryophytes can result in photoinhibition of photosynthesis and growth inhibition (Murray *et al.*, 1993). Therefore, decreased level of chlorophyll *a* fluorescence traits in the present study might be an indication of high light intensity-related negative effects on photochemical activity of PS II.

In general, chlorophyll *a* fluorescence indices were significantly better physiological traits for classification of functional types in comparison to photosynthetic pigment concentrations (Table 3). Only species with different life forms appeared to be significantly discriminated on the basis of chlorophyll *a* and *b* concentrations. However, general probabilities of correct classification of life forms according to physiological traits were highly variable for different life forms, ranging from 0% in the case of weft and 1.6% of rough mat species to 100% for sphagnoid species, with mean value of 35.6%.

Relatively similar pigment/water content ratio of bryophyte species within functional groups is interesting, but physiological basis of species-specific water content/pigment concentration ratio is not clear. Is it possible that chlorophyll concentration is regulated by water content? In an artificial experimental system consisting of a water gradient, tests with five bryophyte species showed that both chlorophyll *a* and chlorophyll *b* concentrations increased with increasing tissue water content (Spitale, 2009), which contradicts the present results. Also, higher chlorophyll concentration has been found in aquatic species than in terrestrial bryophytes (Lopez and Carballeira, 1989). In the study of Spitale (2009) moss samples were acclimated to particular conditions for 20 days, and therefore the described relationship might be attributed to physiological response of the bryophyte species to the hydration level. In the present study, in-

stead of short-term or long-term response of photosynthetic pigment concentration of the particular species to changes in tissue water content, the pigment/water relationship was assessed on a broader scale using a range of bryophyte species in relatively homogeneous field conditions of boreonemoral habitats most likely reflecting long-term physiological adaptations to the prevailing set of environmental conditions.

It is likely that the amount of light available to bryophyte at a particular time, in relation to interaction between natural light and microhabitat conditions, can affect its photosynthetic pigment concentration. It has been shown that decrease in ambient light intensity results in increased chlorophyll content for bryophytes (Sluka, 1983). It is interesting to note that in bryophytes carotenoids can function not only in dissipation of excess energy (Britton, 1995; Heber *et al.*, 2006) but also as accessory pigments in antenna complexes (Czeczuga, 1985). Other environmental variables not estimated in the present study within the habitats generally were similar as they were located not further than 35 km apart. However, microenvironmental heterogeneity associated with substrate topography might be important.

It seems that functional classification systems only partially and to a various extent reflect particular morphological and physiological adaptations. According to the life strategy classification system, all *Sphagnum* species represent the long-lived shuttle type. This type was represented also by epiphytic *Leucodon sciuroides* and *Conocephalum conicum*, the only liverwort species in the present study. It is well-established fact *Sphagnum* species need higher tissue water content to maintain optimum photosynthesis, in comparison to other bryophyte species (Silvola and Aaltonen, 1984). Another well-discriminated species group in our study had a high photosynthetic pigment concentration/water content ratio as well as high photochemical activity of PS II as indicated by chlorophyll *a* fluorescence traits. This particular group consisted of four species, *Plagiomnium undulatum*, *Conocephalum conicum*, *Tetraphis pellucida* and *Atrichum undulatum*. All of them are endohydric, possessing an internal water-conducting system. *Atrichum undulatum*, the species showing the highest concentration of photosynthetic pigments together with a high intensity of fluorescence traits, is known to have high intensity of photosynthetic CO₂ fixation in respect to unit of surface (Krupa, 1984). This physiological feature is associated with a ventilated photosynthesis system due to lamellar structure of leaves. It can be suggested that all members of this group have a similar type of morphological adaptation allowing for efficient photosynthesis at low but relatively stable tissue water content.

One might argue that the distribution of species within the defined continuum of tissue water content, pigment concentration and chlorophyll *a* fluorescence traits may have a stochastic character. Variability of characteristics within particular species was indeed relatively high, as it was already noted in a previous study (Liepiņa and Ievinsh, 2013). It was attempted to eliminate any seasonal dependence of the

measured traits by sampling the same bryophyte species four times during summer. Therefore, a single species was represented by a relatively higher variation within a continuum of water content, photosynthetic pigment and chlorophyll *a* fluorescence traits. Seasonal differences in physiological traits, while well known (e.g. Kershaw and Webber, 1986), are often a neglected aspect in ecophysiological analysis of bryophytes. Sampling of different species only at particular times during the season can potentially result in a significant season-specific component of established relationships. Regarding other potential limitations of the present study, it is necessary to stress out that the particular method used for analysis of relationships between functional traits among functional types of bryophytes can greatly affect the outcome of the analysis. Thus, LDA resulted in a better ability to discriminate between substrates, life forms and life strategies in comparison to PCA, but for habitat and water conducting system the ability was relatively similar. It is generally believed that LDA is a far better method of classification analysis maximizing class separation, but it was also argued that PCA can outperform LDA if the number of samples is relatively small (Martinez and Kak, 2001), which was the case also in the present study. Consequently, PCA in the present study gave more ecophysiological relevant information, showing relationships between various traits for species from different functional groups, while LDA allowed for use of physiological traits in bryophyte functional classification.

CONCLUSIONS

Individual bryophyte species had a clearly distinctive position within the multifactor gradient of tissue water content vs. photosynthetic pigment concentrations and high/low photochemical activity of PS II. The complex use of quantitatively measured functional traits by principal component analysis and linear discriminant analysis allowed to discriminate between functional types of bryophytes within different systems of functional classifications. Inverse relationship between concentration of pigments and water content was found, in general showing that species with higher water storage ability had lower photosynthetic capacity at the level of particular pigment content. Field water content and chlorophyll fluorescence indices field F_v/F_m , equilibrated PI, equilibrated RC/ABS, and field RC/ABS were important functional traits for characterisation of water conducting system, substrate and habitat, and, to a lesser extent, life strategy, while chlorophyll *a* and *b* concentrations were important for discrimination of life form, being highest in dendroid and lowest in sphagnoid species. These particular traits had the highest discriminatory ability within the studied systems of functional classification.

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SAISTĪBA STARP FUNKCIONĀLAJĀM ĪPAŠĪBĀM, FUNKCIONĀLAJIEM TIPIEM UN BIOTOPU BOREONEMORĀLAJIEM SŪNAUGIEM

Pētījuma mērķis bija noteikt sakarību starp fizioloģiskajām īpašībām un funkcionālajiem tipiem briofītiem no pieciem boreonemorālajiem biotopiem, īpašu uzmanību pievēršot šo īpašību izšķiršanas spējai. Paraugus no 25 sūnaugu sugām ievāca četrās reizes vienas veģetācijas sezonas laikā. Gan lauka paraugos, gan paraugos ar līdzsvarotu ūdens saturu noteica ūdens daudzumu, hlorofila *a* fluorescenci un fotosintēzes pigmentu koncentrāciju. Principiālo komponentu analīze atklāja apgrieztu saistību starp pigmentu koncentrāciju un ūdens saturu. Lineāro diskriminantu analīze parādīja, ka salīdzinoši augsta vidējā sekojošās varbūtības klasifikācijas iespēja pēc fizioloģiskajām īpašībām ir ūdens vadīšanas sistēmai, bet mazākā mērā substrātam un biotopam, taču šī iespēja bija ļoti variabla un tipa specifiska dzīvības formām, bet mazāk variabla dzīvības stratēģijām. Lauka ūdens saturam bija augstākā vidējā izšķiršanas nozīme no visām fizioloģiskajām īpašībām, tam sekoja hlorofila fluorescences parametri lauka F_v/F_m , izlīdzinātais PI, izlīdzinātais RC/ABS un lauka RC/ABS. Fotosintēzes pigmentu koncentrācijai bija salīdzinoši maza nozīme funkcionālo tipu klasifikācijā.