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Dead or alive? Testing the use of C:N ratios and chlorophyll fluorescence in vertical shoot profiles to determine depth of vitality and point of senescence in populations of bryophytes.

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Bryophytes with indeterminate growth rarely exhibit clearly identifiable modules or age segments, but can be vertically divided into different physiologically active zones, since physiological activity normally declines vertically along the shoot profile depth. The aim of this study was to investigate whether it is possible to use C:N ratios (C/N) and/or parameters from chlorophyll fluorescence measurements (e.g. Fv/Fm, Fm or qN) to determine if bryophyte tissue is alive, senescent or dead, and at what distance along the shoot segment profile the moss tissue cease to live. Variation in C:N ratios and chlorophyll fluorescence between sites was also examined. This study shows that it is possible to separate alive, senescing and dead parts of the moss shoots in *Pleurozium schreberi*, and that chlorophyll fluorescence is a good method to use, whereas C/N varies between sites and species (for *Hylcomium splendens* and *Racomitrium lanuginosum*) and does not seem to reflect physiological activity to the same degree.

Traditionally, senescence in plants is defined as internally programmed degeneration or age-related declines in survival and reproduction, leading to death or variation in mortality patterns (Roach 2003). Plants with indeterminate growth, such as clonal plants or bryophytes, may escape determinacy of death or aging by continuous growth with production of new meristems, thereby becoming potentially immortal and escaping whole organism senescence (Watkinson and White 1986). While the life span of clonal plants can be reflected in birth and death rates of its specific modules (i.e. repeated, structural and functional subunits of plants, de Kroon et al. 2005), it is rare that bryophytes with indeterminate growth exhibit clearly identifiable modules or age segments, and it is also difficult to know if translocation of resources takes place or not between such modules. One exception is *Hylocomium splendens* (Hedw.) Schimp. where most shoots express sympodial or monopodial growth patterns with clearly distinct modules (Økland 1995).

Clonal bryophytes often exhibits different active zones vertically along their shoots or turfs. For example, in environments with little physical disturbance it is possible to find intact moss shoots that are 5–15 cm long, with green active upperparts, while the lowest parts disintegrate into brown humus. Bryophyte shoots can be vertically divided into different regions of physiologically active zones, where the youngest and apical shoot parts are the most physiologically active with a clear fresh green color. The physiological activity usually declines vertically in the shoot profile depth with senescence, aging and final decomposition (Bates 1979, Økland 1995, Tobias and Niinemets 2010). Thus, bryophytes continues to grow upwards from the apical regions concurrently with being decomposed from underneath (Glime 2007). Obviously, the growth of the shoot apex must be greater than the decomposition rate in order for the bryophyte shoot to continue to stay alive, photosynthesize, and avoid burial by the surrounding shoots (Økland 2000).

Three different physiological zones in the shoot profile depth of clonal bryophytes can be described:

1) Active apical zone

   In the first and uppermost zone of the moss shoot, the green moss apex is physiologically active with high amounts of chlorophylls and nitrogen (Tobias and Niinemets 2010), and low carbon:nitrogen ratio, i.e. C/N (Nakatsubo 1990). Observations by Bates (1979) and Tobias and Niinemets (2010) from single populations in UK and Estonia, respectively, confirms that physiological activity (in e.g. phytosynthesis and chlorophyll content) in the uppermost 2 cm of the peren-

nial bryophyte *Pleurozium schreberi* (Willd. ex Brid.) Mitt. is substantially higher compared with other depths of the shoot segments.

2) Aging and dead zone
   At some distance from the green active apical region, there is a decline in physiological activity with aging (Bates 1979). When the older parts starts to age and senescence, chlorophyll (Tobias and Niinemets 2010) and nitrogen content decrease (or is relocated) and C/N increases (Nakatsubo 1990).
3) Decomposition zone
   In the oldest and most decomposed parts of the shoot profile depth, increased microbial activity (mineralisation and immobilisation) may cause a decrease in C/N (Nakatsubo 1990).

Unfortunately, the underlying causes of rates of senescence and aging in moss tissue are generally poorly understood, but may be related to e.g. growth rates and moss shoot density (Økland and Økland 1996) or dependent upon the shortage of light (van der Hoeven et al. 1993). Bates (1979) studied several physiological characters in 2 cm long shoot segments of *Pleurozium schreberi* to determine patterns of physiological decline with aging and concludes no clear division between living and dead shoot segments was possible.

Tobias and Niinemets (2010) investigated acclimation in photosynthetic characteristics of *P. schreberi* and found that variation in chemical and physiological traits within the depth of the moss canopy layer is a balance between acclimation and senescence and that canopy layers deeper than ca 2 cm reflect senescence by changes in pigment, nitrogen concentration and the photosynthetic capacity.

Currently, there are very few data explaining the diverse patterns of physical zones in mosses found in the field such as e.g. observations of persistent green color in very old moss tissue commonly found in more harsh environments such as northern regions (Tamm 1953). If it would be possible to measure which parts of the moss are dead or alive, it would greatly facilitate experiments involving bryophytes since the size of the physiologically active modules could be determined and controlled. The lack of knowledge of the physiological vitality and inability to determine age at different depths of the vertical shoot profile make it unreliable to use bryophytes in experiments where physiological measurements or growth measurements are used as response variables, since the ratio of living to dead material is unknown (see also Bates 1979). Such variation may interfere with the experimental outcome since a shoot with a higher ratio of living tissue will have higher rates of photosynthesis and therefore be able to have higher growth rates compared to a shoot with a lower amount of living tissue. Additionally, some bryophytes may translocate nitrogen (Eckstein and Karlsson 1999). Hence, it is possible that shoots with a lower amount of living tissue and a higher amount of old or dead tissue will have access to more nitrogen and therefore be able to translocate it to the physiologically active parts and gain benefits that also could induce higher growth rates. Consequently, there is a great need for further development within this research area in order to answer the basic question of what moss parts are dead or alive.

I have identified two reliable methods, analyses of C:N ratio and use of chlorophyll fluorescence, that hitherto, to my knowledge, have not been used on clonal bryophytes to investigate aging and senescence and I therefore wanted to investigate if it is possible to determine whether moss tissue is alive, senescent or dead.

C:N ratio (or C/N) has previously been used in *Sphagnum* peat as an indication of decomposition down the peat profile by Malmer and Holm (1984) and Kuhry et al. (1992), but has not been used as an indicator of vitality in pleurocarpous bryophytes (cf. Zotz and Kahler), although nitrogen content in *P. schreberi* was analysed by Tobias and Niinemets (2010) as a measure of acclimation. Decomposition rates are mainly determined by the physical environment, resource quality and soil organisms (Swift et al. 1979), and the carbon to nitrogen quotient (i.e. C:N ratios) can be used as a measure of resource quality (Coulson and Butterfield 1978, Berg and Staaf 1980).

A previous study showed that there were large variations in C/N between different species of high Arctic bryophytes when comparing between the green and brown moss parts (Pakarinen and Vitt 1974). C/N in the shoot profile depth for 1-cm shoot segments in three shoots of *Racomitrium lanuginosum* (Hedw.) Brid. showed very consistent variation down the aging shoot segment profile depth (Nakatsubo 1990) with the physiological zones down the shoot profile depth. In this study, I was interested in using C/N to see if it was possible to identify the boundaries between zones of physiologically active tissue, aging and decomposing tissue.

In physiological terms, senescence reflects the decreasing ability of plant tissue to photosynthetize as tissue ages. Leaf senescence can therefore be measured in terms of chlorophyll loss since senescence is dominated by the breakdown of chlorophyll and chloroplasts (Noodén et al. 1997). Chlorophyll fluorescence analysis presents a powerful and widely used technique to study photosynthetic performance of plants and bryophytes and may also be used for determining the senescence of plant tissue. When actinic light from the chlorophyll fluorescence meter reaches the chlorophyll molecules, three different responses may occur: 1) light is used to drive the photosynthesis, 2) excess energy can be dissipated as heat or 3) the light can be re-emitted as fluorescence (Maxwell and Johnson 2000).

Accordingly, chlorophyll fluorescence analysis will give a relative measure on the overall function of the photochemistry that may be compared with the changes in heat dissipation between physiologically active and non-active

samples. A relative comparison of chlorophyll fluorescence and heat dissipation for dark-adapted samples will reveal that when the tissue starts to senescence, values of the photochemical quenching (qP) will decrease due to less chlorophyll molecules available, while the values of the non-photochemical quenching (qN or NPQ) will show a relative increase due to a higher amount of heat dissipation (Lu and Zhang 1998). Therefore, studies of relative changes of the non-photochemical quenching can be used to analyse patterns of senescence in bryophyte tissue. Yet, until now, the technique seems to have been mainly used for studying senescence in vascular plants (Lu and Zhang 1998, Lu et al. 2001), but chlorophyll fluorescence (Fv/Fm) in the aquatic liverwort *Jungermannia exsertifolia* Stephani was significantly higher in younger shoots compared to older shoots (Arróniz-Crespo et al. 2008). Furthermore, chlorophyll fluorescence induced by ultraviolet light have been used to determine whether bryophyte spores were normal or aborted (Glime and Bisang 2014).

The aim of this study was to investigate whether it is possible to use C:N ratios and/or parameters from chlorophyll fluorescence measurements to determine if bryophyte tissue is alive, senescing or dead, and at what distance along the shoot segment profile the moss tissue cease to be alive. Since previous studies have focused on only one population and for example, the onset of senescence and decomposition may vary with localities or habitats (Glime 2007), I decided to also examine variation in C:N and chlorophyll fluorescence between populations exposed to different environmental conditions.

Material and methods

Species

Hylocomium splendens, *Pleurozium schreberi* and *Racomitrium lanuginosum* are three widely distributed species that are often forming continuous moss mats to such extent that they will be very dominant in certain ecosystems. Bryophytes in general have very slow decomposition rates, so when they are growing in intact turfs or moss mats, it is possible to collect individual shoots that are very long and therefore represent many years of growth. The species in this study were chosen since it was possible to find sites with intact and thick moss mats which was considered important in order to analyse C/N at different vertical distances from the apical region.

The species have a life-strategy classification as perennial stayers (sensu During 1979) and vegetative propagation is the main mode of reproduction. All three species are common in the northern hemisphere and they can be found in different types of ecosystems ranging from the temperate zone to high altitude mountain tops, or in the High Arctic tundra. *Hylocomium splendens* and *P. schreberi* are common moss species in the forest floor where they form loose turfs. *Racomitrium lanuginosum* prefers more exposed and sunny habitats and can therefore be found at high altitudes in mountains where there is a lack of light competition from vascular plants, on uninhabited early successional land, summits, upland grasslands and heaths, and peatland hummocks (Tallis 1958). *Racomitrium lanuginosum* often grows in compact turfs dominated by vertical growth patterns, but may also switch to a more branched growth form when growing more horizontally.

The species are ecophysiologically different, *H. splendens* and *P. schreberi* are temperate moss species with the relative highest growth rates around 15–25°C (Furness and Grime 1982), while *R. lanuginosum* is more cold-adapted with temperature optima of 10–20°C (Furness and Grime 1982), or 8–10°C (Tallis 1959). Previous studies have shown that *R. lanuginosum* plants have very high C/N compared to the levels in *H. splendens* (Jägerbrand et al. 2005).

Sampling and sites

Sampling of *H. splendens* and *R. lanuginosum* was performed at five sites situated in Iceland (Thingvellir, Armansfell and Audkuluheidi) and Sweden (Abisko, Latnjajaure low and Latnjajaure high) at different altitudes (Table 1). Sampling was carried out in June and August 1996 by collecting 10 cores of 7 cm in diameter at even distances along a 19-m transect running in a west–east direction. The samples consisted of a various amount of moss shoots. Samples used for C:N ratio were 6–8 per site (for more information see Jägerbrand et al. 2005).

Populations distributed along natural temperature gradients (altitudes) provide unique opportunities to capture ecological variation and have previously been used for bryophytes in ecological studies (Zechmeister 1995, Jägerbrand et al. 2014). *Pleurozium schreberi* was therefore collected at four different altitudinal sites (460, 670, 1055 and 1350 m a.s.l.), 5–20 m away from the hiking path at Mt Oakan, Hokkaido, northern Japan, in June 2007 (Table 1). Even though samples from the Mt. Oakan area are from the same geographical site, they are geographically separated by several kilometres and the altitudinal collection areas are therefore called sites hereafter. At each site, 10 randomly distributed samples of approximately 10 cm in diameter were carefully collected, ensuring that no shoots were damaged; however only a few shoots were actually used in the analysis.

The genetic structure of the *P. schreberi* populations from the same sites used in this study have been determined previously (Korpelainen et al. 2012).

Sites differed largely in their geographical locations as well as in temperature and precipitation (Table 1). Western Iceland has an oceanic climate (cool summers and mild winters), the Abisko area in northern Sweden has a

Table 1. Overview of sites, latitude, longitude, altitude (m a.s.l.), location of species samples (x), yearly mean temperature and total yearly precipitation. HS = *Hylocomium splendens*, PS = *Pleurozium schreberi*, RL = *Racomitrium lanuginosum*. [1] Calculated with a lapse rate of 0.53°C/100 m.

Site	Long.	Lat.	Altitude (m a.s.l.)	Location of sampling		Yearly mean temp (°C)	Total yearly precipitation (mm)	Closest weather station
Iceland								
Thingvellir	64°17'N	21°05'W	120	x	x	3.60[1]	1889	Irafoss 1974–1995
Audkuluheidi	65°10'N	20°15'W	480	x	x	– 1.15	755	Hveravellir 1966–1995
Armansfell	64°18'N	21°01'W	610		x	1.01[1]	1858	Haa-sula 1960–1993
Sweden								
Abisko	68°20'N	18°45'E	380	x		– 0.45	313	Abisko 1961–1995
Latnjajaure low elevation	68°21'N	18°30'E	980	x	x	– 2.66	836	Latnjajaure 1993-1995
Latnjajaure high elevation	68°21'N	18°30'E	1290	x	x	– 4.30[1]	836	Latnjajaure 1993-1995
Japan								
Mt. Oakan 460	43°45'N	144°16'E	460		x	3.7[1]	1161.7	Lake Oakan 1979-2007
Mt. Oakan 670	43°45'N	144°16'E	670		x	2.6[1]	1161.7	Lake Oakan 1979-2007
Mt. Oakan 1055	43°45'N	144°16'E	1055		x	0.6[1]	1161.7	Lake Oakan 1979-2007
Mt. Oakan 1350	43°45'N	144°16'E	1350		x	– 1.0[1]	1161.7	Lake Oakan 1979-2007

relatively more continental climate but sites at high altitudes are more similar to high Arctic tundra even though they are geographically situated in the sub-Arctic zone. Mt. Oakan has a more oceanic climate and is situated in the eastern part of Hokkaido, north Japan, with cold winters and relatively warm summers.

Analysis

For *H. splendens* and *R. lanuginosum*, five shoots from each of 6–8 samples from each of the sites were divided into four segments at the following intervals from the apex: 1: 0–0.5, 2: 0.5–2.0, 3: 2.0–5.5, 4: 5.5–13 cm. The choice of segment length was determined after examining shoots visually since the length represented: 1) the active and bright green apex, 2) green and active apical segment, 3) still green but starting to senescence and loosing the green colour, and 4) very brown part, probably decomposing. Material from the moss shoots was ground in liquid nitrogen to a fine-grained powder and dried to constant dry weight at 60°C for 24 h. Samples of 2 mg were analysed on a CN-analyser.

For *P. schreberi*, chlorophyll fluorescence analyses were performed 7–13 November 2007 on samples that were collected in June 2007. All samples were kept dark and dry between sampling and analysis. Bryophytes are poikilohydric (Hosokawa et al. 1964) and may therefore survive desiccation in a state of rest. Photosynthesis stops when the bryophyte tissue is dry, and restarts and recovers when remoisted. Bryophytes may therefore be kept alive under dark and dry conditions for several months or years and still start to photosynthesize when remoisted, although the recovery may not reach the same level of photosynthetic activity as previously. In this study, if there were any effect of the handling on the outcome of the chlorophyll fluorescence measurements, it was assumed to be of similar magnitude for all included samples.

One shoot from each of six samples per site was chosen based on the criterion of being the longest shoot in the sample. The shoots were cut into 1-cm segments, rewetted by spraying evenly with distilled water until the shoots were soaked through. The segments were then directly placed in dark leaf clips for 10 min for dark-adaptation. Samples were handled in a dark-room except for a few minutes when cutting the dry shoots into segments since visual light was necessary to perform the task. Chlorophyll fluorescence was measured with a mini-PAM (i.e. miniaturized pulse-amplitude-modulated photosynthesis yield analyser). The mini-PAM measures the minimum chlorophyll fluorescence (Fo) after a period of dark-adaptation with a weak beam of actinic light, (standard settings were used ca 0.15 µmol quanta m^{-2} s^{-1} and was ensured to not have any photochemical effects), and then flashes a saturation pulse to determine maximal fluorescence yield (Fm). Maximum fluorescence was driven by a 3 µs pulse of 18 000 µmol m^{-2} s^{-1} PAR (photosynthetically active radiation) with a halogen lamp. Fv/Fm (maximum quantum yield of photosystem II), qP (proportion of open PSII or photochemical quenching), NPQ, and qN (both are measures on the non-photochemical quenching) were constructed using the scripting facility on the mini-PAM (Heinz Walz GmbH 1999).

For *P. schreberi*, one shoot from four different samples from each site was carefully divided into 1-cm segments, ground and dried for 24 h. Different samples were used for chlorophyll fluorescence and C/N analyses. However, since samples were lost when the analyser was malfunc-

tioning, this left only two shoot profile samples for the 670 m a.s.l. site. Analyses of C/N in *P. schreberi* were performed on CHNOS elemental analyser. C/N was calculated as mg carbon g^{-1} dry weight divided by mg nitrogen g^{-1} dry weight.

Data analysis

C/N was analysed separately for the three species due to different segment unit lengths and because C/N values between *H. splendens* and *R. lanuginosum* was very high. C/N data for *H. splendens* and *R. lanuginosum* was log-transformed to meet assumptions of normality and then analysed by ANOVA (analysis of variance) and the Tukey–Kramer post hoc test. Due to missing values for the last segments in Latnjajaure low of *R. lanuginosum,* it was necessary to conduct two separate one-way ANOVAs to analyse differences among sites and segments, while two-way ANOVA was used to analyse differences among sites and segments for *H. splendens.*

C/N in *P. schreberi* were analysed using LMM (linear mixed-effects models), assuming a linear relationship between C/N and the independent variables (i.e. site and shoot segment). LMM was preferred as an alternative to ANOVA since C/N in *P. schreberi* had an unbalanced replication. Samples from within one single sample were assumed to represent a sample taken from a normal distributed population and were therefore included as a random factor. The LMM model tested C/N ~ site, C/N ~ shoot segment, and C/N ~ site + shoot segment, and were constructed and analysed by LMM fit by REML (restricted maximum likelihood, a variant of standard maximum likelihood), with Gaussian distribution and log-link function. The quality of fit for the models was chosen based on the lowest values of AIC (Akaike's information criteria) and checked by viewing of plots of predicted and observed residuals. NPQ and qN could not be transformed to meet assumptions of normal distribution, therefore, to investigate inter-correlations between the different chlorophyll fluorescence parameters, a non-parametric correlation analysis (Spearman's rho) was used. qN was analysed by the non-parametric test Kruskal–Wallis to test for significant differences between sites, samples and shoot segments. For shoot segments, significant differences in qN between groups was then analysed by the Mann–Whitney U-test. ANOVAs were performed in Statview (5.0.1), LMM analyses were run with the nlme package (Pinheiro et al. 2013) in R ver. 2.15.2 (<www.r-project.org>), while Spearman's rho, Kruskal–Wallis tests, and Mann–Whitney U-tests were conducted in SPSS 19.

Table 2. ANOVAs of C/N of the shoot profile depth of *Hylocomium splendens* and *Racomitrium lanuginosum* from different sites (Iceland and Sweden).

Variable	DF	F	p
Hylocomium splendens (two-way ANOVA)			
Site	4	25.63	<0.0001
Segment	3	40.96	<0.0001
Site × Segment	12	3.43	0.0002
Residual	133		
Racomitrium lanuginosum (one-way ANOVAs)			
Site	4	7.76	<0.0001
Residual	142		
Segment	3	11.93	<0.0001
Residual	143		

Results

Significant differences in C/N were found among sites and segments in both *Hylocomium splendens* and *Racomitioum lanuginosum* (Table 2). In *H. splendens*, shoot segments at 0–0.5 cm had the lowest C/N values, and the segments

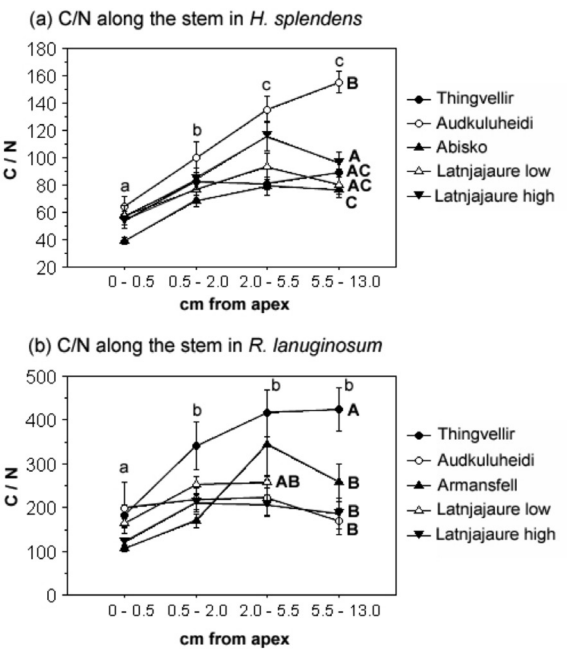

Figure 1. C/N (mean values ± 1 SE) for the sites at different intervals in the shoot profile depth (cm from apex) of (a) *Hylocomium splendens*, and (b) *Racomitrium lanuginosum*. Capital letters show significant (ANOVAs; $p < 0.05$) differences among sites, and lower-case letters show significant differences among the segments down the stem. n = 6–8.

Table 3. Results of linear mixed effects model for C/N in 1-cm shoot segments of *Pleurozium schreberi* from different sites in Mt Oakan, Hokkaido, northern Japan. Significant parameters, estimates, standard errors (SE), and p-values (only significant parameters are shown).

Parameter	Estimates	SE	p
Intercept	34.7	6.9	<0.0001
Shoot segments			
5–6 cm	14.9	7.3	0.043
6–7 cm	21.9	7.8	0.0063
7–8 cm	27.3	8.7	0.0025

0–0.5 cm and 0.5–2.0 cm from the apex had significantly lower C/N compared to the segments 2.0–13.0 cm from apex (Fig. 1). In *R. lanuginosum,* only the apical segment part (0–0.5 cm) had significantly lower C/N compared to the other segments (0.5–13.0 cm).

The best fit LMM of C/N in *P. schreberi* based on AIC (C/N ~ site, AIC = 898; C/N ~ shoot segment, AIC = 839; and C/N ~ site + shoot segment, AIC = 845) was shoot segments with sample as random effect variable (Table 3). Results from LMM shows that the shoot segments from 5 cm to 8 cm in the vertical shoot profile have significantly higher C/N values compared to the other shoot segments (Table 3, Fig. 2), mainly due to higher N values in the younger tissue.

Correlation coefficients showed that Fo (the minimum chlorophyll fluorescence) and Fm (the maximal fluorescence yield) was highly significantly correlated, while the relationship between Fo, Fm and Fv/Fm (the maximum

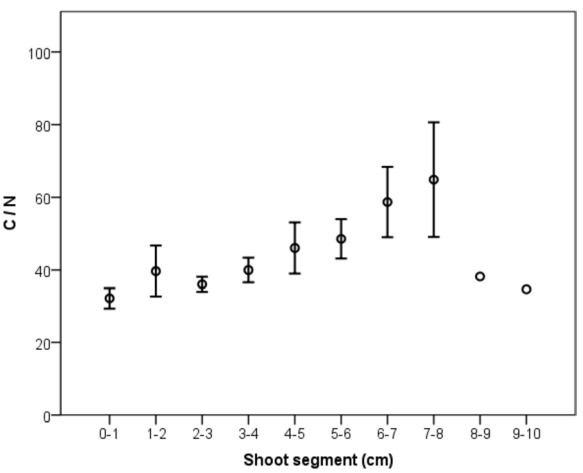

Figure 2. Mean values (± 1 SE) of C/N (carbon:nitrogen ratios) in 1-cm shoot segments in the shoot profile depth of *Pleurozium schreberi* from Mt Oakan, Hokkaido, northern Japan. Samples included four shoots from each of the four sites, except for the site 670 m a.sl. (only two), yielding a total of 100 C/N measurements.

Table 4. Correlation coefficients between chlorophyll fluorescence parameters measured in 1-cm shoot segments in *Pleurozium schreberi* from different sites in Mt Oakan, Hokkaido, northern Japan. Spearman's rho correlation, ** p < 0.01 (two-tailed). n = 209.

	Fo	Fm	Fv/Fm	qP	qN	NPQ
Fo	1					
Fm	0.98**	1				
Fv/Fm	0.06	0.23**	1			
qP	0.33**	0.42**	0.23**	1		
qN	–0.87**	–0.88**	–0.11	–0.52**	1	

quantum yield of photosystem II) and qP (proportion of open PSII or photochemical quenching) was less linear (Table 4). qN or NPQ (considered as approximately similar measurements of non-photochemical quenching, since they have a correlation coefficient of 1.0) was the best proxy for a relative measure of photosynthetically less active samples since they both had strong negative correlation with Fo and Fm (correlation coefficients of –0.87 and –0.88, respectively, Table 4).

Fv/Fm and qN along the shoot segments in *P. schreberi* intersected between 3–4 cm and 4–5 cm (Fig. 3), and the intersections between Fm and qN were also found at this distance (Fig. 4). Fv/Fm shows inaccurate high values of the maximum quantum yield of photosystem II in the lower segment of the shoots (9–11 cm) due to extremely low amounts of Fo and Fm (Fig 3).

The non-photochemical quenching, qN, was significantly different between shoot segments (Kruskal–Wallis test, p < 0.05; Fig. 3, Fig. 4) and increased with segment age, but was not significantly different between sites or

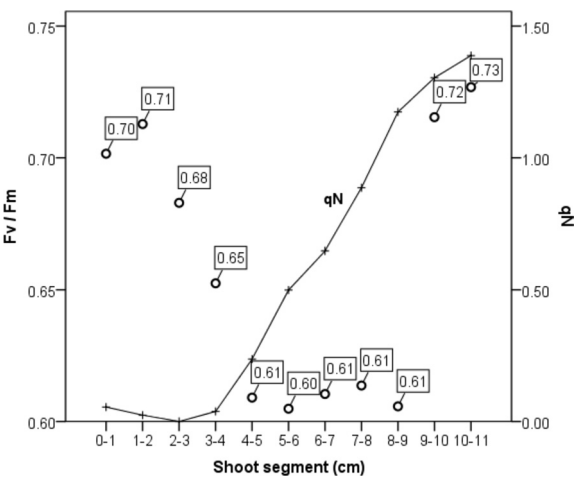

Figure 3. Mean values for 1-cm shoot segments in the shoot profile depth of *Pleurozium schreberi* from Mt Oakan, Hokkaido, northern Japan. Figure with dual axes showing Fv/Fm, maximum quantum yield of PS II, on left (open circles and values within the rectangle connected to the circle), and qN, the non-photochemical quenching on right (indicated by the line and +). Samples included six shoots for each of the four sites, giving 209 measurements of chlorophyll fluoresence.

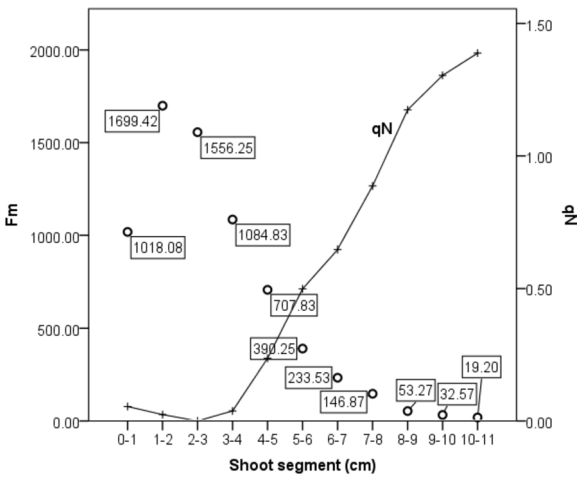

Figure 4. Mean values for 1-cm shoot segments in the shoot profile depth of *Pleurozium schreberi* from Mt Oakan, Hokkaido, northern Japan. Figure with dual axes showing Fm, maximum fluorescence on left (open circles and values within the rectangle connected to the circle), and qN, the non-photochemical quenching on right (indicated by the line and +). Samples included six shoots for each of the four sites, giving 209 measurements of chlorophyll fluoresence.

samples. Test of the non-photochemical quenching qN between the shoot segments (Mann–Whitney U-test) show significant differences between the segments of 0–1 cm and 5–11 cm, 1–2 cm and 4–11 cm, 2–3 cm and 4–11 cm, 3–4 cm and 5–11 cm, 4–5 cm and 6–11 cm, and between 5–6 cm and 7–11 cm (Table 5).

Discussion

This study shows that it is possible to separate living, senescing and dead parts of the moss shoots in *Pleurozium schreberi*, and that chlorophyll fluorescence is a good method to use, whereas C/N varies between sites and species and does not seem to reflect physiological activity to the same degree. The photosynthetically active parts are mainly the uppermost 4 cm (with Fv/Fm above 0.65 and Fm above 700), the senescing or aging parts are between 4–6 cm (indicated by the intersection between Fv/Fm, Fm and qN), while the dead zone starts around 5–6 cm depths (higher values of qN). These results are also in accordance with the significantly higher C/N at 5–8 cm depth for *P. schreberi* that indicates the start of the dead zone.

Previous studies have investigated responses of Fv/Fm in bryophytes to, for example, desiccation (Stark et al. 2013, Hájek and Vicherová 2014) UV-B (Hui et al. 2014) or restoration (drained and rewetted) effects (Kangas et al. 2014). It is difficult to compare Fv/Fm levels from this study with others because researchers include various stem lengths and use various methods for Fv/Fm measurements (for example dark-adaptation and prior handling procedures). The highest Fv/Fm values reported here for the active segments was 0.71 (in the top 1–2 cm of *P. schreberi*), and Fv/Fm subsequently decreased with shoot depth, with the lowest values around 0.60–0.61. Maximum quantum yield of PSII (Fv/Fm) was reported to be around 0.65 in old shoots and 0.70 in young shoots in Jungermannia exsertifolia Stephani grown under controlled conditions (Arróniz-Crespo et al. 2007), but it is unknown if the old shoots showed signs of senescence. Kangas et al. (2014) showed that Fv/Fm in the top 2 cm of *P. schreberi* was 0.76, which is substantially higher than the values reported here. However, in this study, the samples were stored for 5 months before rewetted which is a long time period. Kangas et al. (2014) stored the moss samples for 2–3 days before measuring Fv/Fm. Additionally, it is difficult to draw any conclusions based on values of Fv/Fm from other species since *P. schreberi* have been found to have significantly different Fv/Fm values compared to *Sphagnum girgensohnii* Russow (Kangas et al. 2014).

Observations made by Bates (1979) and Tobias and Niinemets (2010) showed that physiological activity was mainly situated in the uppermost 2 cm of *P. schreberi*. Results in this study agrees partly with that but also shows that the photosynthetic activity is prolonged until at least 4 cm, and thenceforth declines. Still, in Bate's study (1979), rate of photosynthesis declined at a shoot length of 4–6 cm from the apex and was undetectable after 8 cm. Conversely, Tobias and Niinemets (2010) argues that moss depths deeper than ca 2 cm reflect senescence by changes in pigment, nitrogen concentration and the photosynthetic capacity, but they only included the uppermost 0–5 cm in their analysis. Since all previous studies and this one have based measurements on samples collected from field conditions, it is difficult to know for sure whether differences originate in the various methodologies used or are due to different environmental conditions at the collection sites. For example, abiotic factors such as light conditions (Zotz and Kahler 2007, Tobias and Niinemets 2010) or characteristics of the moss canopy such as density (van der Hoeven et al. 1993, Rice et al. 2011) may influence the physiological zone length in moss shoots. Likewise, growth rates, ramification frequency, size of the shoots and disturbances may also play important roles (Økland 1995, Rydgren et al. 2001).

This study included *P. schreberi* samples from four sites originating from different environmental conditions that were separated geographically along an altitudinal gradient (460–1350 m a.s.l.). Nonetheless, site differences in both qN and C/N in *P. schreberi* were shown to be insignificant, which indicates, together with the results of Bates (1979), that the findings of the lengths of the different living, senescing and dead zones can perhaps be generalized for *P. schreberi*. Future studies are needed to confirm if this holds true.

Table 5. Half-matrix of results for the Mann–Whitney U-tests on differences in qN between shoot length (in 1-cm segments, and (n) number of replicates). Values indicates Z, and significance level ($p < 0.05$). n.s.= non significant. Shoot lengths below 11 cm from the apex had fewer than five measurements and was therefore removed from the analysis.

	Shoot number (in 1-cm segments from the apex)										
Shoot nr	0–1 (24)	1–2 (24)	2–3 (24)	3–4 (24)	4–5 (24)	5–6 (24)	6–7 (19)	7–8 (15)	8–9 (11)	9–10 (7)	10–11 (5)
0–1											
1–2	n.s.										
2–3	n.s.	n.s.									
3–4	n.s.	n.s.	n.s.								
4–5	n.s.	–2.1, 0.037	–2.6, 0.010	n.s.							
5–6	–3.2, 0.001	–3.6, <0.0001	–3.9, <0.0001	–3.2, 0.001	n.s.						
6–7	–4.31, <0.0001	–4.6, <0.0001	–4.9, <0.0001	–4.4, <0.0001	–2.9, 0.004	n.s.					
7–8	–4.9, <0.0001	–5.2, <0.0001	–5.4, <0.0001	–5.0, <0.0001	–3.6, <0.0001	–2.2, 0.03	n.s.				
8–9	–5.4, <0.0001	–5.5, <0.0001	–5.7, <0.0001	–5.4, <0.0001	–4.4, <0.0001	–2.8, 0.005	–2.1, 0.035	n.s.			
9–10	–4.95, <0.0001	–5.2, <0.0001	–5.4, <0.0001	–5.0, <0.0001	–4.0, <0.0001	–3.6, 0.001	–2.5, 0.014	n.s.	n.s.		
10–11	–4.6, <0.0001	–4.9, <0.0001	–5.3, <0.0001	–4.6, <0.0001	–3.77, <0.0001	–3.5, <0.0001	–2.8, 0.006	–2.1, 0.03	n.s.	n.s.	

In this study, I used 1-cm shoot segments that are possible to handle for analysis of Fv/Fm in dark-leaf clips. This enabled visibility of small changes in chlorophyll fluorescence variation and it was therefore possible to identify the point of intersection between Fv/Fm, Fm and qN, which is assumed to indicate the aging zone, prior to the dead zone. Still, it is clear from the figures it would have been beneficial with an even finer resolution of 0–0.5 cm long shoot segments. Using larger shoot segment samples would make it difficult to separate the physiological zones.

Samples of *H. splendens* and *R. lanuginosum* were taken at different intervals along the shoot profile depth in order to represent different physiological or aging stages. However the C/N shoot profile curve is unsophisticated and did not succeed in showing the fine-scaled patterns that are visible in the 1-cm shoot segments in *P. schreberi*.

Bates (1979) discusses that the nearest approximation between living and dead material was found to be around 4–8 cm in the vertical shoot depth, which was also around the point where photosynthesis became undetectable. This is fully in agreement with the results in this study since I found that the dead tissue zone starts at 5–6 cm.

The results shows that chlorophyll fluorescence methodology is reflecting physiological activity along the shoot segments, while C/N varies greatly between sites and species and does not seem to reflect physiological activity to the same degree. Similarly, nitrogen content was not significantly different along the shoot profile of the acrocarpous moss Syntrichia ruralis (Hedw.) F. Weber & D. Mohr, while chlorophyll content decreased along the stem (Zotz and Kahler 2007).

Even though it is common to use chlorophyll fluorescence as a measure on the photochemistry and the state of the photosynthesis (even in bryophytes), few studies have been conducted to make use of the chlorophyll fluorescence in the opposite way, i.e. to measure the relative non-photochemical quenching (but see Müller et al. 2001). In this study, when moss shoot segments were photosynthetically active, qN was closer to 0, and qP, Fo and Fm showed higher values. It should therefore be possible to use any of these photochemistry-related parameters to determine if moss shoot segments are photosynthetically active or not, as long as measurements are conducted under dark-adaptation. However, since both NPQ and qN are measuring heat dissipation relative to the dark-adapted state it is not recommended to compare values between e.g. species or samples that have been treated differently. Furthermore, chlorophyll fluorescence is cheaper and easier to use than performing C/N analyses.

C/N in *H. splendens* and *R. lanuginosum* was significantly different between sites and segments, while C/N in *P. schreberi* did not show significant differences between sites but did for shoot segments. For all three species, there are clearly different trends in the C/N shoot profile depth, but these trends do not seem at all as consistent as were found in the three shoots of *R. lanuginosum* by Nakatsubo

(1990). Instead, C/N in the shoot profile depth showed a range of different patterns, from a continuous increase (*H. splendens* from Audkuluheidi), a slow increase (*R. lanuginosum* in Thingvellir), a more or less flat curve (e.g. *P. schreberi*), or a decrease (for one site of *P. schreberi*). Since this study included three different species and several sites with different abiotic and biotic conditions it is perhaps not surprising that the C/N of the shoot profile depth shows a diversity of patterns, since decomposition rates are dependent on many factors. For example, Pakarinen and Vitt (1974) showed that bryophytes have different C/N depending on habitat (hydric, xeric and mesic) and that the C/N depends largely on the nitrogen content since the carbon content is relatively constant. Additionally, transport of nitrogen along the stem may further complicate patterns of C/N. Currently however, the extent of such transports and possible influence on C/N is unknown.

The C/N for the shoot profile depth in *H. splendens* seems to be following two of the identified theoretically different zones since there were significant differences between the segment intervals 0–0.5 (the apical part, low values of C/N), 0.5–2.0 cm (higher values of C/N) and the even higher values in the other part of the shoots (2.0–13 cm). Hence, it is likely that the third phase with decomposition accompanied by lower C/N was not included in the lengths sampled. For *R. lanuginosum*, C/N levels are consistently very high along the shoot profile depth and the only part with low values are the 0.5 cm of the apex. Due to the high C/N values the curve in the shoot profile depth is flattening out and the third decomposition phase (with lower C/N) is probably not included, although the C/N is lower in the last segment for Armansfell and Audkuluheidi, even though it was clearly seen at 5–10 cm depth in shoots from Mt. Fuji (Nakatsubo 1990). It is possible that differences in growth rates at the various sites may influence the position of the segments that are in the decomposition phase.

The C/N in the shoot profile depth in *P. schreberi* from different sites in Mt. Oakan was found to be significantly higher at 5–8 cm and this was also consistent with the higher qN values at ca 5–9 cm. The higher values of C/N and qN indicates that at this specific point in the shoot, senescence and decomposition begins, and that the apical ca 5 cm is ecophysiologically active. The third phase with lower C/N values (and active decomposition) was not clearly visible, although a decrease in C/N was indicated in *H. splendens* at Latnjajaure low and Latnjajaure high, in *R. lanuginosum* at Armansfell and at Mt. Fuji (Nakatsubo 1990), and in *P. schreberi* at site 1055.

For decomposition, it is necessary to make analysis on relatively short shoot segments (1 cm), in order to fully see the total C/N curve to be able to determine when there is a switch from active shoot segments to non-active shoot segments. Determination of the physiological activity of the zone will be greatly facilitated if measurements on chlorophyll fluorescence are performed in the same shoot segments. A problem with the analysis is that very thin and fragile bryophytes or very decomposed material will be difficult to handle (i.e. ground and weighted) and it will also be difficult to get enough material to perform carbon and nitrogen analysis.

For future work it would be interesting to investigate the physiological zones in bryophyte species with various growth forms originating from areas reflecting environmental differences, since this would enable comparisons of the physiological zones between species, sites and morphological or taxonomical groups of bryophytes. Such knowledge would also make it possible to more fully understand how the physiological zones of the moss shoot segments are affected by abiotic and biotic factors.

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References

Arróniz-Crespo, M. et al. 2008. Age-specific physiological responses to UV radiation in the aquatic liverwort J*ungermannia exsertifolia* subsp. *cordifolia*. – Cryptogamie Bryol. 29: 115–126.

Bates, J. W. 1979. The relationship between physiological vitality and age in shoot segments of *Pleurozium schreberi* (Brid.) Mitt. – J. Bryol. 10: 339–351.

Berg, B. and Staaf, H. 1980. Decomposition rate and chemical changes in decomposing needle litter of Scots pine. II. Influence of chemical composition. – In: Persson, T. (ed.), Structure and function of northern coniferous forest. – Ecol. Bull. 32: 373–390.

Coulson, J. C. and Butterfield, J. 1978. An investigation of the biotic factors determining the rates of plant decomposition on blanket bog. – J. Ecol. 66: 631–650.

de Kroon, H. et al. 2005. A modular concept of phenotypic plasticity in plants. – New Phytol. 166: 73–82.

During, H. J. 1979. Life strategies of bryophytes: a preliminary review. – Lindbergia 5: 2–18.

Eckstein, L. R. and Karlsson, P. S. 1999. Recycling of nitrogen among segments of *Hylocomium splendens* as compared with *Polytrichum commune*: implications for clonal integration in an ectohydric bryophyte. – Oikos 86: 87–96.

Furness, S. B. and Grime, J. P. 1982. Growth rate and temperature responses in bryophytes. II. A comparative study of species of contrasted ecology. – J. Ecol. 70: 525–536.

Glime, J. M. 2007. Chapter 13. Decomposition. Bryophyte ecology. Vol. 1. Physiological ecology. – Ebook by Mich. Tech. Univ. and Int. Ass. Bryol., pp. 1–10.

Glime, J. M. and Bisang, I. 2014. Chapter 3-3. Sexuality: size and sex differences. Vol. 1. Physiological ecology. – In: Glime, J. M. (ed.), Bryophyte ecology. Ebook by Mich. Tech. Univ. and Int. Ass. Bryol., p. 17.

Hájek, T. and Vicherová, E. 2014. Desiccation tolerance of *Sphagnum* revisited: a puzzle resolved. – Plant Biol. 16: 765–773.

Heinz Walz GmbH. 1999. Photosynthesis yield analyzer mini-PAM. Portable chlorophyll fluorometer. Handbook of operation. – Heinz Walz GmbH.

Hui, R. et al. 2014. Photosynthesis of two moss crusts from the Tengger Desert with contrasting sensitivity to supplementary UV-B radiation. – Photosynthetica 52: 36–49.

Hosokawa, T. et al. 1964. Causality of distribution of corticolous species in forests with special reference to the physio-ecological approach. – Bryologist 67: 396–411.

Jägerbrand, A. K. et al. 2005. Phenotypic variation at different spatial scales in relation to environment in two circumpolar bryophyte species. – Lindbergia 30: 125–142.

Jägerbrand, A. K. et al. 2014. Variation in responses to temperature treatments ex situ of the moss *Pleurozium schreberi* (Willd. ex Brid.) Mitt. originating from eight altitude sites in Hokkaido, Japan. – J. Bryol. 36: 209–216.

Kangas, L. et al. 2014. Photosynthetic traits of Sphagnum and feather moss species in undrained, drained and rewetted spruce swamp forests. – Ecol. Evol. 4: 381–396.

Korpelainen, H. et al. 2012. Genetic structure of mosses *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Racomitrium lanuginosum* (Hedw.) Brid. along altitude gradients in Hokkaido, Japan. – J. Bryol. 34: 309–312.

Kuhry, P. et al. 1992. Peatland development in relation to Holocene climate changes in Manitoba and Saskatchewan (Canada). – Can. J. Earth Sci. 29: 1070–1090.

Lu, C. and Zhang, J. 1998. Changes in photosystem II function during senescence of wheat leaves. – Physiol. Plant. 104: 239–247.

Lu, C. et al. 2001. Xanthophyll cycle, light energy dissipation and photosystem II down-regulation in senescent leaves of wheat plants grown in the field. – Aust. J. Plant Physiol. 28: 1023–1030.

Malmer, N. and Holm, E. 1984. Variation in the C/N-quotient of peat in relation to decomposition rate and age determination with ^{210}Pb. – Oikos 43: 171–182.

Maxwell, K. and Johnson, G. N. 2000. Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. 51: 659–668.

Müller, P. et al. 2001. Non-photochemical quenching. A response to excess light energy. – Plant Physiol. 125: 1558–1566.

Nakatsubo, T. 1990. Primary production and nitrogen economy of *Racomitrium lanuginosum* at the subalpine lava field of Mt Fuji. – Proc. Bryol. Soc. Jap. 5: 65–70.

Noodén, L. D. et al. 1997. Senescence mechanisms. – Physiol. Plant. 101: 746–753.

Økland, R. H. 1995. Population biology of the clonal moss *Hylocomium splendens* in Norwegian boreal spruce forests. I. Demography. – J. Ecol. 83: 697–712.

Økland, R. H. 2000. Population biology of the clonal moss *Hylocomium splendens* in Norwegian boreal spruce forests. 5. Vertical dynamics of individual shoot segments. – Oikos 88: 449–469.

Økland, R. H. and Økland, T. 1996. Population biology of the clonal moss *Hylocomium splendens* in Norwegian boreal spruce forests. II. Effects of density. – J. Ecol. 84: 63–69.

Pakarinen, P. and Vitt, D. H. 1974. The major organic components and caloric contents of high arctic bryophytes. – Can. J. Bot. 52: 1151–1161.

Pinheiro, J. et al. 2013. nlme: linear and nonlinear mixed effects models. – R Package ver. 3.

Rice, S. K. et al. 2011. Relationships among shoot tissue, canopy and photosynthetic characteristics in the feathermoss *Pleurozium schreberi*. – Bryologist 114: 367–378.

Roach, D. A. 2003. Evolutionary and demographic approaches to the study of whole plant senescence. – In: Noodén, L. (ed.), Plant cell death processes. Academic press, pp. 331–348.

Rydgren, K. et al. 2001. Effects of fine-scale disturbances on the demography and population dynamics of the clonal moss *Hylocomium splendens*. – J. Ecol. 89: 395–405.

Stark, L. R. et al. 2013. Physiological history mask the inherent inducible desiccation tolerance strategy of the desert moss *Crossidium crassinerve*. – Plant Biol. 16: 935–946.

Swift, M. J. et al. 1979. Decomposition in terrestrial ecosystems. Studies in ecology; vol 5. – Blackwell.

Tallis, J. H. 1958. Studies in the biology and ecology of *Rhacomitrium lanuginosum* Brid. I. Distribution and ecology. – J. Ecol. 46: 271–288.

Tallis, J. H. 1959. Studies in the biology and ecology of *Rhacomitrium lanuginosum* Brid. II. Growth, reproduction and physiology. – J. Ecol. 47: 325–350.

Tamm, C. O. 1953. Growth, yield and nutrition in carpets of a forest moss (*Hylocomium splendens*). – Meddel. från Statens Skogsforskningsinst.

Tobias, M. and Niinemets, Ü. 2010. Acclimation of photosynthetic characteristics of the moss *Pleurozium schreberi* to among-habitat and within-canopy light gradients. – Plant Biol. 12: 743–754.

van der Hoeven, E.C. et al. 1993. Vertical profiles of biomass, light intercepting area and light intensity in chalk grassland mosses. – J. Hatt. Bot. Lab. 74: 261–270.

Watkinson, A. R. and White, J. 1986. Some life-history consequences of modular construction in plants. – Phil. Trans. R. Soc. B 313: 31–51.

Zechmeister, H. G. 1995. Growth rates of five pleurocarpous moss species under various climatic conditions. – J. Bryol. 18: 455–468.

Zotz, G. and Kahler, H. 2007. A moss "canopy" – small-scale differences in microclimate and physiological traits in *Tortula ruralis*. – Flora 202: 661-666.