



# Performance of late succession species along a chronosequence: Environment does not exclude *Sphagnum fuscum* from the early stages of mire development

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Bog moss; Chlorophyll fluorescence; Competition; Mire succession; Peatland; Photosynthesis

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## Introduction

Long-term mire succession is generally a process where a wetland ecosystem develops from a minerotrophic fen stage towards an ombrotrophic bog stage (Rydin & Jeglum 2006). During succession, the increasing elevation of the mire surface leads to a gradual decrease in the supply of mineral-rich water from the upland catchment area. As a

## Abstract

**Questions:** *Sphagnum fuscum* (Schimp.) Klinggr. is a characteristic species of the later stages of mire development. It is hypothesized that competition limits its success in earlier successional stages where higher mineral nutrient availability and wetter conditions favour species with a higher production rate and, therefore, a stronger ability to compete. We aim to quantify the importance of different site factors and the effect of neighbouring plants on the performance of *S. fuscum*.

**Location:** A mire chronosequence from a land uplift coast towards inland on the Finnish side of Bothnia Bay, Baltic Sea.

**Methods:** We transplanted *S. fuscum* to seven sites along a mire chronosequence for a period of two growing seasons. Neighbouring plants were removed from half of the locations/transplants. We measured several growth-related parameters, such as rate of photosynthesis, chlorophyll fluorescence ( $F_v/F_m$ ) and abundance of mosses.

**Results:** *Sphagnum fuscum* samples did not survive in the recently exposed sandy shore stage and in fen flarks with extensive sedge cover. Photosynthesis and abundance of transplanted *S. fuscum* were highest in the wet meadows, the stages that immediately follow the sandy shore stage. Photosynthetic rate and  $F_v/F_m$  were low in the late succession bog site compared to the younger successional sites. The occurrence of neighbouring plants decreased the abundance of the transplanted mosses but improved the physiological state. The impact was similar throughout the successional chronosequence.

**Conclusions:** The site factors favourable for rapid *Sphagnum* growth in general also favoured *S. fuscum*. The low photosynthesis, respiration and  $F_v/F_m$  of intact *S. fuscum* growing in its natural habitat compared to the surviving transplants in the younger sites suggests that in order to occur in its natural habitat, *S. fuscum* needs to allocate resources for photoprotective mechanisms. Our results indicate that under better environmental conditions, these resources can be allocated to the photosynthetic apparatus to increase growth.

result, in the latest stage of mire succession (i.e. the bog stage), precipitation and chemical elements are only received from atmospheric deposition. The most notable changes during succession are decreased water level, decreased nutrient availability and increased acidity. These changes are mainly caused by the accumulation of the peat layer (Frolking et al. 2010). Colonization by *Sphagnum* mosses is a critical point in mire succession as they

enhance peat accumulation, disconnection to ground water and acidification (Clymo & Hayward 1982; Van Breemen 1995; however see Soudzilovskaia et al. 2010 for the mechanism) and hence limit the growth of other species (Rydin et al. 1999). Although *Sphagnum* mosses are also present in the relatively young fens, they are only abundant in the late stages of peatland succession (Klinger & Short 1996).

The distribution of *Sphagnum* species in different mires is mainly explained through their niche relative to abiotic site conditions, such as pH, nutrient content and water level (e.g. Clymo & Hayward 1982; Andrus et al. 1983; Andrus 1986; Granath et al. 2010). The biotic factor 'competition' is usually linked with relative distance to the water level. It has been established that hollow species are superior competitors in wetter habitats due to their faster growth rate (Rydin & McDonald 1985; Rydin 1986; Asada et al. 2003; Laine et al. 2011). This implies that the hummock species, *Sphagnum fuscum* (Schimp.) Klinggr. is restricted to drier sites by interspecific competition (Rydin & McDonald 1985; Rydin 1986). However, there is evidence to suggest that *S. fuscum* and other hummock species may perform as well as hollow species under moist conditions (Rydin 1993; Robroek et al. 2007; Granath et al. 2010). This indicates that the mechanisms behind the performance are not yet fully understood.

*Sphagnum fuscum* dominates bog communities in the later successional stages (Vitt et al. 2003) and represents a major carbon sink at a global level (Clymo & Hayward 1982). In Northern Europe, it is the key species necessary for a successional shift from minerotrophic fen to ombrotrophic bog (Svensson 1988). Currently, a large part of the mires in northern boreal and arctic zones, the so-called aapa mires whose central areas are generally lower than the surrounding mineral ground (in contrast to raised bogs), are still in a minerotrophic stage. A changing climate, which is predicted to lead to increased evaporation and altered water table levels in the boreal region, may enhance the ombrotrophication process (Tahvanainen 2011). Since changes in ecosystems related to climate are first seen in the ecotones (Lowe 1991), the border zone between the raised bog and aapa mire regions is likely to be most sensitive for the regime shift. Therefore, it is important to understand which factors currently limit the distribution of *S. fuscum* in the earlier successional stages. Huston & Smith (1987) argued that a key for understanding natural systems lies in understanding the life history and physiological traits of a single species; thus individual-based models could explain the complex variety of successional dynamics that community-based models usually fail to explain.

The aim of this study is to quantify the importance of abiotic site factors and neighbouring plants for the

performance of *S. fuscum* during mire development. We hypothesize that other plants limit the performance at the earlier successional stages, when higher mineral nutrient availability and wetter conditions favour species with a stronger competitive ability due to higher primary production rates. To address our hypothesis, we conducted a transplantation experiment with the removal of neighbouring plants over a mire chronosequence from the early paludification stage to the ombrotrophic bog stage. The level of performance was measured as survival, growth and ecophysiological parameters related to photosynthesis that best describe the ability of species to outcompete others.

## Methods

### Study sites

The study area is located by the Gulf of Bothnia in Siikajoki, Finland (64°45' N, 24°42' E). In the area, a successional gradient of ecosystems is formed as land emerges from the sea due to a post-glacial land uplift of 7.54 mm-yr<sup>-1</sup> (Ekman 1996). During the study years, precipitation in the area was 634 and 613 mm, and annual average temperature was 3.6 and 3.8 °C for 2007 and 2008, respectively. These are higher than the 30-yr (1979–2009) averages of 539 mm and 2.6 °C. The average temperature during the growing seasons (1 May to 30 September) was 12.0 °C and 10.6 °C for 2007 and 2008, respectively (Revonlahti, Siikajoki, 64°41' N, 25°05' E, 48 m a.s.l., Finnish Meteorological Institute).

Seven different mire basins along the successional mire chronosequence were selected for the experiment. The sites were named SJ 0 to SJ 6, with SJ referring to the Siikajoki area and the number referring to the location along the chronosequence, similar to Tuittila et al. (2013). Each site represents a different successional stage from a recently exposed sandy shore to a rainwater-fed ombrotrophic bog. The sites are described in more detail in Tuittila et al. (2013), and due to the similar composition of plant residues in the bottom peat layers are expected to have had similar soil conditions at the initial stage of paludification.

The youngest site, SJ 0, is a recently exposed sandy shore, where vegetation is dominated by graminoids (e.g. *Juncus gerardii* and *Festuca rubra*), and no organic layer has yet developed on the sandy soil due to the young age of the site (ca. 70 yr). The vegetation of sites SJ 1 and SJ 2 resembles wet meadows and is dominated by sedges and grasses. These sites differ from the youngest site in the presence of a thin peat layer (<10 cm) and a patchy moss carpet consisting mainly of *Warnstorfia exannulata*. The sites SJ 3 and SJ 4 are fens dominated by sedges and have well-developed peat and moss layers. Site SJ 5 is at a fen–bog transition stage, with drier hummocks covered by ombrotrophic bog vegetation and wetter surfaces with typical fen

species. SJ 6 is fully covered with ombrotrophic bog vegetation. Site characteristics are given in Table 1.

### Environmental variables

We used a set of six to ten perforated tubes to measure the water levels on each site. The tubes covered the small-scale variation in surface topography (flarks to hummocks) typical of each site. Measurements were made bi-weekly over the growing season.

Between six to ten peat or peat/soil profile samples were collected in autumn 2007 from each site. The samples were handled and analysed at the laboratory of the Finnish Forest Research Institute (Metla), Parkano Research Unit. Samples were analysed for total nitrogen (N) concentration (for peat or soil surface) using dry combustion with a LECO CHN-2000 analyser (LECO Corp., St. Joseph, MI, US). Soil pH was analysed from the samples according to international standards (ISO 10390, 1994).

### Moss sampling and treatments

We collected patches of *S. fuscum* from site SJ 6 and transferred 24 patches to each of the seven sites at the beginning of June 2007. The patches stayed in the sites until August 2008, when the destructive measurements were made (sampling started at 21 August); see below for description of the measurements. The *S. fuscum* patches were cut from the moss carpet using a round, sharp-edged cylinder of 5.3 cm (22 cm<sup>2</sup>) diameter and ca. 4-cm deep. When transferred to a new site, the mosses were spread evenly to cover an area of 14 cm × 14 cm (196 cm<sup>2</sup>); and this loose patch of moss fragments was then covered with a mesh fabric, with 1-cm × 1-cm holes, which was pinned down to the surface with iron wires. Using loose fragments represents diaspore coloni-

zation much more than using a compact clump of mosses. The ideal way would be transplantation of individual shoots; however, tracing them back at the end of the experiment would be difficult. Even with this compromised technique we were not able to find all of the transplanted patches after two growing seasons; they had disappeared amongst vegetation or been carried away by floods. The *S. fuscum* patches were planted in pairs (with and without removal of neighbouring plants;  $n = 12$  for each treatment) into locations with different water levels typical of each site. In the neighbouring plant removal treatment all vascular plants and the capitula of native *Sphagnum* mosses were removed in the close vicinity of the transplants and were kept clear of other species throughout the growing seasons. At site SJ 0, where a water level gradient was absent, samples were planted randomly on the even surface. For the other sites, several pairs were placed into two to three water level locations as follows: sites SJ 1 and SJ 2 WT 0, -2 and -4 cm; site SJ 3 WT +7, -3 and -28 cm; site SJ 4 WT +9 and -15 cm; site SJ 5 WT +1, -15 and -24 cm; and site SJ 6 WT -8, and -47 cm. The WT values are growing season averages.

### Visual measurements

The growth of *S. fuscum* in transplanted patches (14 cm × 14 cm) was estimated as the area covered by the transplanted *S. fuscum* using a percentage scale. In addition, we counted the number of capitula. To evaluate the vitality of the mosses, we estimated their appearance using a scale that ranged from 1 to 4. The different vitality categories are described as follows; 4: *S. fuscum* patches with normal, healthy looking capitula growing upwards. 3: patches with viable capitula pointing upwards but little or no growth. 2: patches with capitula still pointing

**Table 1.** Site characteristics: N% is the total N content in the top 20 cm peat layer. N concentration per dry mass unit (g·g<sup>-1</sup>) was detected using a LECO CHN analyser. Peat pH was measured in water suspension (soil:water suspension = 1:2 (v/v); Merilä et al. 2006). The estimated age for sites SJ 0, SJ 1, SJ 2, SJ 3 and SJ 6 is based on the land uplift rate and elevation, and for sites SJ 4 and SJ 5 on <sup>14</sup>C dating. The most common moss species for each site are given.

|                        | SJ0                           | SJ1  | SJ2  | SJ3   | SJ4  | SJ5  | SJ6   |
|------------------------|-------------------------------|--|--|---|--|--|---|
| Type                   | Sandy beach                   | Wet meadow   | Wet meadow   | Mesotrophic fen   | Oligotrophic fen   | Fen-Bog transition   | Bog   |
| Age                    | ca. 40 yr                     | ca. 180 yr   | 200 yr   | 700 yr  | 1070 ± 70 yr BP  | 2520 ± 50 yr BP  | 3000 yr BP  |
| Location, m a.s.l      | ca. 0.5                       | 1.5-2.0  | 1.5-2.0  | 7   | 12   | 25   | 26  |
| Peat Depth, cm         | 0                             | <10  | 10   | 44  | 87   | 190  | 215   |
| pH                     | 6.3                           | 5.6  | 5.1  | 4.7   | 5.1  | 4.5  | 4.3   |
| N%                     | 0.06                          | 0.2  | 0.4  | 1.6   | 1.7  | 1.3  | 1.0   |
| Moss Species (Cover %) | <i>Drepanocladus</i> spp. (2) | <i>Warnstorfia exannulata</i> (24),<br><i>Calliergon cordifolium</i> (8) | <i>W. exannulata</i> (37),<br><i>Scapania paludicola</i> (7) | <i>Sphagnum fimbriatum</i> (33),<br><i>S. obtusum</i> (8) | <i>S. papillosum</i> (39),<br><i>S. subsecundum</i> (13) | <i>S. papillosum</i> (18),<br><i>S. balticum</i> (13),<br><i>S. majus</i> (16),<br><i>S. fuscum</i> (11) | <i>S. fuscum</i> (61),<br><i>S. balticum</i> (31) |

horizontally and no growth. 1: patches with dead mosses with no viable capitula. The cover for each vascular plant and moss species was determined for the samples without plant removal.

### Gas exchange and chlorophyll fluorescence measurements

To evaluate the ecophysiological state of the mosses, we measured their carbon dioxide (CO<sub>2</sub>) exchange and chlorophyll fluorescence. Measurements were made at the end of August 2008 using an open, fully controlled flow-through gas exchange fluorescence measurement system (GFS-3000; Walz, Effeltrich, Germany). Net CO<sub>2</sub> exchange ( $P_N$ ), quantum yield of PSII ( $\Phi_{PSII}$ ) and maximum quantum yield of photochemistry ( $F_v/F_m$ ) were measured as in Laine et al. (2011). The measurements were made for an even layer of *Sphagnum* capitula placed upright on a net placed between the needle cuvette jaws.  $P_N$  was measured under seven levels of photosynthetic photon flux density (PPFD) ranging from 0 to 2000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  to produce a light response curve of photosynthesis.  $F_v/F_m$  was measured after a 20-min period of darkness. Gross photosynthesis ( $P_G$ ) at each light level was calculated by subtracting the dark respiration ( $R_D$ ) from the CO<sub>2</sub> exchange ( $P_N$ ) values. We used a sign convention at which the gain of CO<sub>2</sub> was positive and release negative.

Samples were weighed before and after measurements. After the measurements, the samples were dried at 60 °C for >24 h, and the dry mass measured. The water content of the mosses before the measurements was, on average,  $1616 \pm 438\%$  ( $\pm\text{SD}$ ) and after measurements  $743 \pm 406\%$ . The variability in water content did not have a significant impact on  $P_N$  (Table 2).

### Data analysis

The data were analysed in a hierarchical manner. First, we analysed the probability of the moss samples dying under different conditions. This was made using mixed effects logistic regression. Site SJ 0 was omitted from the analysis for practical reasons as all the samples were dead and, therefore, clearly differed from the others, where the majority of samples survived. This enabled us to test possible differences among other sites without the additional confusion caused by the evidently significant difference between SJ 0 and the other sites. In the model, site, treatment and water level were used as a fixed effect and a random constant was assigned for each subplot (pair of samples with and without vegetation removal). Treatment contrasts were used for the site, using samples from the original donor site SJ 6 as reference. Models were fitted using function glmer in the lme4 package of

R (cran.r-project.org/package=lme4; accessed 15 Aug 2014; R Foundation for Statistical Computing, Vienna, AT).

In order to see how well the surviving plants performed, the following analyses were carried out for the samples that were alive. We tested for differences in the number, cover and vitality of capitula,  $F_v/F_m$  and  $\Phi_{PSII}$  at PPFD 300 and 1000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  using linear mixed effects models. For cover, we used the logit transformation, which provided a fit with no visible nonlinear trends in the residuals. For  $F_v/F_m$ , the third power was used to better meet the assumptions of normally distributed residuals. Other variables were modelled without transformations.

With all variables, we started with a full model including site, vegetation removal treatment and water level as fixed predictors and random constant for each pair of plots. Water level as included as a continuous variable. Fixed effects were omitted from the models, one at a time, if not found significant using conditional *F*-tests ( $P > 0.05$ ). The residual variance was modelled using a power function whenever the residual plots indicated that the starting assumption on constant variance was not met. Models were fitted using the function lme in the nlme package of R.

To determine the effects of site, vegetation removal treatment and moisture content of the moss samples on photosynthetic capacity, light-use efficiency at low light and respiration, we applied a nonlinear mixed-effects model with the hyperbolic light saturation curve (i.e. Larcher 2003):

$$A_{ksi} = R_{ks} + \frac{\text{PMAX}_{ks} \text{PPFD}_{ksi}}{\alpha + \text{PPFD}_{ksi}} + e_{ksi} \quad (1)$$

where the response  $A_{ksi}$  is the observed net photosynthesis expressed on a dry weight basis, and the predictor  $\text{PPFD}_{ksi}$  is the photosynthetic photon flux density for measurement  $i$  of sample  $s$  on site  $k$ . The parameters to be estimated are respiration ( $R_{ks}$ ), photosynthetic capacity, i.e. the maximum rate of light saturated gross photosynthesis ( $\text{PMAX}_{ks}$ ), and the maximum quantum yield of CO<sub>2</sub> assimilation ( $\alpha$ ). The residual ( $e_{ksi}$ ) is normally distributed, with mean zero and constant variance. Parameters  $\alpha$ ,  $R_{ks}$  and  $\text{PMAX}_{ks}$  were initially written as linear functions of fixed predictors site, treatment and moisture, and bivariate normal sample-specific random effects in  $\text{PMAX}$  and  $\alpha$ . These linear submodels were included in the light saturation curve (1), and all coefficients were estimated in one step. Thereafter, insignificant terms were dropped, one at a time, based on the approximate *F*-test ( $P > 0.05$ ). The reference samples (not transplanted) from site SJ 6 are used as a contrast to which other sites were compared.

**Table 2.** Conditional  $F$ -tests on terms in the full linear mixed-effects models for the differences in capitulum count, *S. fuscum* cover, maximum potential quantum yield of PSII ( $F_v/F_m$ ) and quantum yield of PSII ( $\Phi$ PSII) at PPFD 1000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and ANOVA results of the hyperbolic light saturation model (Eq. 1) for differences in maximum quantum yield of  $\text{CO}_2$  assimilation, maximum photosynthesis (P<sub>MAX</sub>) and dark respiration ( $R$ ). Site refers to the six different study sites (SJ 1 to SJ 6), neighbour to the vegetation removal treatment at which all neighbouring plants were removed, WT denotes water table level, and moisture is moisture content of the mosses after  $\text{CO}_2$  assimilation measurement. Marginal tests were performed, i.e. each term in turn was dropped from the full model and the  $F$ -statistics and  $P$ -values report results from comparison of these two models. All three factors were tested for each parameter and significant values were included in the model.

| Source    | Capitulum Count                |     |         |         | Cover                          |     |         |         |
|-----------|--------------------------------|-----|---------|---------|--------------------------------|-----|---------|---------|
|           | ndf                            | ddf | F-value | P-value | ndf                            | ddf | F-value | P-value |
| Intercept | 1                              | 51  | 3.2     | 0.078   | 1                              | 51  | 2       | 0.228   |
| Site      | 5                              | 51  | 2.3     | 0.055   | 5                              | 51  | 1.9     | 0.096   |
| Neighbour | 1                              | 30  | 14.1    | 0.0007  | 1                              | 30  | 30      | <0.0001 |
| WT        | 1                              | 30  | 2.3     | 0.133   | 1                              | 30  | 1.4     | 0.253   |
| Source    | F <sub>v</sub> /F <sub>m</sub> |     |         |         | ΦPSII@PPFD 1500                |     |         |         |
|           | ndf                            | ddf | F-value | P-value | ndf                            | ddf | F-value | P-value |
| Intercept | 1                              | 41  | 33      | <0.0001 | 1                              | 41  | 177.78  | <0.0001 |
| Site      | 5                              | 41  | 3       | 0.020   | 5                              | 41  | 5.20    | 0.0009  |
| Neighbour | 1                              | 27  | 20      | 0.0001  | 1                              | 27  | 59.44   | <0.0001 |
| WT        | 1                              | 41  | 1       | 0.313   | 1                              | 41  | 14.74   | 0.0009  |
| Source    | α <sub>ks</sub>                |     |         |         | P <sub>MAX</sub> <sub>ks</sub> |     |         |         |
|           | ndf                            | ddf | F-value | P-value | ndf                            | ddf | F-value | P-value |
| Intercept | 1                              | 359 | 56.80   | <0.0001 | 1                              | 356 | 16.90   | <0.0001 |
| Site      | 5                              | 359 | 2.50    | 0.029   | 5                              | 356 | 31.70   | <0.0001 |
| Neighbour | 1                              | 359 | 19.40   | <0.0001 | 1                              | 356 | 4.20    | 0.040   |
| Moisture  | 1                              | 359 | 13.40   | 0.0003  | 1                              | 356 | 0.02    | 0.892   |
| Source    | R <sub>ks</sub>                |     |         |         |                                |     |         |         |
|           | ndf                            | ddf | F-value | P-value |                                |     |         |         |
| Intercept | 1                              | 356 | 8.80    | 0.003   |                                |     |         |         |
| Site      | 5                              | 356 | 8.80    | <0.0001 |                                |     |         |         |
| Neighbour | 1                              | 356 | 0.5     | 0.501   |                                |     |         |         |
| Moisture  | 1                              | 356 | 2.60    | 0.106   |                                |     |         |         |

Final submodels for the photosynthesis parameters in Eq. 1 are defined below (see Table 4 for the final model results).

$$R_{ks} = S_{ks} \quad (2)$$

$$\text{P}_{\text{MAX}}_{ks} = S_{ks} + T_{ks} + a_{ks} \quad (3)$$

$$\alpha_{ks} = S_{ks} + T_{ks} + M_{ks} + b_{ks} \quad (4)$$

where  $S_{ks}$ ,  $T_{ks}$  and  $M_{ks}$  are effects for sites (six levels), treatment (two levels) and moisture content of the samples (continuous), respectively. The last terms in equations (3) and (4) are random effects for sample, with bivariate normal distribution ( $a_{ks}$ ,  $b_{ks}$ )' ca.  $MVN(0, \Sigma_{ks})$ . The random effects take into account such variability in P<sub>MAX</sub> and  $\alpha$  that was not explained by the fixed effects. Nested random

effects were originally included in all three models at two nested levels (site and sample), but only those shown were significant in the final models. The model was fitted and the tests performed using package nlme of the R software, following the procedures of Pinheiro & Bates (2000, Chapter 8).

Principal components analysis (PCA) was used for exploring correlations between the different variables that describe *S. fuscum* success after transplantation and their relationship with environmental variables (growing season median and autumn WT, soil pH, N concentration in the soil and the cover of vascular plants) and the treatments (sites and vegetation removal). For success variables, we took growth and vitality parameters (percentage cover, capitulum count and vitality) and ecophysiological parameters related to photosynthesis ( $P_G$  and  $P_N$  at full light, respiration and  $F_v/F_m$ ). We applied redundancy analysis

(RDA) and variation partition for testing and quantifying the effect of the two treatments (site and vegetation removal) and their interaction for *S. fuscum* success. As the *S. fuscum* transplanted on SJ 0 were all dead, samples on this site were excluded from the data set enabling more accurate and detailed analysis. The data were centred and standardized in order to make different variables comparable. Monte Carlo permutation was used for testing the statistical significance of treatments for the success. The analyses were conducted using Canoco for Windows 5.01 (Leps & Smilauer 2003).

**Results**

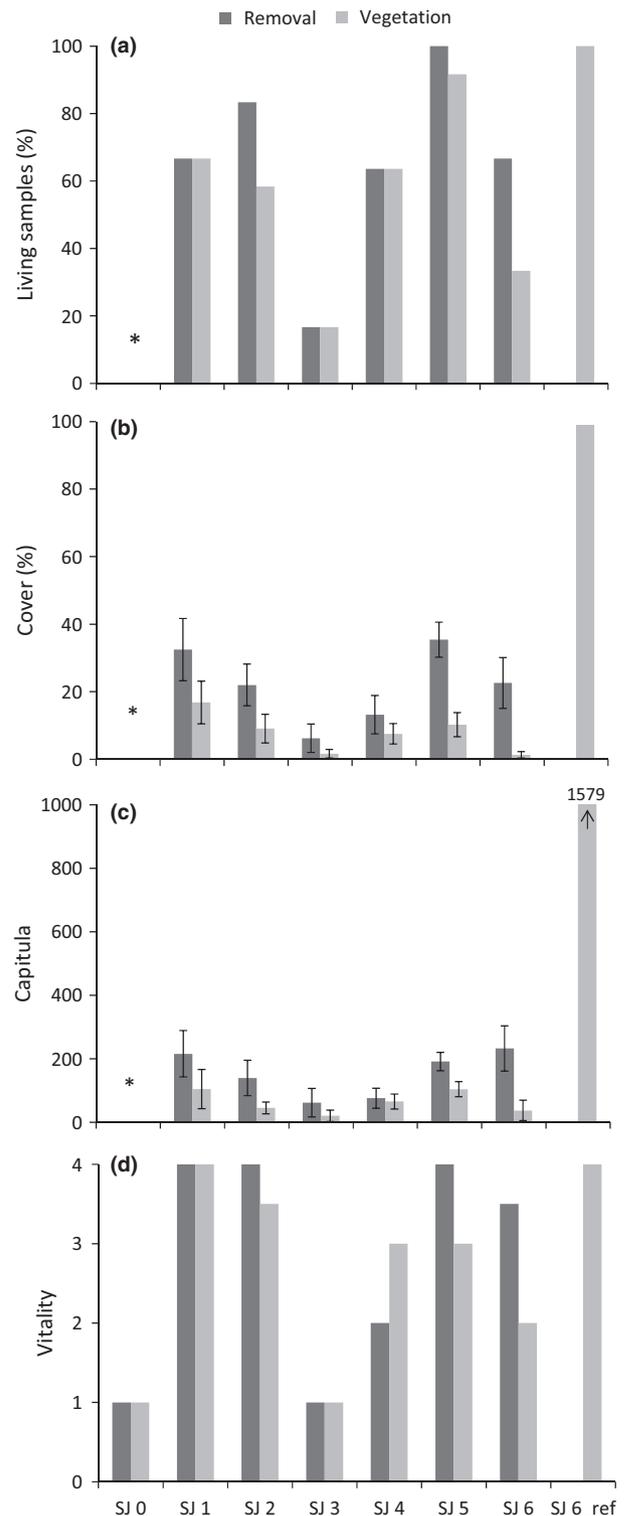
**Survival of *S. fuscum***

All *S. fuscum* individuals transplanted to SJ 0 died over the two growing seasons. Also, at the fen sites (SJ 3 and SJ 4) several submerged samples had died and living capitula were only found in 28 out of 46 sample plots. Survival was best at site SJ 5 (Fig. 1a). Compared to site SJ 6, the probability that samples would die was significantly higher in SJ 3 ( $P < 0.01$ ) and the probability was 0.17, 0.15, 0.97, 0.33, 0.00 and 0.16 for SJ 1, SJ 2, SJ 3, SJ 4, SJ 5 and SJ6, respectively (Fig. A1.1a in App. S1). Vegetation removal treatment or water level did not impact on the probability that transplanted samples would die.

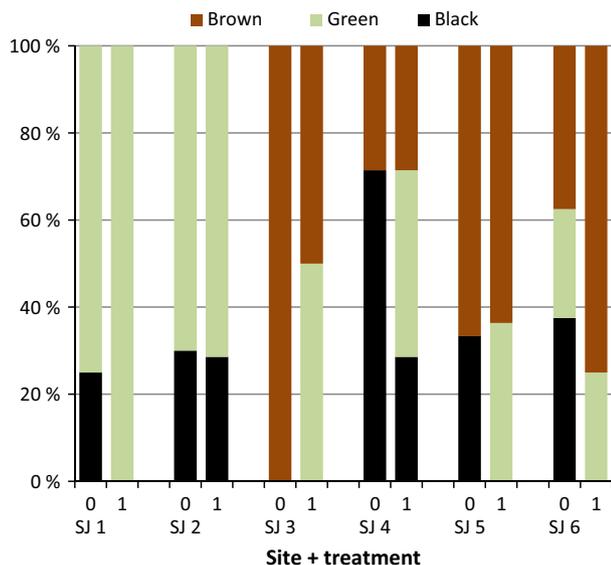
**Visual estimates**

After focusing on the probability of death, we analysed the performance of the survivors. The cover was not affected by site (Fig. 1b, Table 2, Fig. A1.1c), but site had an impact on the number of capitula, which was significantly higher in the intact *S. fuscum* in SJ 6 than in any transplant ( $P = 0.0004$  ( $P$ -values given for final models), Fig. 1c, Table 2, Fig. A1.1b in App. S1). The vegetation removal treatment decreased the number of capitula (on average by  $0.5 \text{ capitula} \cdot \text{cm}^{-2}$ ;  $P = 0.0007$ ) and the cover of capitula ( $P < 0.0001$ ; Table 2, Fig. A1.1b,c in App. S1). Water level had no significant impact on performance of the living

*S. fuscum* samples in terms of number or cover of capitula. The vitality of the living capitula was not affected by site ( $P = 0.66$ ), vegetation removal treatment ( $P = 0.93$ ) or water level ( $P = 0.28$ ; Fig. 1d).



**Fig. 1.** Parameters based on visual estimation of the *S. fuscum* transplants for each site in plots with vegetation removal (dark bars) and with prevailing vegetation (light grey bars). (a) Number of samples with living capitula at the end of experiment. Means and SE for (b) cover and (c) number of capitula (in 14 cm × 14 cm plot, max for SJ 6 reference is 1579) and (d) median of vitality class (1 = worst/dead to 4 = best). Site number refers to the location along the mire chronosequence. SJ 0 is a sandy beach, recently exposed from the sea, and SJ 6 is a bog site. SJ 6\_ref denotes mosses that grow naturally on the bog site and are not transplanted. Cases where all samples have died are indicated by \*. Sample size for the transplanted mosses is 12 for each treatment per site, and 4 for SJ 6\_ref.



**Fig. 2.** Percentage of *S. fuscum* samples of different colour in each site. In x-axis 0 = vegetation removal treatment and 1 = with vegetation. Samples in site SJ 0 are not included since they were all dead.

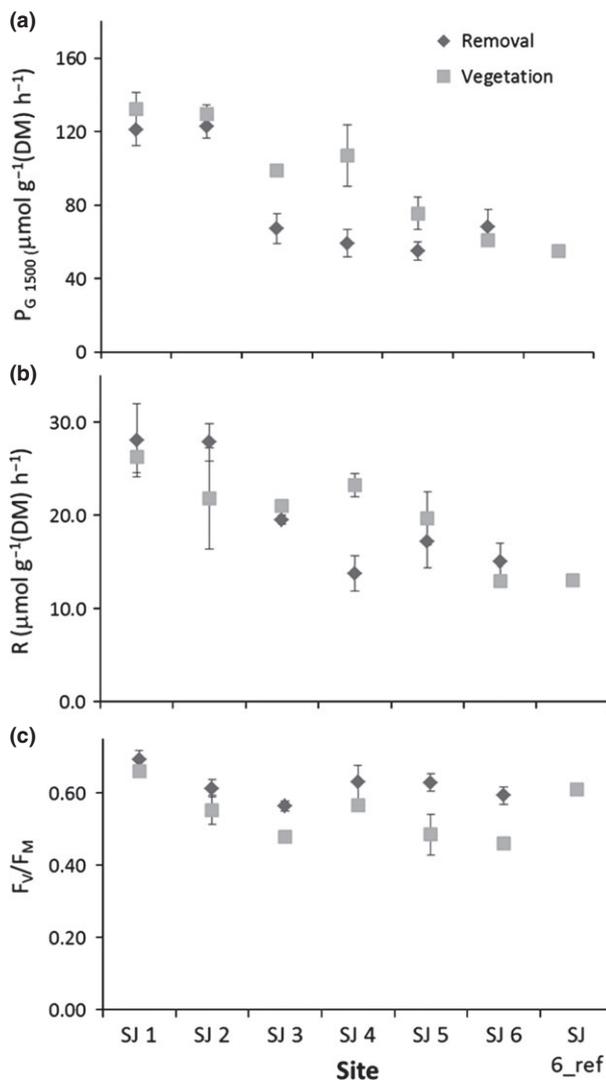
The original brown colour of *S. fuscum* changed after transplantation at some of the sites. At sites SJ 1, SJ 2 and SJ 4 most capitula were black or green, while at sites SJ 3, SJ 5 and SJ 6 brown samples still dominated (Fig. 2).

### The impact of transplantation

Based on survival rates and all the visual parameters, the transplanted samples in all sites performed less well than the intact mosses in site SJ 6 (Fig. 1). This was also the case in site SJ 6, where the transplants generally looked even drier than in other sites during repeated field visits over the two growing seasons.

### Gas exchange and chlorophyll fluorescence measurements

As only living samples were used for the photosynthesis and chlorophyll fluorescence measurements, no results are given for site SJ 0, and sample size varies between the sites and treatments. We expect that samples from SJ 0 would differ from other sites in all respects, as all of them died during the first growing season. The light response of *S. fuscum* photosynthesis varied between sites and vegetation removal treatments (Fig. A2.1 for measured, and Fig. A1.2 for modelled light response curve, in App. S2). The maximum photosynthesis ( $PMAX_{ks}$ ) varied significantly between the sites (Table 2, see Fig 3a for differences in measured  $P_G$ ), being higher in samples transplanted to sites SJ 1, SJ 2, SJ 3 and SJ 4 than in SJ 6 (*post-hoc*



**Fig. 3.** Parameters related to carbon metabolism along the chronosequence. (a) Gross photosynthesis ( $P_G$ ) at PPFD 1500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , (b) dark respiration and (c)  $F_v/F_m$  for *S. fuscum* samples transplanted to each site and for intact reference samples in site SJ 6. Averages and SE are shown for samples with vegetation removal (dark grey) and with prevailing vegetation (light grey). Sample size varies from one to 12.

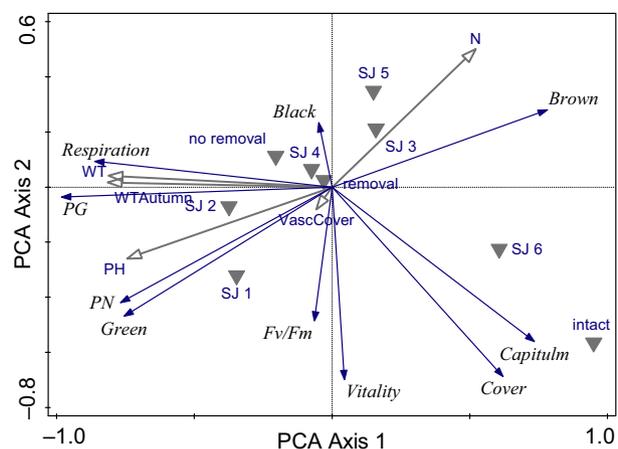
comparisons,  $P < 0.001$ , except SJ 3,  $P = 0.08$ ; Figs A1.1e and A1.2 in App. S1). The vegetation removal treatment decreased  $PMAX_{ks}$  ( $P < 0.005$ ). Moisture content of the moss samples did not explain the variation in  $PMAX_{ks}$ . The light-use efficiency of photosynthesis ( $\alpha_{ks}$ ) varied between sites and was lower in SJ 2 than in the control site ( $P = 0.006$ ; Table 2, Fig. A1.1f in App. S1). Vegetation removal treatment and moisture content decreased  $\alpha_{ks}$  ( $P < 0.0001$  and  $P < 0.001$ , respectively). Respiration rate ( $R_{ks}$ ) differed significantly between sites (Table 2), being higher in SJ 1 and SJ 2 ( $P < 0.0001$ ) than in SJ 6 (Fig. 3b

for measured, and Fig. A1.1g (App. S1) for modelled respiration rate). Vegetation removal treatment or moisture content did not impact  $R_{ks}$ .

The maximum quantum yield of photochemistry,  $F_v/F_m$ , varied significantly between sites (Fig. 3c, Table 2), being lower in SJ 3 than in the reference samples ( $P < 0.05$ ). However, in contrast to the other photosynthetic parameters, there was no clear decreasing trend in  $F_v/F_m$  as the mire succession proceeded, however the vegetation removal treatment increased  $F_v/F_m$  ( $P = 0.0001$ ; Fig. 3c, Fig. A1.1c in App. S1). The quantum yield of PSII ( $\Phi_{PSII}$ ) at PPFD  $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  was lower in all other sites when compared to SJ 6, and was significantly different for sites SJ 1 and SJ 2 (*post-hoc* comparisons,  $P < 0.01$ ; Fig. A1.1d in App. S1). The vegetation removal treatment and water level significantly increased  $\Phi_{PSII}$  ( $P = 0.0001$  and  $P = 0.0004$ , respectively).

### Multivariate analysis

Principal components analysis (PCA) showed a strong correlation between carbon gas exchange parameters and moss colour with median water level, pH and peat/soil N concentration (Fig. 4). Respiration,  $P_G$  and  $P_N$  at PPFD  $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  increased towards wetter, less acid conditions and decreasing N in the soil, as found in the early successional stages. The frequency of the typically brown



**Fig. 4.** Principal components analysis of parameters related to the success of transplanted *S. fuscum* along the mire chronosequence. Parameters are based on visual estimation (cover, capitula, vitality and colour) or carbon metabolism measurements [gross photosynthesis ( $P_G$ ) and net photosynthesis ( $P_N$ ) at full light ( $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), respiration,  $F_v/F_m$ ]. Supplementary environmental variables pH, N content, average water level (WT) and WT in autumn, vascular plant cover, site (SJ 0–SJ 6) and vegetation removal treatment (no removal, removal, intact at site SJ 6) are shown in the ordination space. Eigenvalues and the explained cumulative variation for the first and second axis are 0.44 and 0.21 and 44% and 65%, respectively.

colour of *S. fuscum* increased towards conditions that prevail in the later successional stages. Co-varying capitulum number and cover had no correlation with carbon gas exchange parameters. Parameters related to the state/condition of the mosses,  $F_v/F_m$  and vitality appeared to be intermediate between these two (Fig. 4). While the carbon gas exchange parameters linked with axis 1 and the successional chronosequence, the vegetation removal treatment formed axis 2 and was related to the amount of *S. fuscum*.

Variation partition (based on RDA) between site and vegetation removal treatment impacts showed that site and vegetation removal together explained 32.9% of the adjusted variation of the parameters describing different aspects of *S. fuscum* success (results not shown). Site alone explained 25.7% and the vegetation removal 7.3%.

### Discussion

In this study, the success of *S. fuscum* was impacted by both abiotic site factors and neighbouring plants; however, the effect of site was much stronger. Although *S. fuscum* is naturally abundant in only ombrotrophic hummocks, in our study it performed best when transplanted to the young wet meadows (SJ 1 and SJ 2), where the natural *Sphagnum* cover is very sparse and no *S. fuscum* is found. The success of transplanted *S. fuscum* seemed to correspond to the performance of sphagnum that occur naturally on the succession chronosequence. Laine et al. (2011) measured the highest photosynthetic rates from the early succession species *S. fimbriatum*, while *S. fuscum* and *S. balticum* from the ombrotrophic bog (SJ 6) had the lowest photosynthesis and showed the highest stress (low  $F_v/F_m$ ). In its natural habitat, *S. fuscum* is adapted to the nutrient-poor, acidic and exposed conditions. The low photosynthesis, respiration and  $F_v/F_m$  of intact *S. fuscum* growing in its natural habitat (SJ 6) compared to the surviving transplants in the younger sites indicates that in order to occur in its natural habitat, *S. fuscum* needs to allocate resources to protective mechanisms. Our results indicate that under more favourable environmental conditions, these resources can be allocated to the photosynthetic apparatus to increase growth. *S. fuscum* seems to be rather plastic in its responses to changing conditions and is shown to increase photosynthesis under increased N levels (Granath et al. 2012). In our study, the highest photosynthesis was correlated with increased moisture levels, pH and the green colour of *S. fuscum*, which may indicate increased shading and a lower need for photoprotective mechanisms. This is in line with results of Laing et al. (2014), who found that the photosynthetic performance of *Sphagnum* is most strongly affected by water and light availability, and

thereby moist and shaded conditions, where the requirement for protective mechanisms is low, are optimal for growth, while mosses of open bog plateau had the lowest metabolic rates.

The survival of *S. fuscum* was poorest in the sandy beach site (SJ 0), which lacked an organic soil layer and had extreme water level fluctuations, with occasional flooding. In addition, submergence in the fen flarks (SJ 3 and SJ 4) with minerogenic water was fatal for *S. fuscum*. Although, some studies have reported that *S. fuscum* suffers when grown within a few centimetres from the water level (Wallén et al. 1988), *S. fuscum* in this study performed well when submerged in water in a less nutrient-rich site (SJ 5), and water level had no impact on the measured parameters (except  $\Phi_{PSII}$  at PPFD  $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , which increased with increasing wetness). Also, other studies have found flooding to be advantageous for *S. fuscum* establishment and growth (Rochefort et al. 2002; Campeau et al. 2004), and therefore, submergence alone cannot explain the poor performance of *S. fuscum* in the fen flarks. Our results support the finding of Granath et al. (2010) that the quality of the water is important and *S. fuscum* growth is negatively affected only when submerged in calcareous and high pH water in a rich fen. In addition to the poor performance in the sandy beach site and under minerotrophic water, the mosses that were transplanted back to dry hummocks in the bog (SJ 6) either died or grew poorly. We expect that drought was the main reason for poor performance, as the loose transplant patches most likely did not have similar capillary water transport as natural hummock mosses, with their dense growth form (Hayward & Clymo 1982; Titus & Wagner 1984).

Our hypothesis that other plants limit the success of *S. fuscum* in the earlier successional stages through the rapid growth of early succession species (Laine et al. 2011), which is in agreement with the common paradigm and with Rydin & McDonald (1985) and Rydin (1986), was not supported by our results. The presence of neighbouring plants had no impact on the survival of the adult *S. fuscum* transplants; this result may, however, be partly hampered by the relatively short study period. We hypothesized that the impact of neighbouring plants on *S. fuscum* growth would be particularly strong in the younger sites where *S. fuscum* is missing, but found that the impact was similar in all study sites, and the occurrence of other plants decreased the abundance of transplanted *S. fuscum* capitula throughout the chronosequence. The impact of the neighbouring plants on the physiology of the transplanted mosses along the successional gradient appeared to be less predominant than the impact on moss abundance, but still did not support our hypothesis. Generally, the occurrence of neighbouring plants was favourable for photosynthesis, especially in the fen sites (SJ 3 and SJ 4) with tall sedge veg-

etation and adequate nutrient status. This supports the findings of Hajek et al. (2009) and Laing et al. (2014) who suggested that sphagna growing on open mires suffer from chronic photoinhibition at high light levels and found higher photosynthetic efficiency in mosses of shaded habitats. Shading by vascular plants is the likely reason for the positive impact, as the beneficial effect of neighbouring plants disappeared in site SJ 6 with sparse vascular vegetation. In other sites there was a site-specific increasing trend between maximum photosynthesis and vascular plant cover, which was most pronounced in the fen sites (SJ 3 and SJ 4; results not shown). This positive trend between photosynthetic performance and vascular cover agrees with the finding of Rydin (1997), who suggested that when grown in their realized niche, sphagna compete mainly with each other. Only under very high vascular plant densities, reached with unnatural levels of nutrients (fertilization, pollution; Limpens et al. 2011), does shading have a negative impact on *Sphagnum* growth (Heijmans et al. 2002; Malmer et al. 2003).

Another explanation for the complex impact of neighbouring plants may be the positive effect of the surrounding sphagna on the moisture conditions of the transplanted *S. fuscum* mosses. Due to a lack of stomata, mosses are not able to control their transpiration rate, and therefore photosynthetic activity in mosses is likely more constrained by water availability than in vascular plants (Silvola 1990; Schipperges & Rydin 1998). Moisture availability is one of the most limiting factors for *Sphagnum* growth. The neighbouring mosses increase the ability of the transplants to transport water through capillary action and to retain moisture (Hayward & Clymo 1982; Titus & Wagner 1984), thereby increasing photosynthetic capacity and alleviating the negative impact of competition. However, competition lowered  $F_v/F_m$ , which suggests increased stress. However, Robroek et al. (2007) showed that in the case of a limited water source, the impact of neighbouring species with better water-holding capacity might be negative. In this study, *S. fuscum* was transplanted in loose patches (from 22 to 196 cm<sup>2</sup>) that decreased its ability to take up water in a manner characteristic of species when growing in dense stands in hummocks, which made it more vulnerable to drought.

## Conclusions

The number of surviving *S. fuscum* and the photosynthetic capacity of the surviving individuals appeared to be affected by different factors. Survival of transplants was weakest in the site next to the seashore, where none of the transplants survived. The site factors favourable for rapid *Sphagnum* growth in general appeared to be conducive for *S. fuscum* as well. The low photosynthesis, respiration and  $F_v/F_m$  of intact *S. fuscum* growing in its natural habitat (SJ

6) compared to the surviving transplants in the younger sites indicate that in order to occur in its natural habitat, *S. fuscum* needs to allocate resources for protective mechanisms. Our results suggest that under more favourable environmental conditions in terms of water level, acidity and shading, these resources can be allocated to the photosynthetic apparatus to increase growth.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Figures showing the model estimates and *post-hoc* contrast for the linear and non-linear models for those parameters that had a significant site impact in the model.

**Appendix S2.** Figure of measured light response of *Sphagnum fuscum* photosynthesis in different sites and under different treatments.