

Effects of shading on relative competitive advantage of three species of *Sphagnum*

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SUMMARY

- (1) *Sphagnum* is an important genus of bryophytes holding 10–15 % of the terrestrial carbon stock. With climate change a drier surface may increase the abundance of vascular plants on peatlands, so shading of *Sphagnum* may increase. Here we describe growth cabinet experiments to reveal the effects of shading on interactions among mixtures of three species: *S. capillifolium*, *S. palustre* (hummock species), and *S. fallax* (a hollow species). We measured the six traits: growth in length, growth as increase in dry mass, side-shoot production, nitrogen and carbon proportion of the capitulum dry mass, and C:N ratio in the capitulum.
- (2) Shading had no effect on biomass production or side-shoot production but increased height increment in all three species. It also increased the C and N proportions of total dry mass but decreased C:N ratio in the capitula.
- (3) Neighbours of a different species reduced biomass and side-shoot production in the two hummock species but had no effect on the hollow species.
- (4) All three species showed interaction between shading and neighbour in two or more plant traits. *S. fallax* showed competitive advantage over *S. palustre* in no-shading treatments and over *S. capillifolium* in moderate shading treatments. In addition, under deep shading, *S. fallax* showed a competitive advantage over both hummock species. A clear competitive hierarchy *S. fallax* > *S. capillifolium* > *S. palustre* emerged which was consistent with the hierarchy of side-shoot production.
- (5) The results suggest that all the species appear to tolerate deep shade (for a few months at least). In a shaded environment, especially under deeply shaded conditions, *S. fallax* retains its dominance in hollow habitats (if water availability is guaranteed) by virtue of its advantage in side-shoot production.
- (6) If shading increases then the abundance of different *Sphagnum* species is likely to change.

KEY WORDS: competitive hierarchy; light competition; peatland; side-shoot production

INTRODUCTION

Sphagnum, a genus of bryophytes, is dominant in many peatlands, holds 10–15 % of the terrestrial carbon stock and plays an important role in global

carbon cycling (Clymo & Hayward 1982). *Sphagnum* usually co-exists with vascular plants (Malmer *et al.* 2003), and is shaded by vascular plants and their litter. Most *Sphagnum* species are adapted to low light. They have one-cell-thick

leaves, poorly-developed or non-existent cuticle (Glime 2007), and low chlorophyll a:b ratio (Marschall & Proctor 2004). Their light saturation point is generally at low photon flux density (below 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in laboratory) and rates of CO_2 uptake increase only slightly above 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Harley *et al.* 1989).

Climate warming, nitrogen deposition (Berendse *et al.* 2001, Malmer *et al.* 2003, Bubier *et al.* 2007) and anthropic drying (Hájek *et al.* 2009) tend to promote the expansion of vascular plants in peatlands. Vascular plants at lower densities may be beneficial to *Sphagnum* species, as they reduce evaporation rates (Rincon & Grime 1989, Pedersen *et al.* 2001, van der Wal *et al.* 2005), thereby preventing drought at the capitulum level. But light can be a limiting resource for *Sphagnum* under dense vascular plant cover, which may increase as a result of environmental change (Chapin & Shaver 1985, Kuiper *et al.* 2014). Shading decreases biomass production (Malmer *et al.* 2003, Bonnett *et al.* 2010) and leads to etiolation (Hayward & Clymo 1983). However, *Sphagnum* may acclimatise to shading by changing its morphology, for example by increasing its height growth rate; or by changing its physiology, for example by enhancing chlorophyll concentration and reducing chlorophyll a:b (Hayward & Clymo 1983, Hájek *et al.* 2009, Bonnett *et al.* 2010). Besides morphological and physiological traits, chemical element concentrations may also be affected by shading. Herms & Mattson (1992) indicate that shading limits carbon fixation and hence decreases C:N ratio. In a greenhouse experiment, shading increased nitrogen proportion and decreased C:N ratio in *S. capillifolium* (Bonnett *et al.* 2010).

In their natural habitats, *Sphagnum* species occupy different positions along a light availability gradient at a broad scale from open areas to forest margins, and along a small-scale water table gradient from hummocks to hollows. In general, light flux reduces from open areas to forest margins and the *Sphagnum* species present in open areas, such as *S. fallax*, are considered less shade-tolerant (Fenton & Bergeron 2006), having lower

photosynthetic capacity and maximum quantum yield (Hájek *et al.* 2009), than the species in the forest margins (such as *S. capillifolium*).

As a key process in plant communities, plant-plant interaction has been an important issue in ecological studies since the work of Harper five decades ago (Brooker 2006). In a *Sphagnum* community, the individuals grow so densely that the role of plant-plant interaction may be crucial. Furthermore, *Sphagnum* species are ideal material for experimental research because they can easily be transplanted, with or without artificial mixing of species, to glasshouses (Granath *et al.* 2012) and in the field (Mulligan & Gignac 2002, Rixen & Mulder 2005, Bu *et al.* 2011).

Most pairwise inter-specific competition is asymmetric, where one species reduces the performance of another and may (or may not) increase its own performance. In natural communities, plant species can be ranked in a competitive hierarchy (an ordered ranking from competitive dominant to competitive subordinate) based on either competitive effect or response (Keddy *et al.* 1994). Although it is difficult to determine a clear competitive hierarchy among bryophytes in general (review in Rydin 2009), it does exist in peatlands. Competitive hierarchies are well-known among sphagna. For example, oceanic sphagna are stronger competitors regionally than widespread species, as inferred from their dominance in oceanic peatlands (Gignac 1992). Due to their higher growth rate, hollow species are regarded as superior competitors when and where water is readily available (Gignac 1992, Twenhoven 1992, Bragazza 1997).

All organisms live in changing environments, and inter-specific interactions between co-existing species could be altered by environmental change (Brooker 2006). Competitive hierarchies among bryophytes can also be changed. In wet environments, hollow species can expand in many peatlands by virtue of their competitive advantage (e.g. Limpens *et al.* 2003) but under drier conditions they will be restrained by hummock species (Granath *et al.* 2010). In a simulated warming

experiment the hollow species *S. balticum*, which is normally a strong competitor, also lost its competitive advantage in a high temperature treatment (Breeuwer *et al.* 2008). *S. fallax*, an open area hollow species, is characterised as a competitor (Twenhoven 1992). Whether or not it will lose its competitive advantage with increased shading is unknown.

In this study, we carried out a growth chamber experiment with three *Sphagnum* species, *S. fallax* (Klinggr.) Klinggr. (in our study area an open-area hollow species), *S. palustre* L. (an open-area hummock species), and *S. capillifolium* (Ehrh.) Hedw. (a forest-margin hummock species) to test the hypotheses listed below.

- (1) Shading will decrease biomass and side-shoot production but increase height increment in the three bryophytes.
- (2) Shading is expected to decrease photosynthesis in *Sphagnum*, resulting in less C fixation. This would lead to a reduced C:N ratio. Yet, N proportion has also been reported to increase in *Sphagnum* tissue as a result of translocation of N to photosynthetic tissue. Indeed, the latter would also reduce the C:N ratio. So, shading will increase N proportion and decrease C proportion and C:N ratio.
- (3) The hollow species *S. fallax* will inhibit the hummock species through its superior competitive ability under non-shaded conditions.
- (4) Under shading, especially deep shading, *S. fallax* will lose its competitive advantage when it co-exists with *S. capillifolium* since the latter is more shade tolerant, but it will keep the competitive advantage when *S. palustre* is its neighbour due to its strong resource competitive ability. Thus, the competitive hierarchy among the species or ecological groups will change.

METHODS

Plant materials

We collected shoots of two hummock species (*S. palustre* and *S. capillifolium*) and one hollow species (*S. fallax*) from Hani Peatland (42°13'N, 126°31'E, Figure 1) in the Changbai Mountains, north-east China, in early October 2009. The peatland, is located at an altitude of 900 m.a.s.l. and covers an area of 17 km². The electrical conductivity of peatland water is $53 \pm 3 \mu\text{S cm}^{-1}$ (mean \pm SE, $n=95$), pH is 6.0 ± 0.1 and calcium ion concentration is $9.1 \pm 0.6 \text{ mg L}^{-1}$. The dominant vascular plants include *Larix olgensis* A. Henry, *Betula fruticosa* Pall. var. *ruprechtiana* Trautv., *Vaccinium uliginosum* L., *Carex lasiocarpa* Ehrh., *Phragmites australis* (Clav.) Trin. and others (Bu *et al.* 2011). Common bryophytes are *Sphagnum palustre*, *S. fallax*, *S. magellanicum* Brid., *S. fuscum* (Schimp.) Klinggr., *S. capillifolium*, *Polytrichum strictum* Menz. ex Brid. and *Aulacomnium palustre* (Hedw.) Schwägr.. The peatland is clearly differentiated into forest margin and open areas. *S. capillifolium* is mainly distributed in the forest margin, while *S. palustre* and *S. fallax* are the dominant bryophytes in the open area. At some transitional locations, they co-exist.

Experimental design

The collected *Sphagnum* shoots were cut to 9.0 cm long and then inserted at natural density into 9.0 cm tall translucent cylindrical polypropylene (PP) pots with 6.3 cm outer diameter. Each of these PP pots was put into a transparent cylindrical PVC pot with 6.3 cm inner diameter (the same as the contained PP pot) but 16.0 cm tall. When the inserted *Sphagnum* shoots were of the same species we describe the treatment as monoculture; when the inserted *Sphagnum* shoots were pairwise mixtures we describe it as mixed culture. In the mixed culture, the mass of either species is half that of the species in monoculture, each species being arranged in a semicircle (Figure 2). Ten vigorous shoots (9.0 cm long/tall, without side-shoots or multiple capitula) of each species were held together with a thin rubber

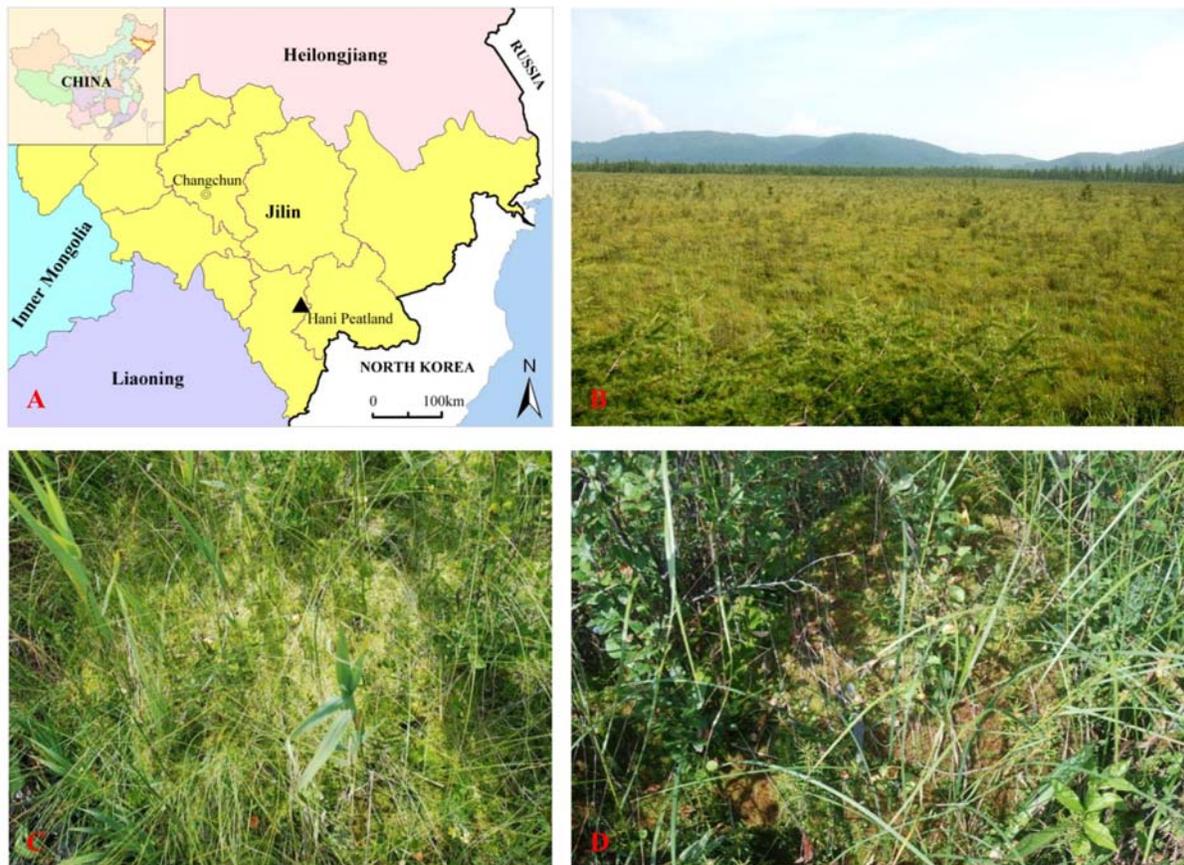


Figure 1. The study area. A: Location of Hani Peatland in north-east China; B: landscape of Hani Peatland; C: vegetation in the open area of Hani Peatland, where *S. palustre* and *S. fallax* are the dominant bryophytes; D: vegetation in the forest margin of Hani Peatland, where *S. capillifolium* is mainly found.

band at the bottom. This band also acted as a marker. The marked bundles were inserted into the centre of the *Sphagnum*-filled pots after removing a corresponding number of the same species shoots to retain the original density. One or two marked bundles were inserted into the mono- or mixed culture, respectively. We used a full factorial design with 3 levels of species (*S. palustre*, *S. capillifolium* and *S. fallax*), 2 levels of neighbour (mono- and mixed culture) and 3 levels of shading (no shading, moderate shading and deep shading), with 5 replicates; a total of 90 pots.

The bryophyte pots were cultured in a HPG-400HX growth chamber (Harbin Donglian

Electronic & Technology Development Co. Ltd, China). To simulate natural conditions, we cultured the bryophyte samples with 16:8 hours day:night and temperature 27:20 °C. Photon flux density was 180 (no shading), 115 (moderate shading) and 40 (deep shading) $\mu\text{mol m}^{-2}\text{s}^{-1}$ during daytime. The photon flux density of the no shading and deep shading treatments was similar to the settings of Fritz *et al.* (2014) and Bonnett *et al.* (2010), respectively. In our experiment, *S. capillifolium* developed a red colour in the no shading treatment as it does in the field (Bonnett *et al.* 2010). All pots with the same shading treatment were in the same layer of the growth chamber, but species and

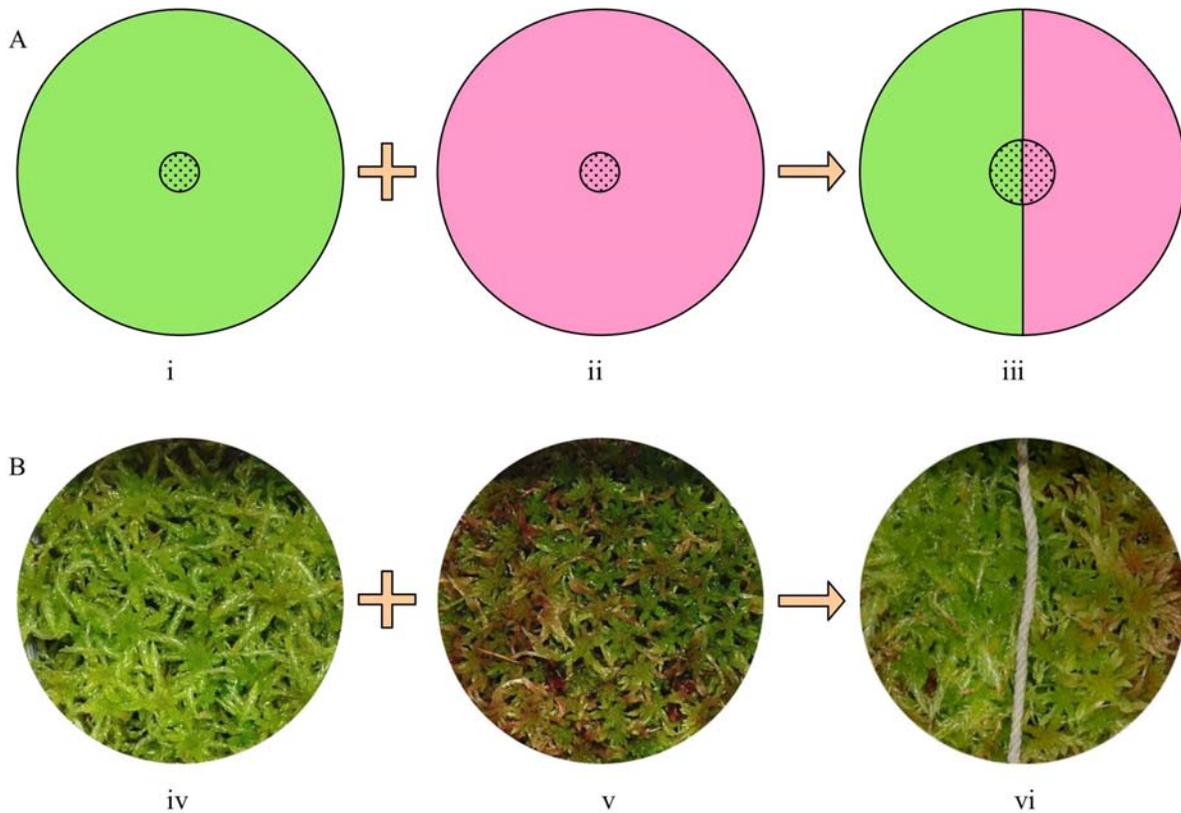


Figure 2. Line diagrams (A) and photographs of examples (B) of mono- and mixed cultures. In the line diagrams, i and ii indicate monoculture and iii indicates mixed culture. In the mixed culture (iii), the mass of species i and ii over the whole area (the large circle) is half that of each species in monoculture. The small circle or semicircle in the centre (marked with dots) indicates the marked bundle. Photos iv and v are monocultures of *S. palustre* and *S. capillifolium*, while vi is a mixed culture of *S. palustre* and *S. capillifolium*. In the photograph, marked plants are difficult to distinguish from the surrounding *Sphagnum*. The string in vi indicates the dividing line between *S. palustre* and *S. capillifolium*.

neighbour treatments were randomised in position. Every second day, pots in the same layer were re-randomised and the whole layers (plants and shading treatments) were interchanged. At the same time, 4 ml of distilled water was sprayed onto the capitula to guarantee their well-being. A preliminary experiment with the water level at 1.5, 3.0, 4.5, 6.0 and 7.5 cm below the surface had shown that the 6.0 cm treatment was suitable for the all three species, although it was close to hollow conditions (Zheng *et al.* 2012). On each ‘second day’ maintenance visit, therefore, distilled water was added below the water surface to maintain the water

level at 6.0 cm below the plant surface. Every week we added 6.0 ml Rudolph’s nutrient medium (Rudolph *et al.* 1988) and the medium in the outer PVC pot was replaced completely every four weeks.

Biological and chemical measurements

After 11 weeks of culture, the height increment of each of the 10 marked plants in a pot was measured and averaged, and the new side shoots in the shoot bundles were counted. The parts between the capitulum (top 1 cm) and the lower 8.0 cm of stems and branches in the shoot bundles were cut off to be oven dried at 70 °C for 24 hours prior to weighing.

(For example, measuring from the original base, and supposing that a plant that began 9 cm long has grown to 13 cm long, we took the section between 8 cm and 12 cm, measured from the bottom, as our measure of biomass increase. Mass that has been passively carried up by internode elongation of the original capitulum is thus included, but the new capitulum is excluded). Dry mass represented biomass production. All the capitula of one species in each pot were used to determine C and N proportions of total dry mass by dichromate oxidation and the Kjeldahl method, respectively.

Data processing and analysis

To test neighbour effect and interaction type, we used the index of relative neighbour effect (RNE) (Markham & Chanway 1996, Callaway *et al.* 2002, Dormann 2007): $RNE = (B_0 - B_w) / \max(B_0, B_w)$, where B_0 and B_w are biomass production (dry mass increase) of plants in mono- and mixed culture, respectively. Negative RNE values indicate facilitation while positive values indicate competition. A significant difference of RNEs for pairs of species indicates asymmetric inter-specific interaction. We calculated RNE for biomass production for each pair of species.

Normality of data was determined using the One-Sample Kolmogorov-Smirnov Test; Pearson correlation analysis was used to determine relationships between biomass production and the other traits (side-shoot production, height increment, carbon proportion, nitrogen proportion and C:N ratio). Two-way ANOVA was used to examine the main and interactive effects of shading and inter-specific interaction on plant traits of each species. One-way ANOVA was used to determine the difference of plant traits among the three *Sphagnum* species and the effect of neighbour on plant traits in the same shading treatment. A *t*-test was used to determine the difference between RNEs for pairs of species. For multiple comparisons we used Tukey HSD *post hoc* tests. The significance level for all tests was set at $\alpha = 0.05$. All statistical analyses were performed with SPSS 16.0 for Windows.

RESULTS

Overall performance

Differences are seen among the three *Sphagnum* species in biomass production, side-shoot production, N proportion and C:N ratio (Table 1). *S. palustre* showed the largest biomass production and C:N ratio, but least side-shoot production and N proportion (Table 1). *S. fallax* produced 3.6 and 2.0 times more side shoots than *S. palustre* and *S. capillifolium*, respectively (Table 1). Biomass production was positively correlated with side-shoot production in all three species (Pearson correlation: $r = 0.82, 0.78$ and 0.79 for *S. palustre*, *S. capillifolium* and *S. fallax*, respectively; $n = 45$, $P < 0.001$ for all). Biomass production was also positively correlated with height increment in *S. fallax* ($n = 45$, $r = 0.57$, $P < 0.001$).

Effect of shading

Shading had no significant effect on biomass production or side-shoot production but increased height increment in all three species (Figure 3A–C, Table 2). It also increased C and N proportion in the capitula but decreased C:N ratio in all species (Figure 3D–F, Table 2). Deep shading affected all the traits except biomass and side-shoot production, while moderate shading affected only C proportion (Figure 3).

Effect of neighbour

Biomass and side-shoot production in each of the three species showed similar responses to neighbours of different species (Figure 4A and B, Table 2). Neighbours reduced biomass and side-shoot production in the two hummock species but had no effect on *S. fallax*. Both *S. capillifolium* and *S. fallax* had a negative effect on *S. palustre* while only *S. fallax* had a negative effect on *S. capillifolium*. Neighbours also led to an increased height increment in *S. capillifolium* (Figure 4C, Table 2). In monoculture, *S. capillifolium* (mean \pm SE, 3.5 ± 0.22) showed lower height increment than *S. palustre* (5.2 ± 0.30 , $P = 0.001$) and *S. fallax* (4.7 ± 0.42 , $P = 0.029$). When it grew with

Table 1. The difference in overall performance (mean \pm 1 SE, $n = 45$) between the three *Sphagnum species* in biomass production, side-shoot production, height increment, carbon and nitrogen proportion of mass and C:N ratio. Biomass and side-shoot production are dry mass increase and number of side shoots produced per 10 shoots in the centre of each pot, respectively. Height increment is the mean of the central 10 plants. Bold values indicate significant ($P < 0.05$) values from one-way ANOVA. Superscripts with different letters indicate significant ($P < 0.05$) differences, analysed by Tukey HSD tests.

Trait	Species			F	P
	<i>S. palustre</i>	<i>S. capillifolium</i>	<i>S. fallax</i>		
Biomass production	0.144 \pm 0.0077 ^b	0.103 \pm 0.0042 ^a	0.109 \pm 0.0051 ^a	14	<0.001
Side-shoot production	3.2 \pm 0.34 ^a	5.8 \pm 0.38 ^b	11.7 \pm 0.88 ^c	55	<0.001
Height increment	4.9 \pm 0.20	4.3 \pm 0.18	4.7 \pm 0.22	2.3	0.101
Carbon proportion of mass	44.6 \pm 0.39	44.8 \pm 0.37	44.8 \pm 0.42	0.12	0.886
Nitrogen proportion of mass	0.93 \pm 0.031 ^a	1.04 \pm 0.028 ^b	1.00 \pm 0.035 ^{ab}	3.3	0.041
Carbon nitrogen ratio	50 \pm 1.5 ^b	44 \pm 1.0 ^a	47 \pm 1.5 ^{ab}	4.9	0.009

S. palustre or *S. fallax* the inter-specific difference in height increment disappeared. Carbon proportion in mixed cultures was lower than in monocultures of all three sphagna (Figure 4D, Table 2). *S. fallax* had a positive neighbour effect on N proportion in *S. palustre* (Figure 4E, Table 2) and a negative neighbour effect on C:N ratio in both hummock species (Figure 4F, Table 2).

Interactive effect of shading and neighbour

With increased shading, biomass production of *S. capillifolium* decreased when *S. palustre* and *S. fallax* were neighbours, but N proportion increased (Figure 5A and E, Table 2). Under no-shading conditions, C proportion was reduced in all three species in the presence of a different species, but in moderate shade this effect disappeared and even led to a positive effect under deep shade (Figure 5B–D, Table 2). An interactive effect of shading and neighbour on C:N ratio was

also found in *S. palustre* and *S. fallax* (Figure 5F and G, Table 2).

Competitive hierarchy

Figure 6 illustrates the strength and direction of species interactions. Compared with *S. palustre*, the competitive advantage for *S. capillifolium* was observed under moderate and deep shading conditions while that for *S. fallax* was observed in the no shading and deep shading treatments. For no shading and moderate shading cases, no clear competitive hierarchy emerged due to similar performance of *S. capillifolium* and *S. fallax* under no shading conditions and of *S. palustre* and *S. fallax* under moderate shading conditions. Shading increased competitive advantage in *S. fallax* when *S. capillifolium* was its neighbour. Under deep shading conditions, there was a clear competitive hierarchy *S. fallax* > *S. capillifolium* > *S. palustre*.

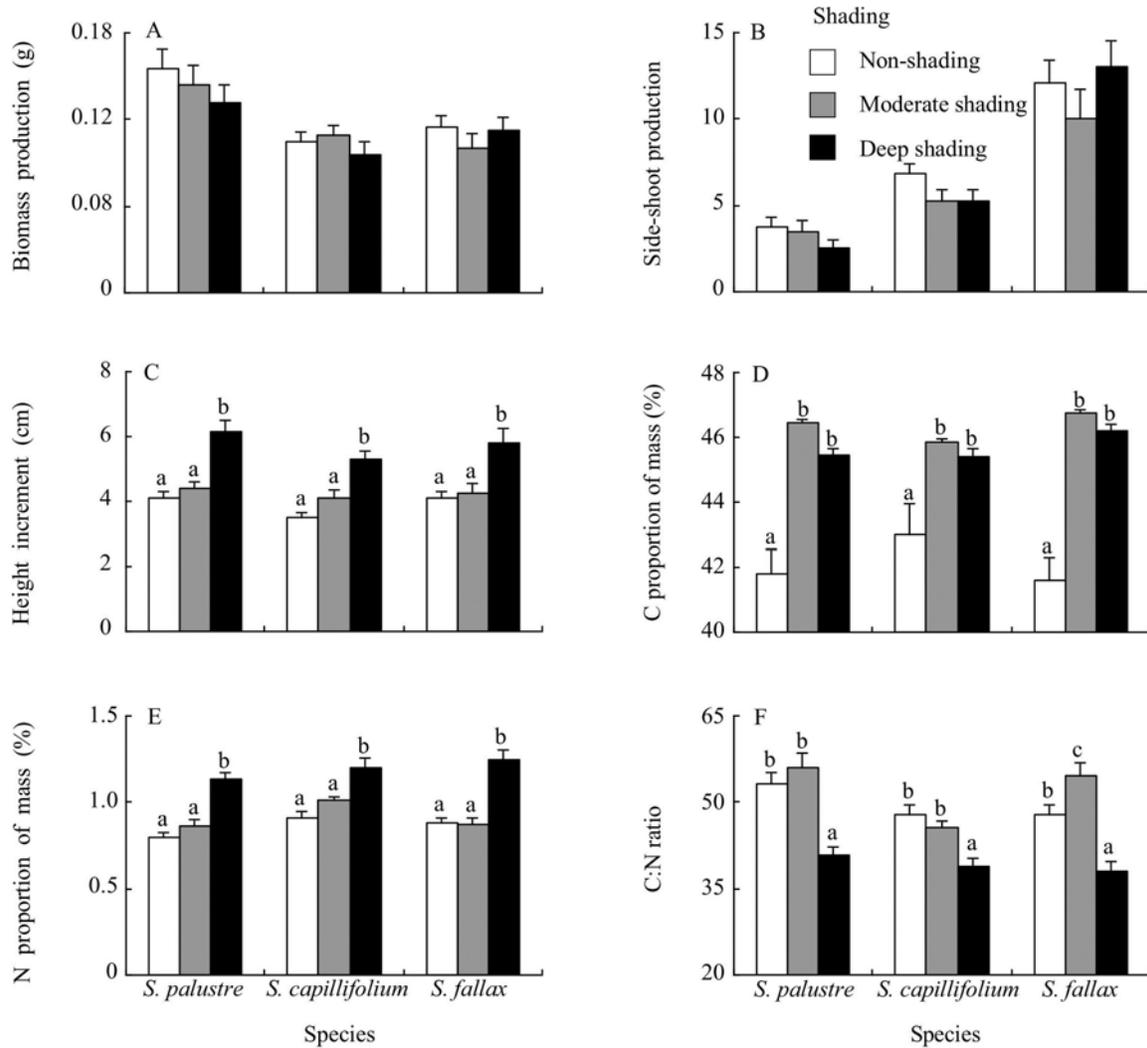


Figure 3. Main effects (mean + 1 SE, $n = 15$) of shading on biomass production (A), side-shoot production (B), height increment (C), carbon proportion of mass (D), nitrogen proportion of mass (E) and carbon : nitrogen ratio (F) in *S. palustre*, *S. capillifolium* and *S. fallax*. Biomass and side-shoot production are dry mass increase and number of side shoots produced per 10 shoots in the centre of each pot, respectively. Height increment is the mean for the central 10 plants. Bars with different letters indicate significant ($P < 0.05$) differences, analysed by Tukey HSD tests. Note that the y-axes in D and F do not start at zero.

Table 2. Two-way ANOVA for effects of shading and neighbour on the performance of three *Sphagnum* species in terms of biomass production, side-shoot production, height increment, carbon and nitrogen proportion of mass and C:N ratio. Biomass and side-shoot production are dry mass increase and number of side shoots produced per 10 shoots in the centre of each pot, respectively. Height increment is the mean of the central 10 plants. Neighbour effect denotes a difference among performances with and without a different neighbour species. Bold values indicate significant ($P < 0.05$) values from two-way ANOVA.

Species	Trait	<i>n</i>	Shading		Neighbour		Shading× Neighbour	
			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>S. palustre</i>	Biomass production	5	2.1	0.143	35	<0.001	0.40	0.810
	Side-shoot production	5	1.8	0.186	12	<0.001	1.2	0.340
	Height increment	5	19	<0.001	2.0	0.148	0.74	0.574
	Carbon proportion of mass	5	73	<0.001	5.3	0.010	12	<0.001
	Nitrogen proportion of mass	5	30	<0.001	7.3	0.002	2.3	0.073
	Carbon : nitrogen ratio	5	27	<0.001	11	<0.001	3.3	0.021
<i>S. capillifolium</i>	Biomass production	5	1.3	0.278	8.3	0.001	3.5	0.017
	Side-shoot production	5	2.6	0.088	6.6	0.004	2.2	0.090
	Height increment	5	26	<0.001	20	<0.001	0.57	0.689
	Carbon proportion of mass	5	47	<0.001	25	<0.001	48	<0.001
	Nitrogen proportion of mass	5	18	<0.001	2.0	0.146	2.8	0.042
	Carbon : nitrogen ratio	5	12	<0.001	4.3	0.022	1.7	0.170
<i>S. fallax</i>	Biomass production	5	0.73	0.488	0.65	0.529	1.5	0.214
	Side-shoot production	5	0.99	0.383	0.51	0.603	0.93	0.459
	Height increment	5	8.0	0.001	0.70	0.505	1.2	0.348
	Carbon proportion of mass	5	134	<0.001	8.8	0.001	19	<0.001
	Nitrogen proportion of mass	5	28	<0.001	0.25	0.779	1.9	0.130
	Carbon : nitrogen ratio	5	27	<0.001	0.041	0.960	5.1	0.002

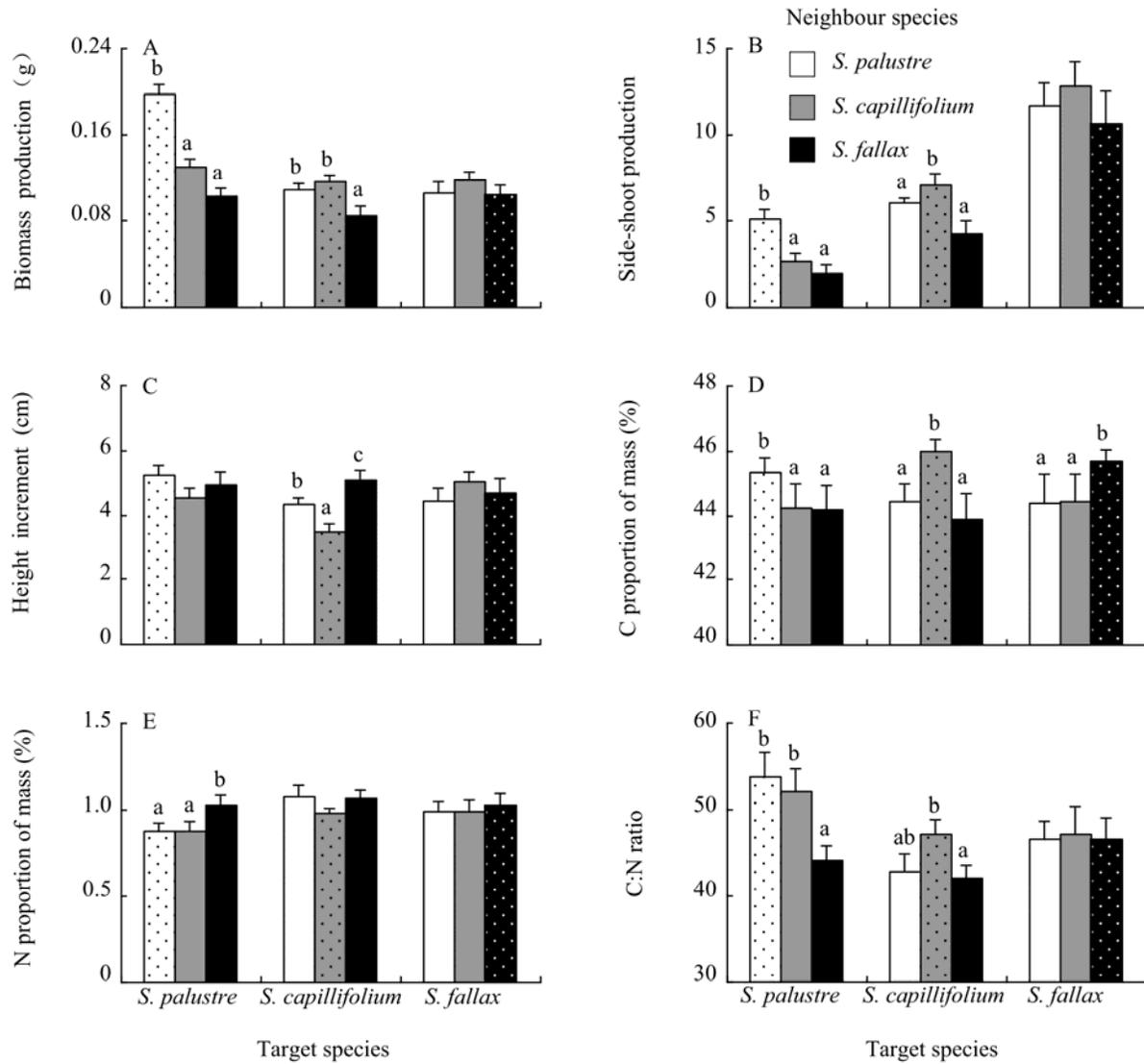


Figure 4. Main effects (mean + 1 SE, $n = 15$) of neighbour on biomass production (A), side-shoot production (B), height increment (C), carbon proportion of mass (D), nitrogen proportion of mass (E) and carbon nitrogen ratio (F) in *S. palustre*, *S. capillifolium* and *S. fallax*. Biomass and side-shoot production are dry mass increase and number of side shoots produced per 10 shoots in the centre of each pot, respectively. Height increment is the mean of the central 10 plants. Monocultures are marked with dots inside the bar. Bars with different letters indicate significant ($P < 0.05$) differences analysed by Tukey HSD tests. Note that the y-axes in D and F do not start at zero.

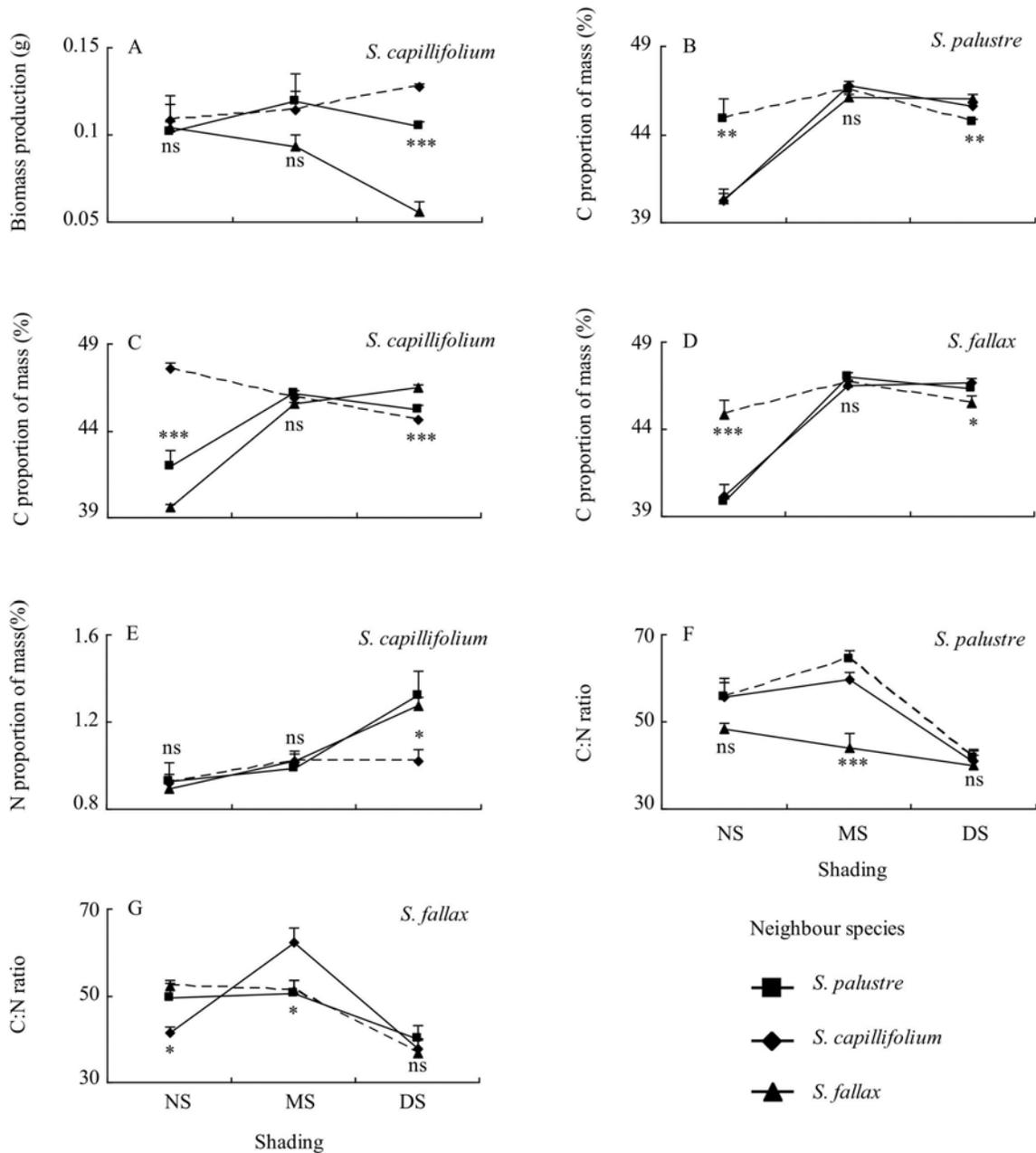


Figure 5. Significant interactions between shading and neighbour in *S. palustre*, *S. capillifolium* and *S. fallax*. Diagrams without significant interactions between shading and neighbour are omitted. Abbreviations indicate the shading in a pot: NS = no shading, MS = moderate shading, DS = deep shading. Bars indicate + 1 SE, $n = 5$. Asterisks denote significant difference among performances under different neighbour conditions in the same shading treatment. * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and ns means no significant difference. Note that the y-axes do not start at zero.

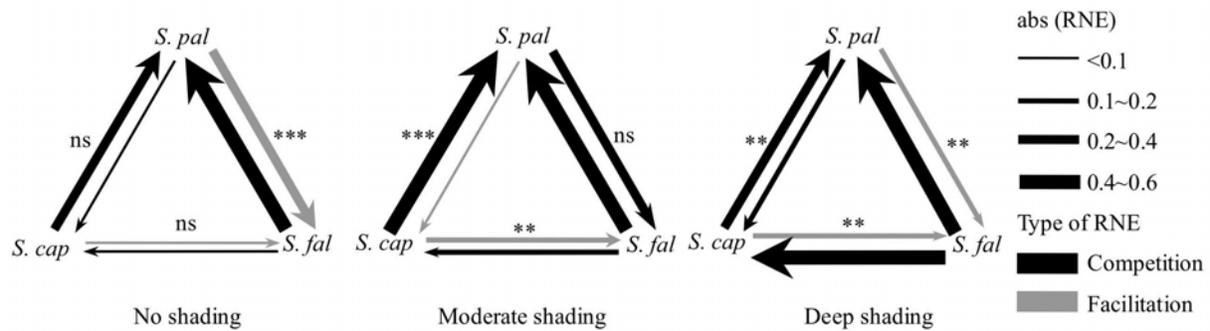


Figure 6. Relative neighbour effects ($RNE = (B_0 - B_w) / \max(B_0, B_w)$, with $B =$ dry mass increase, and subscripts '0' = species in monoculture and 'W' = species in mixed culture) among three sphagna under three shading treatments, showing differences of inter-specific interactions, and a competitive hierarchy. Negative RNE values (grey arrows) indicate facilitation; positive values (black arrows) indicate competition. Species: *S. pal* = *S. palustre*; *S. cap* = *S. capillifolium*; *S. fal* = *S. fallax*. *S. fal*→*S. cap* indicates effect of species *S. fal* (neighbour) on target species *S. cap*. Line width of arrows indicates intensity of effect. Asterisks denote significant difference of RNEs for pairs of species; * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and ns means no significant difference.

DISCUSSION

All three species showed increased height increment in response to shading. However, partly contrary to our first hypothesis, shading had no effect on biomass or side-shoot production. Constant biomass and side-shoot production indicates that under the manipulated shading conditions the plants can make a net gain from photosynthesis. Thus, the constant biomass and side-shoot production may result from the negative effect of decrease of light availability being counteracted by increase of water availability due to evaporation decrease (Rincon & Grime 1989, Pedersen *et al.* 2001, van der Wal *et al.* 2005). It suggests that all the sphagna appear to tolerate deep shade - at least for the 11 weeks of our experiment.

Sphagnum has an optimum N proportion of about 1.3 % in the capitulum of both *S. fallax* and *S. fuscum* (Hájek 2014), and it may be the same in other sphagna. When it is below this optimum, photosynthetic rate increases with N proportion (Granath *et al.* 2012). The capitulum, composed of dense young leaves and branches, is the main location for photosynthesis (Hájek *et al.* 2009). The

exogenous nutrient input is small in peatlands. In *Sphagnum*, nutrients in senescent leaves and branches are transported towards the capitulum (Rydin & Clymo 1989, Aldous 2002). In this study, shading increased N proportion in all three species, suggesting that *Sphagnum* adapts to shade by transporting N from old tissue to the capitula. A similar result was reported by Bonnett *et al.* (2010). These authors found that shading rather than exogenous N input increased N proportion in the capitula of *S. capillifolium* and they explained it by more N in old tissue being transferred to chlorophyll, thus increasing light assimilation. In our study, the colour of *S. capillifolium* changed from red to green in deep shading, and the other two sphagna changed from light green to dark green. Shading increased the allocation of N to chlorophyll (Bonnett *et al.* 2010), thus increasing photosynthetic carbon fixation and counteracting the negative effect of decrease of light. However, the increase in N proportion was greater than in C proportion. Thus, overall, shading tended to decrease C:N ratio in the three sphagna, which is consistent with our second hypothesis.

Sphagnum shoots usually grow similarly in height, thus avoiding shading and drying (Rydin 1993), so cover change rather than height increment is a good index of inter-specific interaction (Rydin 1993, Mälson & Rydin 2009). Side-shoot production may reflect clonal expansion as well as change in cover of bryophytes (Bu *et al.* 2011), and more productive side shoots can overtop and shade neighbours. The three species in the current study differed in side-shoot production suggesting a hierarchy in the potential for clonal expansion, *S. fallax* > *S. capillifolium* > *S. palustre*. This hierarchy is consistent with that indicated by the RNEs (Figure 6) that were calculated for biomass production. Hence, side-shoot production may be a good surrogate for more general competitive ability in *Sphagnum*.

In the shading experiment, all the species pairs showed asymmetric competition along the gradient of shading. The hollow species *S. fallax* showed competitive advantage over *S. palustre* in all the shading treatments, which was consistent with our hypothesis. However, in contrast to the third and fourth hypotheses, in open (no shading) conditions *S. fallax* had no competitive advantage over the forest-margin hummock species *S. capillifolium*, but under moderate and deep shade it did. This shows that *S. fallax* is a superior competitor because of its greater growth rate (Gignac 1992, Bragazza 1997), especially under shade.

As a hollow species, *S. fallax* may utilise more photosynthetic products for growth while hummock species may allocate more photosynthetic products to creating their own environment (Hájek 2014). This could explain why the rate of carbon fixation is greater in *S. fallax* than in *S. capillifolium* (Titus *et al.* 1983). What is more, shading may create a wetter niche by decreasing water loss as evaporation (Rincon & Grime 1989, Pedersen *et al.* 2001, van der Wal *et al.* 2005), thus benefiting the hollow species *S. fallax*. Hence, *S. fallax* still showed a competitive advantage over *S. capillifolium* and *S. palustre* in the shading treatments.

S. fallax is not usually regarded as a shade tolerator because it is mainly distributed in open

areas (Gignac 1992, Fenton & Bergeron 2006) and has lower photosynthetic capacity (Hájek *et al.* 2009). *S. fallax* dominates hollow habitats in many northern peatlands and has even expanded in Europe because N deposition enhances its growth enabling it to outcompete other species of *Sphagnum* (Limpens *et al.* 2003). Our study shows that shading can also lead *S. fallax* to outcompete hummock species. Since nitrogen deposition can facilitate vascular plants (Bubier *et al.* 2007), the advantage in light competition may be another reason for *S. fallax* to displace some other sphagna and to flourish in peatlands. Nevertheless, *S. fallax* is seldom found in forest margins, probably because the water table is too low for it to grow well. Robroek *et al.* (2007a) indicated that sustained periods of drought could cause hollow species to lose competitive advantage. Many aspects of *Sphagnum* performance connected with water constraints, such as drainage (Hájek *et al.* 2009) and warming (Breeuwer *et al.* 2008), are detrimental to *S. fallax*. If water were not limiting, *S. fallax* would be a better competitor under shade and might then appear in forest margins.

Although all three species can endure shading stress, the effect of shading will be more serious if water stress is added. *Sphagnum* has a very good (for its size) water transport system, but this is in the capillary spaces between leaves outside the plant (it is ectohydric; Hayward & Clymo 1982). Thus, any change in water-holding capacity resulting from bulk density alteration will affect water transport around *Sphagnum* (Robroek *et al.* 2007b). A large height increment and stable biomass and side-shoot production under shaded conditions will lead to a low bulk density. This may make *Sphagnum* more susceptible to disturbance by factors such as drought and change the competitive balance between *Sphagnum* and vascular plants (Heijmans *et al.* 2002) so as to exacerbate shading. With increase in vascular plants, *S. fallax* may be expected to dominate hollow habitats as the superior competitor, and *S. capillifolium* to increase its distribution and abundance because this species has competitive advantage over *S. palustre* in shaded environments

and *S. fallax* cannot endure water stress. But, if the climate becomes drier, abundance of the three species will decrease, especially *S. fallax*.

We found that inter-specific neighbours had a negative effect on C proportion in all three sphagna. Such an effect was also found in an earlier drought experiment (Bu *et al.* 2013). Being rich in secondary phenolic compounds, *Sphagnum* may excrete carbon-rich phenolic compounds (Verhoeven & Liefveld 1997) that suppress neighbours in mixed cultures. However, the negative effect on C proportion disappeared with deeper shading. This result is not consistent with competitive intensity increasing in severity as shading increases. The reason is probably that, with less light for C fixation, the plants allocate more C to height increment, thus acquiring light rather than investing in excreting 'expensive' phenolic compounds. In addition, *S. fallax* as a neighbour increased N proportion in the capitula of *S. palustre* (Figure 4E) and this effect occurred mainly in deep shade (not shown in this article). Similarly, N proportion in *S. capillifolium* increased when this species was mixed with the other two bryophytes in deep shade (Figure 5E). The reason is probably that strong light competition by neighbours results in more N transport to the capitula, thus maintaining photosynthesis. Further experiments are needed to discover the mechanism(s) that cause the response of C and N proportion to shading and neighbours in *Sphagnum*.

In summary, our data showed that shading might enhance the competitive advantage of *S. fallax* in hollow habitats. Under deep shading, a clear competitive hierarchy of biomass production (*S. fallax* > *S. capillifolium* > *S. palustre*) emerged, which is consistent with the hierarchy of side-shoot production. This reveals that side-shoot production may be a good index of inter-specific competition in *Sphagnum* at least in deep shade. Our study also suggests that in shade, especially in deep shade, *S. fallax* can retain its dominance of hollow habitats if water availability is guaranteed. If shading increases following climate change then the abundance of different *Sphagnum* species is likely to change.

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