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Short-term effect of deep shade and enhanced nitrogen supply on *Sphagnum capillifolium* morphophysiology

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Abstract *Sphagnum capillifolium* mesocosms collected from an ombrotrophic blanket bog were subjected to controlled photon flux densities (control and shaded) and nitrogen (low and high) treatments between November 2003 and August 2004. Shading significantly reduced biomass of *S. capillifolium* ($P < 0.001$), whilst nitrogen (N) supply significantly increased biomass ($P < 0.05$) suggesting that *S. capillifolium* was limited by N. There was no significant interaction between shading and N on biomass. *S. capillifolium* responded to shading via morphophysiological and biochemical alterations to the photosynthetic tissues such as (1) break down of anthocyanins involved in photoprotection of chloroplasts, (2) translocation of N from mineralized N or old tissues and (3) allocation of translocated N to photosynthetic pigments. The results suggest that *S. capillifolium* can tolerate both low and high light intensities, as well as high N supply via morphophysiological responses but does not acclimate to deep shade, since biomass was reduced. Anthocyanins rather than carotenoids appear to play an essential role in photoprotection with translocation serving as the important source of N. It has been suggested that global change in temperature and N availability may lead to increased vascular plant growth that could increase shade leading to a shift from *Sphagnum* spp. to vascular species in peatlands. However, the species *S. capillifolium* appears to tolerate deep shade and high N deposition due to the mechanisms shown here suggesting that this species may continue to persist in peatland ecosystems.

Keywords *Sphagnum capillifolium* (Ehrh.) Hedw · Shading · Nitrogen · Biomass · Photosynthetic pigments · Anthocyanin

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Introduction

Sphagnum species promote conditions that strongly favour carbon sequestration in peatlands such as waterlogged, nutrient-poor and acidic conditions (Van Breeman 1995), and yet they thrive due to a variety of adaptive physiological mechanisms, including efficient use of nutrients (Aldous 2002a). More than half of the world's peat originated from

Sphagnum spp., representing 10–15% of the terrestrial carbon stock, with more carbon held in dead and living *Sphagnum* than is fixed annually by all terrestrial vegetation (Clymo and Hayward 1982). Thus understanding the environmental conditions that affect the morphophysiological responses of *Sphagnum* is critical to our understanding of carbon sequestration in peatland ecosystems.

Plants have evolved mechanisms for acclimatizing to different environments, and may optimize growth under the prevailing environmental conditions by reallocating resources and/or changing their morphology (Arp 1991). Mosses are the simplest land plants and therefore central to the study of plant acclimation, particularly in incident light and UV radiation (Cove et al. 1997). Bryophytes are generally considered to be shade-adapted plants, reaching photosynthetic light saturation at low irradiances between 30 and 300 μmol m^{-2} s^{-1} (Davey and Rothery 1997), although species of open, sun-exposed habitats may not reach saturation until 1,000 μmol m^{-2} s^{-1} (Proctor 2002). However, *Sphagnum* species such as *S. rubellum* which is closely related to *S. capillifolium* have been found to saturate at intermediate irradiances of approximately 550 μmol m^{-2} s^{-1} (Marschall and Proctor 2004). During periods of bright, dry sunny weather bryophytes will generally be dry and metabolically inactive (Marschall and Proctor 2004). Most of their photosynthesis takes place in rainy or cloudy weather, when irradiance may often be <20% of full sunlight. Shade adapted plants have thinner leaves, larger chloroplasts, are richer in chlorophyll and contain a higher proportion of chlorophyll *b* relative to chlorophyll *a* (Boardman 1977). Also, carotenoids called xanthophylls play an essential role as photoprotective agents by rapidly quenching the excited state of chlorophyll (Taiz and Zeiger 1998).

Sphagnum capillifolium, like several species in Sect. *Acutifolia*, often exhibits a distinctive reddish-violet colour due to the presence of secondary cell-wall pigments known as Sphagnorubins (Gerdol et al. 1998). Mues (2002) states that the typical red pigments reported from certain *Sphagnum* species may have a chemical relation to 3-deoxyanthocyanidins. Anthocyanins generally accumulate in peripheral tissues exposed to high irradiance or in obligatory shade plants due to an imbalance between light capture, CO_2 assimilation and carbohydrate utilization (Steyn et al. 2002). They absorb strongly in the visible region of the spectrum with a tail in the UV (Cockell and Knowland 1999). Studies have shown that they have antioxidant activity and thus may indirectly increase tolerance to UVB radiation by neutralizing free radicals (Husain et al. 1987). They significantly modify the quantity and quality of light incident on chloroplasts (Krol et al. 1995), thereby reducing the risk of photo-oxidative damage (Steyn et al. 2002). Thus, they may provide a more important role in the photoprotection of *S. capillifolium* than carotenoids.

Nitrogen (N) deposition in the UK has increased over the last century and currently averages 1.7 g N m^{-2} $year^{-1}$, with a range of 0.5–8 g N m^{-2} $year^{-1}$ (NEGTAP 2001; Skiba et al. 2004). Nitrogen is thought to limit plant growth in many terrestrial systems (Turetsky 2003). However, a number of studies have reported decreases in bryophyte growth with N addition (Gunnarsson et al. 2004; Limpens and Berendse 2003; Limpens et al. 2003). The tissue N concentration of *Sphagna* at Moor House National Nature Reserve (NNR), UK, has increased by 62% over the past 30 years which has resulted in appreciable species loss and decrease in bryophyte cover (Pitcairn et al. 1995). *Sphagnum* species not only have very low nutrient demands due to low tissue nutrient concentrations, high nutrient use efficiency and tight nutrient cycling but also have a very low threshold for high atmospheric deposition when compared to vascular plants (Turetsky 2003). Lamers et al. (2000) and Berendse et al. (2001) proposed mechanisms of how and when increasing N deposition affects *Sphagnum*. They argue that *Sphagnum* is limited by N when deposition is low and, consequently, the moss takes up all available N for growth and maintenance. At high N deposition, *Sphagnum* is saturated and cannot retain the entire deposited N. The N filter fails, and N becomes available for vascular plants who respond by increasing in cover. Studies examining the effects of enhanced N deposition, elevated carbon dioxide and temperature on plant species composition have shown that vascular plants will increase in dominance due to climate change, and reduce *Sphagnum* growth due to the shade effect (e.g. Lamers et al. 2000; Berendse et al. 2001; Bridgham 2002; Fenner et al. 2007). Growth under low light intensity greatly increases the partitioning of N into chlorophyll and thylakoids

(Evans 1989). Thus, the morphophysiological response of *Sphagnum* spp. to the interactive effect of shading and N deposition will be critical to the survival and growth of these species and ultimately affect the future carbon sequestration of *Sphagnum* dominated peatlands.

The aim of this research was to determine the interactive effect of shading and N supply on *Sphagnum capillifolium* biomass, capitulum N concentration and pigmentation. It was hypothesized that (1) *S. capillifolium* contains anthocyanins as a mechanism to reduce the incident light on chlorophyll, and hence prevent photo-oxidative damage that Murray et al. (1993) showed can occur in other *Sphagnum* species; (2) shading increases the allocation of N to photosynthetic pigmentation to increase photosynthetic carbon fixation; and (3) N was hypothesized to increase photosynthetic pigmentation under shaded conditions.

Materials and methods

Experimental design

Forty peatland mesocosms were collected from Moor House NNR, Upper Teesdale, UK in November 2003. The site (Latitude: 54° 24′ 36″ North; Longitude: 357° 22′ 12″ East) is at an altitude of 500 m and is dominated by ling heather (*Calluna vulgaris* L.), cotton grass (*Eriophorum* spp.) and bryophytes (*Sphagnum capillifolium*, *Sphagnum cuspidatum* and *Sphagnum papillosum*), and is described within the National Vegetation Classification (Rodwell 1991) as *C. vulgaris–Eriophorum vaginatum* blanket mire with *Empetrum nigrum* sub-community (M19b). Nomenclature was from Daniels and Eddy (1990). *S. capillifolium* (Ehrh.) Hedw., from Sect. *Acutifolia* is distinguished in the field by morphological characters and habitat preferences. It is common in open and dryer habitats and forms hummocks. Pitcairn et al. (2006) estimated total annual N deposition as between 1.8 and 4 g N m^{-2} $year^{-1}$ at Great Dunfell near Moor House, UK. Data collected by the UK ECN (Environmental Change Network) showed that between 1994 and 2003, the average annual atmospheric input of N to Moor House was 1.03 ± 0.13 g m^{-2} (Cundill et al. 2007). Photosynthetic photon flux density (PPFD) ranged between 100 and 1,500 μmol m^{-2} s^{-1} at the site in 2004 (Bonnett 2005). More detailed descriptions of the Moor House climate may be found in Holden and Adamson (2002).

The mesocosms were collected from patches (hummocks) with a total areal cover of *S. capillifolium* only, and were made of PVC tubing measuring 400 mm in depth × 110 mm in diameter. The mesocosms were collected by first cutting the peat around the base of the tube and then carefully pushing down on the tube into the peat. The mesocosms were then removed from the peat by digging around the cores and removing the surrounding peat. The bases of the mesocosms were tightly sealed with polythene bags and tape to prevent soil aeration.

The 40 mesocosms were split into two groups of 20 replicates depending on the quality of plant material and placed in a greenhouse. Twenty replicates (control) were placed under high light intensity bulbs with a timer set for a 12-h photoperiod during day time [>300 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD)] in a lattice square design. The remaining 20 replicates were placed in a lattice square design 1 m from the control treatment to maintain similar climatic conditions but enclosed beneath shading mesh to maintain PPFD from all angles at <40 μmol m^{-2} s^{-1}. During the spring months (after April) when days were brighter and longer (100–1,500 μmol m^{-2} s^{-1}), supplementary lighting for the control treatment was not required. The minimum average temperature was 8.01 ± 0.05°C in February, and the maximum was 21.62 ± 0.18°C in August.

All mesocosms were watered automatically using peristaltic pumps for 30 min each day with an artificial rainwater solution that matched Moor House rainwater containing 1.38 mg NH_4NO_3 l^{-1}, 0.48 mg NH_4Cl l^{-1}, 0.4 mg KCl l^{-1}, 3.26 mg $MgSO_4 \cdot 7H_2O$ l^{-1}, 2.76 mg $CaCl_2 \cdot 6H_2O$ l^{-1}, 4.12 mg NaCl l^{-1} and 0.64 mg Na_2SO_4 l^{-1} from November 2003. The flow rate was approximately 42 ± 0.9 ml day^{-1} to each mesocosm. The flow rate was chosen to account for the evapotranspiration rate (determined by measuring the change in weight of randomly selected mesocosms over a couple of days). However, water tables were maintained level with the surface of the peat throughout the experiment by watering with distilled water every other day until saturated. Both treatments received 0.98 g N m^{-2} $year^{-1}$ for 4 months between

November 2003 and March 2004. This low N supply represented the lower estimate of annual N deposition reported by Pitcairn et al. (2006) and Cundill et al. (2007) for the site.

After 4 months incubation, 10 replicates within each light level were subjected to high N supply interspersed within the lattice square design at the start of April 2004. This was a factorial design for shading and N interaction. The high N supply mesocosms received 40 ml of a 0.248 g NH_4NO_3 l^{-1} solution in an artificial rainwater once a week. This gave final concentrations of 0.98 g N m^{-2} $year^{-1}$ for low N supply and 20 g N m^{-2} $year^{-1}$ for high N supply. The mesocosms were subjected to each N supply for 5 months over spring–summer, equivalent to 0.41 g N m^{-2} for the low N supply and 8.3 g N m^{-2} for the high N supply. At the end of the experiment in August 2004, after 9 months, the mesocosms were dismantled.

Biomass, capitulum C and N concentration

Total living *S. capillifolium* biomass (red or green tissue) within each mesocosm was separated from non-pigmented or dead tissue, and dried at 70°C for 3 days. The dry weight of living (pigmented) biomass was weighted and divided by the area of the core (m^2) to give an estimate of *S. capillifolium* biomass per mesocosm (g dry biomass m^2). This is a relative measure of change in pigmented biomass between treatments over the experimental period rather than an absolute measurement of growth. Samples of *S. capillifolium* capitulum were separated from each *Sphagna,* bulked together per mesocosm, ground with a ball mill and analyzed for total C and N on a CN analyzer (Vario EL Elemental Analyser System E GmbH, Hanau, Germany) and expressed as mg C or N g^{-1} dry biomass.

Capitulum pigmentation

Chlorophyll *a*, *b* and carotenoids were determined by the methods of Pietrini et al. (2002) and Lovelock and Robinson (2002). A total of 0.02 g of dry, ground *S. capillifolium* capitulum were dissolved in 2 ml acetone–water (80% v/v) in centrifugation vials for 24 h at 4°C followed by centrifugation at 13,000 rpm for 10 min. The absorbance of the supernatant was measured with a spectrophotometer at 470, 643 and 661 nm. Chlorophyll *a*, chlorophyll *b* and carotenoids were determined in units of μmol ml^{-1} using the equations of Lichtenthaler (1987) and converted to μg g^{-1} dry biomass using the following molecular weights: Chl $a = 893.5$ g mol^{-1}, Chl $b = 907.5$ g mol^{-1} and carotenoids = 550 g mol^{-1}.

Anthocyanins in *S. capillifolium* capitulum were determined by the method of Pietrini et al. (2002). A total of 0.02 g of dry, ground *S. capillifolium* capitulum were dissolved in 2 ml of 1% HCl in methanol in a centrifugation vial for 4 h at 4°C to avoid degradation of chlorophylls followed by centrifugation at 13,000 rpm for 10 min. The absorbance of the supernatant was measured using a spectrophotometer at 534 and 661 nm, and $A_{534} - 0.25 \times A_{661}$ was used to account for interference from chlorophylls (Mancinelli 1984). Anthocyanin concentration (μg g^{-1} dry biomass) was calculated as cyanide-3-glucoside using 29,600 as extinction coefficient and 445 MW (Wrolstad 1976).

Statistical analysis

All statistical analyses were performed with Minitab Release 15 (Minitab Inc.). Data were tested for normality using Kolmogorov–Smirnov normality test. Distributions that were non-normal were log transformed. Differences between treatments were determined using two-way ANOVA with interaction. Pearson correlation analysis was used to determine relationships between N, photosynthetic pigments and anthocyanin using normal and log transformed data.

Results

Biomass, capitulum C and N concentration

Biomass was significantly lower (35%) in the shaded treatment relative to the control, whilst biomass was significantly higher (168%) in the high N supply treatment relative to the low N supply treatment (Fig. 1a; Table 1). Shading and N did not have an interactive effect on biomass. Shading and N did not significantly affect the capitulum C concentration (Fig. 1b). However, there was a significant interactive effect of shading and N on the capitulum N concentration (Fig. 1c) as well as a significant effect of shading as a main effect. This suggests that shading

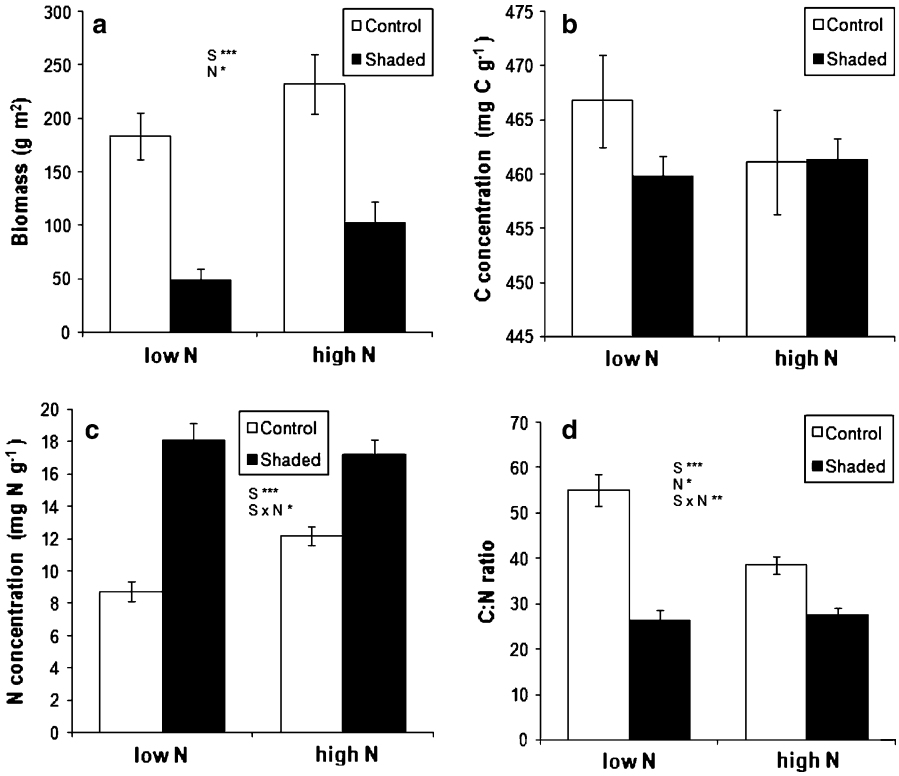

Fig. 1 Effect of shade and enhanced N supply on *S. capillifolium* (**a**) mesocosm biomass, (**b**) capitulum C concentration, (**c**) capitulum N concentration and (**d**) capitulum C:N ratio. Significant effects of shade (S), nitrogen (N) and the interaction (S × N) are shown as *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. Data are mean values ± standard error ($n = 10$)

Table 1 Two-way ANOVAs with interaction for each parameter

	Shade		N		Shade × N		R^2
	F	*P*	*F*	*P*	*F*	*P*	
Biomass	**39.85**	**0.001**	**5.94**	**0.021**	0.01	0.914	60
C concentration	0.98	0.329	0.36	0.551	1.10	0.303	7
N concentration	**73.44**	**0.001**	2.33	0.137	**6.48**	**0.016**	71
C:N ratio	**81.14**	**0.001**	**6.26**	**0.018**	**10.92**	**0.002**	75
Chlorophyll concentration	**61.53**	**0.001**	0.06	0.806	**5.35**	**0.029**	72
Chlorophyll a:b ratio	**12.63**	**0.001**	2.65	0.116	2.03	0.166	42
Chlorophyll:N ratio	**24.97**	**0.001**	0.10	0.757	1.25	0.274	51
Carotenoid concentration	**18.40**	**0.001**	0.46	0.505	0.76	0.391	43
Carotenoid:chlorophyll ratio	1.54	0.226	0.720	0.405	0.07	0.795	9
Carotenoid:N ratio	**5.79**	**0.024**	1.98	0.172	1.18	0.288	26
Anthocyanin concentration	**11.06**	**0.003**	0.95	0.340	0.13	0.722	34

Bold represents significance at $P > 0.05$ with the associated *F* value and percentage variation explained (R^2; $n = 40$)

significantly increased capitulum N concentration (174%), whilst N only increased the capitulum N concentration of the control treatment (139%). Shading and N had a significant interactive effect on the C:N ratio due to the effects on capitulum N concentration (Fig. 1d).

Pigmentation

Shading and N had a significant interactive effect on the chlorophyll concentration (Fig. 2a; Table 1) as well as a significant effect of shading as a main effect. This shows that shading significantly increased

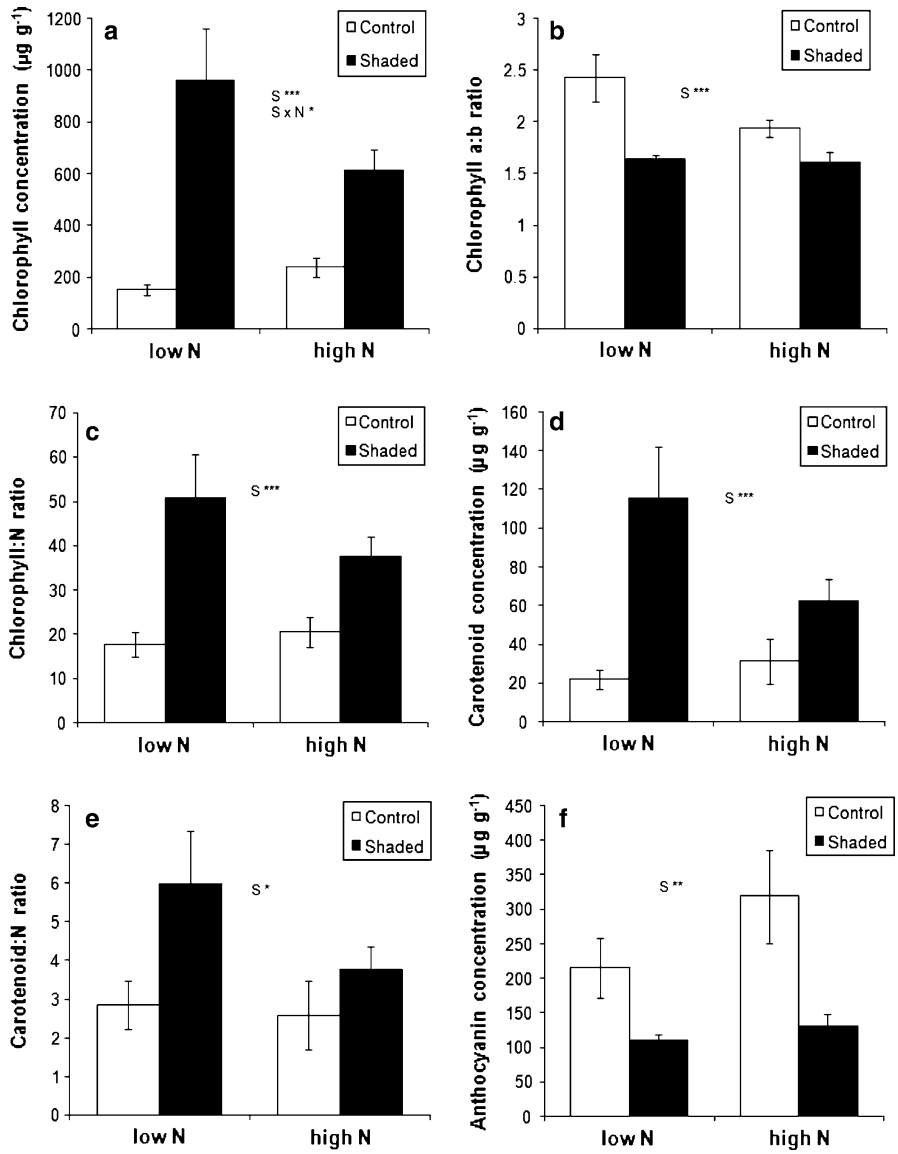

Fig. 2 Effect of shade and enhanced N supply on *S. capillifolium* capitulum pigmentation: (**a**) chlorophyll concentration, (**b**) chlorophyll *a*:*b* ratio, (**c**) chlorophyll:N ratio, (**d**) carotenoid concentration, (**e**) carotenoid:N ratio and (**f**) anthocyanin concentration. Significant effects of shade (S), nitrogen (N) and the interaction (S × N) are shown as *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. Data are mean values ± standard error ($n = 10$)

the chlorophyll concentration under low (635%) and high N supply (256%). The interaction suggests that N supply only increased the capitulum chlorophyll concentration in the control treatment (158%) whilst the chlorophyll concentration was reduced by N supply (64%) in the shaded treatment. These results are comparable to the effects of shading and N on the capitulum N concentration and are supported by the significant correlations between chlorophyll and capitulum N concentration (Table 2). Shading significantly reduced the chlorophyll *a*:*b* ratio (Fig. 2b), increased the chlorophyll:N ratio (Fig. 2c), increased the carotenoid concentration (Fig. 2d), increased the carotenoid:N ratio (Fig. 2e) and reduced the anthocyanin concentration to approximately 46% of the control (Fig. 2f). There were no significant effects of shade or N on the carotenoid:chlorophyll ratio (Table 1). Carotenoid concentration correlated positively with capitulum N concentration, whilst anthocyanin was negatively correlated with capitulum N concentration (Table 2).

Table 2 Pearson correlation coefficients between capitulum N concentration and pigments where *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$ ($n = 40$)

	N	Chlorophyll	Chlorophyll *a*	Chlorophyll *b*	Carotenoid
Chlorophyll	0.818***				
Chlorophyll *a*	0.818***	0.998***			
Chlorophyll *b*	0.814***	0.993***	0.983***		
Carotenoid	0.663***	0.822***	0.819***	0.822***	
Anthocyanin	−0.383*	−0.293	−0.290	−0.294	−0.264

Data were log transformed except capitulum N concentration

Discussion

Biomass, capitulum C and N concentration

Annual N deposition at Moor House has been estimated at between 1.8 and 4 g N m^{-2} $year^{-1}$ in 1995 (Pitcairn et al. 2006) and 1.29 g N m^{-2} $year^{-1}$ in 2001 (Cundill et al. 2007). In the UK, mean annual N deposition averages 1.7 g N m^{-2} $year^{-1}$, but varies geographically between 0.5 and 8 g N m^{-2} $year^{-1}$ (NEGTAP 2001; Skiba et al. 2004). The increase in biomass between N levels is, therefore, surprising considering that the high N supply (20 g N m^{-2} $year^{-1}$) was significantly higher than estimates at Moor House as well as mean annual deposition for the UK. However, the high N supply was within the range of published atmospheric deposition experiments examining *Sphagnum* productivity across Europe that range from 0.3 to 23 g N m^{-2} $year^{-1}$ (Limpens et al. 2003; Van der Heijden et al. 2000; Aldous 2002a, b). Whilst it is recognized that the relative measure of biomass used in this study is not an accurate measure of absolute growth, it does suggest that *S. capillifolium* was limited by N supply in the short-term under both light levels. At N-limited sites, increased N deposition usually results in increased growth, up to a certain capitulum concentration (Vitt et al. 2003). Above this point, *Sphagnum* growth has been found to be limited by other factors, such as phosphorus (Aerts et al. 1992), and the high N concentration may result in a nutrient imbalance or even be toxic (Bridgham 2002). Studies have shown that *Sphagnum* spp. usually respond to high N supply within the first year, but growth is usually reduced in the following years (Arróniz-Crespo et al. 2008; Breeuwer et al. 2009; Gunnarsson and Rydin 2000). Thus, it is possible that the biomass of *S. capillifolium* in this study might have been reduced by high N supply over successive growing seasons.

Sphagnum growth has been found to decrease when the capitulum N concentration becomes too high, owing to moderate influxes (*c.* 1 g N m^{-2} $year^{-1}$) over long periods (Gunnarsson and Rydin 2000; Bragazza et al. 2004), or high influxes (>15 g N m^{-2} $year^{-1}$) over a shorter period (Van der Heijden et al. 2000). The capitulum N concentration of *S. capillifolium* grown under control conditions was significantly increased by high N supply from 8 to 12 mg N g^{-1} dry biomass since *Sphagnum* species have no known mechanism for regulating their uptake of N (Jauhiainen et al. 1998). Lamers et al. (2000) argued that *Sphagnum* saturate at an N deposition of 1.8 g N m^{-2} $year^{-1}$, reaching a threshold concentration of 12–13 mg N g^{-1}. However, fertilization experiments in the Netherlands with artificial N supply up to 10-fold greater than deposition have reported capitulum N concentrations of up to about 20 mg g^{-1} (e.g. Van der Heijden et al. 2000; Berendse et al. 2001). Since biomass was higher under the high N supply treatment, this suggests that 12 mg N g^{-1} was not detrimental to growth. However, considering that the N supply was at the high end of published studies, this suggests that the tissue may have reached saturation.

Shading and N supply did not have an interactive effect on *S. capillifolium* biomass suggesting that deep shade (<40 μmol m^{-2} s^{-1}) will reduce biomass irrespective of the N supply, although high N supply may partially offset some of the reduction in biomass due to shading. Shading in the field may not have as dramatic impact on biomass and, therefore, the results from this study focus on mechanisms rather than potential impacts in the field. However, PPFDs

as low as 100 μmol m^{-2} s^{-1} have been reported at Moor House at mid-day (Bonnett 2005). The capitulum N concentration in the shaded treatment averaged 18 mg N g^{-1} resulting in a significant reduction in the capitulum C:N ratio. This increase in capitulum N concentration was due to shading, and was not affected by N supply suggesting that the high capitulum N concentration was the result of translocation within the plants, even exceeding the high N supply treatment. Aldous (2002a, b) found that *S. capillifolium* can translocate N from old to young tissues from mineralized N. Nitrogen retention from atmospheric deposition satisfies only a small proportion of the annual N budget for moss production with the remainder supplied from internal N cycling particularly mineralized N (Aldous 2002a). Hence, in the short-term under shaded conditions, high capitulum N concentrations do not necessarily mean toxic effects, and translocation of N may be a more important source of N than atmospheric deposition for some *Sphagnum* species.

Sphagnum capillifolium grown under shaded conditions were limited by N, since biomass was higher under high N supply. Thus, the translocation of N and subsequent allocation to photosynthetic pigments, thylakoids and/or soluble protein under shaded conditions may have been affected by high N supply. Rice et al. (2008) showed that the surface of the capitulum functions similarly to a vascular plant leaf, in which strong linear relationships exist between N and both the enzyme 1,5-bisphosphate carboxylase oxygenase (Rubisco) and the chlorophyll (Evans 1989). However, bryophytes tend to become less efficient at retaining N under high deposition (Aerts et al. 1992; Woodin and Lee 1987) indicating N saturation in tissues. Translocation within individuals is also affected by N deposition (Aldous 2002b). Bragazza et al. (2005) and Limpens et al. (2003) found that the relative difference in N concentration between capitulum and stem decreased with increasing N deposition, suggesting a possible metabolic mechanism that reduces excessive N accumulation in the capitulum. This mechanism may explain why the capitulum N concentration in the high N supply treatment under shaded conditions was not significantly different to the low N supply despite higher biomass. *Sphagnum* plants adapt to changing atmospheric chemistry through, for example, higher N tissue content, reduced N uptake, reduced nitrate reductase activity and increased free amino acid concentration in tissue (Limpens and Berendse 2003; Bragazza et al. 2004). Thus, N may have been translocated and subsequently allocated to stem growth and elongation. Rice et al. (2008) suggested that instead of translocation of N to keep it within high light regions, certain *Sphagnum* species differentially concentrate mass and its associated N in the capitulum, allowing the shoot of some species to use N within the canopy effectively for net carbon uptake. This character is associated with rapid stem elongation, leading to a strong correlation between plant height and photosynthetic assimilation. Bridgham (2002) stated that efficient scavenging of atmospheric N and translocation of internal N can supply almost all of the nutrient demand of *S. capillifolium* under at least some circumstances. Thus translocation of N may serve as an important mechanism permitting the co-existence of *S. capillifolium* with vascular plants under shaded and enhanced N deposition.

Pigmentation

The increase in capitulum N concentration under shaded conditions via translocation was associated with an increased requirement for compounds associated with photosynthetic capacity. Photosynthetic capacity in vascular plants is related to the N concentration primarily as the proteins of the Calvin cycle and thylakoids represent the majority of leaf N (Evans 1989). Total chlorophyll and carotenoids were significantly increased by shading in both N supply levels. In many species, growth under shading greatly increases the partitioning of N into chlorophyll and thylakoids, whilst the electron transport capacity per unit of chlorophyll declines (Evans 1989). Also, chlorophyll and carotenoids correlated significantly with the capitulum N concentration. Thus, the translocated N was allocated to photosynthetic pigmentation to increase the absorption of photons. Granath et al. (2009) found that mosses not only allocated more N into the photosynthetic apparatus but also allocated a higher proportion of N to chlorophyll. However, in comparison to this study, the driver of the allocation in their study was N not shading. They questioned whether the shading effect of vascular plant coverage increasing with N deposition is strong enough to explain the increase in

chlorophyll N. The results presented here suggest that there are mechanisms that can increase allocation of N to chlorophyll under shaded conditions.

The remaining proportion of translocated N may have been allocated to soluble protein (i.e. Rubisco) and pigment–protein/reaction centre complexes that contain the majority of thylakoid N (60–85%; Evans 1989). Since biomass was relatively higher under high N supply for shade grown plants, this suggests that under low N supply, N may have been translocated to photosynthetic pigments at the expense of soluble protein involved in the dark reactions of photosynthesis. Under high N supply, allocation of N to soluble protein relative to chlorophyll may have increased growth due to greater N availability. In vascular plants, with increasing N per unit leaf area, the proportion of total leaf N in the thylakoids remains the same, whilst the proportion in soluble protein increases (Evans 1989). However, since capitulum N and chlorophyll concentrations were not significantly different, the higher biomass in this treatment may also be explained by stem elongation.

The chlorophyll *a*:*b* ratio was significantly reduced by shading under both N supply treatments from 2.4 to 1.6. Bryophytes typically have low chlorophyll *a*:*b* ratios that would generally be regarded as characteristic of shade plants (Valanne 1984) with reported values mostly lying within the range from 1.5 to 3.0 (Marschall and Proctor 2004). This implies that the light-harvesting chlorophyll *a*:*b* protein complex makes up a large proportion of the chlorophyll present (Marschall and Proctor 2004). Martin and Churchill (1982) found generally higher chlorophyll *a*:*b* ratios for mosses on an exposed sandstone outcrop than in an oak-hickory forest in Kansas. The shaded forest mosses also showed a striking increase in chlorophyll concentration as in this study (Plate 1).

Carotenoids were significantly increased by shading suggesting that carotenoids not associated with photoprotection or non-photochemical quenching (NVQ) were increased for absorption of light energy. This is supported by the positive correlation between carotenoids and chlorophyll. Also, there was no significant effect of shade or N on the carotenoid:chlorophyll ratio. Marschall and Proctor (2004) found that high chlorophyll:carotenoid ratios were associated with bryophytes growing in more or less deep shade. Therefore, it is hypothesized that *S. capillifolium* has characteristics of intermediately shaded species, and may increase carotenoids not associated with NVQ under shaded conditions to increase the efficiency of light capture. Rosevear et al. (2001) concluded that at any level of shade tolerance a similar range of carotenoid concentrations occur in all species, indicating that the ability to produce carotenoids, in general, does not play a major role in determining tolerance of high light or shade. However, Rice et al. (2008) found that photosynthetic assimilation was negatively correlated with carotenoid concentration. Under light intensities of 800 μmol m^{-2} s^{-1} *Sphagnum* undergoes photoinhibition that reduces rates of growth (Murray et al. 1993). Thus under high light, carotenoids may function to dissipate excess light energy within this species expressed through xanthophyll cycle interconversions, whilst under low light carotenoids function as accessory pigments for photosynthetic carbon gain.

Anthocyanins were significantly reduced by shading under both N supply treatments. The loss of anthocyanins occurred in the capitulum and upper light-exposed stem where photosynthesis occurs (Plate 1). It is proposed that for *S. capillifolium,* anthocyanins play an important role in photoprotection rather than carotenoids. Anthocyanins absorb

Plate 1 Observable change in biomass, chlorophyll, anthocyanin pigmentation and alteration to the morphology of *S. capillifolium* by shading after 4 months. The control treatment is *left* and the shaded treatment is *right*

light in blue wavelengths that could be captured by free chlorophyll, and hence anthocyanins localized in the cell vacuole, may be poised to scavenge oxygenated radicals leaking from chloroplasts as well as mitochondria and peroxisomes (Grace and Logon 2000). Attenuation by anthocyanins may help to re-establish a balance and therefore reduce the risk of photo-oxidative damage (Steyn et al. 2002). Dunn and Robinson (2006) found varying but significant species specific responses in UV absorbing pigments and anthocyanin concentration of three moss species to seasonal variation of UVB radiation. Bryophytes may be particularly susceptible to UVB damage due to their simple structure, with most lacking differentiation and the protective cuticle or epidermal layer of higher plants.

The control of anthocyanin accumulation or breakdown appears unrelated to the controls on photosynthetic pigmentation due to the lack of correlations between anthocyanins and photosynthetic pigments. However, the breakdown of anthocyanin may have been partially associated with changes in capitulum N concentration that was associated with photosynthetic pigments. Marschall and Proctor (2004) stated that the major physiological requirement of bryophytes during transient exposures to bright sunshine as the plant dries out is likely to be for photoprotection rather than energy capture. Marschall and Proctor (2004) showed that other *Sphagnum* species saturate at relatively low PPFDs, but have high NVQ indicating a high level of photoprotection consistent with their unshaded habitat. However, anthocyanins appear to be the critical mechanism of photoprotection in *S. capillifolium* rather than carotenoids that may principally serve as accessory pigments.

Conclusions

This study has shown that deep shade can significantly reduce biomass of *S. capillifolium*. High N supply increased biomass in the short-term suggesting that *S. capillifolium* was limited by N. *S. capillifolium* responded to shading via morphophysiological and biochemical alterations to the photosynthetic tissues, such as (1) break down of anthocyanins involved in photoprotection of chloroplasts, (2) translocation of N from mineralized N or old tissues and (3) allocation of translocated N to photosynthetic pigments. High N supply may have partially ameliorated the negative effect of shading by allocation to photosynthetic pigments, soluble protein or stem elongation, though productivity was primarily limited by light intensity. Overall these results suggest that *S. capillifolium* can tolerate both low and high light intensities, as well as high N supply via morphophysiological responses but does not acclimate to deep shade since biomass was reduced. *S. capillifolium* grows in non-shaded habitats in competition with vascular plants, and are thus exposed to a wide range of light intensities. Anthocyanins rather than carotenoids appear to play an essential role in photoprotection with translocation serving as the important source of N. It has been suggested that global change in temperature and N availability may lead to increased vascular plant growth that could increase shade leading to a shift from *Sphagnum* spp. to vascular species in peatlands. However, the species *S. capillifolium* appears to tolerate deep shade and high N deposition due to the mechanisms shown here suggesting that this species may continue to persist in peatland ecosystems.

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