Photosynthetic responses of two pleurocarpous mosses to low-level nitrogen addition: a study in an old-growth fir forest

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We examined photosynthetic responses of two dominant pleurocarpous mosses, Actinothuidium hookeri (Mitt.) Broth. and Hylocomium splendens (Hedw.) Schimp. to low-level nitrogen (N) addition. The study was conducted in an old-growth fir forest on the eastern Tibetan Plateau. The added N, 1 g N/m², was mainly absorbed by the new-growth. The concentrations of chlorophyll a and b both increased 8 days after N addition. The quantum yield of Photosystem II (ΦPSII) also increased. However, no significant changes were found in terms of gas exchange parameters. The mass-based CO₂ assimilation rate, chlorophyll a content, and chlorophyll a/b ratio (which is related to antenna size of the photosystem), of H. splendens were all higher than those of A. hookeri. Shoot mass per area (SMA) of H. splendens was lower than that of A. hookeri. We conclude that the photosynthetic rate was less sensitive to low-level N addition than chlorophyll contents and chlorophyll fluorescence parameters, suggesting other limiting factors in the photosynthetic process. Additionally, the faster growing H. splendens has a higher photosynthetic capacity than A. hookeri, allocating fewer resources to structural tissue.

Keywords: Actinothuidium hookeri, Chlorophyll fluorescence, Gas exchange, Hylocomium splendens, Nitrogen allocation, Photosynthetic rate

Introduction

Nitrogen (N) deposition rate in China has increased significantly in the past decade (Lu & Tian, 2007). Elevated N inputs affect plant composition (Wedin & Tilman, 1996), biodiversity (Stevens et al., 2004), and ecosystem productivity (Gough et al., 2000), especially in N poor ecosystems, such as boreal and subalpine forest, because they are mainly N limited (Vitousek & Howarth, 1991). These changes can be attributed to direct physiological effects and indirect effects caused by alteration of species composition (Manning et al., 2006). The direct physiological effects of N addition on plants could be stronger than the indirect effects (Manning et al., 2006). The physiological effects of N enrichment on photosynthesis have been extensively studied (e.g. Granath et al., 2009b, 2012). The photosynthetic rate should theoretically increase after N addition, since more N can be allocated to the photosynthetic apparatus (e.g. pigments and Rubisco) when Sphagnum experiences elevated N availability (Granath et al., 2012). Given the positive correlation between photosynthetic capacity and leaf N concentration (Wright et al., 2004; Granath et al., 2012), the photosynthetic capacity of vascular plants is stimulated by low level N deposition, even a short period after N fertilization (Gough et al., 2004). To gain information about the effects of N deposition in subalpine forest, data on the responses of the dominant mosses in the forest are required.

Ground-dwelling mosses are a ubiquitous and dominant feature of understory communities in boreal and subalpine forest (Liu & Bao, 2011). The mosses, especially pleurocarpous mosses, form continuous dense carpets with large areas of ground cover over the soil surface (Lindo & Gonzalez, 2010). Due to the lack of effective cuticles, mosses are very efficient in taking up N deposition, and the entire plant is able to absorb nutrients (Turetsky, 2003). Thus, mosses are expected to be sensitive to N addition, especially physiologically (Manning et al., 2006; Granath et al., 2012). Granath et al. (2009b) found a unimodal response of photosynthesis with a N concentration optimum for photosynthetic capacity at around 1.3% for Sphagnum balticum. Positive relationships were also observed in a greenhouse experiment (Granath et al., 2012) and along a
latitudinal nitrogen deposition gradient (Granath et al., 2009a). These results suggest that mosses may respond with an increased photosynthetic rate to low doses of N addition or deposition (e.g. less than 1 g N/m²/year; Granath et al., 2009b). No significant effect of N addition on Fv/Fm of Sphagnum spp. was found (Carfrae et al., 2007; Granath et al., 2012). However, Granath et al. (2009b) reported increased Fv/Fm under elevated N deposition. The effective quantum yield of Photosystem II (PSII) is an important parameter to evaluate the efficiency of photochemistry (Maxwell & Johnson, 2000). Despite many publications, details about the effects of N addition on photosynthetic rate and chlorophyll fluorescence of ground-dwelling mosses in subalpine forest are still sparse. Furthermore, with regard to the direct effects of N addition, moss species with different growth rates and nutrient acquisition strategies showed divergent responses to N addition (Paulissen et al., 2004; Koranda et al., 2007).

The species studied were Actinotrichium hookeri (Mitt.) Broth. and Hylocomium splendens (Hedw.) Schimp. These species were widespread and dominant in the ground layer of an old-growth coniferous forest on the eastern Tibetan Plateau. Both species are carpet forming pleurocarpous mosses. A. hookeri is endemic to eastern Asia, whereas H. splendens is one of the most abundant pleurocarpous mosses in the boreal forest and subalpine forest across North America, north Europe, and the northern part of Asia. Although the life form of the two mosses was similar, the mean growth rate of H. splendens was much higher than that of A. hookeri (0.142 vs. 0.058 mm/day) during the growing season (Wang et al., 2007). The growth rate of A. hookeri is less sensitive to habitat than that of H. splendens (Wang et al., 2007).

Our aim was to explore the short term effects of N addition on the photosynthesis of these two pleurocarpous mosses, using an in situ N addition experiment in an old-growth fir forest. The maximum photosynthetic rate and the chlorophyll fluorescence parameters of both species were measured before and after N addition. Based on existing information regarding the photosynthetic responses to N addition, we expected significant increase in the photosynthetic rate and the yield of chlorophyll fluorescence after N addition. We also hypothesized that the photosynthetic rate of H. splendens would be higher than that of A. hookeri.

Materials and Methods
The experiment was performed in an old-growth fir forest in Dagu Glacier Park, Heishui County in Sichuan Province, China (32° 14′ N, 102° 47′ E, approx. 3700 m a.s.l.). The climate is characterized by wet, short summers and cold, dry winters with an annual average temperature of 4.4 °C and precipitation of 620 mm. The forest is dominated by Abies fargesii var. faxoniana (Rehder & E.H.Wilson) Tang S.Liu and the canopy cover is approximately 50%. Bryophytes are abundant and account for approximately 60% of the ground cover. The most dominant bryophyte species are the pleurocarpous mosses, e.g. A. hookeri, H. splendens, Pleuroziunm schreberi (Brid.) Mitt., and Ptilium crista-castrensis (Hedw.) De Not.

The N addition experiment was set up in August 2012. Five plots (each 2 × 2 m) were established where A. hookeri and H. splendens were co-dominant N was applied on August 23 and 24; 2.86 g NH4NO3 (1 g N/m²) was dissolved in 5 L water (7.14 mmol/l), and applied to each plot using a hand sprayer. The amount of added N was based on the N deposition rate on the eastern Tibetan Plateau (Lu & Tian, 2007). The added water was equivalent to 1.25 mm precipitation.

Moss samples were collected, with the underlying substrate in each plot, both before and 8 days after N addition. Samples were sealed in plastic bags, and brought to the laboratory within 1 hour. Litter and other mixed mosses were carefully removed, dead tissues were eliminated, and only green photosynthetically active tissues were collected as the final samples. The samples were submerged in distilled water for 1 minute, drained and the surface water carefully removed with paper towels. Moist water-absorbing cotton was placed on the bottom of Petri dishes (5 cm in diameter) to prevent desiccation during the measurements. The bryophyte samples were arranged carefully to fill the dishes while avoiding overlapping. A picture of each sample was taken directly over the dish, and ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to calculate the tissue projected area. After 30 minutes of light induction with 150 μmol photons/m²/s photosynthetically active radiation (PAR), the dishes were placed in a Li-6400-22L Lighted Conifer Chamber (Li-Cor, Inc., Lincoln, NE, USA) under conditions of 15 °C chamber temperature, 60–80% relative humidity, and a 400 ppm CO2 concentration with an air flow rate of 300 μmol/s. Twelve steps of light intensity were established: 800, 600, 400, 300, 200, 150, 100, 80, 60, 40, 20, and 0 μmol photons/m²/s PAR. Each light level lasted for 3–5 minutes to allow the assimilative rate to reach a relatively steady state. The light-saturated CO2 assimilation rate per area (Aarea), light saturation point (LSP), and light compensation point (LCP) were estimated by fitting light response curves (Farquhar et al., 2001). Furthermore, nine steps of CO2 concentration were established: 50, 100, 200, 300, 400, 600, 800, 1000, 1400 μmol/m³.
and 1200 ppm. The chamber PAR was set to 400 μmol photons/m²/s. The parameter CO₂ saturation point (Cₛₐₚ) and CO₂ compensation point (Cₛₐₚ) were estimated by fitting the CO₂ response curves (Ye & Gao, 2008). The fitting coefficients were all above 0.99. Each sample was dried at 70°C for 48 hours to determine the dry mass. The SMA (shoot mass per area, g/m) was calculated as the dry mass divided by the tissue projected area. The light-saturated CO₂ assimilation rate per nitrogen, Aₙ, was calculated by dividing Aₙ by N₉₉.

One stem of each species was labelled in each plot for chlorophyll fluorescence measurement, before N addition. Initial fluorescence (Fᵢ), maximal fluorescence (Fₘ), steady-state fluorescence (Fₛ), basic fluorescence after light induction (Fₛ), and maximal fluorescence emission in the presence of quenching (Fₘ) were determined using a PAM-2100 fluorometer (Heinz Walz, Effeltrich, Germany). The labelled shoots were dark-adapted for 30 minutes before being exposed to a saturation pulse lasting 0.8 seconds at an intensity of approximately 8300 μmol/m²/s. The same labelled shoots were measured both before and approximately 8 days after N application in the morning before 11 a.m. More variables were calculated based on the measured parameters: basic fluorescence in dark (Fᵢ=Fₘ-Fᵢ) and light (ΔF=Fₘ-Fᵢ), maximal PSII photochemical efficiency (Fᵥ/Fₘ), effective quantum yield of PSII (Φₛₛ=ΔF/Fₘ), photochemical fluorescence quenching (qP=(Fₘ-Fₛ)/(Fₘ-F₀)), and non-photochemical fluorescence quenching [NPQ=(Fₘ-Fₚ)/(Fₘ-Fₛ); Maxwell & Johnson, 2000].

Sampling and chlorophyll fluorescence measurement were done simultaneously in the same plot, beside the labelled shoots which were used in the chlorophyll fluorescence measurement. Chloroplast pigments were extracted using 95% alcohol for 24 hours in the dark, from samples which had been preserved in liquid-nitrogen. Concentrations of chlorophyll a, chlorophyll b, and carotenoids were determined following the method of Bao & Leng (2005). Total concentrations of carbon (Cₘасс) and nitrogen (Nₘасс) were determined by using a Vario Macro Cube Elemental Analyser (Elementar, Germany). Phosphorus concentration (Pₘасс) was analysed using the Mo-Sb Antispetrophotography Method. The stoichiometric ratios (C/N and N/P) were calculated from the elemental concentrations. The amount of N in chlorophyll (Nₕₗₐₜ) was calculated from the chlorophyll content based on the N concentration in chlorophyll molecules. The proportion of N invested in chlorophyll (Nₕₗₐₜ/Nₕₗₐₜ) was calculated (Granath et al., 2009a). To further investigate the N absorption and allocation, the moss samples were divided into sections. For H. splendens, which forms obvious annual growth increments, the shoots were divided into current-year (S1), last-year (S2), and older section (SO). The shoots of A. hookeri were divided into growing tip, in which the branches elongate successively (S1), older green section (S2), and brown section (SO, Figure 1). The N concentration of each part was determined.

General linear mixed models were used to test the N addition and species effects on photosynthesis and other parameters (Supplementary Material 1); plot (n=5) was treated as a random effect in the analysis. The mixed model analyses used the nlme package in R (Pinheiro et al., 2013). The Wald test was used to investigate that statistical significance of the fixed effects. Standard residual analysis was used to check normality and homogeneity of variance. The different N concentrations of each section before and after N addition were tested with paired t-test. All analyses were performed in R 3.0.1 (R Core Team, 2013).

Results
The N addition effect on tissue Nₘасс was significant (P=0.035), whereas the Cₘасс and Pₘасс changed minimally (Figure 2a–c, Supplementary Material 2). The N content of S1 was increased significantly for both species (Figure 3, Supplementary Material 3). The content of Chl a and Chl b increased after N addition, however, the carotenoid content was minimally changed.
Furthermore, no significant change was found for the two mosses in terms of Chl a/b ratio (Figure 2j), which is a measure of the antenna size of the photosystem. No significant response was found in any of the gas exchange parameters (Figure 4a–g, Supplementary Material 2) to N addition, whereas, the WPSII was significantly increased (P < 0.019) and the NPQ was decreased (P < 0.010) after N addition (Figure 4i and k).

Significant differences were found between the two mosses. The N concentration of H. splendens was higher than that of A. hookeri (Figure 2b, Supplementary Material 2) to N addition, whereas, the \( \Phi_{PSII} \) was significantly increased (P = 0.019) and the NPQ was decreased (P = 0.010) after N addition (Figure 4i and k).

Discussion

Our study demonstrates the sensitivity of ground-dwelling pleurocarpous mosses to the addition of N. Our objectives were to investigate the photosynthetic responses (in terms of gas exchange and chlorophyll fluorescence) after N addition and the photosynthetic differences between two species with similar life forms and distinct growth rates. Our results indicate that added low-level N was mainly absorbed by the new growth, the quantum yield of PSII was stimulated by N addition after 8 days and that the faster growing H. splendens had a higher photosynthetic rate.

Our finding that the tissue N concentration of the moss shoots was stimulated by the N addition is consistent with several other studies on the effect of N addition and N deposition on pleurocarpous moss (Gundale et al., 2011) or Sphagnum spp. (Granath et al., 2009a, 2012). Furthermore, the added N was mainly absorbed by the current-year or the new-growth section (Figure 3). Membrane damage to ‘black’ (lower) tissue has been reported in another
The montane moss *Racomitrium lanuginosum* (Hedw.) Brid (Pearce et al., 2003). Cell membranes play important roles in nutrient uptake and conservation (Glime, 2007). Furthermore, the architecture of the moss canopy may also affect the uptake of N, because the new-growth section may retain the solution in the top of the moss canopy. Thus explaining why little added N was absorbed by the lower tissues of the mosses.

With more available N after N addition, the mosses clearly allocated more N to chlorophyll. This was shown by both the increased chlorophyll content and the $\frac{N_{\text{Chl}}}{N_{\text{tot}}}$ ratio (Figure 2f–h). The $\frac{N_{\text{Chl}}}{N_{\text{tot}}}$ ratio provides an estimate of within-cell allocation to light-harvesting apparatus and the ratio is usually higher in shaded habitats (Evans, 1989; Granath et al., 2009a). In a long-term N fertilization study, overstory coverage increases with N enrichment. Ground-dwelling mosses were more sheltered by higher vascular plants and suffered from a reduction in light availability (e.g. Gunnarsson et al., 2004). Thus, shade-tolerant species may allocate more N into light-harvesting pigments (Evans, 1989). However, the light availability changed minimally in the short term. Our results suggest that the higher $\frac{N_{\text{Chl}}}{N_{\text{tot}}}$ ratio was caused by the elevated N level. This coincides with the results of Granath et al. (2009a) who found that the shading effect may be less effective in explaining the increased $\frac{N_{\text{Chl}}}{N_{\text{tot}}}$ ratio.

At variance with our first hypothesis, the data showed no significant effect of N addition on $A_{\text{mass}}$. Typically, the allocation of N to chlorophyll improves $A_{\text{mass}}$ (Granath et al., 2009a), and the chlorophyll content was a good predictor of photosynthetic rate (Gaberscik & Martinec, 1987; Granath et al., 2009a). However, instead of the photosynthetic rate, $\Phi_{\text{PSII}}$ increased after N addition (Figure 4a and i). $\Phi_{\text{PSII}}$, which measures the efficiency of PSII photochemistry, is reported to be correlated with CO2 fixation in laboratory conditions (Maxwell & Johnson, 2000). Light photosynthetic electron transport may also be promoted by N addition, which could also be measured by $\Phi_{\text{PSII}}$. CO2 fixation is typically favoured by elevated N availability. For example, the photosynthetic rate of *Sphagnum* species was reported to be stimulated by low level N addition or N deposition (Granath et al., 2009a, 2012). The mismatch between $\Phi_{\text{PSII}}$ and $A_{\text{mass}}$ may be caused by other limiting factors in photosynthetic process, such as CO2 assimilation and carboxylation. The mosses studied were shade-tolerant species which were adapted to the shade habitat of forest floor. Shade-tolerant plants tend to partition more N into thylakoids, where light harvesting and primary reaction occur (Evans, 1989). Thus, the light-saturated CO2 assimilation, $A_{\text{mass}}$, might have been limited by the key enzymatic processes of the Calvin cycle (Evans, 1989). van de Weg et al. (2013) reported no significant increase of the carboxylation efficiency of Rubisco following N addition, which confirmed our inference. Furthermore, the photosynthetic tissues need to be well hydrated to function properly, but the presence of thin films of liquid water form a barrier to CO2 diffusion (Hanson et al., 2014). The mosses could not increase the gas exchange surface area in the short time after N addition. Thus, CO2 might be another factor limiting $A_{\text{mass}}$. Thus, instead of $A_{\text{mass}}$, $\Phi_{\text{PSII}}$ is sensitive to N addition. Another important chlorophyll fluorescence parameter, $\frac{F_{\text{i}}}{F_{\text{m}}}$, showed little change after N application (Figure 4h). This mirrored the results that the maximum fluorescence intensity $\frac{F_{\text{i}}}{F_{\text{m}}}$ is insensitive to chlorophyll content unless the Chl a/b ratio is affected (Dinç et al., 2012). Our results show that the chlorophyll contents and $\Phi_{\text{PSII}}$ were increased after N addition. The chlorophyll fluorescence parameter, $\Phi_{\text{PSII}}$, may be used as a rapid and efficient tool to monitor the ecosystem N condition.

Consistent with our second hypothesis, $A_{\text{mass}}$ of the faster growing species, *H. splendens*, is higher than
Species with higher SMA typically have a higher investment in structural tissue (Rice et al., 2008; Waite & Sack, 2010). *H. splendens*, with smaller SMA, invested more N in chlorophyll than *A. hookeri* (Figure 2f and k). Species with less structural tissue allocation allow deep light penetration and have greater vertical distribution of photosynthesis (Rice et al., 2008). Our results confirmed the negative relationship between LMA (leaf mass per area, a trait similar to SMA for bryophytes) and A_mass for vascular plants (Wright et al., 2004), as the bryophyte shoot systems are considered analogous to vascular plant leaves (Proctor, 2000). A similar correlation was found in mosses between CMA (canopy mass per area) and A_mass (Waite & Sack, 2010). In vascular plant leaves, LMA is an important trait which interlinked with many other traits (Sack & Holbrook, 2006; Waite & Sack, 2010). Thus, the A_mass discrepancy could be mainly explained by SMA. Our results indicate that the faster growing species, *H. splendens*, has a higher mass-based CO2 assimilation rate, while the area-based CO2 assimilation rates were similar for the two mosses (Figure 4a, Supplementary Material 2). This confirmed the mass-based CO2 assimilation rate relates more closely to growth than the area-based one (Poorter et al., 2014). Given that species with the same life form or adapted to same habitat presumably have a limited range of traits, most interspecific trait relationships studies focused on species with different life forms or from different habitats (Bond-Lamberty & Gower, 2007; Waite & Sack, 2010). Our results highlight the differences in photosynthetic capacity and functional traits for two species with the same life form and that grow in the same habitat.

We therefore conclude that the added N was mainly absorbed by the new growth section of the two mosses, and the chlorophyll contents were increased after N addition. The chlorophyll fluorescence parameters, ΦPSII and NPQ, were affected by N application. However, N addition did not affect the light-saturated photosynthetic rate after 8 days. By
allocating resources differently, the two pleurocarpous mosses, which differ in growth rate, display differences in mass-based photosynthetic rate and chlorophyll contents.

Acknowledgements

We thank the Administration Bureau of Dagu Glacier Park, particularly Huaxiang Tang, for the support and facilities for field research. We also thank anonymous reviewers for their valuable comments and suggestions on a previous version of this manuscript. We acknowledge Wei Wang for improving the language of the manuscript. This work was funded by the Strategic Priority Research Program of the Chinese Academy of Science (No. XDA05070306) and the National Science & Technology Pillar Program in 12th Five-year Plan of China (No. 2011BAC09B0402).

Taxonomic Additions and Changes: Nil.

References

Bao, W.K. & Long, L. 2005. Determination methods for photosynthetic pigment content of bryophytes with special relation of extracting solvents. Chinese Journal of Applied and Environmental Biology, 11(2): 235–7.

Bond-Lamberty, B. & Gower, S. 2007. Estimation of stand-level leaf area for boreal bryophytes. Oecologia, 151(4): 584–90.

Cartrae, J.A., Sheppard, L.J., Raven, J.A., Leith, I.D. & Crossley, A. 2007. Potassium and phosphorus additions modify the response of Sphagnum capillifolium growing on a Scottish ombrotrophic bog to enhanced nitrogen deposition. Applied Geochemistry, 22(6): 1111–21.

Dine, E., Ceppi, M.G., Töth, S.Z., Bottka, S. & Schansker, G. 2012. The chl a fluorescence intensity is remarkably insensitive to the chl a/b ratio remains unaffected. Biochimica et Biophysica Acta (BBA) – Bioenergetics, 1817(5): 770–9.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C-3 plants. Oecologia, 78(1): 9–19.

Farquhar, G.D., von Caemmerer, S. & Berry, J.A. 2001. A model of photosynthesis. Plant Physiology, 125(1): 207–16.

Gabersek, A. & Martincic, A. 1987. Seasonal dynamics of net photosynthesis and productivity of Sphagnum papillosum. Lindbergia, 13(3): 105–10.

Glimé, J.M. 2007. Bryophyte ecology [online]. [accessed 3 December 2012]. Available at: <http://www.bryocol.nmt.edu/br.html>.

Gough, C.A., Seiler, J.R. & Maier, C.A. 2004. Short-term effects of fertilization on loblolly pine (Pinus taeda L.) photosynthesis, Plant Cell and Environment, 27(7): 876–86.

Gough, L., Osenberg, C.W., Gross, K.L. & Collins, S.L. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. Oikos, 89(3): 428–39.

Granath, G., Strengbom, J., Breeuwer, A., Heijmans, M.M.P.D., Berendse, F. & Rydin, H. 2009a. Photosynthetic performance in Sphagnum transplanted along a latitudinal nitrogen deposition gradient. Oecologia, 158(4): 705–15.

Granath, G., Wiedermann, M.M. & Strengbom, J. 2009b. Physiological responses to nitrogen and sulphur addition and raised temperature in Sphagnum balticum. Oecologia, 161(3): 481–90.

Granath, G., Strengbom, J. & Rydin, H. 2012. Direct physiological effects of nitrogen on Sphagnum: a greenhouse experiment. Functional Ecology, 26(2): 353–64.

Gundale, M.J., Delucia, T.H. & Nordin, A. 2011. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. Global Change Biology, 17(8): 2743–53.

Gunnarsson, U., Granberg, G. & Nilsson, M. 2004. Growth, production and interspecific competition in Sphagnum: effects of temperature, nitrogen and sulphur treatments on a boreal mire. New Phytologist, 163(2): 349–59.

Hanson, A.D., Rovraglia, R. & Villarreal, J.C. 2014. Diffusion limitation and CO2 concentrating mechanisms in bryophytes.

In: D.T. Hanson & S.K. Rice, eds. Photosynthesis in bryophytes and early land plants. Dordrecht: Springer, pp. 95–111.

Koranda, M., Kerschbaum, S., Wanek, W., Zeichmeister, H. & Richter, A. 2007. Physiological responses of bryophytes Thuidium tamariscinum and Hylocomium splendens to increased nitrogen deposition. Annals of Botany, 99(1): 161–9.

Lindo, Z. & Gonzalez, A. 2010. The bryosphere: an integral and influential component of the earth’s biosphere. Ecosystems, 13(4): 612–27.

Liu, X. & Bao, W.K. 2011. Community structure and vascular plant species composition of primary spruce forest near timberline in the eastern Tibetan plateau. Biodiversity Science, 19(1): 34–40.

Lu, C.Q. & Tian, H.Q. 2007. Spatial and temporal patterns of nitrogen deposition in China: synthesis of observational data. Journal of Geophysical Research: Atmospheres, 112(D22): D22206.

Manning, P., Newington, J.E., Robson, H.R., Saunders, M., Eggers, T., Bradford, M.A., Bardgett, R.D., Bonkowski, M., Ellis, R.J., Gange, A.C., Grayston, S.J., Kandeler, E., Marhan, S., Reid, E., Tscherko, D., Godfray, H.C.J. & Rees, M. 2006. Decoupling the direct and indirect effects of nitrogen deposition on ecosystem function. Ecology Letters, 9(9): 1015–24.

Maxwell, K. & Johnson, G.N. 2000. Chlorophyll fluorescence — a practical guide. Journal of Experimental Botany, 51(345): 659–68.

Paulissen, M.P.C.P., van der Ven, P.J.M., Dees, A.J. & Bobrink, R. 2004. Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. New Phytologist, 164(3): 451–8.

Pearce, I.S.K., Woodin, S.J. & van der Wal, R. 2003. Physiological and growth responses of the montane bryophyte Racomitrium lanuginosum to atmospheric nitrogen deposition. New Phytologist, 160(1): 145–55.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. 2013. nlme: linear and nonlinear mixed effects models [online]. [accessed 3 January 2013]. Available at: <http://CRAN.R-project.org/package=nlme>.

Poorter, H., Lambors, H. & Evans, J.R. 2014. Trait correlation networks: a whole-plant perspective on the recently criticized leaf economic spectrum. New Phytologist, 201(2): 378–82.

Proctor, M.C.F. 2000. Physiological ecology. In: A.J. Shaw & B. Goffinet, eds. Bryophyte biology. Cambridge: Cambridge University Press, pp. 225–47.

R Core Team. 2013. R: A language and environment for statistical computing [online]. [accessed 3 January 2013]. Available at: <http://www.R-project.org/>.

Rice, S.K., Aclandler, L. & Hannon, D.T. 2008. Do bryophyte shoot systems function like vascular plant leaves or canopies? Functional trait relationships in Sphagnum mosses (Sphagaceae). American Journal of Botany, 95(11): 1366–74.

Sack, L. & Holbrook, N.N. 2016. Leaf hydraulics. Annual Review of Plant Biology, 57: 361–81.

Stevens, C.J., Dise, N.B., Mountford, J.O. & Gowing, D.J. 2004. Impact of nitrogen deposition on the species richness of grasslands. Science, 303(5655): 1876–9.

Turetsky, M.R. 2003. The role of bryophytes in carbon and nitrogen cycling. Bryologist, 106(3): 395–409.

van de Weg, M.J., Shaver, G.R. & Salmon, V.G. 2013. Contrasting effects of long term versus short-term nitrogen addition on photosynthesis and respiration in the arctic. Plant Ecology, 214(10): 1273–86.

Vitousek, P.M. & Howarth, R.W. 1991. Nitrogen limitation on land and in the sea — how can it occur. Biogeochernistry, 13(2): 87–115.

Walte, M. & Sack, L. 2010. How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. New Phytologist, 185(1): 156–72.

Wang, Q., Wu, N., Luo, Y., Shi, L.S., Bao, W.K. & Shi, F.S. 2007. Moss growth rate and its environmental determinants in subalpine coniferous forest and clear-cut land in eastern Tibetan plateau, China. Acta Phytocoecologica Sinica, 31(3): 464–9.

Wedin, D.A. & Tilman, D. 1996. Influence of nitrogen loading and species composition on terrestrial carbon balance of grasslands. Science, 274(5293): 1720–3.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Caderven-Bares, J., Chapin, T., Corniessien, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gallis, J., Hikosaka, K., Lambers, H., Luy, C., Midtgley, J.J., Navas, M.L., Nielsen, U., Oleksyn, J., Granberg, G., Nilsson, M. & Nilsson, M. 2004. Growth, production and interspecific competition in Sphagnum: effects of temperature, nitrogen and sulphur treatments on a boreal mire. New Phytologist, 163(2): 349–59.
Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. 2004. The worldwide leaf economics spectrum. *Nature*, 428(6985): 821–7.

Ye, Z.P. & Gao, J. 2008. Change of carboxylation efficiency of *Salvia miltiorrhiza* in the vicinity of CO2 compensation point. *Journal of Northwest A & F University. Natural Science Edition*, 36(5): 160–4.