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Research paper

***Sphagnum* can 'filter' N deposition, but effects on the plant and pore water depend on the N form**

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Abstract

The ability of *Sphagnum* moss to efficiently intercept atmospheric nitrogen (N) has been assumed to be vulnerable to increased N deposition. However, the proposed critical load (20 kg N ha^{-1} yr^{-1}) to exceed the capacity of the *Sphagnum* N filter has not been confirmed. A long-term (11 years) and realistic N manipulation on Whim bog was used to study the N filter function of *Sphagnum* (*S. capillifolium*) in response to increased wet N deposition. On this ombrotrophic peatland where ambient deposition was 8 kg N ha^{-1} yr^{-1}, an additional 8, 24, and 56 kg N ha^{-1} yr^{-1} of either ammonium (NH_4^+) or nitrate (NO_3^-) has been applied for 11 years. Nutrient status of *Sphagnum* and pore water quality from the *Sphagnum* layer were assessed. The N filter function of *Sphagnum* was still active up to 32 kg N ha^{-1} yr^{-1} even after 11 years. N saturation of *Sphagnum* and subsequent increases in dissolved inorganic N (DIN) concentration in pore water occurred only for 56 kg N ha^{-1} yr^{-1} of NH_4^+ addition. These results indicate that the *Sphagnum* N filter is more resilient to wet N deposition than previously inferred. However, functionality will be more compromised when NH_4^+ dominates wet deposition for high inputs (56 kg N ha^{-1} yr^{-1}). The N filter function in response to NO_3^- uptake increased the concentration of dissolved organic N (DON) and associated organic anions in pore water. NH_4^+ uptake increased the concentration of base cations and hydrogen ions in pore water though ion exchange. The resilience of the *Sphagnum* N filter can explain the reported small magnitude of species change in the Whim bog ecosystem exposed to wet N deposition. However, changes in the leaching substances, arising from the assimilation of NO_3^- and NH_4^+, may lead to species change.

Keywords: Manipulation experiment, Tissue N, Dissolved organic nitrogen, Base cations, N uptake

1. Introduction

Elevated atmospheric nitrogen (N) deposition and its ecological impact is an issue of widespread concern. Peatlands have a significant impact on the global C cycle and there is estimated to be 500 Pg C stored in northern peatlands (Yu, 2012), one-third of the global surface soil C pool (Gorham, 1991). *Sphagnum* moss plays a central role in peatland sustainability and carbon (C) sequestration. *Sphagnum* species in peatland are described as ‘ecosystem engineers’ creating acidic, nutrient-poor, and water saturated soils, enabling them to outcompete other plants (Van Breemen, 1995). The impact of elevated atmospheric N deposition on peatland ecosystems is therefore likely to be mediated through effects on *Sphagnum* species.

Understanding how *Sphagnum* removes inorganic N from precipitation and the effects of increasing N inputs on this process is key to predicting N effects on peatland ecosystems. *Sphagnum* mosses are adapted to nutrient-limited conditions (Van Breemen, 1995). Having no rhizoids and internal water-conducting tissue like other non-vascular plants, *Sphagnum* efficiently intercepts nutrients, including N, coming from the atmosphere (Bobbink *et al.*, 1998; Van Breemen, 1995). The efficient N removal by *Sphagnum* has been likened to a filter effect (Lamers *et al.*, 2000). However, there are limits to the capacity of this filter; as atmospheric N deposition increases the *Sphagnum*

N filter fails and mineral N levels in the rhizosphere increase (Bragazza *et al.*, 2005; Lamers *et al.*, 2000). The increased N availability in the rhizosphere can promote the growth of vascular plants (Berendse *et al.*, 2001; Bragazza *et al.*, 2012; Heijmans *et al.*, 2001; Limpens *et al.*, 2003). Thus, elevated atmospheric N deposition can lead to species change, changes in decomposition rates and ultimately reduce C accumulation in peatland ecosystems (Berendse *et al.*, 2001; Bragazza *et al.*, 2012; Heijmans *et al.*, 2001; Limpens *et al.*, 2011; Sheppard *et al.* 2014).

Sphagnum moss has been assumed to be vulnerable to increased N deposition. *Sphagnum* N status across a natural gradient of ambient atmospheric N deposition revealed that elevated atmospheric N deposition increased tissue N concentrations of *Sphagnum* (e.g. Malmer and Wallén, 2005; Pitcairn *et al.*, 1995; Wiedermann *et al.*, 2009) leading eventually to N saturation of *Sphagnum* (Bragazza *et al.*, 2004; Bragazza *et al.*, 2005; Limpens *et al.*, 2011; Lamers *et al.*, 2000; Harmens *et al.*, 2014). The critical load causing N saturation of *Sphagnum* with increased N availability in the rhizosphere has been proposed at 20 kg N ha^{-1} yr^{-1} (Harmens *et al.*, 2014; Lamers *et al.*, 2000). However, the proposed critical load of N deposition to exceed the capacity of the *Sphagnum* N filter has not been confirmed.

Long-term and realistic *in situ* manipulation studies are urgently needed to elucidate the above question. However, few such studies have been conducted at the proposed critical N load (20 kg N ha^{-1} yr^{-1}) and below except for those by Granath *et al.* (2009) and Xing *et al.* (2010). In many N manipulation studies, the N concentration of *Sphagnum* moss exposed to 30-50 kg N ha^{-1} yr^{-1} of N addition increases to 15-20 mg g^{-1} for short-term (2-3 years, Berendse *et al.*, 2001; Fritz *et al.*, 2012; Nordbakken *et al.*, 2003; Tomassen *et al.*, 2003) and long-term (12 years, Granath *et al.*, 2009) experiments, and often greatly exceeds 20 mg g^{-1} (Heijmans *et al.*, 2001). N addition (40-80 kg N ha^{-1} yr^{-1} for up to 4 years) also increased mineral N concentrations in pore water (Limpens *et al.*, 2003; Limpens *et al.*, 2004; Limpens & Berendse, 2003). However, many manipulation studies in peatland have conducted short-term, high N dose experiments that do not simulate the effect of long-term elevated N deposition on peatland ecosystems and thus are unable to assess the *Sphagnum* N filter function in response to increased N deposition. It is likely that the *Sphagnum* N filter function may be vulnerable to acute increases in N availability caused by low frequency N applications at high concentrations that compromise it in a way that frequent small inputs do not.

Since N deposition contains two forms of mineral N in varying proportions (Stevens *et al.*, 2011), we also need to understand the respective effects of reduced (NH_4^+) versus oxidized (NO_3^-) N on the N status of the *Sphagnum* moss and the

Sphagnum N filter function. NH_4^+ is more detrimental to *Sphagnum* than NO_3^- (Manninen *et al*., 2011; Sheppard *et al*., 2014), possibly due to the greater toxicity of NH_4^+ (Gerendás *et al*., 1997; Krupa, 2003; Stevens *et al*., 2011; Limpens and Berendse, 2003) coupled to preferential uptake of NH_4^+ by *Sphagnum* (Fritz *et al*., 2014; Liu *et al*., 2013; Wiedermann *et al*., 2009). For example, Manninen *et al.* (2011) found that NH_4^+ addition increased shoot N concentration of *Sphagnum* and decreased photosynthetic variables (F_v/F_m) and shoot dry weight of *Sphagnum*.

Pore water that has passed through the *Sphagnum* filter may differ in terms of water quality including pH (Manninen *et al*., 2011; Sheppard *et al*., 2014) when *Sphagnum* is exposed to NH_4^+ and NO_3^- separately. This could be caused by the different exchange processes of *Sphagnum* with respect to N assimilation between NH_4^+ and NO_3^-. In higher plants, NH_4^+ uptake is usually accompanied by cation leaching (Krupa, 2003; Li *et al*., 2013; Staelens *et al*., 2008; Stevens *et al*., 2011) and hydrogen ion (H^+) leaching (Krupa, 2003; Liu *et al*., 2013; Manninen *et al*., 2011; Paulissen *et al*., 2004; Stevens *et al*., 2011; Tomassen *et al*., 2003). In contrast, NO_3^- uptake is accompanied by hydroxyl ion (OH^-) loss generated by nitrate reduction (Manninen *et al*., 2011; Stevens *et al*., 2011). However, few manipulation studies have evaluated the form of reactive N in wet deposition (Blodau *et al*., 2006; Paulissen *et al*., 2004; Sheppard *et* *al*., 2014; Sheppard *et al*., 2013; Van den Berg *et al*., 2008).

Sheppard *et al*. (2014) found that 9 years of these treatments significantly reduced the cover of *Sphagnum*, but that the magnitude of change was small, especially at N loads below 32 kg N ha^{-1} yr^{-1} (N additions of 24 kg N ha^{-1} yr^{-1} plus ambient N deposition of 8 kg N ha^{-1} yr^{-1}). They concluded that *S. capillifolium* is relatively resilient to wet N deposition. In addition, Sheppard *et al*. (2014) showed that although the magnitude of change is small, the effects of wet N deposition on species change on the peatland were different depending on the N form. We suggest the reasons for this may be due to different interactions between *Sphagnum* and N form resulting from NH_4^+ and NO_3^- assimilation.

This study addresses these gaps in our understanding, assessing the *Sphagnum* N filter function in response to increased wet N deposition supplied separately as NH_4^+ or NO_3^-. The specific objectives were as follows: 1) to assess the *Sphagnum* N filter function in response to 11 years of increased wet deposition, including the proposed critical load of N deposition (20 kg N ha^{-1} yr^{-1}), 2) to evaluate the sensitivity of the *Sphagnum* filter function to different N forms, NO_3^- and NH_4^+, and 3) to evaluate the quality of pore water, including pH and base cations, that has passed through the *Sphagnum* filter. These objectives were addressed using the ongoing N manipulation experiment, established in 2002 on Whim bog in SE Scotland: 8, 24, and 56 kg N ha^{-1} yr^{-1} of wet NH_4^+ (as NH_4Cl) and wet NO_3^- (as $NaNO_3$) has been sprayed separately on each plot of peatland for more than a decade. The experiment has been conducted under 'real' world conditions, where N additions were automated and coupled to rainfall, facilitating frequent small N inputs at concentrations more closely resembling those in wet deposition (Sheppard *et al*., 2004; Sheppard *et al*., 2014).

2. Materials and methods

2.1. Study Site

The study was conducted at Whim bog (282 m a.s.l., 3°16'W, 55° 46'N) on 3-6 m of deep peat in the Scottish Borders, 30 km south of Edinburgh, Scotland. No active management has been conducted for at least 70 years. The most common species on this bog, *Calluna vulgaris, Eriophorum vaginatum*, *Sphagnum capillifolium*, *Hypnum jutlandicum*, *Pleurozium schreberi* and *Cladonia portentosa* occur widely on similar habitats through the northern hemisphere (Gore, 1983). *S. capillifolium* is a hummock forming species. Mean annual air temperature and annual precipitation between 2003 and 2013 were 7.9 (5.9 – 9.0) °C and 1124 (734 – 1486) mm, respectively. Annual wet N deposition for NH_4^+ and NO_3^- at this site is *ca.* 3 and 3 kg N ha^{-1} yr^{-1}, respectively, with dry deposited NH_3 contributing a further 2 kg N ha^{-1} yr^{-1}, so that the total atmospheric N deposition is *ca.* 8 kg N ha^{-1} yr^{-1} (Leith *et al.*, 2004; Sheppard *et al.*, 2004; Sheppard *et al.*, 2014).

2.2. Treatments

An area of bog was divided into four replicated blocks each containing eleven 12.8 m^2 circular plots. Plots are 3 m apart to avoid contamination from adjacent plots. The treatments, replicated in four plots, have been supplied to each plot from a central spinning disc generating fine rain droplets all year round since June 2002 (Sheppard *et al.* 2004). Three doses of N were used: 8, 24, and 56 kg N ha^{-1} yr^{-1}, as either reduced N in NH_4Cl or oxidized N in $NaNO_3$, referred to as N_{red} Y and N_{ox} Y respectively, where Y represents the annual dose applied excluding ambient deposition, *e.g.* N_{red} 56. Solution concentrations for N doses of 8, 24, and 56 kg N ha^{-1} yr^{-1} are < 0.57, 1.71, and 4.0 mM, respectively. The wet N treatments are in addition to the estimated ambient deposition of *ca.* 8 kg N ha^{-1} yr^{-1} and supplied 10 % additional rainwater (Sheppard *et al.*, 2014). A rainwater only control per each of the four replicated blocks was also provided. Treatments are applied automatically when weather conditions meet the criteria of: air temperature > 0 °C and wind speed < 5 m s^{-1}, coupling application to real world conditions with a realistic frequency, *ca.* 120 applications yr^{-1} (Sheppard *et al.*, 2014).

2.3. Sphagnum *pore water*

Pore water samples from the open *Sphagnum* moss layer were collected using mini rhizon suction samplers (Rhizon MOM, Eijkelkamp Agrisearch Equipment, Wageningen, The Netherlands) attached to a 20 mL plastic syringe inserted into the *Sphagnum* layer (5cm). In August 2013, one collector was placed in each plot and the collector location for *Sphagnum* pore water was fixed until October 2013. The syringe and connectors attached to the rhizon samplers were wrapped in thin foil to exclude light and to keep them cool.

Pore water samples were collected weekly during the period from August 2013 to October 2013 and immediately transported back to the nearby laboratory where aliquots were filtered through a 0.45 µm membrane filter (Puradisc™, Whatman Inc., NJ, USA, and stored in the dark at 4°C. The following chemical determinations were carried out on the filtered pore water samples: pH by glass electrode (MP220, Mettler Toledo, Leicester, UK), major ions (Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Ca^{2+}, and Mg^{2+}) by ion chromatography (CH-9101, Metrohm, Herisau, Swizerland), NH_4^+ by AMmonia Flow Injection Analyser (AMFIA, ECN; Wyers *et al.* 1993), and dissolved total nitrogen by

HPLC with nitrogen specific detector (Model 8060, Antek Instruments, Houston, USA). Dissolved inorganic N (DIN) concentrations were calculated as the sum of NO_3^- and NH_4^+. The concentrations of dissolved organic nitrogen (DON) were calculated by subtracting DIN from the total N concentrations. Anion deficits were calculated by subtracting total anion (Cl^- + NO_3^- + SO_4^{2-}) concentration from total cation (H^+ + Na^+ + NH_4^+ + K^+ + Mg^{2+} + Ca^{2+}) concentration.

2.4. Tissue nutrient concentrations of Sphagnum *moss*

To evaluate the nutrient status of *Sphagnum* treated for 11 years, vegetation samples were collected from *Sphagnum* pore water sampling locations at the beginning of December 2013. The collected *Sphagnum* were thoroughly cleaned of litter using tweezers before separating into capitula (0-1 cm) and stem fractions (>1 cm). These were then dried at 70 ºC for 72 h and ball milled. C and N in the samples were measured with a CN analyzer (CN corder MT-700, Yanaco Co., Ltd., Tokyo, Japan). For P, the dried samples were ignited at 550 ºC for 2 h then digested using potassium peroxodisulfate ($K_2S_2O_8$), and P measured using molybdenum blue (ascorbic acid) spectrophotometric method (UV mini-1240, Shimadzu, Kyoto, Japan). Standard reference material (NIST Apple Leaves 1515, National Institute of Standards and

Technology, Maryland, USA) was analyzed along with *Sphagnum* samples to ensure accuracy within 5% of known N and P concentrations.

2.5. Calculation and statistical analysis

Uptake per volume of NO_3^- and NH_4^+ by *Sphagnum* was calculated by the following equations:

$$NO_3^-\ uptake_{plot\ x} = (Na^+_{plot\ x} - Na^+_{control}) - NO^-_{3\ plot\ x}$$

$$NH_4^+\ uptake_{plot\ y} = (Cl^-_{plot\ y} - Cl^-_{control}) - NH^+_{4\ plot\ y}$$

where the unit is μmol l^{-1} for the ions in *Sphagnum* pore water. $Na^+_{control}$ and $Cl^-_{control}$ concentrations were averages over the 4 control plots.

In calculating the uptake per volume of N from the treatments in each plot, it was assumed that the counter ions (Na^+ for NO_3^- and Cl^- for NH_4^+) were not retained by *Sphagnum*, but acted as conservative tracers. This assumption is based on the premise that Na^+ and Cl^- in pore water are derived only from atmospheric deposition, such as rainfall deposition, and the treatment supplied in this study. Conservative tracer behavior has been shown for both Na^+ (Eppinga *et al*., 2008; Staelens *et al*., 2008) and Cl^- (Appelo & Postma, 1994; Bragazza *et al*., 2005).

The Kruskal-Wallis H test was used to determine the differences of DIN, DON, the sum of K^+, Mg^{2+}, Ca^{2+}, and H^+ concentrations, and anion deficits in the pore water of the *Sphagnum* layer among the treatments. Spearman's rank correlation coefficient (*r*) was used to examine the relationships between uptake per volume of NO_3^- or NH_4^+ and DON and the sum of K^+, Mg^{2+}, Ca^{2+}, and H^+ concentrations. All statistical analyses were carried out using SPSS 22.0J (SPSS Japan Inc.).

3. Results

3.1. Nutrient status of Sphagnum *moss*

Tissue N concentrations in the capitulum (Fig. 1a) and stem (Fig. 1b) of *Sphagnum* moss exposed to different levels of N addition after 11 years of treatment increased with N deposition, linearly for N_{red} (NH_4^+) and logarithmically for N_{ox} (NO_3^-). In contrast, tissue P concentrations in the capitulum (Fig. 1c) and stem (Fig. 1d) of *Sphagnum* did not change with N addition for treatments with either N_{red} or N_{ox}. Consequently, the N:P ratio of capitulum (Fig. 1e) and stem (Fig. 1f) of *Sphagnum* increased with N deposition, although the linear relationship between N:P ratio of stem for N_{red} and N deposition was not significant (P = 0.069).

3.2. Sphagnum *pore water*

Dissolved inorganic N (DIN) concentrations were less than 20 µmol l^{-1} in pore water from the *Sphagnum* layer exposed to 11 years treatment with different levels of N addition, except for the highest dose (Fig. 2a). DIN (Fig. 2a), DON (Fig. 2b), the sum of the cations (Mg^{2+} + Ca^{2+} + K^{+} + H^{+}) (Fig. 2c), and anion deficits (Fig. 2d) were significantly different [P = 0.018 for DIN, 0.007 for DON, 0.027 for the sum of the cations (Mg^{2+} + Ca^{2+} + K^{+} + H^{+})] among the treatments.

There was a significant linear relationship with a gradient of 0.24 between the uptake per volume of NO_3^- by *Sphagnum* and the DON concentration in pore water (P < 0.05; Fig. 3a) and there was also a significant linear relationship between anion deficit and DON concentration in pore water for N_{ox} (P <0.05; Fig. 4a). In contrast, DON concentration did not increase with increasing uptake per volume of NH_4^+ (Fig. 3b) and there was no significant relationship between anion deficit and DON concentration in pore water for N_{red} (Fig. 4b). However, there was a significant linear relationship with a gradient of 0.84 between the uptake per volume of NH_4^+ by *Sphagnum* and the sum of the base cations and H^+ concentrations in pore water (P < 0.05; Fig. 3d).

4. Discussion

4.1. Sphagnum *N-filter function in response to 11 years of increased wet N deposition*

The critical load causing N saturation of *Sphagnum* and increased N availability in the rhizosphere is proposed at 20 kg N ha^{-1} yr^{-1} (Harmens *et al*., 2014; Lamers *et al*., 2000). However, our long-term (11 years) and realistic N manipulation study indicated that the *Sphagnum* N filter is more resilient to wet N deposition than previously inferred. The increase in tissue N concentrations in the capitula of *Sphagnum* with increasing N deposition (Fig. 1a) was consistent with other N manipulation studies (Table 1). However, DIN concentrations in pore water did not increase by similar proportions (Fig. 2a). Our results suggest that *Sphagnum* exposed to up to 32 kg N ha^{-1} yr^{-1} (N additions of 24 kg N ha^{-1} yr^{-1} plus ambient N deposition of 8 kg N ha^{-1} yr^{-1}) in this study still retained the capacity to take up almost all deposited N, even when deposition exceeded the critical load. Xing *et al.* (2010) also showed that N loading of 16 kg ha^{-1} yr^{-1}, close to the critical load, for 7 years did not increase NH_4^+ and NO_3^- concentrations in pore water of *Sphagnum*-dominated peatland. Our study deals with the hummock forming *S. capillifolium* and it is possible that the response of other *Sphagnum* species may be different or less sensitive, especially the lawn species. However, our study showed that the hummock *S. capillifolium* N filter capacity is not impaired by wet N deposition at the proposed critical load (20 kg N ha^{-1} yr^{-1}) and above (32 kg N ha^{-1} yr^{-1}) for at least a decade.

The resilience of the *Sphagnum* N filter to elevated wet N deposition can help to explain the small magnitude of species change in the Whim bog ecosystem reported by Sheppard *et al.* (2014). Their work demonstrated the long-term (9 years) consequence of N addition on the cover of key components including *Sphagnum*, showing that N addition could significantly reduce the cover rate of *Sphagnum*, but that the magnitude of change was small, especially at N loads below 32 kg N ha^{-1} yr^{-1} (N additions of 24 kg N ha^{-1} yr^{-1} plus ambient N deposition of 8 kg N ha^{-1} yr^{-1}). They concluded that *S. capillifolium* is relatively resilient to wet N deposition. Dry deposited ammonia (NH_3) however, caused significantly more damage to *Sphagnum* per unit N deposited than the corresponding inputs of wet N deposition as NH_4^+ and NO_3^- (Sheppard *et al*., 2011; Sheppard *et al*., 2013; Sheppard *et al*., 2014). Therefore, the elevated mineral N concentration in pore water from the N-saturated *Sphagnum* layer observed by Lamers *et al*. (2000) could reflect exposure to elevated NH_3 concentrations, especially in areas where agriculture dominates the landscape.

Elevated tissue N concentration in *Sphagnum* for N_{red} 56 (Fig. 1a), together with higher DIN concentrations in pore water for N_{red} 56 (Fig. 2a) suggest that after 11 years, the highest dose of reduced N has saturated the *Sphagnum* filter, causing it to leak mineral N to the pore water (Berendse *et al*., 2001; Limpens *et al*, 2003). The N concentration of *Sphagnum* for N_{red} 56 (18.4 mg g^{-1}) was comparable with that of other N manipulation studies where tissue N concentration of *Sphagnum* moss exposed to 30-50 kg N ha^{-1} yr^{-1} of N addition increased to 15-20 mg g^{-1} (Table 1) but was much higher than the threshold of 12-13 mg g^{-1} suggested by Lamers *et al*. (2000) for N-saturated *Sphagnum* (Table 1).

N saturation of *Sphagnum* for the N_{red} 56 treatment is also corroborated by the high stem N concentration (17.1 mg g^{-1}), being similar to that of the capitulum (18.4 mg g^{-1}) of *Sphagnum* (Fig. 1ab). No significant difference was found between the stem and capitulum of *Sphagnum* for N_{red} 56 (P = 0.119), while N concentrations in the capitulum were significantly higher than in the stem for other N dose plots. In 'clean' environments, N concentrations in the capitulum exceed those in the stem parts (Aldous, 2002a; Bragazza *et al*., 2005; Fritz *et al*., 2012; Gunnarsson & Rydin, 2000; Van der Heijden *et al*., 2000; Tomassen *et al*., 2003), reflecting the reallocation of N from old *Sphagnum* branches to new photosynthetically active branches (Aldous, 2002b; Gunnarsson & Rydin, 2000). However, as N deposition increases, *Sphagnum* stored N in the stem as a means of avoiding excessive N accumulation in the capitulum (Aldous, 2002a; Bragazza *et al*., 2005; Limpens *et al*., 2003; Limpens & Berendse, 2003).

No significant difference in tissue P concentrations of *Sphagnum* among the treatments (Fig. 1cd) indicates that elevated N deposition does not affect P concentration of *Sphagnum*, i.e. does not reduce or increase growth. Other studies have also found that P concentrations in *Sphagnum* are not influenced by elevated N (Bragazza *et al*., 2004; Gunnarsson & Rydin, 2000; Limpens *et al*., 2003).

Increase in the N:P ratio of *Sphagnum* also supports N saturation occurring for the N_{red} 56 treatment. An N:P ratio of 30 is proposed as an index of P limitation of *Sphagnum* species due to N saturation (Bragazza *et al.,* 2004; Güsewell *et al*., 2003). In this study, the N:P ratio of the capitulum (44, Fig. 1e) and stem (46, Fig. 1f) of *Sphagnum* exposed to N_{red} 56 where DIN concentrations were elevated (Fig. 2a), were more than 30. Furthermore, the N:P ratio of *Sphagnum* exposed to N doses up to 32 kg N ha^{-1} yr^{-1} (N additions of 24 kg N ha^{-1} yr^{-1} and ambient N deposition of 8 kg N ha^{-1} yr^{-1}), where almost all the DIN was taken up (Fig. 2a), was below 30 (Fig. 1ef), suggesting N saturation is not occurring.

4.2. Sensitivity of Sphagnum filter function to different N forms: NO_3^- vs NH_4^+

The linear response for N_{red} and logarithmic response for N_{ox} in tissue N concentrations of *Sphagnum* (Fig 1ab) indicate either higher uptake of NH_4^+ or reduced growth and less growth dilution, and imply a greater effect of NH_4^+ than NO_3^- on *Sphagnum* for high N dose. NH_4^+ is preferentially taken up by *Sphagnum* (Fritz *et al*., 2014; Liu *et al*., 2013; Wiedermann *et al*., 2009) because *Sphagnum* has a high cation exchange capacity (Bates, 1992; Gunnarsson & Rydin, 2000). In addition, NH_4^+ has greater toxicity (Gerendás *et al*., 1997; Krupa, 2003; Limpens and Berendse, 2003; Stevens *et al*., 2011). Van der Weijden (2015) showed higher concentrations of N-rich amino acids like glutamine, arginine and asparagine in capitulum tissue of *Sphagnum* exposed to wet NH_4^+ addition after 11 years of treatment at Whim bog than those for wet NO_3^- addition because of detoxification of NH_4^+ stored in the plant into N-rich amino acids.

The enhanced DON leaching from *Sphagnum* when NO_3^- is taken up, as discussed later, may reduce tissue N concentration of *Sphagnum* and be a means of counteracting effects of high NO_3^- deposition. These observations indicate that the effect of N on *Sphagnum* will be highly sensitive to the relative proportions of reduced and oxidized N in precipitation.

4.3. Changes in pore water quality as a result of passing through the Sphagnum *filter, differentiated by N form*

The significant positive relationship between NO_3^- uptake per volume by *Sphagnum* and DON concentration in pore water (Fig. 3a) and no increase in DON concentration with uptake per volume of NH_4^+ by *Sphagnum* (Fig. 3b) indicate DON leaching into pore water from *Sphagnum* in response to NO_3^- uptake by *Sphagnum*. Kivimäki (2011) showed higher DON concentration in pore water from *Sphagnum* in response to wet NO_3^- addition in Whim bog. Results from this study lend support to the possible transformation of experimentally added NO_3^- to DON in peatlands (Blodau *et al*., 2006). Bragazza and Limpens (2004) demonstrated that the DON concentration in pore water from the *Sphagnum* layer increased with N deposition and was a major component of total dissolved nitrogen.

The process of DON leaching from plants is poorly understood (Cape *et al*., 2010). The enhanced anion deficit for N_{ox} plots (Fig. 2d) and significant positive relationship between anion deficit and DON concentration (Fig. 4a) indicates the presence of organic N anions to retain the charge balance in pore water when NO_3^- is taken up. Anion deficits have been reported in stream water and lake water, and are ascribed to the presence of organic anions, or bicarbonate for samples with sufficiently high pH (Driscoll et al., 1989; Kopáček et al., 2000). The pH of all pore water samples in this study was less than pH 6, so the contribution of bicarbonate ions will have been small. NO_3^- acquisition by shoots of land plants yields OH^- and the neutralization of OH^- leaves –COO^- (Raven, 1988). This study is consistent with NO_3^- uptake causing the leaching of carboxylate anions as well as OH^- from *Sphagnum*.

The enhanced DON leaching from *Sphagnum* when NO_3^- is taken up may alleviate N saturation of *Sphagnum*. The slope value of 0.24 between NO_3^- uptake per volume and DON concentration (Fig. 3a) was high enough to alleviate increased tissue N concentration of *Sphagnum* exposed to NO_3^- dose. In support of this view, the N concentration of *Sphagnum* capitulum and stem was higher for N_{red}56 than for N_{ox} 56 (Fig. 1a).

The significant positive relationship with slope value of 0.84 between NH_4^+ uptake per volume by *Sphagnum* and the sum of the base cations and H^+ concentrations in pore water (Fig. 3d) indicates that NH_4^+ uptake by *Sphagnum* can be explained by the leaching of base cations and hydrogen ions though ion exchange. Reduction of tissue concentrations of K, Ca, and Mg of *Sphagnum* moss exposed to experimental NH_4^+ treatments (Kivimäki, 2011; Manninen *et al*, 2011) support the observation of base cations leaching from *Sphagnum* in this study. The leaching of base cations and hydrogen ions is reflected in higher concentrations of the sum of Mg^{2+}, Ca^{2+}, K^+, and H^+ concentrations in pore water (Fig. 2c). Tomassen *et al*. (2003) also reported lower pH in peat moisture due to the uptake of NH_4^+ by *Sphagnum*. In contrast, higher pH in soil pore water was observed for NO_3^- treatments (Sheppard *et al*., 2014), probably due to hydroxyl ion (OH^-) loss generated by nitrate reduction (Manninen *et al*., 2011; Stevens *et al*., 2011). The changes in concentrations of these components, especially H^+ (soil acidity), could affect species change. Sheppard *et al*. (2014) showed that the effects of wet N deposition on species change on the peatland were different according to the N form. Our study indicates that the *Sphagnum* N filter could affect species change through leaching substances from *Sphagnum* resulting from NH_4^+ and NO_3^- assimilation.

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Figure Captions

Fig. 1. Relationship of nitrogen (N) deposition (the sum of ambient N deposition and applied N) with *Sphagnum* N concentration of a) capitulum and b) stem, P concentration of c) capitulum and d) stem, and N:P ratio of e) capitulum and f) stem. Background N deposition is *ca.* 8 kg N ha^{-1} yr^{-1} (Leith *et al.*, 2004; Sheppard *et al.*, 2004). Bars represent standard error (n = 4). The fitted model is: capitulum N concentration for N_{red} = 8.76 + 0.15 × (N deposition) (P < 0.05); capitulum N concentration for N_{ox} = 7.0 + 1.63 × $\log_e$ (N deposition) (P < 0.05); stem N concentration for N_{red} = 4.7 + 0.19 × (N deposition) (P < 0.05); stem N concentration for N_{ox} = 1.7 + 2.26 × $\log_e$ (N deposition) (P < 0.05); capitulum NP ratio for N_{red} = 19 + 0.37 × (N deposition) (P < 0.05); capitulum NP ratio for N_{ox} = 23 + 0.21 × (N deposition) (P < 0.05); stem NP ratio for N_{ox} = 7.7 + 6.10 × $\log_e$ (N deposition) (P < 0.05).

Fig. 2. *Sphagnum* pore water of concentrations of a) dissolved inorganic nitrogen (DIN, NO_3^- + NH_4^+), b) dissolved organic nitrogen (DON), c) base cations (K^+ + Mg^{2+} + Ca^{2+}) + protons (H^+), and d) anion deficit. Bars represent standard error (n = 4). DIN concentrations were calculated by summing NO_3^- and NH_4^+ concentrations. Treatment was significant for DIN, DON, and the sum of the cations (Mg^{2+} + Ca^{2+} + K^+ + H^+), P = 0.018, 0.007, and 0.027, respectively.

Fig. 3. Relationship between DON concentration and uptake per volume of a) NO_3^- and b) NH_4^+. Relationship between base cations and protons concentration and uptake per volume of c) NO_3^- and d) NH_4^+.

Fig. 4. Relationship between anion deficits and DON concentration for a) N_{ox} and b) N_{red}.

Table 1

Capitulum N concentration of *Sphagnum* moss exposed to increased atmopsheric N deposition

N concentration (mg g^{-1})	N deposition (kg N ha^{-1} yr^{-1})	Years	Experiment type	Reference
12-13	18	-	Ambient N deposition	Lamers *et al*. (2000)
13	20	-	Ambient N deposition	Bragazza *et al* (2005)
18	30 (2)	12	NH_4NO_3 addition	Granath *et al*. (2009)
15	40	3	NH_4NO_3 addition	Tomassen *et al* (2003)
15	40 (1-2)	3	NH_4NO_3 addition	Fritz *et al* (2012)
20	40 (5)	3	NH_4NO_3 addition	Nordbakken *et al* (2003)
20	50 (39)	2-3	NH_4NO_3 addition	Berendse *et al*. (2001)
24	50 (52)	2	NH_4NO_3 addition	Heijmans *et al* (2001)
16	40 (40)	1.5	NH_4NO_3 addition	Limpens *et al* (2003)
18	56 (8)	11	NH_4Cl addition	This study
13	56 (8)	11	$NaNO_3$ addition	This study

Number of parenthesis shows ambient N deposition.

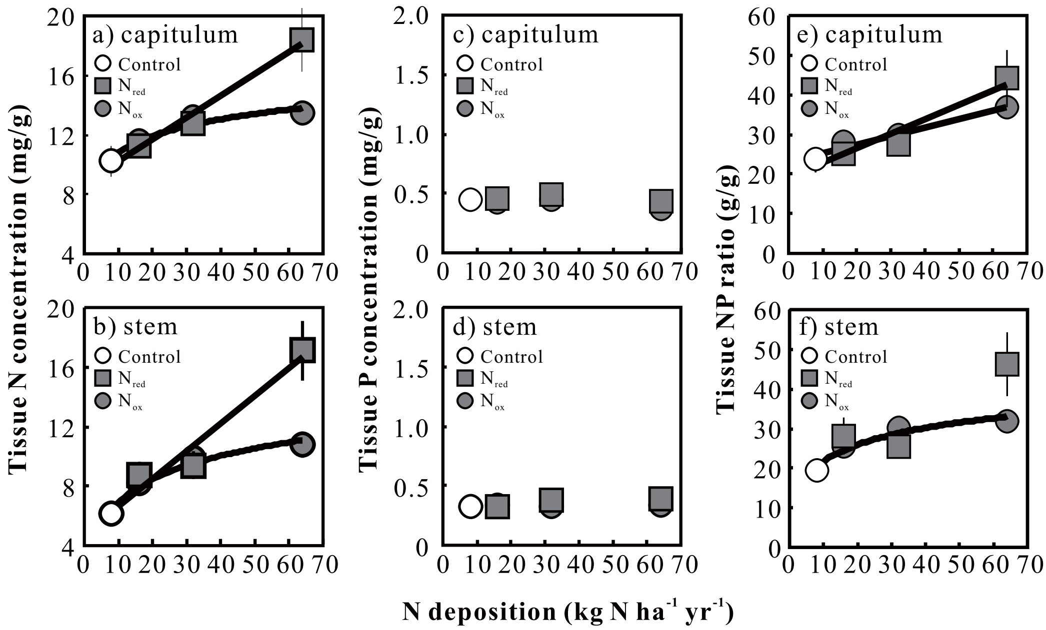

Fig. 1
Chiwa et al.

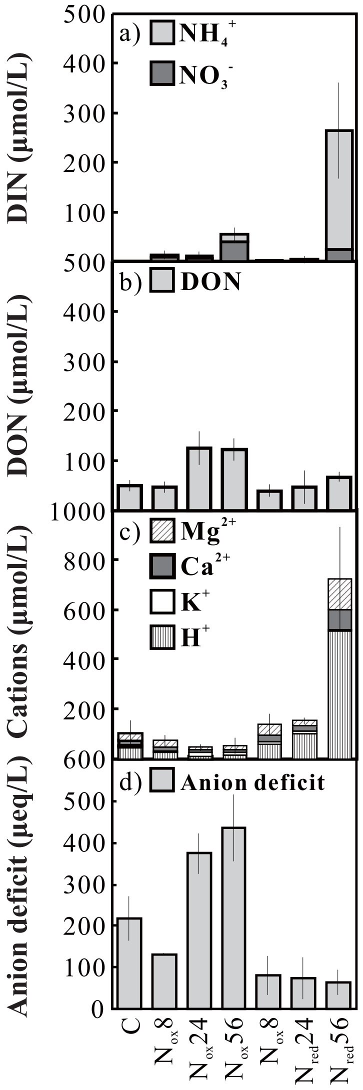

Fig. 2
Chiwa et al.

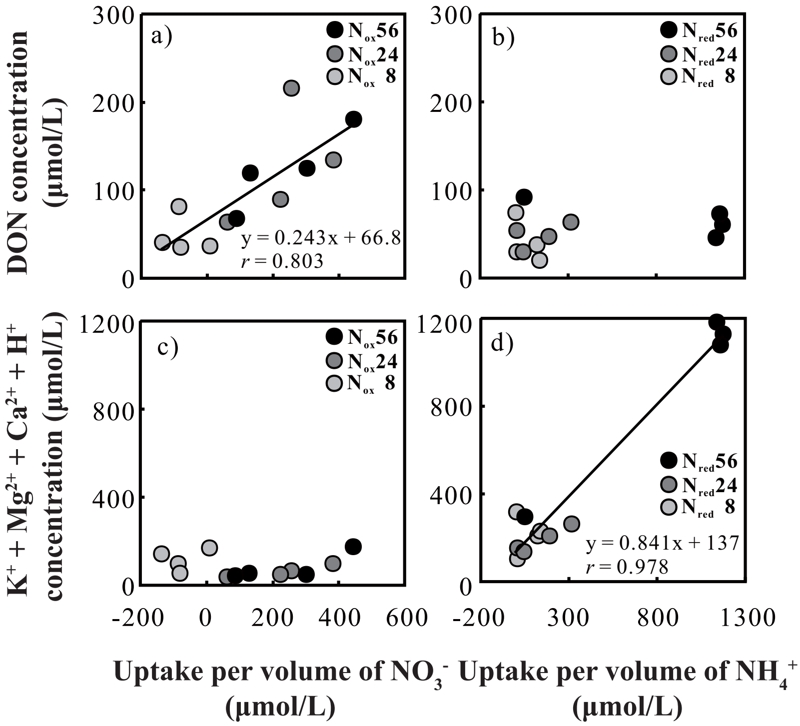

Fig. 3
Chiwa et al.

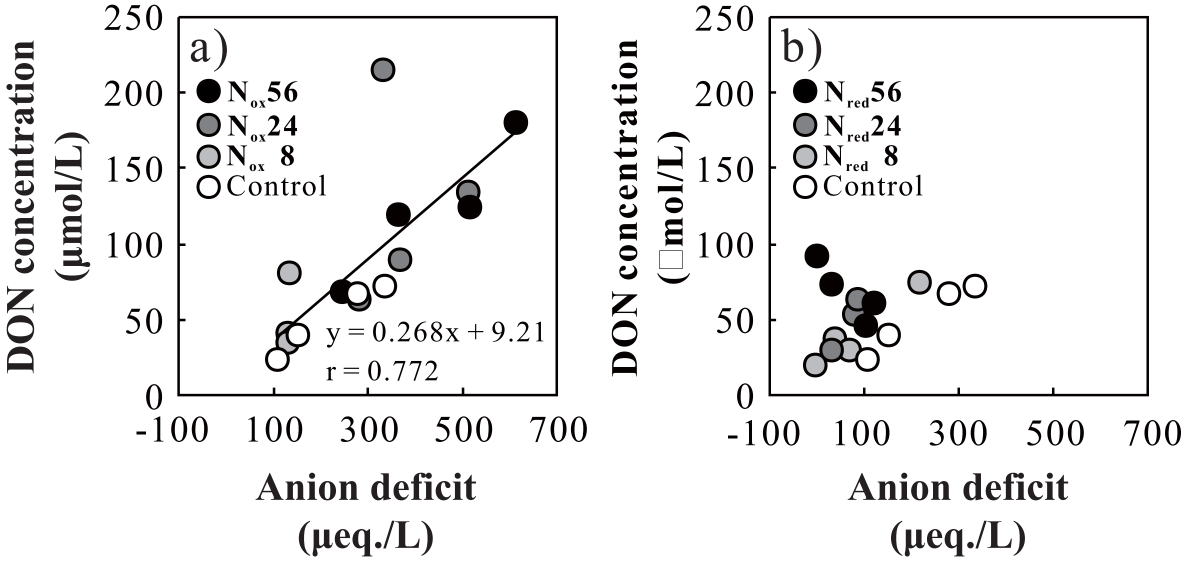

Fig. 4

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