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2016-11-15
Elsevier B.V.
http://hdl.handle.net/10138/297767
Manninen, S, Kivimäki, S, Leith, I D, Leeson, S R & Sheppard, L J 2016, 'Nitrogen deposition does not enhance Sphagnum decomposition', The Science of the Total Environment, vol. 571, pp. 314-322. https://doi.org/10.1016/j.scitotenv.2016.07.152

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Nitrogen deposition does not enhance *Sphagnum* decomposition

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Abstract

Long-term additions of nitrogen (N) to peatlands have altered bryophyte growth, species dominance, N content in peat and peat water, and often resulted in enhanced *Sphagnum* decomposition rate. However, these results have mainly been derived from experiments in which N was applied as ammonium nitrate (NH_4NO_3), neglecting the fact that in polluted areas, wet deposition may be dominated either by NO_3^- or NH_4^+. We studied effects of elevated wet deposition of NO_3^- vs. NH_4^+ alone (8 or 56 kg N ha^{-1} yr^{-1} over and above the background of 8 kg N ha^{-1} yr^{-1} for 5 to 11 years) or combined with phosphorus (P) and potassium (K) on *Sphagnum* quality for decomposers, mass loss, and associated changes in hummock pore water in an ombrotrophic bog (Whim). Adding N, especially as NH_4^+, increased N concentration in *Sphagnum*, but did not enhance mass loss from *Sphagnum*. Mass loss seemed to depend mainly on moss species and climatic factors. Only high applications of N affected hummock pore water chemistry, which varied considerably over time. Overall, C and N cycling in this N treated bog appeared to be decoupled. We conclude that moss species, seasonal and annual variation in climatic factors, direct negative effects of N (NH_4^+ toxicity) on *Sphagnum* production, and indirect effects (increase in pH and changes in plant species dominance under elevated NO_3^- alone and with PK) drive *Sphagnum* decomposition and hummock C and N dynamics at Whim.

Keywords: ammonium, decomposition, nitrate, peatlands, hummock pore water, *Sphagnum*

1 Introduction

Sphagnum mosses dominate boreal and subarctic peatlands, which contain up to 30% of the global soil carbon (C) pool (Yu *et al.*, 2010). Peatlands store C because biomass production exceeds C losses. The environmental conditions, high water table, anoxia, acidity, that characterise peatlands mean C losses are smaller than C fixation, productivity, facilitating C storage (van Breemen, 1995). Nitrogen (N) deposition may modify these conditions improving the likelihood of C loss in several ways: increasing vascular plant productivity and thus transpiration, drying the soil out and affecting the heat balance; reducing the CN ratio: changing pH and the fungi to bacteria ratio (Treseder, 2008; see also Bragazza *et al*., 2012; Bubier *et al*., 2007; Gunnarsson *et al*., 2002). Carbon is lost from peatlands as carbon dioxide (CO_2), methane (CH_4) and dissolved organic C (DOC) (Charman *et al.*, 1999; Chasar *et al*., 2000). Emissions of CO_2 and CH_4 from peatlands, as well as the amount of DOC in pore water, may be altered under the changing climate due to changes in temperature, hydrology and organic matter quality (Gong *et al*., 2013; Treat *et al*., 2014; White *et al*., 2008). The capacity of peatlands to store C may be further threatened by the increasing atmospheric reactive nitrogen (N) deposition (Galloway *et al*., 2004), which can decrease *Sphagnum* biomass production both directly and indirectly (Bubier *et al*., 2007; Hautier *et al*., 2009; Limpens and Berendse 2003a; Limpens *et al*., 2011). Moreover, if increased N deposition improves *Sphagnum* "quality" for decomposers by decreasing its C:N ratio (Bragazza *et al*., 2006; Gerdol *et al*., 2007; Limpens and Berendse, 2003b), the role of peatlands as key C stores may change.

Biological N_2-fixation by prokaryotes associated with *Sphagnum* mosses may fully account for the N input needed to sustain high rates of C sequestration in pristine ombrotrophic bogs (Damman, 1978; Hemond, 1983; Vile *et al*., 2014). In addition, rootless *Sphagnum* mosses derive N from atmospheric deposition of reactive N compounds such as soluble ammonium (NH_4^+) and nitrate (NO_3^-) (Hemond, 1983; Soares and Pearson, 1997). While ammonia (NH_3) emissions from agriculture are the main precursor for NH_4^+ in particulate matter and precipitation, fossil fuel combustion is the main source of N oxides (NO_x) and thus NO_3^- produced through atmospheric reactions (Galloway *et al*., 2004). Phosphorus (P) supply to pristine bogs is also mainly atmospheric (precipitation), but quite low (Rydin and Jeglum, 2013). Phosphorus availability can regulate N impacts both on *Sphagnum* growth (Aerts *et al*.,

1992; Kivimäki, 2011; Limpens *et al*., 2004; Toberman *et al*., 2015) and breakdown (Damman, 1988; Hogg *et al*., 1994).

Sphagnum shoots have a high C:N ratio under naturally acidic, nutrient-limited conditions, hindering decomposition (Gorham, 1991). Thus increased N deposition leading to decreased litter C:N ratio (Bragazza *et al*., 2006; Gerdol *et al*., 2007; Limpens and Berendse, 2003a) should accelerate decomposition of *Sphagnum* litter. However, experimental and field results on the effects of elevated N(PK) deposition on *Sphagnum* decay are inconsistent: Bragazza *et al*. (2006) measured (laboratory incubation) enhanced decomposition rates (higher CO_2 emissions and DOC release) for *S. fuscum* and/or *S. capillifolium* and *S. magellanicum*, formed under higher N deposition in the field (deposition range ≈ 2 to 20 kg N ha^{-1} yr^{-1}); Bragazza *et al*. (2012) reported accelerated mass loss of *S. fuscum* exposed to elevated N deposition (30 kg N ha^{-1} yr^{-1} for 5 years) in the field after 1 year, but not after 3 years. Siegenthaler *et al*. (2010), in turn, found no effect of enhanced N deposition (30 kg N ha^{-1} yr^{-1} for 1 year) on the decomposition of *S. fallax*. No effects of 5-years of N amendments with or without P and potassium (K) (16, 32 or 64 kg N ha^{-1} yr^{-1} + 50 kg P ha^{-1} yr^{-1} and 63 kg K ha^{-1} yr^{-1}) were likewise found on decomposition of *S. capillifolium* in an ombrotrophic bog in Canada (Bubier *et al*., 2007).

Given the contrasting results on *Sphagnum* decomposition rate under elevated N deposition and the fact that the results on N effects in peatlands have mainly been derived from experiments in which N was applied as NH_4NO_3 (see Limpens *et al*., 2011), we wanted to differentiate the long-term effects of elevated NO_3^- vs. NH_4^+ deposition alone or combined with PK on *Sphagnum* mass loss and associated changes in hummock pore water chemistry. As uptake rates for soluble NH_4^+ exceed those for NO_3^- in *Sphagnum* (Lütke Twenhöven, 1992; Wiedermann *et al*., 2009), elevated NH_4^+ supply may increase *Sphagnum* N concentration more than that of NO_3^- (e.g. Paulissen *et al*., 2004) leading to greater decreases in the C:N ratio. Thus it was hypothesized that adding NH_4^+ will enhance *Sphagnum* decomposition more than adding NO_3^- in an acidic bog despite the fact that NO_3^- increases and NH_4^+ decreases pH both within the plant and the soil (Bobbink *et al*., 1998; Raven, 1988).

2 Material and methods

2.1 Experimental set-up

The *in situ* study was performed at Whim, an ombrotrophic peat bog in the Scottish Borders, where the N addition experiment has been running since 2002 (Sheppard *et al*., 2004). Whim is situated ca. 30 km south of Edinburgh, Scotland (3°16'W, 55°46'N) and represents a transition between a lowland raised bog and a blanket bog. It stands 280 m a.s.l., with an annual rainfall of ca. 900 mm (Lindsay, 1995; Ratcliffe, 1964). However, the years 2007-2009 and 2014 were wet with a rainfall of 1161-1476 mm vs. 944 mm in 2013. Annual mean temperatures ranged between 6.9°C (2013) and 8.4°C (2014) (Fig. 1). The water table fluctuations were similar in all plots with an average -7 cm during the 2007-2009 incubations. Although the measured water table was sometimes above the ground level, *S. capillifolium* in hummocks was never submerged under water.

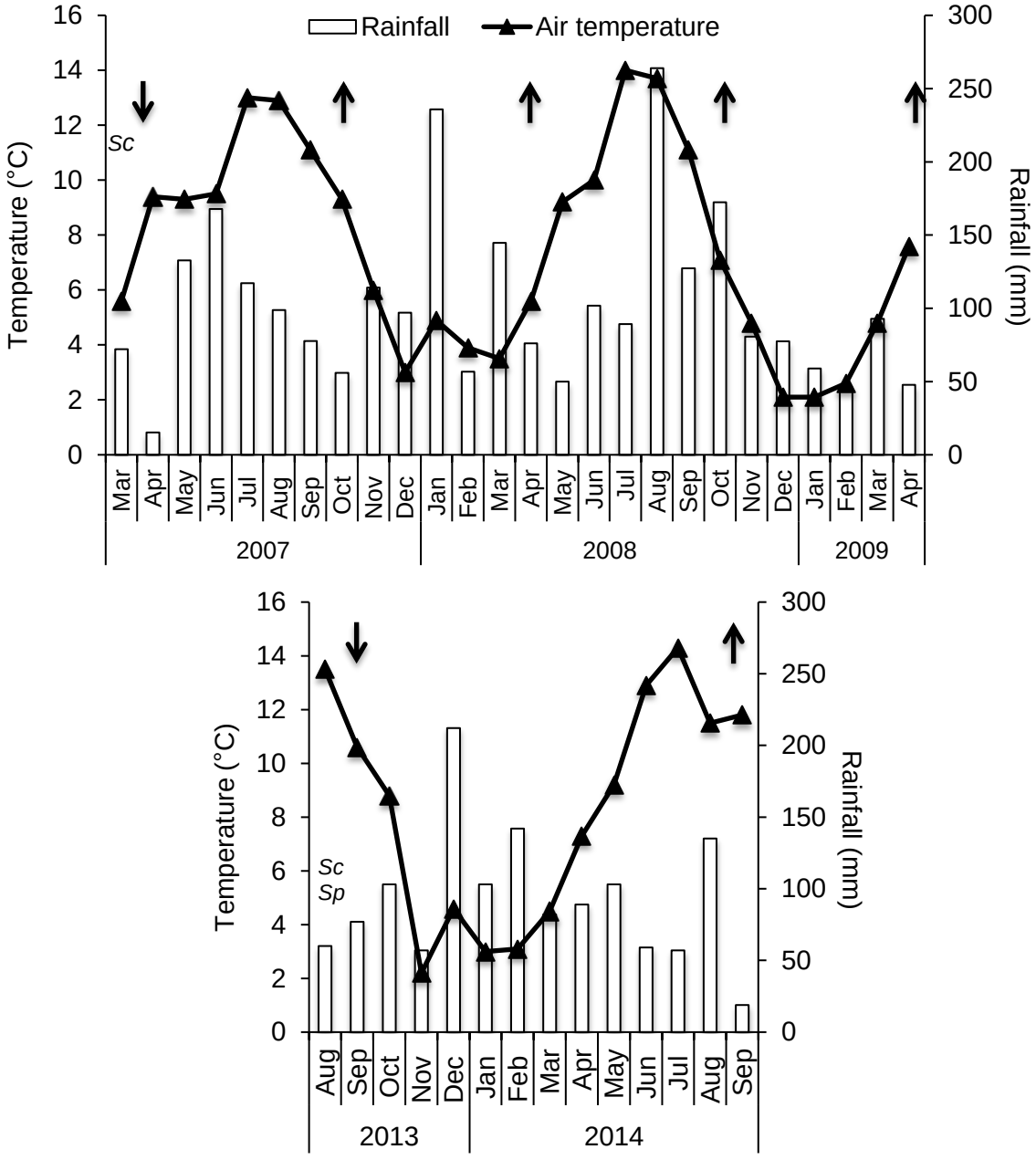


Fig. 1. Monthly mean temperatures and rainfall at Whim in March 2007 – April 2009 and August 2013 – September 2014. Abbreviations indicate when *S. capillifolium* (*Sc*) and/or *S.*

papillosum (*Sp*) were sampled for preparation of litterbags, and arrows indicate when litterbags were placed in (🡻) and removed from (🡹) hummocks.

The hummock vegetation is dominated by *Calluna vulgaris* (L.) Hull. and *Sphagnum capillifolium* (Ehrh.) Hedw. In hollows, *Eriophorum vaginatum* L. is a common vascular species, while *S. capillifolium* is replaced by *S. fallax* (Klinggr.) Klinggr. and *S. papillosum* Lindb. (Mizunuma, 2008). Other common species at Whim are hypnaceous mosses *Hypnum jutlandicum* Holmen & Warncke and *Pleurozium schreberi* (Brid.) Mitt. (Sheppard *et al*., 2004). The peat is very acidic, with a pH_{H2O} ca. 3.4 (range 3.27-3.91) and 10% base saturation. The average concentrations of available P (10 mM citric acid) and K in 2002-2011 were 43 mg kg^{-1} and 90 mg kg^{-1} of air-dry peat, respectively (Sheppard *et al*., 2014).

The study area is divided into four blocks and the treatments are randomly assigned inside the blocks, each block containing one plot (13 m^2) of every N(PK) treatment and a control plot. The round plots are 3 m apart to avoid contamination from the next plot (Sheppard *et al*., 2004). All the plots received the background N deposition ca. 8 kg ha^{-1} yr^{-1} (Sutton *et al*., 2003). Additional reactive N was applied either as nitrate ($NaNO_3$) identified as NOd or ammonium (NH_4Cl) identified as NHd where d = 8 or 56 kg N ha^{-1} yr^{-1}. There were also treatments with NO_3^- or NH_4^+ added at 24 kg N ha^{-1} yr^{-1}, but they were excluded from this study as they did not get PK added together with N. PK was supplied as K_2HPO_4 maintaining a P:N ratio of 1:14 (NH8 PK and NO8 PK plots received 0.6 kg ha^{-1} yr^{-1} of P and NH56 PK and NO56 PK plots received 4 kg ha^{-1} yr^{-1} of P). The nutrients were added to rain water collected on site and supplied by plastic pipe joined to a central rotating disc at the centre of every plot, which produced a fine spray across the plot area. Control plots received additional rainwater only. Wet treatments were supplied automatically when it was raining, increasing precipitation to the plots by ~10% (Table S1). Meteorological parameters were monitored at 15 minute or more frequent intervals. The site and experimental set-up are described in more detail in Sheppard *et al*. (2004).

2.2 *Sphagnum* quality and decomposition

Sphagnum litterbags were incubated in hummocks twice, first for 2 years (April 2007 – April 2009) and then for 1 year (September 2013 – September 2014). The latter incubation was performed because the results on *S. capillifolium* mass loss from 2007-2009 contradicted our hypothesis. Moreover, as the decay of *Sphagnum* varies depending on species and the

151 microenvironment (Belyea, 1996; Hájek, 2009; Johnson and Damman, 1991), we incubated
152 litter of both *S. capillifolium* (hummock species) and *S. papillosum* (hollow species) in 2013-
153 2014 to assess the role of litter quality vs. environment on decomposition. Treatments with PK
154 additions were not included in 2013-2014 because PK was not found to affect *Sphagnum* mass
155 loss in 2007-2009. As mass loss of uniform standard litter (Rooisbosh tea leaves) had only
156 been found to be favoured in autumn by 56 kg N ha^{-1} yr^{-1} of both N forms (Sheppard *et al*.,
157 2013a), the 2013-2014 incubation was started in September. Moreover, the 2013-2014
158 incubation lasted only for 1 year because the duration of incubation did not affect mass loss in
159 2007-2009. A more detailed description of the incubations is given below.
160 *Sphagnum capillifolium* shoots for the 2007-2009 incubations were collected early March
161 2007, while *S. capillifolium* and *S. papillosum* for the latter incubations were sampled late
162 August 2013 (Fig. 1). The moss samples were thoroughly picked clean, photosynthetically vital
163 tissue excluded, and the stem part (3-8 cm below top of the capitulum) retained. The shoot parts
164 from each plot were mixed and divided into five portions: one was oven-dried for 48 hours at
165 70°C, ground with a ball mill and analysed for total C and N with a Carlo Erba NA2500 total
166 C/N analyser; the remaining four portions were air-dried for ≥76 hours before being transferred
167 to litterbags made from 0.71 mm polypropylene mesh. Polypropylene is fairly resistant to acidic
168 conditions and the mesh size retains the material but excludes macrofauna. An aliquot of
169 approximately 0.5 g (0.3-0.9 g) of air-dried sample was transferred to every litterbag. In April
170 2007, four replicate litterbags of *S. capillifolium* were placed in same plots at the same depth
171 from where the mosses had been sampled. One litterbag was removed from each plot after 6,
172 12, 18 and 24 months (i.e. October 2007, April 2008, October 2008, and April 2009) (Fig. S1).
173 *Sphagnum capillifolium* and *S. papillosum* were sampled for the 2013-2014 incubations
174 from the control treatment and N8 and N56 treatments without PK in early August 2013.
175 Samples from different treatments were analysed for C and N prior to incubations and litterbags
176 prepared as in 2007. In September 2013, litterbags were placed both in the plots of treatment
177 origin and in control plots. *Sphagnum* from control plots was also incubated in the N8 and N56
178 treatments (Figs. S2a-S2c). Given the low abundance of *S. papillosum* at Whim, *S. papillosum*
179 litterbags were incubated in *S. capillifolium* 'environment' instead of the hollows they were
180 collected from. For the same reason, the total C and N concentrations were only analysed for
181 pooled samples of *S. papillosum* from different plots within each treatment. All the litterbags,
182 which were placed in hummocks in September 2013, were removed in September 2014. The
183 sampling of material for incubations, and placing and removal of litterbags were always done
184 on days when it was not raining, i.e. N was not applied.

In the laboratory, extraneous material, i.e. vascular plant litter that had fallen in, moss penetration and fine roots of vascular plants were carefully removed. Mass remaining (%) from the samples was established by air-drying to constant weight. The samples were then oven-dried, ground with a ball mill and analysed for C and N. In addition to C:N ratios, we calculated relative decomposition efficiency (RDE) of N and C, i.e. the ratio of percent mass loss of N or C to percent mass loss of *S. capillifolium* (Siegenthaler *et al*., 2010). RDE < 1 means relative accumulation of an element throughout the decomposition and RDE > 1 relative loss of it.

2.3 *Sphagnum* hummock pore water

Rhizon samplers comprising a 0.45 µm filter (Rhizon, Eijkelkam, B.V., The Netherlands) were placed adjacent to the moss bags to collect water samples from within the hummock, 5-15 cm below the moss surface. A syringe was used to apply suction to the tube, drawing water and nutrients through the filter into the syringe until full. Water samples from the syringes were collected twice a month if possible, but at least once every month between July 2007 and November 2008. Because the amount of water in the syringe depended on the amount of rain and the ability of the hummock to retain moisture, it was not always possible to obtain sufficient sample from each plot for all the different analyses, so not every variable was measured for each date. The sample was halved and a subsample used for measuring DOC was frozen immediately until analysis. The other half was further divided with one part used for measuring pH (Mettler Toledo MP220 pH meter) and the other filtered and then frozen. DOC was analysed using a DC-80 Total Organic Carbon (TOC) analyser (Rosemount-Dohrmann) using a sub sample of 200 µl and total N, NH_4^+, and NO_2^-+NO_3^- were measured by continuous flow analyser (San ++ Continuous flow analyser, Skalar analytical BV, The Netherlands). Concentration of dissolved organic N (DON) was calculated as the difference between total dissolved N (TDN) and inorganic N (NO_2^-+NO_3^- + NH_4^+). Hummock pore water samples were only collected in 2007-2008.

2.4 Statistical analyses

Data was tested for normality (Kolmogorov-Smirnov) and when necessary, log-transformed to meet the assumption of normal distribution. *Sphagnum capillifolium* mass loss during the 2007-2009 incubation was analysed with GLM Mixed ANOVA (Repeated Measures) using time as within-subject factor, and treatment, N form (NHd or NOd), and PK (no PK or PK) as between-

subjects factors, with Tukey's HSD as a *post-hoc* test (SPSS 23 for Windows). Differences in the mass loss between *Sphagnum* origins (control or N treatments), incubation environments (control or N treatments) and *Sphagnum* species in 2013-2014 were analysed with Student's t-test. GLM ANOVA with Tukey's HSD as a *post-hoc* test was used to test the differences between treatments in *Sphagnum* quality. As there were problems with the CN analyses of samples removed after the 18- and 24-month incubations, they were excluded from the analyses. Likewise, as there were long breaks in water table measurements in the autumn 2013, only data from 2007-2009 was used in statistical analyses. As samples on hummock pore water were not always obtained for each plot due to the low water table, it was not possible to analyse effect of time on hummock pore water variables, and thus plot means averaged across samplings within each treatment were used in the analyses. Differences in variables between N forms, PK applications or years were analysed using Student's t-test, or Mann-Whitney U test when the assumption of normal distribution was not met by log-transformations. Relationships between variables were studied with Spearman's rank correlation analysis. Results were considered as significant at $P < 0.50$ if not otherwise noted. All data is presented without transformation. Number of replicate plots per treatment was always used as n.

3 Results

3.1 *Sphagnum* quality prior to the incubations

The highest average N concentrations were measured in *S. capillifolium* from the NH56 treatment prior to both incubations (Figs 2 and 3). Overall, the ranges for shoot N concentrations and C:N ratios in *S. capillifolium* were larger, and the average C concentration across treatments (control and elevated N treatments without PK) higher prior to the 2013-2014 than 2007-2009 incubations ($t = 9.42$, $P < 0.001$). Adding PK resulted in a lower average N concentration and a higher average C:N ratio in *S. capillifolium* prior to the 2007-2009 incubations (both $P < 0.05$).

The species did not differ from each other in terms of litter quality prior to the 2013-2014 incubations. *Sphagnum papillosum* shoots had the highest N concentration in the NO56 treatment though. Moreover, the C concentration of *S. papillosum* decreased with increasing N concentration ($r_S = -0.90$, $P = 0.037$).

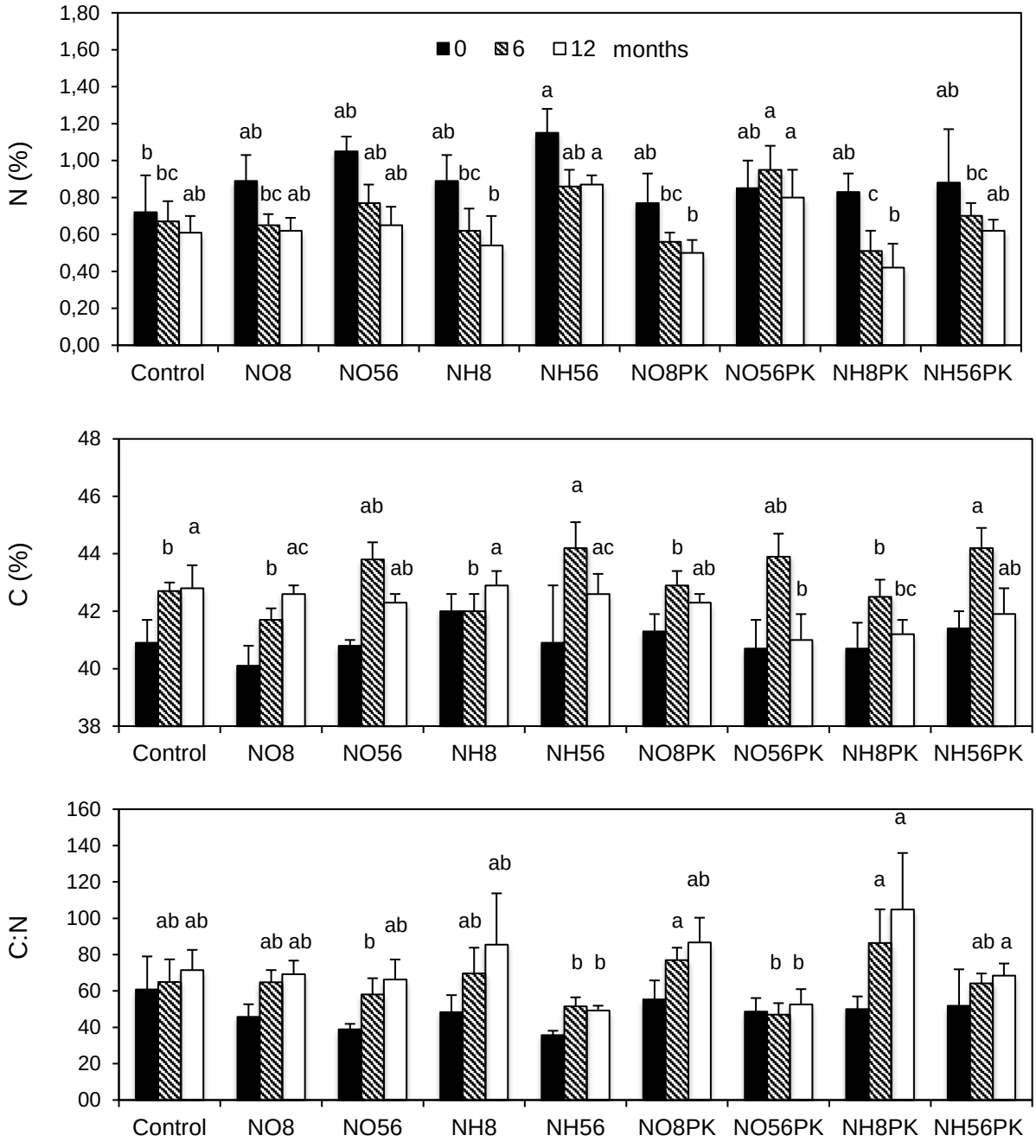


Fig. 2. Treatment means±SDs for *Sphagnum capillifolium* total N and C concentrations and C:N ratios prior to the 2007-2009 incubations and after 6- or 12-month incubation. Letters indicate differences between treatments within given time at $P < 0.05$ (GLM ANOVA, Tukey's HSD post-hoc test, n = 4).

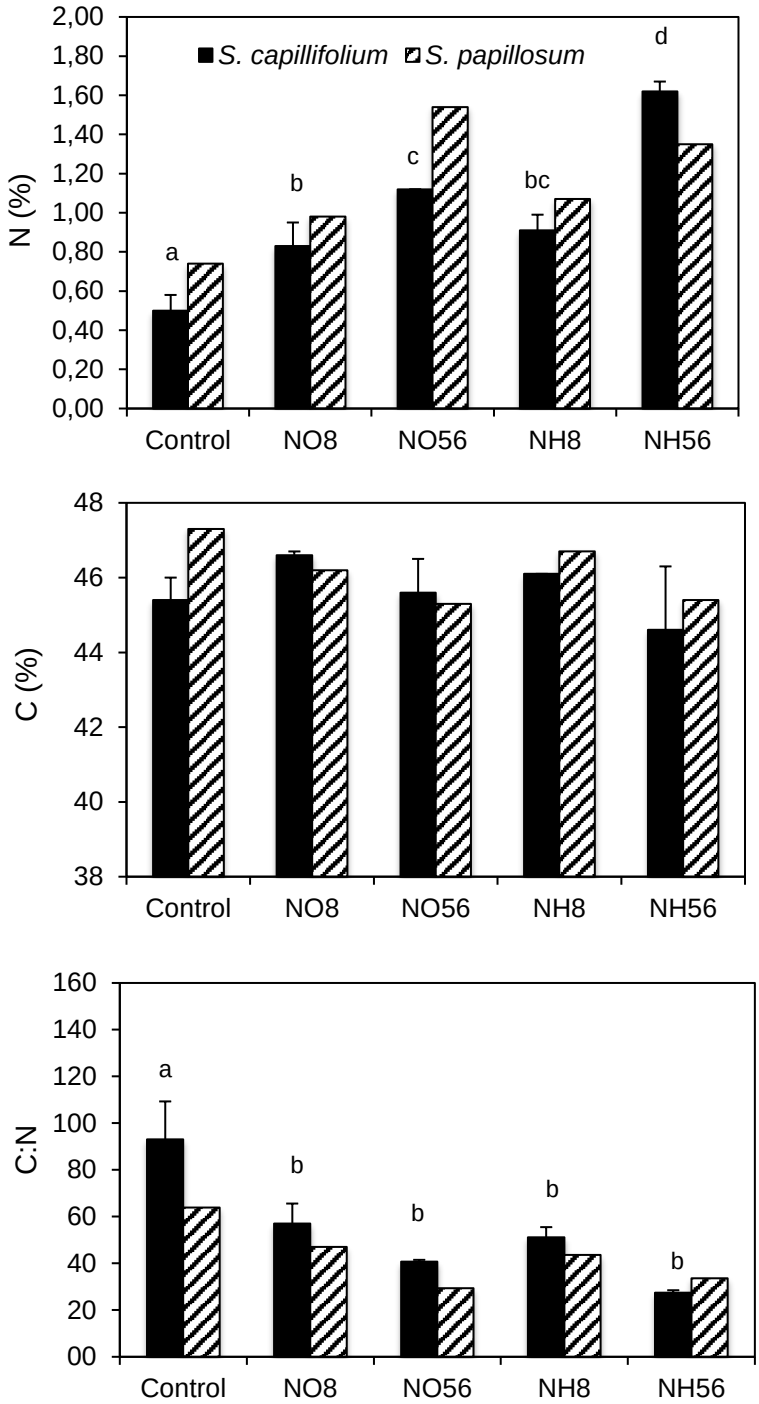


Fig. 3. Treatment means±SDs for *Sphagnum* total N and C concentrations and C:N ratio prior to the 2013-2014 incubations. Letters indicate differences between the treatments within each variable at $P < 0.05$ in *S. capillifolium* (GLM ANOVA, Tukey's HSD post-hoc test, n = 4). Results on *S. papillosum* are based on one pooled sample per treatment.

3.2 *Sphagnum* mass loss and changes in *Sphagnum* quality during incubation

The average mass losses of *S. capillifolium* litter in the control treatment were 14.3-18.6% at individual removals in 2007-2009. There were no main effects of time (F = 1.437, *P* = 0.238) or treatment (F = 0.78, *P* = 0.63) on decomposition rate (Fig. 4). Neither the form of N (F = 0.122, *P* = 0.73) nor PK (F = 0.782, *P* = 0.384) affected mass loss. In 2013-2014, neither moss origin nor the decomposing environment affected mass loss for either *Sphagnum* species (Figs 5a-c). However, decaying *S. capillifolium* lost weight faster in the N (without PK) enrichment plots over the 12-month incubations in September 2013 - September 2014 than April 2007 - April 2008 (15.9% vs. 8.3%, t = 4.83, *P* < 0.001) when incubated in plots of treatment origin. The mass loss of *S. papillosum* likewise was unaffected by N additions, but it did decompose faster than *S. capillifolium* in the treatments of litter origin (t = 2.03, *P* = 0.10), the control treatment (t = 2.58, *P* = 0.033), and especially when litters from the control treatment were incubated in the elevated N treatments (t = 6.52, *P* = 0.001).

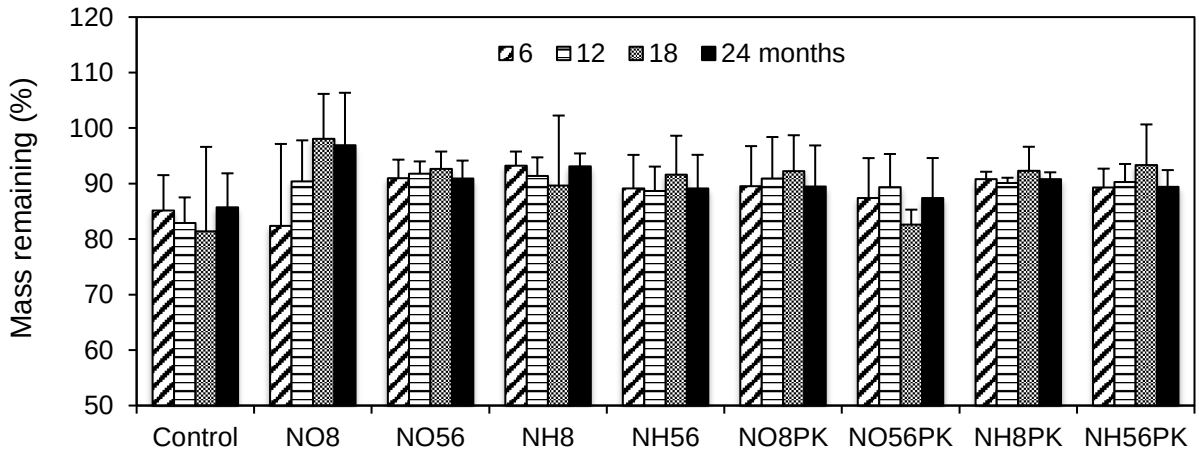
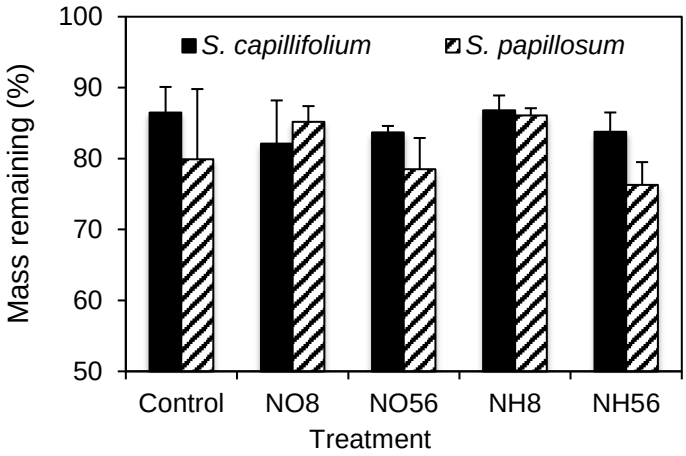
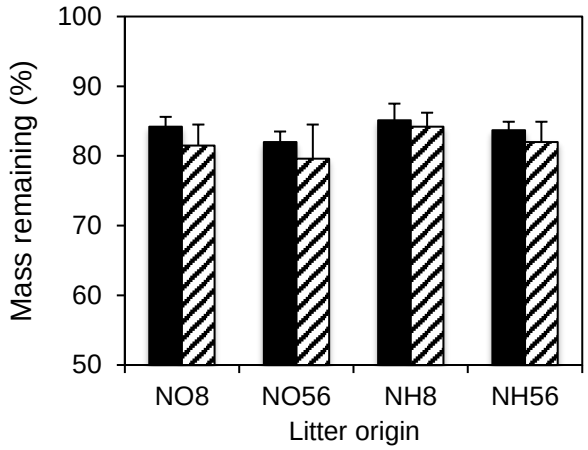


Fig. 4. Means+SDs for mass remaining after 6-, 12-, 18 and 24-month incubations of *Sphagnum capillifolium* in the treatments of litter origin in 2007-2009 (n = 4).

a)



b)



c)

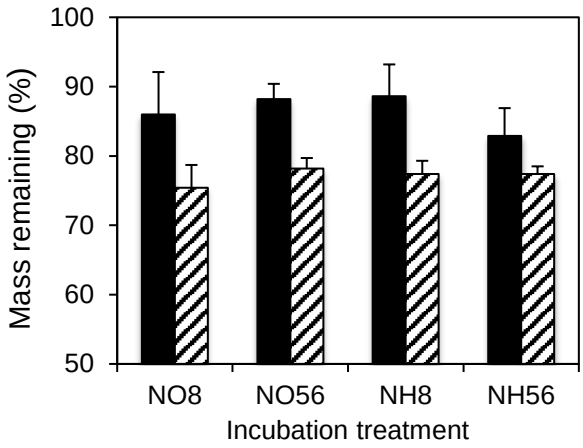


Fig. 5. Means+SDs for mass remaining after 12-month incubations in September 2013 – September 2014, when *Sphagnum capillifolium* and *S. papillosum* a) litters from different treatments were incubated in the treatments of litter origin (both species n = 4) or b) litters from elevated N (alone) treatments were incubated in the control treatment (both species n = 4) , and c) litter from the control treatment were incubated in the N8 and N56 treatments (without PK) (*S.capillifolium* n = 4, *S. papillosum* n = 2).

Most of the N loss from decaying *S. capillifolium* occurred between April and October 2007 ($t = 4.63$, $P < 0.001$) (Fig. 2, Table 1), and the losses during the early stage of decomposition were greater from *Sphagnum* in the NHd than NOd treatments (RDE of N: $t = 2.24$, $P = 0.033$). In contrast, C accumulated in decaying *S. capillifolium* in summer 2007 (April vs. October 2007: $t = -8.75$, $P < 0.001$), and the C concentrations were still higher in April 2008 than April 2007 ($t = 5.58$, $P < 0.001$). Mass losses of *S. capillifolium* or *S. papillosum* were not correlated with litter quality prior to the incubations.

Table 1. Treatment means±SDs for relative decomposition efficiency (RDE) of N and C during the 6- and 12-month incubations of *Sphagnum capillifolium* between April - October 2007 and April 2007 - April 2008, respectively. Letters indicate differences between treatments within given time at $P < 0.05$ (GLM ANOVA, Tukey's HSD post-hoc test, n = 4).

Treatment	6 months		12 months	
	N	C	N	C
Control	0.76±1.6ab	-0.35±0.3	0.91±1.2b	-0.27±0.1
NO8	2.38±1.6ab	-0.47±0.6	2.55±0.7ab	-
0.11±0.6				
NO56	2.92±0.9ab	-0.92±0.3	4.69±0.9ab	-
0.46±0.1				
NH8	4.82±1.5a	0.07±0.3	5.19±2.2a	-
0.37±0.5				
NH56	2.84±1.3ab	-1.00±0.8	2.20±0.5ab	-0.51±0.6
NO8 PK	2.41±1.0ab	-0.52±0.4	4.40±2.5ab	-0.40±0.3
NO56 PK	-1.42±2.2b	-0.94±0.8	0.98±2.2ab	0.10±0.5
NH8 PK	4.32±1.7a	-0.51±0.3	5.04±2.0ab	-0.14±0.3
NH56 PK	1.33±3.2ab	-0.64±0.1	2.40±2.3ab	-0.08±0.2

3.3 Hummock pore water chemistry

The largest TDN concentration was measured in the NO56 treatment (Table 2). Adding NO_3^- at 56 kg ha^{-1} yr^{-1} resulted in an average 240-fold $NO_2^-+NO_3^-$ concentration compared to that in the control treatment, while adding NH_4^+ at 56 kg ha^{-1} yr^{-1} only increased NH_4^+ concentration 3-fold. DON was the dominant N fraction in hummock pore water in all but the NO56 treatment. Nitrogen plus PK decreased hummock pore water $NO_2^-+NO_3^-$ concentration ($P = 0.072$).

DOC concentrations and pH were higher in the NOd than NHd treatments ($t = 2.56$, $P = 0.002$ and $t = 6.94$, $P < 0.001$, respectively) (Table 2). There was also a dose effect on pH:

adding 56 kg N ha^{-1} yr^{-1} as NO_3^- alone or together with PK increased pH, while pH values were more acidic when 56 kg N ha^{-1} yr^{-1} was added as NH_4^+ alone or together with PK.

Table 2. Treatment means±SDs for hummock pore water chemistry between January 16th and October 21st, 2008. Letters in indicate differences between treatments at $P < 0.10$ (Tukey HSD post-hoc test, n = 4).

Treatment	TDN (mg l^{-1})	$NO_3^- + NO_2^-$ (mg l^{-1})	NH_4^+ (mg l^{-1})	DON (mg l^{-1})	DOC (mg l^{-1})	pH
Control	0.54±0.2bc	0.007±0.006b	0.09±0.06b	0.44±0.3ab	23.0±8.6b	4.68±0.3b
NO8	0.43±0.2bc	0.017±0.031b	0.09±0.04b	0.32±0.2b	28.1±9.8b	4.94±0.3b
NO56	3.32±1.6a	1.684±0.858a	0.09±0.05b	1.44±0.7a	19.6±1.8b	5.84±0.2a
NH8	0.42±0.3bc	0.004±0.008b	0.04±0.05b	0.37±0.3b	25.8±14.3b	4.26±0.1bc
NH56	1.30±0.8b	0.086±0.032b	0.31±0.16a	0.91±0.7ab	20.8±6.1b	3.87±0.0c
NO8 PK	0.22±0.1c	0.005±0.007b	0.04±0.02b	0.17±0.1b	28.0±7.9b	4.66±0.2b
NO56 PK	1.14±0.5b	0.026±0.045b	0.09±0.03b	1.02±0.4a	39.5±16.2a	5.61±0.5a
NH8 PK	0.40±0.2bc	0.006±0.003b	0.13±0.04b	0.27±0.2b	16.7±3.5b	4.37±0.0bc
NH56 PK	0.39±0.2bc	0.003±0.004b	0.07±0.07b	0.31±0.2b	14.2±5.9b	3.92±0.4c

3.4 Relationships between studied variables

The only correlation between hummock pore water chemistry and *Sphagnum* quality or mass loss was that between mass loss after the 18-month incubation (April 2007 – October 2008) and average DOC concentration in August 2007 – November 2008 ($P = 0.069$) (Table 3). Hummock pore water NO_2^-+NO_3^- concentrations were more strongly correlated with N concentration in *S. capillifolium* litter prior to the incubations than were NH_4^+ and DON concentrations, and NO_2^- +NO_3^- concentrations were positively correlated with DON concentrations unlike NH_4^+ concentrations. DON concentration also tended to increase with increasing pH.

Table 3. Spearman correlation coefficients (r_S) for relationships between *S. capillifolium* quality prior to the 2007-2009 incubation and mass loss (after 18 months of incubation) or hummock pore water chemistry (average in July 2007 – November 2008). *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ° $P \leq 0.1$, ns = non significant (n = 35).

	Mass loss	Hummock pore water			
		TDN	NO_2^-+NO_3^-		
	NH_4^+	DON			
Sphagnum					
N	ns	0.43**	0.62***	0.29°	0.31°
Hummock pore water					
NO_2^-+NO_3^-	ns	0.72***	ns	ns	ns

NH_4^+	ns	0.43**	0.44**	ns	ns
DON	ns	0.94***	0.58***	ns	ns
DOC	0.31°	ns	ns	ns	ns
pH	ns	0.29°	ns	ns	0.29°

Hummock pore water chemistry was not correlated with rainfall, although DOC concentrations were temporally variable, being highest when the temperature was highest (air mean, and soil 10 cm and 20 cm temperatures all correlated at $P < 0.001$) and/or water table lowest ($P = 0.002$). Air and soil temperatures were negatively correlated with water table depth (both $P < 0.01$), but water table was not correlated with rainfall (data not shown).

4 Discussion

4.1 Long-term N addition, irrespective of form or improved PK availability, does not increase *Sphagnum* mass loss

Neither the 2- to 3-fold increases in *Sphagnum* N concentration which decreased the C:N ratio under both high NH_4^+ and NO_3^- deposition, nor N-driven changes in the decomposing environment, i.e. NO_3^- increasing and NH_4^+ decreasing pH appreciably affected *Sphagnum* mass loss. Similarly, improving the moss P and K content (Phuyal *et al*., 2008) did not enhance decomposition. However, mass loss in September 2013 – September 2014 exceeded that in April 2007 – April 2008, and the litter of the hollow species lost mass faster than that of the hummock species.

Most of the *Sphagnum* decay takes place in the first 4-6 months (Johnson and Damman, 1991; Rochefort *et al*., 1990). Elevated N supply can accelerate plant litter mass loss in the early stages of decomposition, while retarding degradation of recalcitrant litter (Berg, 2014). Our results suggest that these opposite effects may even cancel each other out, so that there is no N-related net enhancement of mass loss. Supporting this theory, Currey *et al*. (2010) reported significantly enhanced potential mineralization of labile forms of C in both the NO56 and NH56 treatments, while the potential activity of enzymes such as cellobiohydrolase (CBH) involved in the breakdown of more complex forms of C was slightly decreased. Nitrogen would appear to inhibit the activities of extracellular enzymes that decompose recalcitrant C (Fog, 1988; Gallo *et al*., 2004).

Nitrogen form and dose did influence *Sphagnum* decomposition: more N lost early on with NHd vs. NOd additions. Overall, hummock pore water chemistry under elevated N deposition

probably reflects pH-induced changes in vegetation composition and soil, driven by N form (see Sheppard *et al*., 2013b) than solely the complex responses of *Sphagnum* and its decomposition to elevated NH_4^+ or NO_3^- deposition. It is plausible for instance that much of the DON came from *H. jutlandicum* that thrives under high NO_3^- deposition (Sheppard *et al*., 2013a), given its cover correlated positively with DON concentration and pH, while *S. capillifolium* cover only negatively correlated with pH (Lucy Sheppard and Sirkku Manninen, unpublished).

4.2 The role of *Sphagnum* species

The differences in mass loss between hummock and hollow species of *Sphagnum* may be maintained under elevated N deposition (present study; Johnson and Damman, 1991; Breeuwer *et al*., 2008). Although the pH of the hummock environment did not seem to affect *Sphagnum* mass loss, moss species pH may be important: *S. papillosum* is less acidic than *S. capillifolium* (Vuohelainen, 2008). In 2013, neither litter N concentration nor C:N ratio differed between species, but *S. capillifolium* had the highest average N concentration in the NH56 treatment, while *S. papillosum* had that in the NO56 treatment. The difference in moss pH must thus at least partly relate to differences in NH_4^+ vs. NO_3^- uptake between the species. The 3-fold increase in the N concentration of hummock-grown *S. capillifolium* in the NH56 treatment and its overall smaller mass loss under elevated N deposition are attributed to its higher intrinsic concentration of sphagnan, i.e. *Sphagnum* cell wall polysaccharides (Clymo and Hayward, 1982). While phenolic compounds also slow down mass loss of *Sphagnum* (Freeman *et al*., 2001), they are present in much lower concentrations than uronic acids (Rydin and Jeglum, 2013), which can comprise 10-30% of *Sphagnum* dry weight (Clymo and Hayward, 1982). Moreover, uronic acids release hydrogen ions, which are involved in cation exchange and lower pH (Stalheim *et al*., 2009). Given that the mass losses from *S. capillifolium* in the control treatment over 12 months (17% in 2007-2008 and 13% in 2013-2014) were similar to those previously reported for *S. capillifolium* and other hummock species (e.g. *S. fuscum*) in an acidic, oxic surface peat layer (Belyea, 1996; Clymo, 1965; Johnson and Damman, 1991; Rochefort *et al*. 1990; Turetsky *et al*., 2008), our *in situ* findings of long-term effects of NO_3^- and NH_4^+ deposition on *S. capillifolium* mass loss are considered applicable for upland raised bogs in general.

4.3 The role of seasonal (climatic) factors

Not much is known about environmental limitations on the decomposition of specific chemicals in polysaccharide-dominated litter (Hájek *et al*., 2011). At Whim, the activities of both CBH and acetyl-glucosaminidase (NAG) involved in the breakdown of complex forms of C and N, respectively, were ca. 30- and 6-fold, respectively, in autumn vs. spring in the aerobic litter layer (Currey *et al*., 2010). Although the N additions had slight negative effect on potential CHB and NAG activities (Currey *et al*., 2010), Sheppard *et al*. (2013a) also found mass loss (teabags) being greater in high N treatments in autumn than spring-early summer. Thus the greater mass loss of *S. capillifolium* litter in September 2013 – September 2014 than April 2007 – April 2008 is attributed to higher activities of extracellular enzymes in early stage of decomposition. The fact that this seasonal effect on mass loss was not observed in the control treatment may at least partly be due to morphological changes and consequently, decreased water-holding capacity of *S. capillifolium* under high N deposition (Carfrae *et al*., 2007; Manninen *et al*., 2011). The higher C concentration in *S. capillifolium* in autumn may also have stimulated microbial decomposition (Sinsabaugh and Moorhead, 1994), implying it was due to a larger proportion of labile forms of C (see Currey *et al*., 2010).

Although there was no correlation between rainfall and hummock pore water chemistry, the high peak values for moss NH_4^+ concentration (Lucy Sheppard, unpublished) and hummock pore water DON concentration in the NO56 treatment on 14 August 2008 are attributed to the heavy rain (32.6 mm) which led to a huge application of treatment, rapid assimilation of NO_3^- and NO_2^- in moss tissue (Blodau *et al*., 2006; Hemond, 1983; Woodin *et al*., 1985) and subsequent DON release (see Chiwa *et al*., 2016). DON is released from moss both through microbial breakdown and leaching from green, senescing and necrotic segments (Bragazza and Limpens, 2004). Given that environmental influences on vascular plant breakdown do not necessarily transfer to non-vascular plants (see Breeuwer *et al*., 2008), the positive correlation between hummock pore water DOC concentration and *S. capillifolium* mass loss most likely reflects drying-rewetting induced leaching of DOC from *Sphagnum* (Waiser, 2006).

4.4 C cycling in peatlands under elevated N deposition

The capacity of *Sphagnum* and the other bog plants to trap atmospheric N was exceeded in the NO56, NH56 and NO56 PK treatments as shown by the increased concentrations of DON and inorganic N in hummock pore water (see Bragazza and Limpens, 2004). Accumulation of C and loss of N in the decaying *Sphagnum* in all but the control and NO56 PK treatments, infer

increases in the relative proportion of recalcitrant organic compounds and/or accumulation of microbial C. Especially under elevated N deposition, the latter may have been derived (mainly by fungi) from low molecular weight DON (peptides, amino acids) released from the decaying *Sphagnum* (Farrell *et al*., 2014; Lappalainen *et al*., 2013; van Breemen 1995), and small particulate or dissolved organic matter originating e.g. from *C. vulgaris* (Currey *et al*. 2011) and passing through decaying *Sphagnum* (Bragazza and Iacumin, 2009; Scheffer *et al*., 2001; Thormann *et al*., 2004).

The negative correlation between C and N concentrations in *S. papillosum* suggest accelerated cellular C metabolism including enhanced respiratory C losses stimulated by higher N availability (Juutinen *et al*., 2016; Kivimäki, 2011). At any rate, at Whim even the control plots are losing C, i.e. are CO_2 sources (Kivimäki, 2011; Kivimäki *et al*., 2013). This may be due to the background deposition of N (8 kg N ha^{-1} yr^{-1}) and/or the 10% increase in precipitation from the spray. Overall, our results and those of Brock and Bregman (1989), Keller *et al*. (2006), and Verhoeven *et al*. (1990) suggest that the C and N cycles are decoupled in *Sphagnum*-dominated bogs and that the effect of elevated N deposition on C storage reflects *Sphagnum* growth and cover rather than enhanced mass loss.

4.5 Conclusions

Incubation studies such as this one in the field have their limitations being highly susceptible to ingrowth by roots etc. and the restricted numbers of replicates due to high workload. That said, our results from more than 5 and 10 years of treatment do not provide evidence of realistically enhanced N deposition increasing mass loss from *Sphagnum*. We conclude that N-induced reduction in *Sphagnum* growth (NH_4^+) and the less acidic pore water pH associated with increased supply of NO_3^- - which drove alterations in vegetation composition, modified by temperature related changes in water table, are the main drivers of C and N cycling under elevated N deposition. Supporting Hájek (2009) we conclude that permanently aerated and still water-saturated (from the viewpoint of water potential) conditions maximize decomposition in open hummocks. The long-term observations from Whim highlight the need for such experiments coupled with field monitoring on the function of bog ecosystems under elevated N deposition and climate change.

Acknowledgements

Natural Environment Research Council (NERC), Centre for Ecology and Hydrology (CEH) and the EU funded NEU and ECLAIRE programmes (The ECLAIRE project, FP7-Env.2011.1.1.2-1 282910**)** support the Whim site. The 2007-2008 litter and water measurements performed by Sanna Kivimäki were funded by CEH with Scottish Natural Heritage (SNH). Lara Castillo and Erika Hansson prepared the litterbags in 2013 and Dr Susan Owen helped with fieldwork in 2014. Financial support by the Transnational Access to Research Infrastructures activity in the 7th Framework Programme of the EC under the ExpeER project for conducting the fieldwork by Sirkku Manninen is gratefully acknowledged. We also thank Tim Moore for his valuable comments.

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