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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

103 The *in situ* study was performed at Whim, an ombrotrophic peat bog in the Scottish Borders,
104 where the N addition experiment has been running since 2002 (Sheppard *et al*., 2004). Whim
105 is situated ca. 30 km south of Edinburgh, Scotland (3°16'W, 55°46'N) and represents a
106 transition between a lowland raised bog and a blanket bog. It stands 280 m a.s.l., with an
107 annual rainfall of ca. 900 mm (Lindsay, 1995; Ratcliffe, 1964). However, the years 2007-
108 2009 and 2014 were wet with a rainfall of 1161-1476 mm vs. 944 mm in 2013. Annual mean
109 temperatures ranged between 6.9°C (2013) and 8.4°C (2014) (Fig. 1). The water table
110 fluctuations were similar in all plots with an average -7 cm during the 2007-2009 incubations.
111 Although the measured water table was sometimes above the ground level, *S. capillifolium* in
112 hummocks was never submerged under water.
113

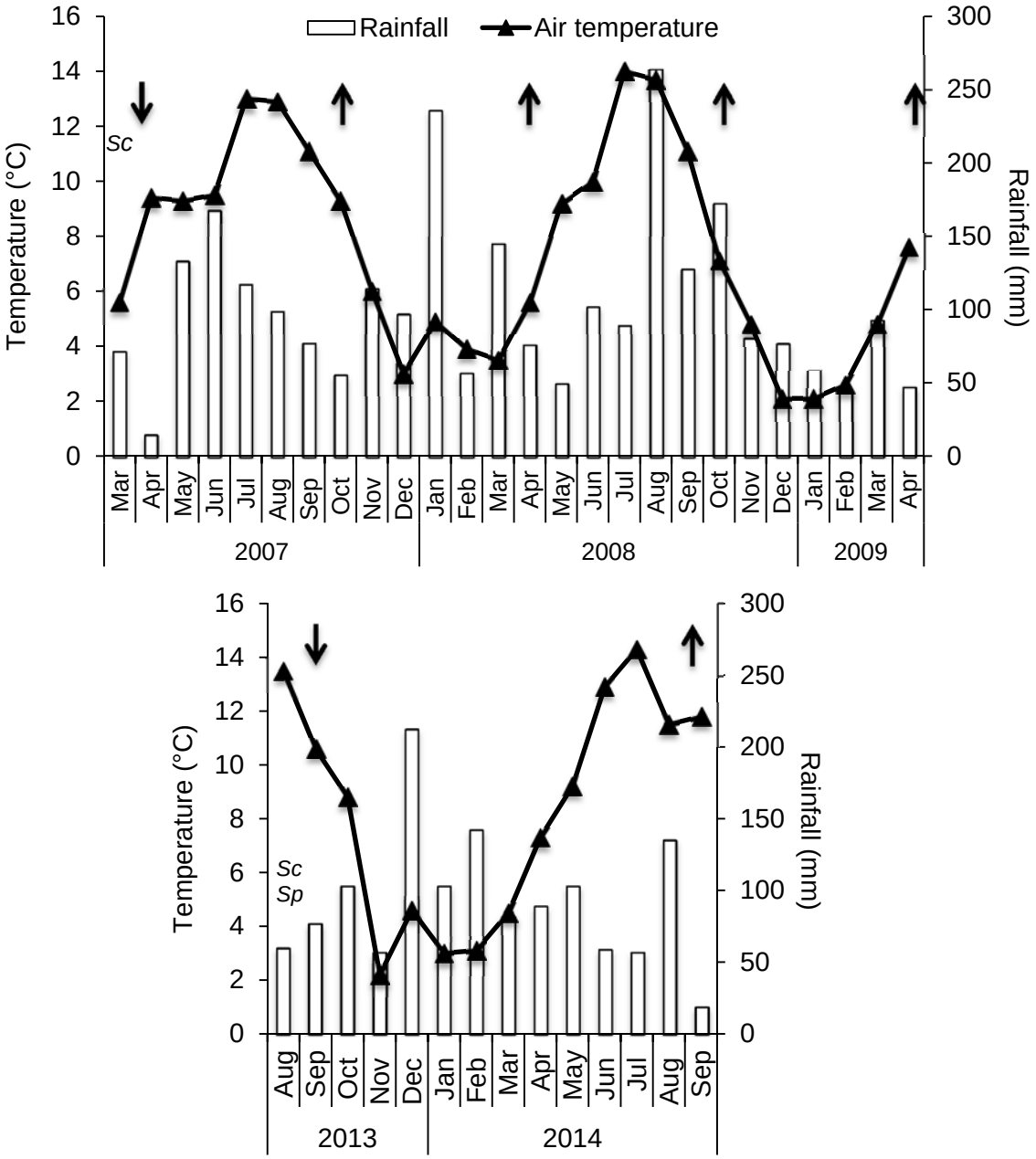

Fig. 1. Monthly mean temperatures and rainfall at Whim in March 2007 – April 2009 and August 2013 – September 2014. Abbreviations indicate when *S. capillifolium* (*Sc*) and/or *S. papillosum* (*Sp*) were sampled for preparation of litterbags, and arrows indicate when litterbags were placed in (🡻) and 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 microenvironment (Belyea, 1996; Hájek, 2009; Johnson and Damman, 1991), we incubated litter of both *S. capillifolium* (hummock species) and *S. papillosum* (hollow species) in 2013-2014 to assess the role of litter quality vs. environment on decomposition. Treatments with PK additions were not included in 2013-2014 because PK was not found to affect *Sphagnum* mass loss in 2007-2009. As mass loss of uniform standard litter (Rooisbosh tea leaves) had only been found to be favoured in autumn by 56 kg N ha^{-1} yr^{-1} of both N forms (Sheppard *et al*., 2013a), the 2013-2014 incubation was started in September. Moreover, the 2013-2014 incubation lasted only for 1 year because the duration of incubation did not affect mass loss in 2007-2009. A more detailed description of the incubations is given below.

Sphagnum capillifolium shoots for the 2007-2009 incubations were collected early March 2007, while *S. capillifolium* and *S. papillosum* for the latter incubations were sampled late August 2013 (Fig. 1). The moss samples were thoroughly picked clean, photosynthetically vital tissue excluded, and the stem part (3-8 cm below top of the capitulum) retained. The shoot parts from each plot were mixed and divided into five portions: one was oven-dried for 48 hours at 70°C, ground with a ball mill and analysed for total C and N with a Carlo Erba NA2500 total C/N analyser; the remaining four portions were air-dried for ≥76 hours before being transferred to litterbags made from 0.71 mm polypropylene mesh. Polypropylene is fairly resistant to acidic conditions and the mesh size retains the material but excludes macrofauna. An aliquot of approximately 0.5 g (0.3-0.9 g) of air-dried sample was transferred to every litterbag. In April 2007, four replicate litterbags of *S. capillifolium* were placed in same plots at the same depth from where the mosses had been sampled. One litterbag was removed from each plot after 6, 12, 18 and 24 months (i.e. October 2007, April 2008, October 2008, and April 2009) (Fig. S1).

Sphagnum capillifolium and *S. papillosum* were sampled for the 2013-2014 incubations from the control treatment and N8 and N56 treatments without PK in early August 2013. Samples from different treatments were analysed for C and N prior to incubations and litterbags prepared as in 2007. In September 2013, litterbags were placed both in the plots of treatment origin and in control plots. *Sphagnum* from control plots was also incubated in the N8 and N56 treatments (Figs. S2a-S2c). Given the low abundance of *S. papillosum* at Whim, *S. papillosum* litterbags were incubated in *S. capillifolium* 'environment' instead of the hollows they were collected from. For the same reason, the total C and N concentrations were only analysed for pooled samples of *S. papillosum* from different plots within each treatment. All the litterbags, which were placed in hummocks in September 2013, were removed in September 2014. The sampling of material for incubations, and placing and removal of litterbags were always done 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.

196

197 2.3 *Sphagnum* hummock pore water

198

199 Rhizon samplers comprising a 0.45 µm filter (Rhizon, Eijkelkam, B.V., The Netherlands)
200 were placed adjacent to the moss bags to collect water samples from within the hummock, 5-
201 15 cm below the moss surface. A syringe was used to apply suction to the tube, drawing water
202 and nutrients through the filter into the syringe until full. Water samples from the syringes
203 were collected twice a month if possible, but at least once every month between July 2007
204 and November 2008. Because the amount of water in the syringe depended on the amount of
205 rain and the ability of the hummock to retain moisture, it was not always possible to obtain
206 sufficient sample from each plot for all the different analyses, so not every variable was
207 measured for each date. The sample was halved and a subsample used for measuring DOC
208 was frozen immediately until analysis. The other half was further divided with one part used
209 for measuring pH (Mettler Toledo MP220 pH meter) and the other filtered and then frozen.
210 DOC was analysed using a DC-80 Total Organic Carbon (TOC) analyser (Rosemount-
211 Dohrmann) using a sub sample of 200 µl and total N, NH_4^+, and NO_2^-+NO_3^- were measured
212 by continuous flow analyser (San ++ Continuous flow analyser, Skalar analytical BV, The
213 Netherlands). Concentration of dissolved organic N (DON) was calculated as the difference
214 between total dissolved N (TDN) and inorganic N (NO_2^-+NO_3^- + NH_4^+). Hummock pore
215 water samples were only collected in 2007-2008.

216

217 2.4 Statistical analyses

218

219 Data was tested for normality (Kolmogorov-Smirnov) and when necessary, log-transformed
220 to meet the assumption of normal distribution. *Sphagnum capillifolium* mass loss during the
221 2007-2009 incubation was analysed with GLM Mixed ANOVA (Repeated Measures) using
222 time as within-subject factor, and treatment, N form (NHd or NOd), and PK (no PK or PK) as
223 between-subjects factors, with Tukey's HSD as a *post-hoc* test (SPSS 23 for Windows).
224 Differences in the mass loss between *Sphagnum* origins (control or N treatments), incubation
225 environments (control or N treatments) and *Sphagnum* species in 2013-2014 were analysed
226 with Student's t-test. GLM ANOVA with Tukey's HSD as a *post-hoc* test was used to test the
227 differences between treatments in *Sphagnum* quality. As there were problems with the CN
228 analyses of samples removed after the 18- and 24-month incubations, they were excluded
229 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 ($r_S = -0.90$, $P = 0.037$).

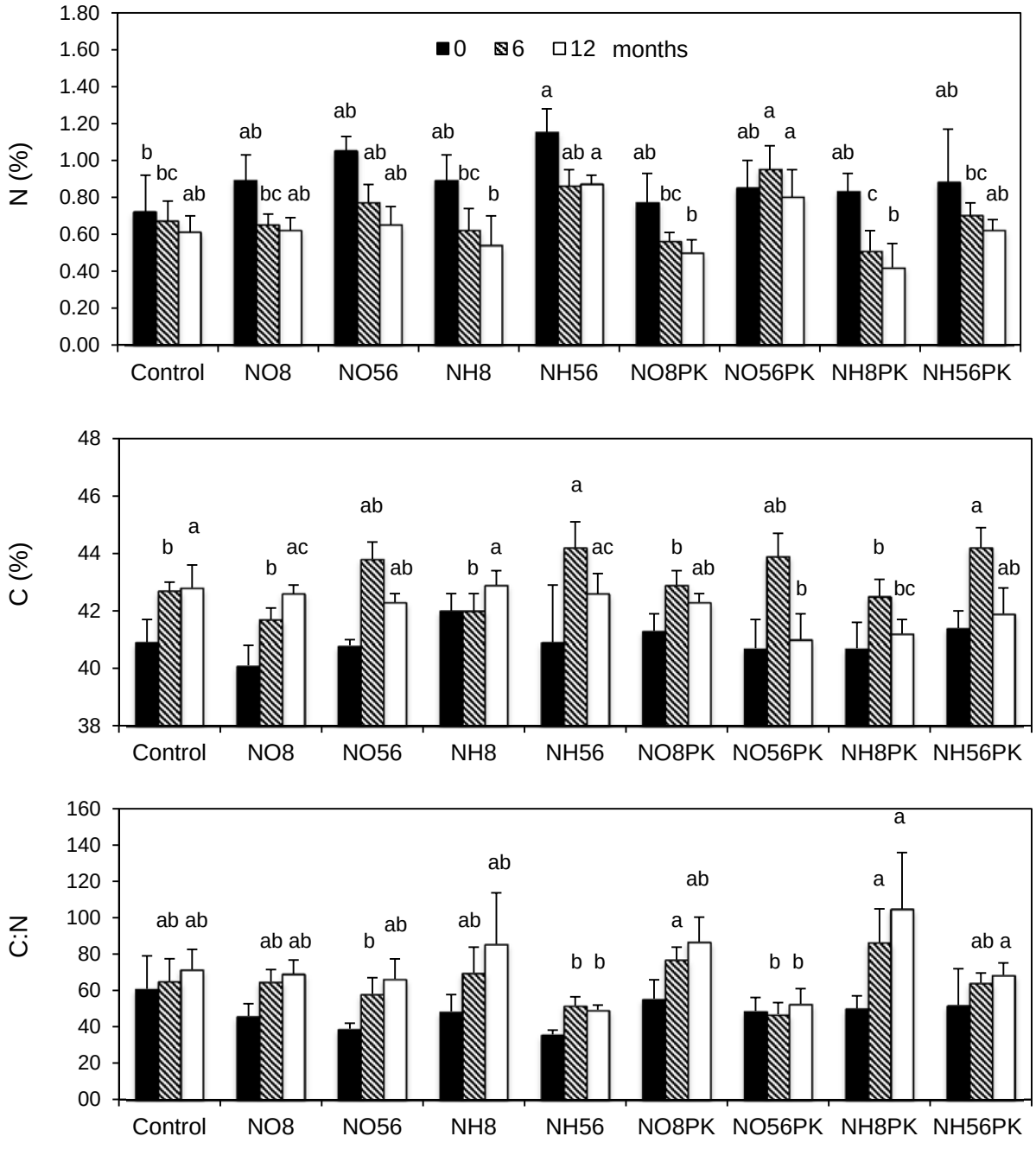

Fig. 2. Treatment means±SDs for *Sphagnum capillifolium* total N and C concentrations and C:N ratios prior to the 2007-2009 incubations and after 6- or 12-month incubation. Letters indicate differences between treatments within given time at $P < 0.05$ (GLM ANOVA, Tukey's HSD post-hoc test, n = 4).

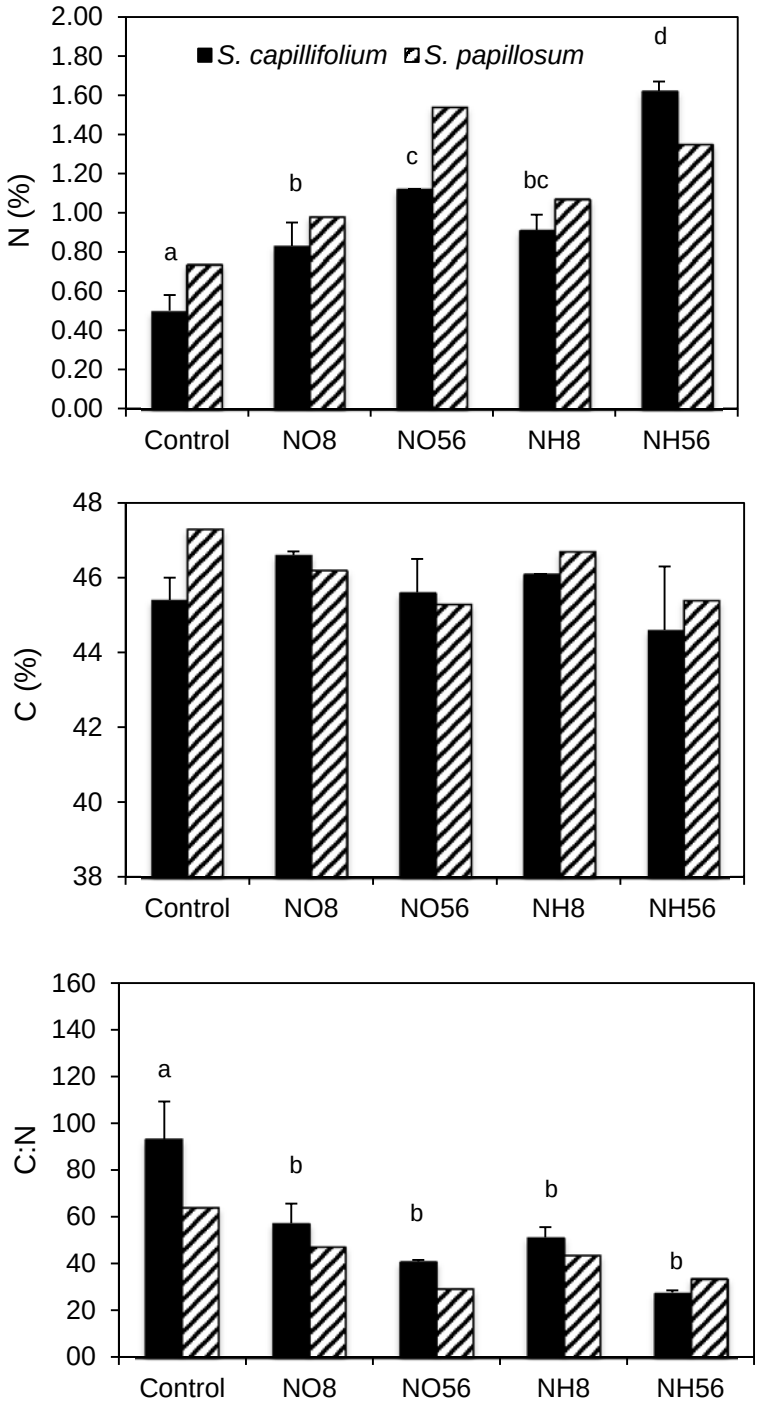

Fig. 3. Treatment means±SDs for *Sphagnum* total N and C concentrations and C:N ratio prior to the 2013-2014 incubations. Letters indicate differences between the treatments within each variable at $P < 0.05$ in *S. capillifolium* (GLM ANOVA, Tukey's HSD post-hoc test, n = 4). Results on *S. papillosum* 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, $P = 0.001$).

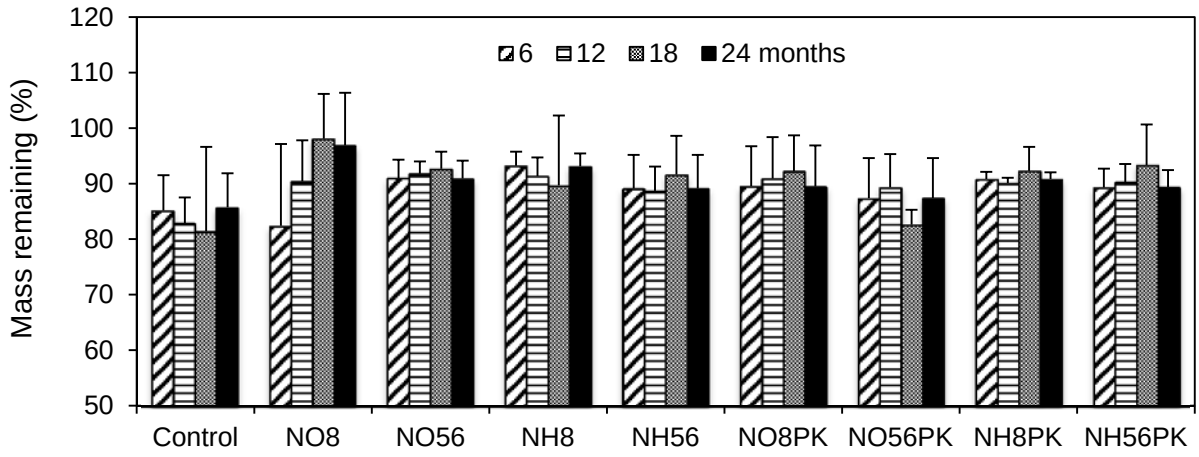

Fig. 4. Means+SDs for mass remaining after 6-, 12-, 18 and 24-month incubations of *Sphagnum capillifolium* in the treatments of litter origin in 2007-2009 (n = 4).

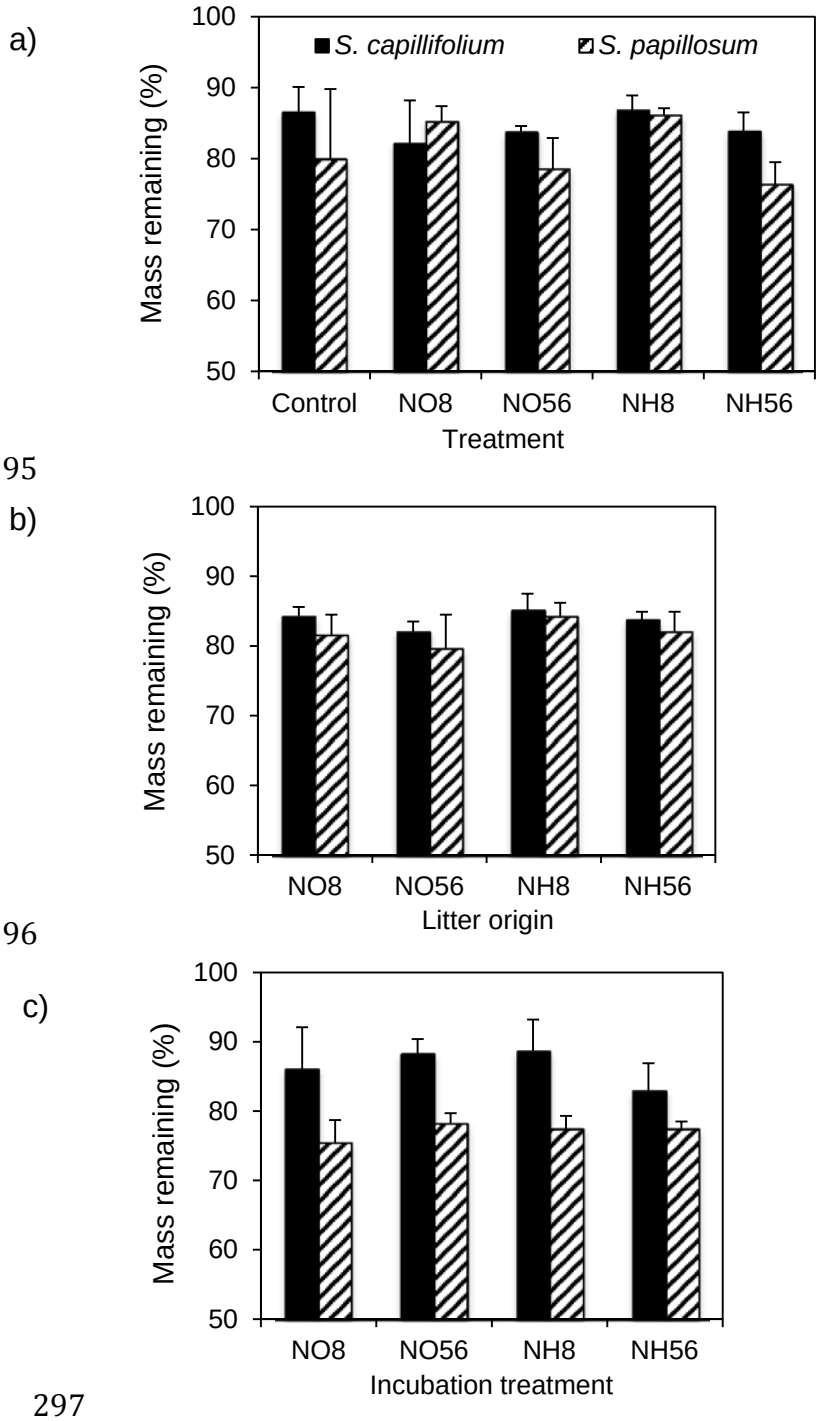

Fig. 5. Means+SDs for mass remaining after 12-month incubations in September 2013 – September 2014, when *Sphagnum capillifolium* and *S. papillosum* a) litters from different treatments were incubated in the treatments of litter origin (both species n = 4) or b) litters from elevated N (alone) treatments 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

459 both CBH and acetyl-glucosaminidase (NAG) involved in the breakdown of complex forms
460 of C and N, respectively, were ca. 30- and 6-fold, respectively, in autumn vs. spring in the
461 aerobic litter layer (Currey *et al*., 2010). Although the N additions had slight negative effect
462 on potential CHB and NAG activities (Currey *et al*., 2010), Sheppard *et al*. (2013a) also
463 found mass loss (teabags) being greater in high N treatments in autumn than spring-early
464 summer. Thus the greater mass loss of *S. capillifolium* litter in September 2013 – September
465 2014 than April 2007 – April 2008 is attributed to higher activities of extracellular enzymes in
466 early stage of decomposition. The fact that this seasonal effect on mass loss was not observed
467 in the control treatment may at least partly be due to morphological changes and
468 consequently, decreased water-holding capacity of *S. capillifolium* under high N deposition
469 (Carfrae *et al*., 2007; Manninen *et al*., 2011). The higher C concentration in *S. capillifolium* in
470 autumn may also have stimulated microbial decomposition (Sinsabaugh and Moorhead,
471 1994), implying it was due to a larger proportion of labile forms of C (see Currey *et al*.,
472 2010).
473 Although there was no correlation between rainfall and hummock pore water chemistry,
474 the high peak values for moss NH_4^+ concentration (Lucy Sheppard, unpublished) and
475 hummock pore water DON concentration in the NO56 treatment on 14 August 2008 are
476 attributed to the heavy rain (32.6 mm) which led to a huge application of treatment, rapid
477 assimilation of NO_3^- and NO_2^- in moss tissue (Blodau *et al*., 2006; Hemond, 1983; Woodin *et*
478 *al*., 1985) and subsequent DON release (see Chiwa *et al*., 2016). DON is released from moss
479 both through microbial breakdown and leaching from green, senescing and necrotic segments
480 (Bragazza and Limpens, 2004). Given that environmental influences on vascular plant
481 breakdown do not necessarily transfer to non-vascular plants (see Breeuwer *et al*., 2008), the
482 positive correlation between hummock pore water DOC concentration and *S. capillifolium*
483 mass loss most likely reflects drying-rewetting induced leaching of DOC from *Sphagnum*
484 (Waiser, 2006).
485
486 4.4 C cycling in peatlands under elevated N deposition
487
488 The capacity of *Sphagnum* and the other bog plants to trap atmospheric N was exceeded in the
489 NO56, NH56 and NO56 PK treatments as shown by the increased concentrations of DON and
490 inorganic N in hummock pore water (see Bragazza and Limpens, 2004). Accumulation of C
491 and loss of N in the decaying *Sphagnum* in all but the control and NO56 PK treatments, infer
492 increases in the relative proportion of recalcitrant organic compounds and/or accumulation of

493 microbial C. Especially under elevated N deposition, the latter may have been derived
494 (mainly by fungi) from low molecular weight DON (peptides, amino acids) released from the
495 decaying *Sphagnum* (Farrell *et al*., 2014; Lappalainen *et al*., 2013; van Breemen 1995), and
496 small particulate or dissolved organic matter originating e.g. from *C. vulgaris* (Currey *et al*.
497 2011) and passing through decaying *Sphagnum* (Bragazza and Iacumin, 2009; Scheffer *et al*.,
498 2001; Thormann *et al*., 2004).
499 The negative correlation between C and N concentrations in *S. papillosum* suggest
500 accelerated cellular C metabolism including enhanced respiratory C losses stimulated by
501 higher N availability (Juutinen *et al*., 2016; Kivimäki, 2011). At any rate, at Whim even the
502 control plots are losing C, i.e. are CO_2 sources (Kivimäki, 2011; Kivimäki *et al*., 2013). This
503 may be due to the background deposition of N (8 kg N ha^{-1} yr^{-1}) and/or the 10% increase in
504 precipitation from the spray. Overall, our results and those of Brock and Bregman (1989),
505 Keller *et al*. (2006), and Verhoeven *et al*. (1990) suggest that the C and N cycles are
506 decoupled in *Sphagnum*-dominated bogs and that the effect of elevated N deposition on C
507 storage reflects *Sphagnum* growth and cover rather than enhanced mass loss.
508
509 4.5 Conclusions
510
511 Incubation studies such as this one in the field have their limitations being highly susceptible
512 to ingrowth by roots etc. and the restricted numbers of replicates due to high workload. That
513 said, our results from more than 5 and 10 years of treatment do not provide evidence of
514 realistically enhanced N deposition increasing mass loss from *Sphagnum*. We conclude that
515 N-induced reduction in *Sphagnum* growth (NH_4^+) and the less acidic pore water pH
516 associated with increased supply of NO_3^- - which drove alterations in vegetation composition,
517 modified by temperature related changes in water table, are the main drivers of C and N
518 cycling under elevated N deposition. Supporting Hájek (2009) we conclude that permanently
519 aerated and still water-saturated (from the viewpoint of water potential) conditions maximize
520 decomposition in open hummocks. The long-term observations from Whim highlight the need
521 for such experiments coupled with field monitoring on the function of bog ecosystems under
522 elevated N deposition and climate change.
523
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525

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535

536 **References**
537

538 Aerts R, Wallén B, Malmer N (1992) Growth-limiting nutrients in *Sphagnum*-dominated bogs
539 subject to low and high atmospheric nitrogen supply. Journal of Ecology 80, 131-140.
540 Belyea LR (1996) Separating effects of litter quality and microenvironment on decomposition
541 rates in a patterned peatland. Oikos 77, 529-539.
542 Berg B (2014) Decomposition patterns for foliar litter – A theory for influencing factors. Soil
543 Biology and Biochemistry 78, 222-232.
544 Blodau C, Basiliko N, Mayer B, Moore TR (2006) The fate of experimentally deposited
545 nitrogen in mesocosms from two Canadian peatlands. Science of the Total Environment
546 364, 215-228.
547 Bobbink R, Hornung M, Roelofs JGM (1998) The effects of air-borne nitrogen pollutants on
548 species diversity in natural and semi-natural European vegetation. Journal of Ecology 86,
549 717-738.
550 Bragazza L, Limpens J (2004) Dissolved organic nitrogen dominates in European bogs under
551 increasing atmospheric N deposition. Global Biogeochemical Cycles, doi:
552 10.1029/2004GB002267
553 Bragazza L, Freeman C, Jones T, Rydin H, Limpens J, Fenner N, Ellis T, Gerdol R, Hájek M,
554 Hájek T, Iacumin P, Kutnar L, Tahvanainen T, Toberman H (2006) Atmospheric nitrogen
555 deposition promotes carbon loss from peat bogs. Proceedings of National Academy of
556 Sciences 103, 19386-19389.
557 Bragazza L, Iacumin P (2009) Seasonal variation in carbon isotopic composition of bog plant
558 litter during 3 years of field decomposition. Biology and Fertility of Soils 46, 73-77.
559 Bragazza L, Buttler A, Habemacher J, Brancaleoni L, Gerdol R, Fritze H, Hanajík P, Laiho R,

560 Johnson D (2012) High nitrogen deposition alters the decomposition of bog plant litter and
561 reduces carbon accumulation. Global Change Biology 18, 1163-1172.
562 Breeuwer A, Heijmans M, Robroek BJM, Limpens J, Berendse F (2008) The effect of
563 increased temperature and nitrogen deposition on decomposition in bogs. Oikos 117, 1258-
564 1268.
565 Brock TCM, Bregman R (1989) Periodicity in growth, productivity, nutrient content and
566 decomposition of *Sphagnum recurvum* var. *mucronatum* in a fen woodland. Oecologia 80,
567 44-52.
568 Bubier JL, Moore TR, Bledzki LA (2007) Effects of nutrient addition on vegetation and
569 carbon cycling in an ombtrotrophic bog. Global Change Biology 13, 1168-1186.
570 Carfrae JA, Sheppard LJ, Raven JA, Leith ID, Crossley A (2007) Potassium and phosphorus
571 additions modify the response of *Sphagnum capillifolium* growing on a Scottish
572 ombrotrophic bog to enhanced nitrogen deposition. Applied Geochemistry 22, 1111-1121.
573 Charman DJ, Aravena R, Bryant CL, Harkness DD (1999) Carbon isotopes in peat, DOC,
574 CO_2, and CH_4 in a Holocene peatland on Dartmoor, southwest England. Geology 27, 539-
575 542.
576 Chasar LS, Chanton JP, Glaser PH, Siegel DI, Rivers JS (2000) Radiocarbon and stable
577 carbon isotopic evidence for transport and transformation of dissolved organic carbon,
578 dissolved inorganic carbon, and CH_4 in a northern Minnesota peatland. Global
579 Biochemical Cycles 14, 1095-1108.
580 Chiwa M, Sheppard LJ, Leith ID, Leeson SR, Tang YS, Cape JN (2016) *Sphagnum* can
581 'filter' N deposition, but effects on the plant and pore water depend on the N form. Science
582 of the Total Environment 559, 113-120.
583 Clymo RS (1965) Experiments on breakdown of *Sphagnum* in two bogs. Journal of Ecology
584 53, 747-758.
585 Clymo RS, Hayward PM (1982) The ecology of *Sphagnum*. In: Smith AJE, editor. Bryophyte
586 Ecology. London: Chapman and Hall. p. 229-289.
587 Currey PM, Johnson D, Sheppard LJ, Leith ID, Toberman H, van der Wal R, Dawson LA,
588 Artz RRE (2010) Turnover of labile and recalcitrant soil carbon in response to nitrate and
589 ammonium deposition in an ombrotrophic peatland. Global Change Biology 16, 2307-
590 2321.
591 Currey PM, Johnson D, Dawson LA, van der Wal R, Thornton B, Sheppard LJ, Leith ID, Artz
592 RRE (2011) Five years of simulated atmospheric nitrogen deposition have only subtle

593 effects on the fate of newly synthesized carbon in *Calluna vulgaris* and *Eriophorum*
594 *vaginatum*. Soil Biology and Biochemistry 43, 495-502.
595 Damman AWH (1978) Distribution and movement of elements in ombrotrophic peat bog.
596 Oikos 30, 480-495.
597 Damman AWH (1988) Regulation of nitrogen removal and retention in *Sphagnum* bogs and
598 other peatlands. Oikos 51, 291-305.
599 Farrell M, Prendergast-Miller M, Jones DL, Hill PW, Condron LM (2014) Soil microbial
600 organic nitrogen uptake is regulated by carbon availability. Soil Biology and Biochemistry
601 77, 261-267.
602 Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic matter.
603 Biological Reviews 63, 433-462.
604 Freeman C, Ostle NJ, Kang H (2001) An enzymatic latch on a global C store. Nature 409,
605 149.
606 Gallo M, Amonette R, Lauber C, Sinsabaugh RL, Zak DR (2004) Microbial community
607 structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils.
608 Microbial Ecology 48, 218-229.
609 Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP,
610 Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR,
611 Vörösmarty CJ (2004) Nitrogen cycles: past, present and future. Biogeochemistry 70, 153–
612 226.
613 Gerdol R, Petraglia A, Bragazza L, Iacumin P, Brancaleoni L (2007) Nitrogen deposition
614 interacts with climate in affecting production and decomposition rates in *Sphagnum*
615 mosses. Global Change Biology 13, 1810-1821.
616 Gong J, Kellomäki S, Wang K, Zhang C, Shurpali N, Martikainen PJ (2013) Modeling CO_2
617 and CH_4 flux changes in pristine peatlands of Finland under changing climate conditions.
618 Ecological Modelling 263, 64-80.
619 Gorham E (1991) Northern peatlands: role in the carbon cycle and probable responses to
620 climatic warming. Ecological Applications 1, 182–195.
621 Gunnarsson U, Malmer N, Rydin H (2002) Dynamics or constancy in *Sphagnum* dominated
622 mire ecosystems? Ecography 25, 685-704.
623 Hájek T (2009) Habitat and species control on *Sphagnum* production and decomposition in a
624 mountain raised bog. Boreal Environment Research 14, 947-958.
625 Hajék T, Balance S, Limpens J, Ziljstra M, Verhoeven JTA (2011) Cell-wall polysaccharides
626 play an important role in decay resistance of *Sphagnum* and actively depressed

627 decomposition in vitro. Biogeochemistry 103, 45-57.
628 Hautier Y, Niklaus PA, Hector A (2009) Competition for light causes plant biodiversity loss
629 after eutrophication. Science 324, 636-638.
630 Hemond HF (1983) The nitrogen budget of Thoreau's Bog. Ecology 64,99–109.
631 Hogg EH, Malmer N, Wallén B (1994) Microsite and regional variation in the potential decay
632 rate of *Sphagnum magellanicum* in south Swedish raised bogs. Ecography 17, 50-59.
633 Johnson LC, Damman AWH (1991) Species-controlled *Sphagnum* decay on a south Swedish
634 raised bog. Oikos 61, 234-242.
635 Juutinen S, Moore TR, Laine AM, Bubier JL, Tuittila E-S, De Young A, Chong M (2016)
636 Responses of the mosses *Sphagnum capillifolium* and *Polytrichum strictum* to nitrogen
637 deposition in a bog: growth, ground cover, and CO_2 exchange. Botany 94, 127-138.
638 Keller JK, Bauers AK, Brigdham SD, Kellogg LE (2006) Nutrient control of microbial
639 carbon cycling along an ombrotrophic-minerotrophic peatland gradient. Journal of
640 Geophysical Research 111, G03006. doi:10.1029/2005JG000152.
641 Kivimäki SK (2011) Changes in carbon and nitrogen dynamics in *Sphagnum capillifolium*
642 under enhanced nitrogen deposition. PhD thesis. University of Edinburgh, Scotland, UK.
643 Kivimäki SK, Sheppard LJ, Leith ID, Grace J (2013) Long-term enhanced nitrogen deposition
644 increases respiration and carbon loss from a *Sphagnum* bog in the Scottish Borders.
645 Environmental and Experimental Botany 90, 53-61.
646 Lappalainen M, Kukkonen JVK, Piirainen S, Sarjala T, Setälä H, Koivusalo H, Finér L,
647 Laurén A (2013) Nitrogen release in decomposition of boreal mor and peat as affected by
648 enchytraeid worms. Boreal Environment Research 18, 181-194.
649 Limpens J, Berendse F (2003a) Growth reduction of *Sphagnum magellanicum* subjected to
650 high nitrogen deposition: the role of amino acid nitrogen concentration. Oecologia 135,
651 339-345.
652 Limpens J, Berendse F (2003b) How litter quality affects mass loss and N loss from
653 decomposing *Sphagnum*. Oikos 103, 537-547.
654 Limpens J, Berendse F, Klees H (2004) How phosphorus availability affects the impact of
655 nitrogen deposition on *Sphagnum* and vascular plants in bogs. Ecosystems 7, 793-804.
656 Limpens J, Granath G, Gunnarsson U, Aerts R, Bayley S, Bragazza L, Bubier J, Buttler A,
657 van den Berg LJL, Francez A-J, Gerdol R, Grosvernier P, Heijmans MMPD, Hoosbeek
658 MR, Hotes S, Ilomets M, Leith I, Mitchell EAD, Moore T, Nilsson MB, Nordbakken J-F,
659 Rochefort L, Rydin H, Sheppard LJ, Thormann M, Wiedermann MM, Williams BL, Xu B

(2011) Climatic modifiers of the response to nitrogen deposition in peat-forming *Sphagnum* mosses: a meta-analysis. New Phytologist 191, 496-507.

Lindsay RA (1995) Bogs: The ecology, classification and conservation of ombrotrophic mires. Scottish Natural Heritage.

Lütke Twenhöven F (1992) Competition between two *Sphagnum* species under different deposition levels. Journal of Bryology 17, 71-80.

Manninen S, Woods C, Leith ID, Sheppard LJ (2011) Physiological and morphological effects of long-term ammonium or nitrate deposition on the green and red (shade and open grown) *Sphagnum capillifolium*. Environmental and Experimental Botany 72, 140-148.

Mizunuma T (2008) Response of ombrotrophic bog vegetation to enhanced nitrogen deposition: the role of multivariate analysis. MSc thesis. University of Edinburgh.

Paulissen MPCP, van der Ven PJM, Dees AJ, Bobbink R (2004) Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. New Phytologist 164, 451-458.

Phuyal M, Artz RRE, Sheppard LJ, Leith ID, Johsson D (2008) Long-term nitrogen deposition increases phosphorus limitation of bryophytes in an ombrotrophic bog. Plant Ecology 196, 111-121.

Ratcliffe DA (1964) Mires and bogs. In: Burnett JH, editor. The vegetation of Scotland. Edinburgh, UK.

Raven JA (1988) Acquisition of nitrogen by the shoots of land plants: its occurrence and implications for acid-base regulation. New Phytologist 109,1–20.

Rochefort L, Vitt DH, Bayley SE (1990) Growth, production, and decomposition dynamics of *Sphagnum* under naturally and experimentally acidified conditions. Ecology 71, 1986–2000.

Rydin H, Jeglum JK (2013) The biology of peatlands, 2nd ed. Oxford: Oxford University Press.

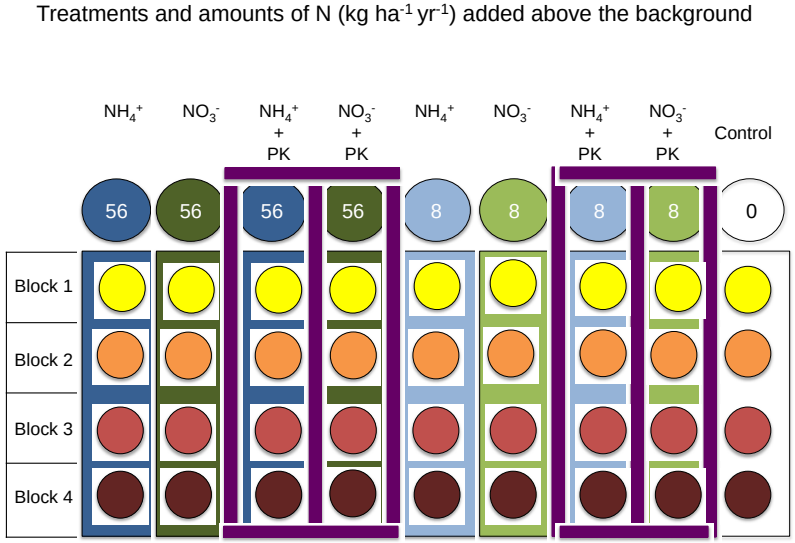
Scheffer RA, van Logtestijn RSP, Verhoeven JTA (2001) Decomposition of *Carex* and *Sphagnum* litter in two mesotrophic fens differing in dominant plant species. Oikos 92, 44-54.

Sheppard LJ, Crossley A, Leith ID, Hargraeves KJ, Carfrae JA, van Dijk N, Cape JN, Sleep D, Fowler D, Raven JA (2004) An automated wet deposition system to compare the effects of reduced and oxidised N on ombrotrophic bog species: Practical considerations. Water, Air and Soil Pollution Focus 4, 197-205.

693 Sheppard L, Leith ID, Leeson S, Mizunuma T, de Bakker R, Elustondo D, Garcia-Gomez H
694 (2013a) PK additions modify the effects of N dose and form on species composition,
695 species litter chemistry and peat chemistry in a Scottish peatland. Biogeochemistry 116,
696 39-53.
697 Sheppard LJ, Leith ID, Leeson SR, van Dijk N, Field C, Levy P (2013b) Fate of N in a
698 peatland, Whim bog: immobilisation in the vegetation and peat, leakage into pore water
699 and losses as N_2O depend on the form of N. Biogeosciences 10, 149-160.
700 Sheppard LJ, Leith ID, Mizunuma T, Leeson S, Kivimäki S, Cale JN, van Dijk N, Leaver D,
701 Sutton MA, Fowler D, van den Berg LJL, Crossley A, Field C, Smart S (2014) Inertia in
702 an ombrotrophic bog ecosystem in response to 9 years' realistic perturbation by wet
703 deposition of nitrogen, separated by form. Global Change Biology 20, 566-580.
704 Siegenthaler A, Buttler A, Bragazza L, van der Heijden E, Grosvernier P, Gobat J-M,
705 Mitchell EAD (2010) Litter- and ecosystem-driven decomposition under elevated CO_2 and
706 enhanced N deposition in a *Sphagnum* peatland. Soil Biology and Biochemistry 42, 968-
707 977.
708 Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme
709 production: a model for nitrogen and phosphorus control of litter decomposition. Soil
710 Biology and Biochemistry 26, 1305-1311.
711 Soares A, Pearson J (1997) Short-term physiological responses of mosses to atmospheric
712 ammonium and nitrate. Water, Air, and Soil Pollution 93, 225-242.
713 Stalheim T, Balance S, Christensen BE, Granum PE (2009) Sphagnan – a pectin-like polymer
714 isolated from *Sphagnum* moss can inhibit the growth of some typical food spoilage and
715 food poisoning bacteria by lowering pH. Journal of Applied Microbiology 106, 967-976.
716 Sutton MA, Cape JN, Rihm B, Sheppard LJ, Smith RI, Spranger T, Fowler D (2003) The
717 importance of accurate background atmospheric deposition estimates in setting critical
718 loads for nitrogen. In: Empirical Critical Loads for Nitrogen, Proceedings of the Expert
719 Workshop, Berne SAEFL Environmental Documentation No. 164, November 2002. p.
720 231–257.
721 Thormann MN, Bayley SE, Currah RS (2004) Microcosm tests of the effects of temperature
722 and microbial species number on the decomposition of *Carex aquatilis* and *Sphagnum*
723 *fuscum* litter from southern boreal peatlands. Canadian Journal of Microbiology 50, 793-
724 802.
725 Toberman H, Tipping E, Boyle JF, Helliwell RC, Lilly A, Henrys PA (2015) Dependence of
726 ombrotrophic peat nitrogen on phosphorus and climate. Biogeochemistry 125, 11-20.

727 Treat CC, Wollheim WM, Varner RK, Grandy AS, Talbot J, Frolking S (2014) Temperature
728 and peat type control CO_2 and CH_4 production in Alaskan permafrost peats. Global
729 Change Biology 10, 2674-2686.
730 Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem
731 studies. Ecology Letters 11, 1111-1120.
732 Turetsky MR, Crow SE, Evans RJ, Vitt DH, Wieder RK (2008) Trade-offs in resource
733 allocation among moss species control decomposition in boreal peatlands. Journal of
734 Ecology 96, 1297-1305.
735 Van Breemen N (1995) How *Sphagnum* bogs down other plants. Trends in Ecology and
736 Evolution 10, 270-275.
737 Verhoeven JTA, Maltby E, Schmitz MB (1990) Nitrogen and phosphorus mineralization in
738 fens and bogs. Journal of Ecology 78, 713-726.
739 Vile MA, Wieder RK Živković T, Scott KD, Vitt DH, Hartsock JA, Iosue CL, Quinn JC,
740 Petix M, Fillingim HM, Popma JMA, Dynarski KA, Jackman TR, Albright CM, Wykoff
741 DD (2014) N_2-fixation by methanotrophs sustain carbon and nitrogen accumulation in
742 pristine peatlands. Biogeochemistry 121, 317-328.
743 Vuohelainen A (2008) The effects of increased nitrogen deposition on *Sphagnum* species in
744 the Whim Moss nitrogen manipulation experiment. BSc thesis. University of Edinburgh.
745 Waiser MJ (2006) Relationship between hydrological characteristics and dissolved organic
746 carbon concentration and mass in northern prairie wetlands using a conservative tracers
747 approach. Journal of Geophysical Resesearch 11(G2):G02024.
748 White JR, Shannon RD, Weltzin JF, Pastor J, Bridgham SD (2008) Effects of soil warming
749 and drying on methane cycling in a northern peatland mesocosm study. Journal of
750 Geophysical Research 113, G00A06. doi:10.1029/2007JG000609.
751 Wiedermann MM, Gunnarsson U, Ericson L, Nordin A (2009) Ecophysiological adjustment
752 of two *Sphagnum* species in response to anthropogenic nitrogen deposition. New
753 Phytologist 181, 208-217.
754 Woodin S, Press MC, Lee JA (1985) Nitrate reductase activity in *Sphagnum fuscum* in
755 relation to wet deposition of nitrate from the atmosphere. New Phytologist 99, 381-388.
756 Yu Z, Loisel J, Brosseau DP, Beilman DW, Hunt SJ (2010) Global peatland dynamics since
757 the Last Glacial Maximum. Geophysical Research Letters 37:L13402.
758 doi:10.1029/2010GL043584.

GRAPHICAL ABSTRACT



The experimental set-up to study effects of wet-deposited N at Whim.

Highlights

- Wet-deposited NH_4^+ or NO_3^- at 56 kg N ha^{-1} yr^{-1} applied alone or with elevated PK for 5 to 11 years did not alter *Sphagnum* mass loss in an ombrotrophic bog
- C accumulation vs. N loss from decaying *Sphagnum* suggest C limitation of decomposition
- High dose of NO_3^- induced greater changes in hummock pore water chemistry than that of NH_4^+
- *Sphagnum* mass loss is affected more by moss species and abiotic factors (season) than moss N concentration or C:N ratio

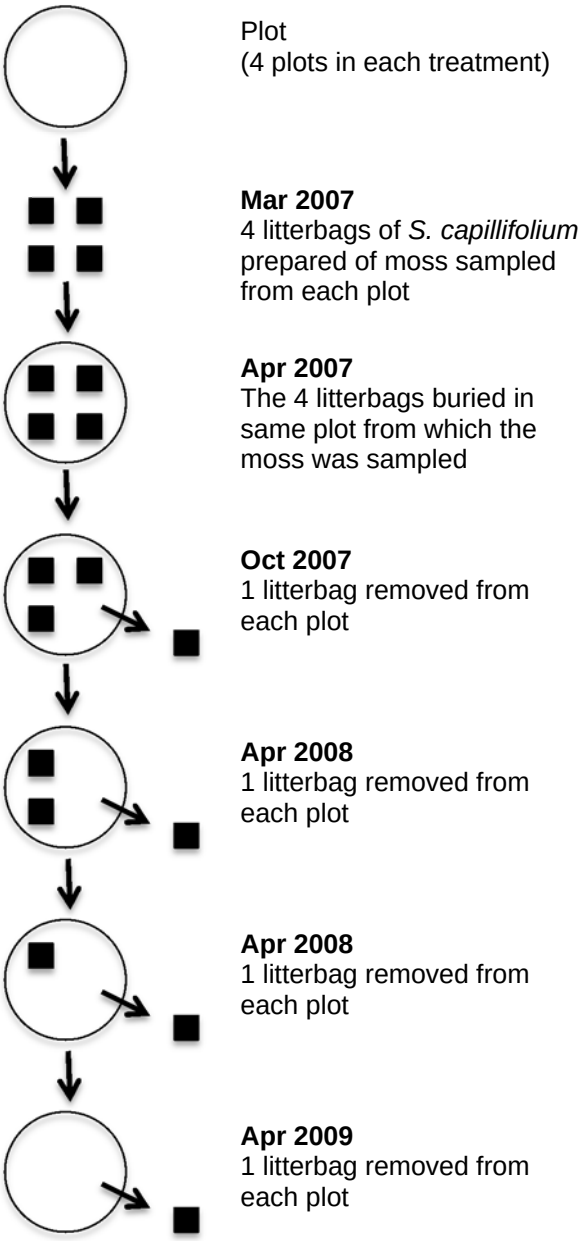


Fig. S1. Incubations of *S. capillifolium* in the plots of treatment origin in April 2007 – April 2009 (n = 4 at each removal, i.e. number of replicate plots in each treatment).

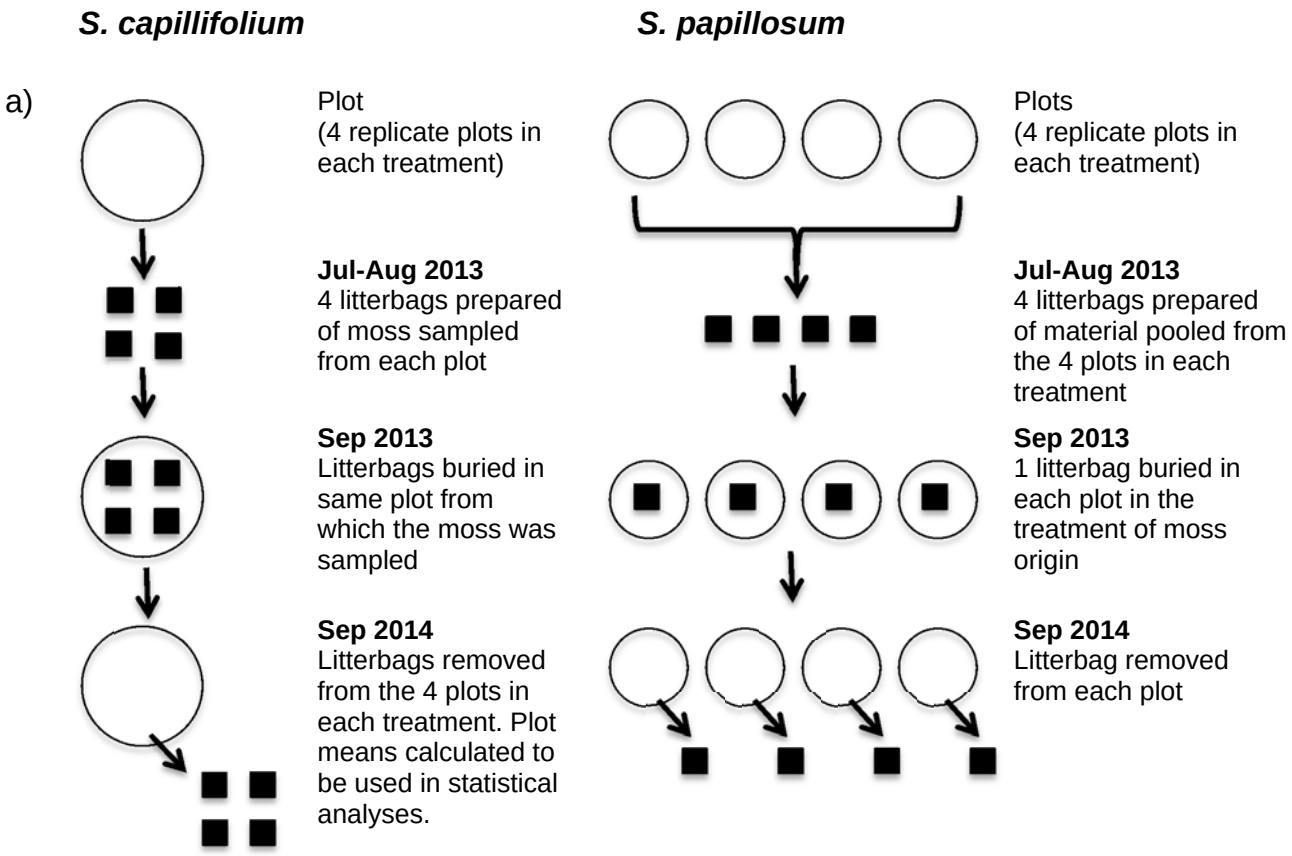


Fig. S2a. Incubations of *S. capillifolium* and *S. papillosum* in the plots of treatment origin in September 2013 – September 2014. *S. capillifolium* n = 4 and *S. papillosum* n = 4, i.e. number of replicate plots in each treatment.

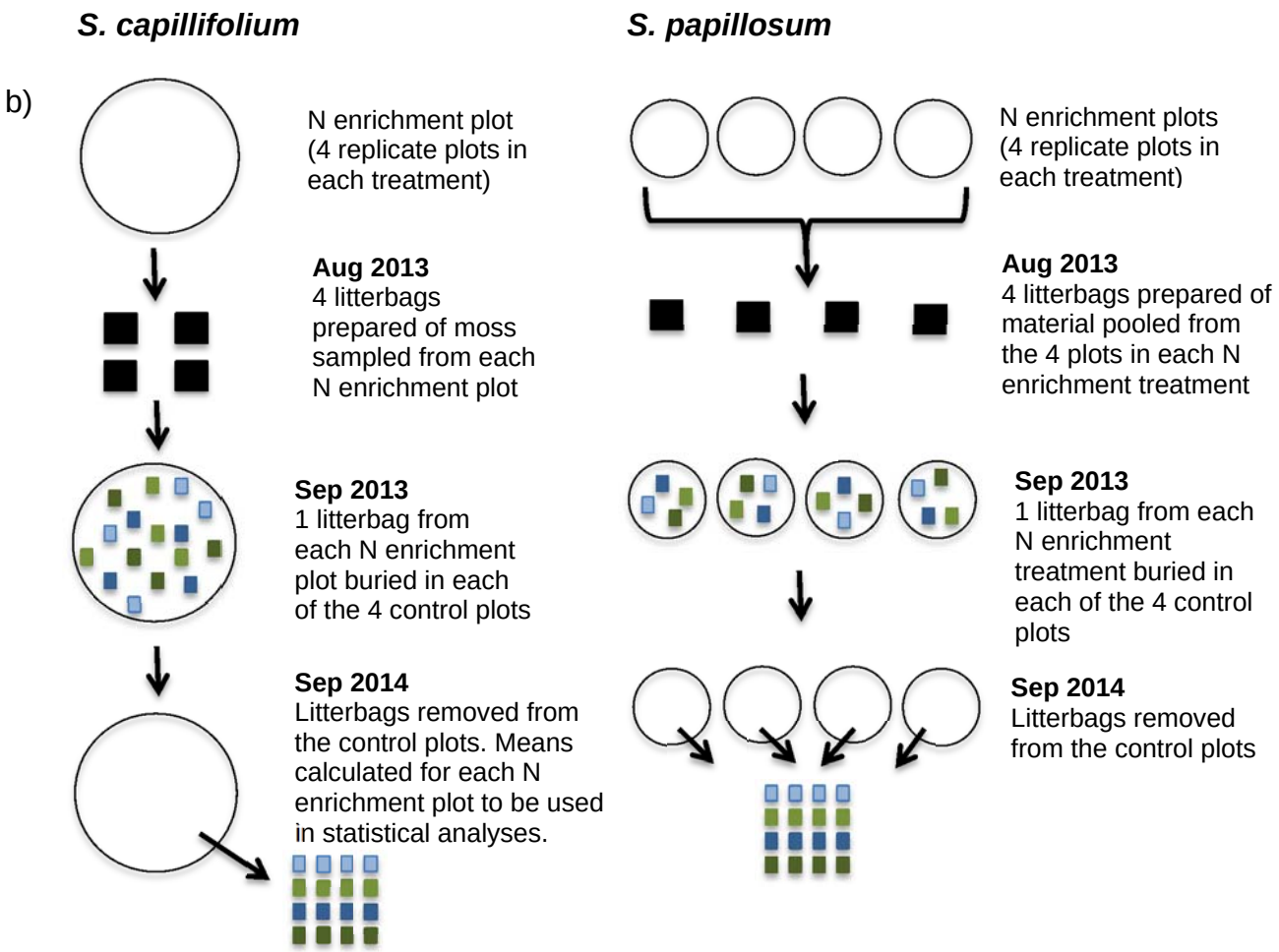


Fig. S2b. Incubations of *S. capillifolium* and *S. papillosum* collected from the N enrichment treatments in the control treatment in September 2013 – September 2014. Different colours indicate litterbags from different N enrichment treatments as follows: = NH8, = NO8, = NH56, = NO56. *S. capillifolium* n = 4, *S. papillosum* n = 4.

c)

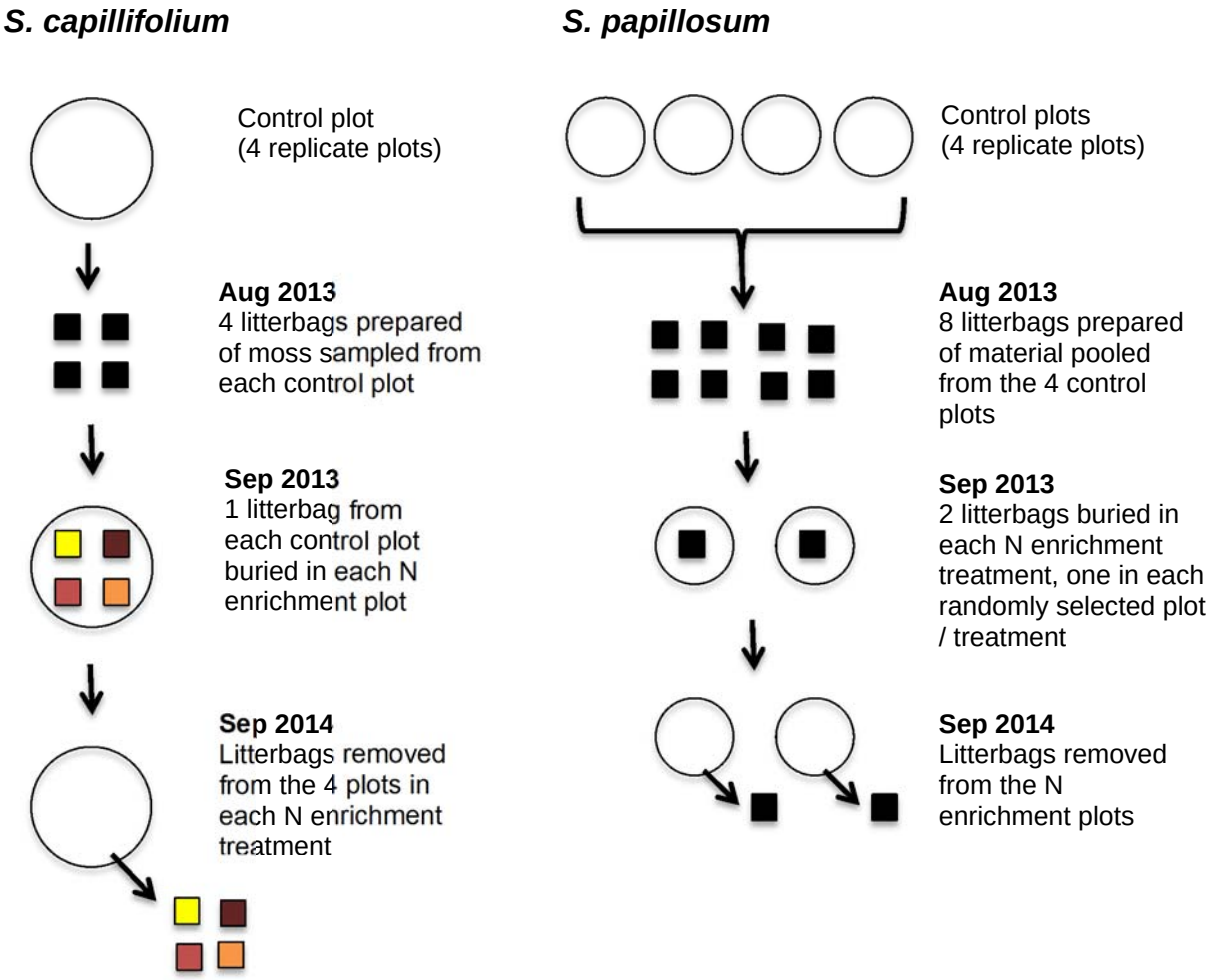


Fig. 3b. Incubations of *S. capillifolium* and *S. papillosum* collected from the control treatment in the N enrichment treatments in 2013-2014. Different colours indicate control litter from the four blocks : = block 1, = block 2, = block 3, = block 4. *S. capillifolium* n = 4, *S. papillosum* n – 2.