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Forest mosses sensitively indicate nitrogen deposition in boreal background areas

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Abstract

Mosses take up nitrogen (N) mainly from precipitation through their surfaces, which makes them competent bioindicators of N deposition. We found positive relationships between the total N concentration (mossN%) of common terrestrial moss species (feather mosses *Pleurozium schreberi* and *Hylocomium splendens*, and a group of *Dicranum* species) and different forms of N deposition in 11–16 coniferous forests with low N deposition load in Finland. The mosses were collected either inside (*Dicranum* group) or both inside and outside (feather mosses) the forests. Deposition was monitored *in situ* as bulk deposition (BD) and stand throughfall (TF) and detected for ammonium ($\text{NH}_4^+\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$), dissolved organic N (DON), and total N (N_{tot} , $\text{kg ha}^{-1}\text{yr}^{-1}$). N_{tot} deposition was lower in TF than BD indicating that tree canopies absorbed N from deposition in N limited boreal stands. However, mossN% was higher inside than outside the forests. In regression equations, inorganic N in BD predicted best the mossN% in openings, while DON in TF explained most variation of mossN% in forests. An asymptotic form of mossN% vs. TF N_{tot} curves in forests and free $\text{NH}_4^+\text{-N}$ accumulation in tissues in the southern plots suggested mosses were near the N saturation state already at the N_{tot} deposition level of 3–5 $\text{kg ha}^{-1}\text{yr}^{-1}$. N leachate from ground litterfall apparently also contributed the N supply of mosses. Our study yielded new information on the sensitivity of boreal mosses to low N deposition and their response to different N forms in canopy TF entering moss layer. The equations predicting the N_{tot} deposition with mossN% showed a good fit both in forest sites and openings, especially in case of *P. schreberi*. However, the open site mossN% is a preferable predictor of N deposition in monitoring studies to minimize the effect of tree canopies and N leachate from litterfall on the estimates.

Keywords: Bryophytes, boreal forest, critical load, dissolved organic nitrogen, canopy effect

Capsule: Mosses take up different N forms from precipitation depending on their supply

1. Introduction

The anthropogenic nitrogen (N) emissions originating from agriculture, animal husbandry and the use of fossil fuels (vehicles, energy production, industry) are dry and wet deposited onto terrestrial ecosystems as oxidized and reduced forms of N (Erisman et al. 2007, Mayer et al. 2015). Excess of N deposition has considered responsible to eutrophication of vegetation and nutrient unbalances in many habitats worldwide (Dirnböck et al. 2018). The effects of atmospheric N deposition on vegetation depend on the cumulative amount of N deposited in the past (Bernhardt-Römermann et al. 2015) and on the forms and amounts of current N deposition. In addition, the effects depend on habitat type and vary among the plant species and guilds (Perring et al. 2018). In this regard, poikilohydric mosses (bryophytes) form a distinctive group among major plant guilds of the understorey vegetation in boreal forests. Mosses lack cuticle layer and many species have large cationic exchange capacities which enable them to take up nutrients through the entire surface (Glime 2007). These properties make mosses one of the most sensitive plant groups to N deposition but also suitable organisms to be used as bioindicators (Pitcairn et al. 2006).

Terrestrial mosses take up most of nitrogen (N) they need from atmospheric wet and dry deposition (Forsum et al. 2006, Hawkins et al. 2018) although some of the N in mosses originates from the soil (Ayres et al. 2006, Liu et al. 2013). Moreover, biological fixation of atmospheric N₂ by moss-associated cyanobacteria contributes to moss N content especially in nutrient-limited northern ecosystems with a low N deposition ($< 3 - 5 \text{ kg ha}^{-1} \text{ yr}^{-1}$) (Rousk et al. 2013, Salemaa et al. 2019). In some experimental exposures, boreal mosses have been

shown to prefer ammonium (NH_4^+) and dissolved organic N (DON) over nitrate (NO_3^-) as N source (Forsum et al. 2006, Nordin et al. 2006). However, mosses have an ability to also take up NO_3^- from spraying solution (Solga and Frahm 2006, Hawkins et al. 2018, van den Elzen et al. 2018), but NO_3^- assimilation of mosses may be suppressed by the high supply of NH_4^+ and DON (Liu et al. 2013). The contribution of DON (especially the amino acid component) to moss N supply is suggested to be important in N limited northern ecosystems (Forsum et al. 2006) and when NH_4^+ deposition is low (Liu et al. 2013). Interestingly, DON has been found to serve as an important source of N also in sub-tropical epiphytic mosses growing in nutrient-poor canopy habitat in cloud forests (Song et al. 2016).

Tree layer controls the amount and forms of atmospheric N deposition entering the moss layer of boreal forests, and more knowledge on the response of terrestrial mosses to different N forms in TF precipitation is needed. Tall tree canopies filter more efficiently atmospheric gases (e.g. NO_2 , NH_3 , and HNO_3) and aerosols (e.g. NO_3^- , NH_4^+) than lower vegetation in open areas due to aerodynamically rough surfaces of trees (Fowler et al. 1999, Neirynck et al. 2007). The dry-deposited gaseous and particulate N compounds are absorbed and retained in the canopy (Fowler et al. 2009), where they are dissolved in water and converted from one form to another. In addition to gaseous N compounds, water-dissolved NH_4^+ and NO_3^- may be taken up by tree foliage in N-limited forests decreasing N deposition to the forest floor. In high-deposition sites, NH_4^+ and NO_3^- are in turn leached from the foliage (de Schrijver et al. 2007, Neirynck et al. 2007), while DON is released in unpolluted or less polluted forests (Mustajärvi et al. 2008, Izquieta-Rojano et al. 2016).

Mosses are widely used as a bioindicator for long-term trends and regional distribution of total N (N_{tot}) deposition in Europe (Harmens et al. 2011, 2014; Skudnik et al. 2014, Meyer et al. 2015, Kosonen et al. 2018). The lowest total N concentrations (<0.6%) of mosses

(mossN%) are measured in background areas in northern Europe (Poikolainen et al. 2009) and Scotland with annual N_{tot} deposition $<3 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Harmens et al. 2011), whereas the highest mossN% ($> 2.5\%$) are measured in areas of intensive agriculture with high NH_3 emissions e.g. in the UK (Pitcairn et al. 2006) and Germany (Harmens et al. 2014, Meyer et al. 2015). In remote boreal areas, a possibility to apply mossN% as bioindicator of N deposition instead of costly maintenance of continuous monitoring systems is of special interest. However, sufficient knowledge is still lacking on how forest structure and tree species composition affect the relationship between atmospheric N deposition and mossN% in field conditions e.g. to enable preparation of appropriate sampling protocols for the purpose.

In this study we investigated and modelled the relationships between the mossN% and the background N deposition in boreal forests of Finland with a low N deposition in the European scale. We compared the relationship between mossN% and N deposition both in forest openings (bulk deposition, BD) and inside forests (TF) including different N fractions in deposition (N_{tot} , $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and DON). We hypothesised that mossN% is equal or higher in the forest than in open sites (Kluge et al. 2013, Skudnik et al. 2014, Meyer et al. 2015) due to 1) canopy drip of DON (Harmens et al. 2014) and 2) N leachate from litterfall shed on the forest floor. In other words, we suggest that DON significantly contributes to mossN% (Kielland 1997, Forsum et al. 2006, Liu et al. 2013) in northern forests with low N deposition, given that the assimilation cost of organic N (amino acids) is expected to be lower than that of inorganic N, especially NO_3^- (Chapin et al. 1993). Furthermore, N leaching by precipitation from litterfall (senescent needles and other canopy material; Ukonmaanaho and Starr 2001) may give additional input of N to mosses, and we predict a positive relationship between the mossN% and the amount of N leachate at a regional scale.

111

112 **2. Materials and methods**

113

114 *2.1. Study sites*

115

116 Moss samples and precipitation (water and snow) were collected from intensively monitored
117 coniferous plots in Finland (UN-ECE ICP Forests Level II network, Merilä et al. 2014) in
118 three different inventories carried out in (1) 16 forest plots in 2009, (2) 11 forest openings in
119 2009–2010, and (3) in 10 forest plots in 2002–2003 (a subset of the plot network studied in
120 2009) (Table 1). The study sites were located along the climatic gradient between the
121 northern latitudes 60–70°. The plots were divided into two groups, hereafter referred as
122 “south” and “north” according to their situation in the boreal vegetation subzones of Finland
123 (Fig. 1, Table 2). The first group consisted of the plots in the southern boreal and the second
124 one those in the middle and northern boreal subzones. In the “north” the N deposition level is
125 lower (Fig. 2), growing season shorter, the soil less-fertile and basal area of forests lower than
126 in the “south” (Hilli et al. 2008). The forest data in 2009 consisted of seven Scots pine and
127 nine Norway spruce dominated plots. Majority of the plots (12) were under conventional
128 forest management and four (plot nos. 3, 19, 20, 21) were located in protected areas. We refer
129 to the plots using their ICP Forests codes (Merilä et al. 2014). Basic characteristics of tree
130 stand, understorey vegetation and soil are given in Table 2. All plots were located in
131 background areas without any substantial nearby N emission sources, except the spruce plot
132 Uus23S in the western coast. We excluded this plot from statistical analyses because it was
133 an outlier due to N emissions from a fox fur farm in vicinity, but tested its effect on the shape
134 of response curves (mossN% vs. N deposition).

135

2.2. Collecting and N% determination of moss samples

We used three subsamples of moss data in investigating the relationship of mossN% and atmospheric N deposition in Finland (Table 1). Forest mosses and stand TF deposition were collected within the same ICP Forests Level 2 plots. Open site mosses and bulk deposition were collected from the two separate forest openings in the vicinity of each other and the Level 2 forest plots within the same study area.

Subsample 1 (forest plots, 2009)

The data consists of three moss species, two feather mosses *Pleurozium schreberi* and *Hylocomium splendens*, and a group of acrocarpous *Dicranum* species (mainly *D. polysetum* in the south and *D. polysetum*, *D. scoparium* and *D. fuscenscens* in the north) collected from 16 ICP Forests Level II plots during Jul–Aug in 2009. The moss layer was partly shaded by tree canopies inside the forests (Table 2). We took 28 area based moss samples (30 cm x 30 cm) from each plot. The samples located systematically along the four sides of the ground vegetation monitoring subplot (30 m x 30 m), and the distance between two samples was 3–6 m. All vegetation including ground litter and humus layer was removed as one piece and stored frozen before biomass separation in a laboratory. Some dwarf shrubs, herbs or grasses grew above the moss layer in most samples.

All plant species were carefully separated wearing clean laboratory gloves on hands.

Altogether 1–8 joint samples were formed per moss species for chemical analysis. Moss thalli were divided into upper green part and lower decomposing yellow-green part. The upper part (length 4 cm) had generally 2.5 - 3 annual growths in Jul–Aug. The samples were dried in

60°C temperature, weighed and grinded, and the mossN% of upper parts was determined using the CHN analyser (LECO) (Parkano lab, the Finnish Forest Research Institute, FFRI, nowadays Luke).

Subsample 2 (forest openings, 2009–2010)

This data consisted of moss samples from forest openings in the vicinity of the ICP Forests Level II plots (*P. schreberi* from 9 and *H. splendens* from 8 sites) collected as a part of "European Moss Survey" (ICP Vegetation 2010, Meyer et al. 2015). The moss samples were collected at least 10 m distance from the nearest trees so that TF precipitation had minimal effect on their N content. Altogether 4–6 moss samples were taken from different points of the open site and combined to form a sample of 2 g dry weight per site. Moss samples with three annual growths were dried at 35°C, grained and mossN% was determined using the modified micro Kjeldahl method (Kubin and Siira 1980) in the Muhos lab, FFRI. The Kjeldahl method might give slightly lower mossN% values than LECO CHN (because it underestimates the amount of NO₃⁻). However, an earlier nationwide survey of forest mossN% in Finland (Poikolainen et al. 2009), basing on the modified Kjeldahl method, provided rather similar values for mossN% we received by LECO CHN for forest mosses in the current study. Furthermore, the Muhos lab participated in the international quality assurance test and its results on mossN% reference material agreed well with recommended values (Harmens et al. 2014). In the data analyses we used the average mossN% of the samples collected monthly during May– Sep, (5 months) in 2009 or 2010. The missing moss data in some open sites was substituted by modelled values basing data from the other sites (details in 2.4.).

Subsample 3 (forest plots, 2002–2003)

Moss samples (*H. splendens*, *P. schreberi* and *Dicranum* spp.) were collected from 10 ICP Forests Level II plots in Jul 2002 or Aug 2003. The sampling protocol was similar as described above for the subsample 1. We reanalysed the total N concentration of stored moss powder in 2017 and determined separately nitrite + nitrate ($\text{NO}_2^- + \text{NO}_3^-$) and NH_4^+ concentrations. For the $\text{NO}_2^- + \text{NO}_3^-$ and NH_4^+ analyses, 50 ml of deionized water was added to 0.25 g of ground moss. The samples were vigorously shaken by hand, after which they were kept (horizontally) in a shaker for 16 h (250 rpm), centrifuged for 15 min (4000 rpm), and filtered through a 0.45- μm Millipore filter (Millex HA, cellulose esters; Merck KGaA, Darmstadt, Germany). The $\text{NO}_2^- + \text{NO}_3^-$ and NH_4^+ concentrations were measured with a Thermo Scientific Gallery Plus Automated Photometric Analyser at the Lammi Biological Station, University of Helsinki, following the SFS-EN ISO 13395 and SFS-EN ISO 11732 standards of the International Organization for Standardization. As the $\text{NO}_2^- + \text{NO}_3^-$ concentrations of moss samples were below the detection limit of the analyser ($<10 \mu\text{g l}^{-1}$, i.e. $<2 \mu\text{g NO}_2^- + \text{NO}_3^- \text{-N g}^{-1} \text{ dw}$), the amount of water-soluble DON was calculated by subtracting $\text{NH}_4^+ \text{-N}$ from total N.

2.3. Precipitation, N deposition measurements and N leaching data

Monitoring data on the amount of precipitation and chemical composition of bulk deposition (BD) in the open areas was available for 14 ICP Level II plots including the outlier plot Uus23S (Mustajärvi et al. 2008). In two areas (Juupajoki plot nos 10, 11, and Punkaharju 16, 17), where pine and spruce plots were located close to each other, there was one common plot for open area deposition measurements for the pine and spruce plots (Suppl. Table S.1).

Stand TF data was available for all 16 plots. As the moss samples for chemical analysis had app. 2.5–3 annual growths, we used the corresponding three-year-period in calculating the mean annual deposition. We used the deposition period of 2007–2009 for the moss data from 2009 and 2010 (growth 2009 was unfinished in the latter year) and the periods of 2000–2002 or 2001–2003 for the moss data from 2002 and 2003, respectively. The use of three-year means diminished the effect of annual variation in the amounts of precipitation and N deposition. There were not increasing or decreasing temporal trends in the N_{tot} deposition either in BD or TF across 2000 – 2010, but annual variation in precipitation controlled the amount of N deposition in collectors (Suppl. Fig. S.1).

TF was collected within the forest stand with systematically located 20 rainfall collectors resulting in an area based estimate for the forest floor, and BD in the nearby open area with 3 rainfall collectors (funnel, diameter 20 cm), during the snow-free period. During winter, 6–10 (TF) and 2 (BD) snow collectors (diameter 36 cm) were used. Besides wet-deposition, the collectors probably caught some dry deposition during the 2–4 weeks collecting periods. Deposition samples were filtered (membrane filter, 0.45 μm). Total N was determined by flow injection analysis (FIA) (the Vantaa lab, FFRI), and $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ by ion chromatography (IC) (the Rovaniemi lab, FFRI), both laboratories were internationally accredited. DON deposition was calculated by subtracting the measured $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ from the total N. The sampling procedure for the deposition measurements, including laboratory analyses, is described in detail in Mustajärvi et al. (2008).

We used available data of the annual mass of litterfall fractions and their N amounts ($\text{kg ha}^{-1} \text{ yr}^{-1}$) for 11 plots (Uus23S excluded) provided by the study of Ukonmaanaho et al. (2008). Based on this data, we modelled the amount of N in the leachate from litterfall ($\text{kg ha}^{-1} \text{ yr}^{-1}$)

as 6% from the total N in litterfall according to a short-term experiment (Aug-Sep)
(Ukonmaanaho and Starr 2001).

3. Data analysis

We used area based N deposition values ($\text{kg ha}^{-1} \text{ yr}^{-1}$) consisting of information on both the N concentration in precipitation and amount of precipitation in the data analysis. The amount of N_{tot} in BD and TF within the study areas (open site vs. forest site) and the amount of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ within BD and TF were compared by paired t tests. Furthermore, we tested the effect of zone (north, south) and tree species on the amount of DON in TF using ANOVA and post-hoc Tukey's t-tests.

We calculated open site/forest site mossN% ratios to nine study areas having moss samples from the both habitats. Then we modelled the missing mossN% values for open sites (five open sites in *H. splendens* and seven in *P. schreberi*) by multiplying forest mossN% values using this ratio (cf. Meyer et al. 2015). We used tree-species specific average ratios for *P. schreberi* and the overall average for *H. splendens*.

We used four-factor ANOVA in testing the effect of moss species (*P. schreberi*, *H. splendens*), habitat (open site, forest), zone (northern, southern part of the country) and dominant tree species (pine, spruce) and their two-factor interactions on the mossN% (13 plots with two coexisting moss species). Similarly, we used three-factor ANOVA in testing the effect of moss species (three coexisting species in forest habitat), zone and tree species and their two-factor interactions on mossN% (13 forest plots, 2009) and on free $\text{NH}_4^+\text{-N}$ concentration in moss tissues (10 forest plots, 2002–2003). Pairwise comparisons between

the moss species after ANOVA were made by post-hoc Tukey's t-tests. Assumption of normal distribution of the data in using parametric tests was checked by Shapiro-Wilk normality tests and the data was $\log(x+1)$ transformed when needed. We analysed the relationship between the averages of mossN% and amount of N leachate calculated separately to the pine and spruce stands in the north and south using Spearman rank correlation. The tests were carried out using the STATISTIX 10.0 program (2013).

We fitted linear logarithmic models (lm) for the three moss species in order to study the response of mossN% (y variable) to different N forms in deposition (x variable). Separate species-specific models were calculated for mossN% in open site vs. BD, and for mossN% in forest site vs. TF. We used total N ($N_{\text{tot}} = \text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N} + \text{DON}$), inorganic N ($N_{\text{inorg}} = \text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) or individually $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and DON as explanatory variables. Interaction between N_{inorg} and DON, tree species (pine, spruce) and N_{tot} , as well as DON and stand basal area were tested. We answered the hypothesis arguing DON contributes significantly to mossN% in forest habitats by comparing two models, the first having only N_{inorg} as a predictor, and the second having both N_{inorg} and DON as predictors of the mossN%. Linear logarithmic models (lm) were used also in analysing the response of $\text{NH}_4^+\text{-N}$ and total N% of tissues of feathermosses (average of *H. splendens* and *P. schreberi*) to increasing N_{tot} in TF deposition (data 2002-2003).

Finally, we studied how well N_{tot} deposition (y) in background forests in Finland could be predicted according to mossN% of the three moss species (x). We made separate lm logarithmic models for open site (BD vs. feather mosses) and forest site (TF vs. feather mosses and *Dicranum* spp.) data. We used the species-specific as well as average mossN% values of *H. splendens* and *P. schreberi* as predictors in the models. The goodness of fit was

evaluated according to adjusted R^2 values in all models. We used the R statistical environment in constructing the models (R core team 2017).

4. Results

4.1. Amount and chemistry of N deposition and N leachate from litterfall

The N_{tot} in BD increased from 1.2 to 4.8 kg ha⁻¹ yr⁻¹ along the latitudinal gradient from north to south (Fig. 2a, Table S.1). The N_{tot} was higher in BD than in TF ($p < 0.01$), but the average difference was smaller in the north (0.3 kg ha⁻¹ yr⁻¹, $p < 0.05$) than in the south (0.8 kg ha⁻¹ yr⁻¹, $p < 0.01$). The absolute (Fig. 2a,b) and relative amounts (Fig. S.2a,b) of different N forms varied between BD and TF. In BD, the proportion of N_{inorg} was 70–85% of N_{tot} , whereas in TF it was ca. 60% in the north and only 40% in the southernmost plots. The amount of NO_3^- -N was higher than that of NH_4^+ -N in both BD and TF ($p < 0.001$). Only in the outlier plot Uus23S (excluded from the tests above) NH_4^+ -N was remarkable higher than NO_3^- -N in TF (Fig. 2b). The amount of DON increased from north to south in TF, and it was higher in spruce than pine stands in the south ($p < 0.05$) (Table S.2a-c). The amount of N leached from the tree litter varied from 0.3 – 0.6 kg ha⁻¹ yr⁻¹ in the north and from 0.7 – 2.3 kg ha⁻¹ yr⁻¹ in the south (Fig. 2c).

4.2. Effect of site, zone, tree species and litterfall on mossN%

In all species sampled from forests, the average mossN% was higher in the southern than northern part of the country ($p < 0.05$) (Fig. 3a, Table 2), and in spruce than pine stands

($p < 0.05$) (Fig. 3b). *H. splendens* and *P. schreberi* had similar average mossN% values, but *Dicranum* spp. had higher mossN% than the feather mosses in forests ($p < 0.05$). The average mossN% of *P. schreberi* and *H. splendens* was higher in forest (range 0.8–1.7%) than in open site habitats (0.5–1.2%) both in pine and spruce dominated areas ($p < 0.001$) (Fig. 3a,b). The mossN% of feather mosses was higher in spruce than pine dominated areas also in open site habitats in the southern part of country ($p < 0.05$) (Fig. 3b, for ANOVA see Table S.3a,b). Moreover, the mossN% of feather mosses tended to increase with increasing amount of N leachate from litterfall ($p < 0.08$) when compared as average values in pine and spruce stands in the south and north. The outlier plot Uus23S was not included in the data analysis presented here.

4.3. Response of mossN% to total N deposition at open and forest sites

In the open sites, the mossN% of both feather moss species increased almost linearly with increasing N_{tot} in BD (Fig. 4a,b). The R^2 value of the open site model (mod1B) was higher for *P. schreberi* (81%) than for *H. splendens* (52%) (Table 3a,b). Similarly, the mossN% of both feather moss species and *Dicranum* spp. sampled from forests increased with increasing N_{tot} in stand TF (Fig. 4a-c). However, here the response followed an asymptotic curve showing a slight decrease in the N accumulation rate at higher TF N_{tot} levels. R^2 value for *P. schreberi* (73%) was higher than those for *H. splendens* (60%) and *Dicranum* spp. (54%) in the mod1TF equations (Table 3a-c). When the outlier plot Uus23S was included to these models, the shape of the curves did not change compared to the basic models without Uus23S (Fig. 4a-c; for Model tables see Suppl. Table S.4a,b). For *P. schreberi* the fit of the mod1B was higher (R^2 81%) than that of mod1TF (73%), but opposite was true for *H. splendens* (Table 3a,b).

4.3.1. The effect of tree species in the response models

The dominant tree species (pine or spruce) surrounding the forest openings had no statistically significant effect when added as the second predictor with N_{tot} in the models of feather mosses (mod2B, Table 3a,b). However, mossN% (especially in *H. splendens*) tended to increase higher with increasing N_{tot} deposition in openings surrounded by spruce than by pine in the southern part of the country (Fig. S.3). Similar effect of the tree species was found in forest sites. Here interaction between N_{tot} and tree species ($p < 0.06-0.08$) in equations of *P.schreberi* and *Dicranum* spp. (mod2TF) showed higher accumulation rate of mossN% in spruce than in pine dominated stands (Table 3a-c, Fig. S.3).

4.3.2. MossN% in relation to different forms of N deposition and stand basal area

N_{inorg} of BD explained most variation in mossN% of the both feather moss species in forest openings in one-predictor models (mod6B). Adding DON of BD as the second predictor was not significant and did not increase the R^2 values of the models (mod7B) (Table 3a,b). On the other hand, N_{inorg} of TF in forest sites had relatively low R^2 values (21 – 39%) in one-predictor models (mod6TF) for all three moss species, whereas TF DON as the second predictor was highly significant ($p < 0.01$) and it explained most variation in mossN% (mod7TF, Table 3). When TF DON was included to the models, TF N_{inorg} changed to be an insignificant predictor. There was no interaction between N_{inorg} and DON in either BD or TF models (not shown). However, the effect of TF DON on mossN% was controlled by the forest structure, being higher in the stands with larger basal area (significant interaction DON: Basal area in modTF8 in all moss species) (Table 4, Fig.S.4). In the case of *P.*

schreberi also N_{inorg} had a small positive effect ($p < 0.10$) on mossN% when the effect of DON was linked in the interaction term with basal area.

The distinct effects of DON in open and forest sites are clearly seen in the shape of the response curves of mossN% vs N form (Fig. 4a-c). The range of DON (on x axis) in the forest sites was twice as wide as that in the open sites. Thus, in the open sites the effect of DON on mossN% was minimal whereas N_{inorg} dominated the N_{tot} deposition pattern. On the other hand, DON determined the shape of mossN% vs. N_{tot} curve in the forest sites. The location of the outlier plot Uus23S was distant from the response curves of mossN% vs BD in the open sites, but it fitted quite well to the curves of mossN% vs. TF N_{tot} or TF N_{inorg} based on the forest data (Fig. 4a-c).

4.4. Free NH_4 -N in moss tissues (forest data 2002-2003)

An analysis of N forms in mosses collected from the forest plots (2002–2003) showed that most N (average 98.8%) was assimilated into organic N compounds. However, we found a small amount of free NH_4^+ -N (1.2%) in tissues. The concentration of NH_4^+ -N was higher in the southern than in northern stands ($p < 0.001$), and in spruce than in pine stands ($p < 0.001$) in each three moss species (Suppl. Fig. S.5, Table S.3c). Average NH_4^+ -N as well as total N concentration of the feather mosses increased with increasing TF N_{tot} deposition according to an asymptotic curve (Fig. 5, Table S.5).

4.5. Predicting N_{tot} deposition for background forest areas by mossN%

Equations predicting the N_{tot} deposition in background forests of Finland according to average mossN% of the feather moss species gave high fit for both open ($R^2=73\%$) and forest site habitats ($R^2=79\%$) (Fig. 6, Suppl. Table S.6). *P. schreberi* predicted slightly higher BD N_{tot} estimates than *H. splendens* for open sites, but the TF N_{tot} estimates of the two species were almost similar for forest sites. *Dicranum* spp. predicted lower TF N_{tot} than feather mosses for forests.

5. Discussion

We found clear positive relationship between the mossN% of and N_{tot} deposition in background forest areas along an extensive north-south gradient in Finland. The N_{tot} deposition was lower in forest TF than in open site BD, indicating that tree canopies filtered and absorbed dry and wet-deposited N in northern N-limited forests (Mustajärvi et al. 2008). On the other hand, mossN% was higher within forest stands than in forest openings. Corresponding difference in mossN% concentrations between the two types of growing sites has also been observed in Slovenia (Skudnik et al. 2014) and in Germany (Kluge et al. 2013, Meyer et al. 2015), but there – unlike in Finland – N_{tot} deposition was much higher in forests (rich with $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) than in open sites. The possible reasons for the observed mossN% pattern in our study are: (1) Mosses prefer certain forms of N (e.g. amino acids in DON) the amounts of which are higher in TF than in BD (cf. Harmens et al. 2014); (2) a part of N accumulating in mosses originates from N leachates of litterfall (Ukonmaanaho and Starr 2001) not included in BD or TF; (3) biological N_2 fixation of moss-associated bacteria (Salemaa et al. 2019); (4) translocation of N from senescing tissues to new moss growth (Eckstein and Karlsson 1999, Salemaa et al. 2008), and (5) differences in moss growth rates

due to differences in abiotic and biotic factors between geographical areas and forest types (Harmens et al. 2011).

5.1. The effect of different N forms on mossN%

Our results suggested that differences in the deposition chemistry and supply of different N forms between the open and forest sites affected the uptake of N from precipitation by mosses. At the open sites inorganic forms of N in deposition explained the accumulation of N in mosses, whereas DON explained almost completely the variation of mossN% in forest sites. These correlative results gave indirect evidence for moss affinity to DON and supported the hypothesis that the canopy drip of DON greatly contributed to mossN% in forests. However, mosses seemed to have an ability to absorb both inorganic and organic N forms depending on the region and stand structure. NH_4^+ -N and NO_3^- -N in BD correlated positively with mossN%, but it is difficult to conclude which inorganic N form was preferred by mosses based on the correlative approach. The effect of N_{inorg} also in forest site was observed in the outlier ‘fox farm’ plot (Uus23S), where the annual NH_4^+ -N deposition was 3.3 kg ha^{-1} higher in TF than in BD most probably because dry-deposited NH_3 and NH_4^+ were washed down from the spruce canopy. Here the mossN% of *P. schreberi* was 2.2% in forest and 1.4% in opening.

Depending on the conditions, amino acids in DON may contribute substantially to N sequestration of mosses (Wiedermann et al. 2009, Liu et al. 2013) although they only account for a small proportion of the bioavailable fraction of DON (Neff et al. 2002). DON usually accounts for about 20 to 65% of total N in wet deposition (Cornell et al. 2003). In the Finnish background forests, DON formed nearly a half (>40%) of the N_{tot} in TF, whereas its share

was only 20% in BD. We did not analyse the composition of DON, but mosses apparently took up amino acids, such as glycine (Song et al. 2016), as they can be absorbed easily into moss cells through cation exchange capacity and proton (H^+) pump (Glime et al. 2007).

5.2. The effect of tree species and stand basal area on mossN%

The average mossN% of the studied moss species was higher in spruce than in pine stands. Furthermore, the accumulation rate of N in mosses in relation to N_{tot} was slightly higher under the spruce than pine canopies and increased with increasing stand basal area. Both the characteristics of tree species and the site fertility regulated the composition of TF in forests. Especially in the southern spruce stands, tree canopies modified the N in TF so that DON leached from the canopies compensated the inorganic N retained therein (Mustajärvi et al. 2008). The greater needle mass (i.e. greater leaf area) of southern spruce stands in comparison to those of pine (Merilä et al. 2014) enable spruce crowns to capture more N_{inorg} from dry and wet deposition than pine crowns. In addition, the higher fertility level of spruce dominated site types (Tamminen 2000, Lukina et al. 2018) may result in larger amount of DON leaching in TF in spruce than in pine stands. N_{inorg} in TF explained the mossN% of *P. schreberi* at forest sites when interaction term DON:basal area was included in the model. This is reasonable particularly in northern stands which are sparser and have smaller canopy coverage (Table 2) in comparison to southern stands. It is noteworthy, that tree species of the surrounding forest also tended to influence mossN% in open site models in the southern part of the country, although the sampling plots were treeless. This kind of tree effect may be caused by litterfall or the fertility level of the site.

5.3. Effect of litterfall, N_2 fixation, N translocation and microclimate on mossN%

459

460 Aboveground litterfall (shed leaves, branches and bark) of trees and understorey vegetation is
461 an important pathway of nutrient return in the forest ecosystem. According to Ukonmaanaho
462 et al. (2008) N return from needle litter of coniferous stands is ca. 5 and 7–14 kg N ha⁻¹ yr⁻¹
463 in the northern and in the southern Finland, respectively. During autumn and winter, litterfall
464 enter new decomposable material and water-extractable N on the forest floor (Ukonmaanaho
465 and Starr 2001, Hilli et al. 2008). As predicted, the modeled estimates for the amount of N
466 leachate for our forest plots tended to show a positive relationship with mossN% at the
467 regional scale. N leachate contributes to internal cycling of N in forests and it may partly
468 explain the higher mossN% at forest than at open sites.

469

470 In a previous study carried out partly in the same forest plots as the present study, the activity
471 of cyanobacteria hosted by mosses was highest in the northern plots having low N deposition,
472 and ceased in the south (Salemaa et al. 2019). Because light level regulates the activity of
473 cyanobacteria, it is probable that N₂ fixation is higher in mosses growing at open than forest
474 sites. In contrast, N translocation to the new growth of mosses may be faster in moist
475 conditions expressing as higher mossN% in forests than at open sites.

476

477 *5.4. The impact of low N deposition on mossN%*

478

479 Large-scale European data has provided evidence of asymptotic relationship between the
480 mossN% and N deposition (Harmens et al. 2014). The data from Finland (open site data of *P.*
481 *schreberi*, also used in our study) represented background areas with the lowest bulk N
482 deposition. The Finnish data was within the initial linear part of the asymptotic response
483 curve and showed a good fit with the model. The models of all three moss species at open

sites had high R^2 values also in the current analyses. The best fit was found for the relationship between N% of *P. schreberi* and the amount of N_{inorg} in BD ($R^2 = 82\%$). Similarly, in a Swiss study by Kosonen et al. (2018) mossN% of *P. schreberi* and *Hypnum cupressiforme* showed a good relationship with N originating from dry deposited NH_3 and from precipitation (DON excluded; $R^2=81\%$). The mossN% was generally slightly higher in Switzerland than in Finland, probably due to the higher contribution of dry-deposited NH_3 to mossN% at the Swiss sites (Kosonen et al. 2018).

Overall, bulk sampler measurements include only a part of the total amount of the reactive N included in fine particles and gases incorporated on foliar surfaces of plants i.e., the sampling method overestimate wet deposition but underestimate dry deposition (Loubet et al. 2009, Meyer et al. 2015). Our sample plots (except one outlier) were located in background areas far from agricultural and livestock sites and roads, and therefore the amount of dry deposition on the plots was most probably low. On the other hand, in natural boreal ecosystems, both low temperatures and rainfall limit the ability of mosses to assimilate excess N_{inorg} and to allocate it into new biomass.

In the response models of the joint European data the mossN% did not greatly respond to increasing NH_4^+ -N deposition of over $12 \text{ kg ha}^{-1}\text{yr}^{-1}$, and wet bulk N deposition of over $20 \text{ kg ha}^{-1}\text{yr}^{-1}$ (Harmens et al. 2014). According to Pitcairn et al. (1998) mossN% is saturated to a level of ca. 2% when the wet BD is ca. $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. However, Pitcairn et al. (2006) concluded mosses cannot reach high N concentrations in the field conditions when precipitation is high, unless rainfall constantly contains large concentrations of N. In Finland, N_{tot} deposition is remarkably lower than the N levels mentioned above. At open sites the relationships between mossN% and N_{tot} in BD were linear and the highest mossN% was 1 –

1.2%. However, at forest sites the accumulate rate of N in mosses started to decline when N deposition was only 3 – 5 kg ha⁻¹ yr⁻¹ and the highest mossN% c.a. 1.4 – 2.0%. Also free NH₄⁺-N accumulated in mosses in the southern forests at this N deposition level suggesting mosses were near the N saturation state at these sites. Thus, our data suggests that the boreal mosses were achieving the N saturated state at much lower level than 20 kg ha⁻¹ yr⁻¹ when growing under the effects of tree canopies and litterfall.

5.5. Predicting N deposition based on mossN%

The nationwide surveys on mossN% in *H. splendens* in Finland reflected well the known decreasing gradient of N deposition from south to north at the country level, but the correlation with the modelled N deposition was fairly weak (Poikolainen et al. 2009). The authors suggested that in addition to N deposition, the variability in a range of site and stand characteristics cause variation in mossN%. In the present study, we were able to quantify the relationship between mossN% and N_{tot} in more detail because N deposition was measured at the moss sampling sites (TF) or in their vicinity (BD).

At open sites, the linear pattern of accumulation of N in mosses with increasing N_{tot} deposition gives evidence of their high bio-indicator value at conditions of fairly low N deposition. This finding justifies using the open site equations in modelling N_{dep} particularly in remote boreal areas, where it is not possible to maintain continuous N_{dep} monitoring systems. Mosses growing in forests took up N from TF precipitation passing tree and dwarf shrub canopies and from litterfall. Because these processes are controlled by many environmental factors (e.g. stand structure, amount of dry deposition and precipitation, and temperature), we recommend using the equations calculated from the open site data. We also

recommend using *P. schreberi* in field studies when possible because its equations showed the best fit between the mossN% and N deposition at both open and forest sites.

6. Conclusions

Given the low precipitation and low N deposition at the Finnish sites as well as higher mossN% in forests than in openings, it is probable that TF at background sites was enriched with N forms (e.g. amino acids in DON) readily taken up by mosses. This was reflected as a lowered N uptake efficiency of mosses at higher N exposure levels. The view is supported by the asymptotic form of the accumulation curves of the N_{tot} and $\text{NH}_4\text{-N}$ in tissues vs. N deposition, suggesting mosses in forests were approaching N saturation at TF N_{tot} of 3–4 kg $\text{ha}^{-1} \text{yr}^{-1}$. Corresponding level of N deposition has been found to turn off N_2 fixation of cyanobacteria associated with mosses (Salemaa et al 2019). The accumulation of $\text{NH}_4^+\text{-N}$ in moss tissue may indicate that the supply of N_{inorg} exceeds the ability of mosses to assimilate and allocate N into biomass production i.e. the moss growth is no longer N limited. However, it should be noted that a part of the N accumulated in mosses apparently came from the N leachate from litterfall emphasizing the effect of internal N flux of the forest ecosystem. According to Bobbink et al. (2010) and Bobbink and Hettelingh (2011) critical N load for eutrophication, i.e. slow changes in the structure and function of boreal forests, may take place already at N deposition levels $< 5 \text{ kg ha}^{-1} \text{yr}^{-1}$. This level is near the suggested N saturation point of the boreal mosses in our study. However, more research is needed for understanding what kind of roles mosses have in the internal N flux and eutrophication process of boreal ecosystems.

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559

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565

566

567 Appendix: Supplementary Figures and Tables

568

References

- Ayres E., van der Wal R., Sommerkorn M., Bardgett R.D., 2006. Direct uptake of soil nitrogen by mosses. *Biology Letters* 2, 286-288.
<http://doi.org/10.1098/rsbl.2006.0455>
- Bernhardt-Römermann, M., Baeten, L., Craven, D., De Frenne, P., Hédli, R., Lenoir, J., ... & Dierschke, H., 2015. Drivers of temporal changes in temperate forest plant diversity vary across spatial scales. *Global Change Biology*, 21(10), 3726-3737.
<https://doi.org/10.1111/gcb.12993>
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., ... & Emmett, B., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological applications*, 20(1), 30-59.
<http://doi.org/10.1890/08-1140.1>
- Bobbink R., Hettelingh J.P. (Eds.), 2011. Review and revision of empirical critical loads and dose-response relationships, Coordination Centre for Effects, National Institute for Public Health and the Environment (RIVM). www.rivm.nl/cce
- Chapin III F.S., Moilanen L., Kielland K., 1993. Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. *Nature* 361, 150-153.
- Cornell S.E., Jickells T.D., Cape J.N., Rowland A.P., Duce R.A. 2003. Organic nitrogen deposition on land and coastal environments: a review of methods and data. *Atmospheric Environment* 37, 2173–2191. [https://doi.org/10.1016/S1352-2310\(03\)00133-X](https://doi.org/10.1016/S1352-2310(03)00133-X)
- De Schrijver A., Geudens G., Augusto L., Staelens J., Mertens J., Wuyts K., Gielis L., Verheyen K., 2007. The effect of forest type on throughfall deposition and seepage

593 flux: a review. *Oecologia* 153, 663-674. <https://doi.org/10.1007/s00442-007->
594 0776-1

595 Dirnböck, T., Pröll, G., Austnes, K....& Forsius, M.; 2018. Currently legislated decreases in
596 nitrogen deposition will yield only limited plant species recovery in European
597 forests. *Environmental Research Letters* 13: 12. <https://doi.org/10.1088/1748->
598 9326/aaf26b

599 Eckstein, R.L., Karlsson, P.S., 1999. Recycling of nitrogen among segments of
600 *Hylocomium splendens* as compared with *Polytrichum commune*: implications for
601 clonal integration in an ectohydric bryophyte. *Oikos*, 86, 87–96.

602 Erisman, J.W., Bleeker, A., Galloway, J., Sutton; M.S., 2007. Reduced nitrogen in ecology
603 and the environment. *Environmental Pollution* 150, 140-149.
604 <https://doi.org/10.1016/j.envpol.2007.06.033>

605 Forsum, Å., Dahlman, L., Näsholm, T., Nordin, A., 2006. Nitrogen utilization by
606 *Hylocomium splendens* in a boreal forest fertilization experiment. *Functional*
607 *Ecology* 20, 421-426. <https://doi.org/10.1111/j.1365-2435.2006.01127.x>

608 Fowler D., Cape J.N., Coyle M., Flechard C., Kuylenstierna J., Hicks K., Derwent D., Johnson
609 C., Stevenson D. 1999. The global exposure of forests to air pollutants. *Water, Air,*
610 *and Soil Pollution* 116, 5-32. <https://doi.org/10.1023/A:1005249231882>

611 Galloway, J. N., Dentener, F. J., Capone D. G., Boyer E. W., Howarth R. W., Seitzinger S. P.,
612 Asner G. P., Cleveland C. C., Green P. A., Holland E. A., Karl D. M., Michaels A. F.,
613 Porter J. H., Townsend A. R., Vöosmarty, C. J., 2004. Nitrogen cycles: past, present,
614 and future. *Biogeochemistry* 70, 153–226. <https://doi.org/10.1007/s10533->
615 004-0370-0

616 Glime J.M., 2007. *Bryophyte Ecology*. Michigan, Michigan Technological University and
617 the International Association of Bryologists. <http://www.bryoecol.mtu.edu>

618 Harmens H., Norris D.A., Cooper D.M., et.al., 2011. Nitrogen concentrations in mosses
619 indicate the spatial distribution of atmospheric nitrogen deposition in Europe.
620 Environmental Pollution 159, 2852–2860.
621 <https://doi.org/10.1016/j.envpol.2011.04.041>

622 Harmens H., Schnyder E., Thöni L., Cooper D.M., Mills G., Leblond S., Mohr K., Poikolainen
623 J., Santamaria J., Skudnik M., Zechmeister H.G., Lindroos A.-J., Hanus-İllnar, A.,
624 2014. Relationship between site-specific nitrogen concentration in mosses and
625 measured wet bulk atmospheric nitrogen deposition across Europe.
626 Environmental Pollution 194, 50–59.
627 <https://doi.org/10.1016/j.envpol.2014.07.016>

628 Hawkins B.J., May, E., Robbins, S., 2018. Nitrate and ammonium uptake in 21 common
629 species of moss from Vancouver Island, British Columbia. Botany 96, 201-208,
630 <https://doi.org/10.1139/cjb-2017-0154>.

631 Hilli, S., Derome, J., 2008. Water-extractable organic compounds in different
632 components of the litter layer of boreal forest soils along a climatic gradient.
633 Boreal Environment Research 13 (suppl. B): 92-106.

634 Izquieta-Rojano S., García-Gomez H., Aguillame L., Santamaría J.M., Tang Y.S., Santamaría
635 C., Valiño F., Lasheras E., Alonso R., Àvila A., Cape J.N., Elustondo D., 2016.
636 Throughfall and bulk deposition of dissolved organic nitrogen to holm oak
637 forests in the Iberian Peninsula: Flux estimation and identification of potential
638 sources. Environmental Pollution 210, 104-112.
639 <https://doi.org/10.1016/j.envpol.2015.12.002>

640 Kielland K. 1997. Role of free amino acids in the nitrogen economy of arctic cryptogams.
641 Ecoscience 4, 75-79 <https://doi.org/10.1080/11956860.1997.11682379>

642 Kluge M., Pesch R., Schröder W., Hoffmann A., 2013. Accounting for canopy drip effects
643 of spatiotemporal trends of the concentrations of N in mosses, atmospheric N
644 depositions and critical load exceedances: a case study from North-Western
645 Germany. *Environmental Sciences Europe* 25, 26.
646 <https://link.springer.com/article/10.1186/2190-4715-25-26>

647 Kosonen Z., Thimonier A., Schnyder E., Thöni L., 2018. Nitrogen concentration in moss
648 compared with N load in precipitation and with total N deposition in
649 Switzerland. *Environmental Pollution* 239, 169-178.
650 <https://doi.org/10.1016/j.envpol.2018.03.063>

651 Kubin, E., Siira, J., 1980. On the suitability of the phenyl hypochlorite procedure for the
652 determination of total nitrogen from plant and soil samples. *Aquilo Ser. Botanica*
653 17, 11-17.

654 Liu X.-Y., Koba K., Makabe A., Li X.-D., Yoh M., Liu C.-Q., 2013. Ammonium first: natural
655 mosses prefer atmospheric ammonium but vary utilization of dissolved organic
656 nitrogen depending on habitat and nitrogen deposition. *New Phytologist* 199,
657 407-419. <https://doi.org/10.1111/nph.12284>

658 Loubet B., Asman W.A.H., Theobald M.R., Hertel O., Sim Tang Y., Robin P., Hassouna M.,
659 Dämmgen U., Genermont S., Collier P., Sutton M.A., 2009. Ammonia deposition
660 near hot spots., in: Sutton M.A., Reis S., Baker, S.M.H. (Eds.), *Atmospheric*
661 *Ammonia*. Springer, The Netherlands, pp. 205–267.

662 Lovett G.M., Lindberg S.E. 1993. Atmospheric deposition and canopy interactions of
663 nitrogen in forests. *Canadian Journal of Forest Research* 23, 1603-1616.

664 Lukina, N.V., Orlova, M.A., Bakhmet, O.N., Tikhonova, E.V., Tebenkova, D.N., Kasakova,
665 A.I., Kryshen, A.M., Gornov, A.V., Smirnov, V.E., Shashkov, M.P., Ershov, V.V.,

666 Knyazeva, S.V., 2019. The influence of vegetation on the forest soil properties in
667 the Republic of Karelia. *Eurasian Soil Science*, 52: 793–807.

668 Merilä P., Mustajärvi, K., Helmisaari, H-S., Hilli, S., Lindroos, A-J., Nieminen, T.M., Nöjd, P.,
669 Rautio, P., Salemaa, M., Ukonmaanaho, L., 2014. Above-and below-ground N
670 stocks in coniferous boreal forests in Finland: Implications for sustainability of
671 more intensive biomass utilization. *Forest Ecology and Management* 311: 17-28.
672 <https://doi.org/10.1016/j.foreco.2013.06.029>

673 Meyer M., Schröder W., Nickel S., Leblond S., Lindroos A.-J., Mohr K., Poikolainen J.,
674 Santamaria J.M., Skudnik M., Thöni L., Beudert B., Dieffenbach-Fries H., Schulte-
675 Bisping H., Zechmeister H.G. 2015. Relevance of canopy drip for accumulation of
676 nitrogen in moss used as biomonitors for atmospheric nitrogen deposition in
677 Europe. *Science of the Total Environment* 538, 600-610.
678 <https://doi.org/10.1016/j.scitotenv.2015.07.069>

679 Mustajärvi K., Merilä P., Derome J., Lindroos A.-J., Helmisaari H.-S., Nöjd P.,
680 Ukonmaanaho L., 2008. Fluxes of dissolved organic and inorganic nitrogen in
681 relation to stand characteristics and latitude in Scots pine and Norway spruce
682 stands in Finland. *Boreal Environment Research* 13 (suppl. B), 3-21.

683 Neff J.C., Holland E.A., Dentener F.J., McDowell W.H., Russell K.M., 2002. The origin,
684 composition and rates of organic nitrogen deposition: A missing piece of the
685 nitrogen cycle? *Biogeochemistry* 57, 99-136.
686 <https://doi.org/10.1023/A:101579162>

687 Neiryneck J. Kowalski A.S., Carrara A., Genouw G., Berghmans P., Ceulemans R. 2007.
688 Fluxes of oxidized and reduced nitrogen above a mixed coniferous forest
689 exposed to various nitrogen emission sources. *Environmental Pollution* 149, 31-
690 43. <https://doi.org/10.1016/j.envpol.2006.12.029>

691 Nordin, A., Strengbom, J., Ericson, L., 2006. Responses to ammonium and nitrate additions
 692 by boreal plants and their natural enemies. *Environmental Pollution* 141, 167–174.
 693 <https://doi.org/10.1016/j.envpol.2005.08.017>

694 Perring M.P. Diekmann, M., Midolo G. et al. 2018. Understanding context dependency in the
 695 response of forest understorey plant communities to nitrogen deposition.
 696 *Environmental Pollution* 242, 1787-1799.

697 Pitcairn, C.E.R., Leith, I.D., Sheppard, L.J., Sutton, M.A., Fowler, D., Munro, R.C.Tang, S.,
 698 Wilson, D., 1998. The relationship between nitrogen deposition, species composition
 699 and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms.
 700 *Environmental Pollution* 102 (S1), 41–48. [https://doi.org/10.1016/S0269-](https://doi.org/10.1016/S0269-7491(98)80013-4)
 701 [7491\(98\)80013-4](https://doi.org/10.1016/S0269-7491(98)80013-4)

702 Pitcairn C., Fowler D., Leith I., Sheppard L., Tang S., Sutton M., Famulari D., 2006.
 703 Diagnostic indicators of elevated nitrogen deposition. *Environmental Pollution* 144,
 704 941-950. <https://doi.org/10.1016/j.envpol.2006.01.049>

705 Poikolainen J., Piispanen J., Karhu J., Kubin E., 2009. Long-term changes in nitrogen
 706 deposition in Finland (1990-2006) monitored using the moss *Hylocomium splendens*.
 707 *Environmental Pollution* 157, 3091-3097.
 708 <https://doi.org/10.1016/j.envpol.2009.05.018>

709 R Core Team, 2017. R: A language and environment for statistical computing. R Foundation
 710 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>

711 Rousk, K., Jones, D.L., DeLuca, T.H., 2013. Moss-cyanobacteria associations as biogenic
 712 sources of nitrogen in boreal forest ecosystems. *Frontiers in microbiology* 4, 150,
 713 <https://doi.org/10.3389/fmicb.2013.00150>

714 Salemaa M., Mäkipää R., Oksanen J., 2008. Differences in the growth response of three
 715 bryophyte species to nitrogen. *Environmental Pollution* 152, 82-91.
 716 <https://doi.org/10.1016/j.envpol.2007.05.019>

717 Salemaa, M., Lindroos, A.-J., Merilä, P., Mäkipää, R. & Smolander, A., 2019. N₂ fixation
 718 associated with the bryophyte layer is suppressed by low levels of nitrogen deposition
 719 in boreal forests. *Science of the Total Environment* 653: 995-1004.
 720 <https://doi.org/10.1016/j.scitotenv.2018.10.364>

721 SFS-EN ISO 11732, 2001. Water quality. Determination of ammonium nitrogen. Method by
 722 Flow Analysis (CFA and FIA) and Spectrometric Detection (ISO 11732:2005).

723 SFS-EN ISO 13395, 2001. Water quality. Determination of Nitrite Nitrogen and Nitrate
 724 Nitrogen and the Sum of Both by Flow Analysis (CFA and FIA) and Spectrometric
 725 Detection (ISO 13395:1996).

726 Skudnik M., Jeran Z., Batic F., Simoncic P., Lojen S., Kastelec D., 2014. Influence of canopy
 727 drip on the indicative N, S and $\delta^{15}\text{N}$ content in moss *Hypnum cupressiforme*.
 728 *Environmental Pollution* 190, 27-35. <https://doi.org/10.1016/j.envpol.2014.03.016>

729 Solga A., Frahm J.-P. 2006. Nitrogen accumulation by six pleurocarpous moss species and
 730 their suitability for monitoring nitrogen deposition. *Journal of Bryology* 28, 46-52.
 731 <https://doi.org/10.1179/174328206X104552>

732 Song L., Lu H.-Z., Xu X.-L., Li S., & Liu, W.-Y., 2016. Organic nitrogen uptake is a
 733 significant contributor to nitrogen economy of subtropical epiphytic bryophytes.
 734 *Nature/ Scientific Reports* 6:30408. <https://doi.org/10.1038/srep30408>

735 STATISTIX 10.0. Analytical Software, 2013. <https://www.statistix.com/>

736 Tamminen, P. 2000. Soil factors. In: Mälikönen E. (ed.), *Forest condition in a changing*
 737 *environment – The Finnish case*, pp. 72-86. Kluwer Academic Publishers.

738 Ukonmaanaho, L. & Starr, M., 2001. The importance of leaching from litter collected in
739 litterfall traps. Environmental Monitoring and Assessment 66, 129 -146.
740 <https://doi.org/10.1023/A:1026432315707>

741 Ukonmaanaho, L., Merilä, P., Nöjd, P., Nieminen, T.M., 2008. Litterfall production and
742 nutrient return to the forest floor in Scots pine and Norway spruce stands in Finland.
743 Boreal Environment Research 13 (suppl. B), 67-91.

744 van den Elzen, E., van den Berg, L.J., van der Weijden, B., Fritz, C., Sheppard, L.J., Lamers,
745 L.P.M., 2018. Effects of airborne ammonium and nitrate pollution strongly differ in
746 peat bogs, but symbiotic nitrogen fixation remains unaffected. Science of the Total
747 Environment 610, 732-740, <https://doi.org/10.1016/j.scitotenv.2017.08.102>

748 Wiedermann M.M., Gunnarsson U., Ericsson L., Nordin A. 2009. Ecophysiological
749 adjustment of two Sphagnum species in response to anthropogenic N deposition. New
750 Phytologist 181, 208-217. <https://doi.org/10.1111/j.1469-8137.2008.02628.x>
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755 Figure 1. Location of the plots and distribution of the vegetation zones in Finland. ICP
 756 Forests plot code shown. In the data analysis “south” = southern boreal and “north” =
 757 middle and northern boreal subzones.

758

a)

b)

c)

Figure 2. Amount of different N forms in a) bulk (BD) and b) throughfall (TF) deposition (averages across 2007-2009, see Suppl. Table S.2). Plots ordered according to N latitude (Table 2) from north to south (left – right). P = Scots pine and S = Norway spruce in the plot names. Plot Uus23S is an outlier and positioned far right. c) Average amount of N_{tot} in TF and in leachate (LE) from litterfall (shed needles and other canopy material) in the southern and northern pine and spruce plots (number of plots in parenthesis). The amount of N in LE was calculated as 6% from the N of litterfall (Ukonmaanaho and Starr 2001).

772 a)

773

774 b)

775

776 Figure 3. Box-plot diagrams of total mossN% of three species (*Pleurozium schreberi*,
 777 *Hylocomium splendens* and *Dicranum* spp.) collected from forests and open sites a) in
 778 northern vs. southern parts of Finland and b) in Scots pine vs. Norway spruce dominated
 779 areas. Middle line in a box is a median, the box shows 75 % and 25 % percentiles, and the
 780 whiskers denote 97.5 % and 3.5 % percentiles.

781

782 a) *Pleurozium schreberi*

783

784 b) *Hylocomium splendens*

785

786

787 c) *Dicranum* spp.

788

789 Figure 4. Total mossN% in relation to different N forms (N_{tot}, N_{inorg} and DON) in bulk
 790 (samples from openings) and in throughfall (TF) deposition (samples from forests) for a)
 791 *Pleurozium schreberi*, b) *Hylocomium splendens* and c) *Dicranum* spp. (only forest samples).
 792 Dominant tree species in the sampling site given in the point colour. The outlier plot Uus23S
 793 (near a fox farm), marked as a point in figures, is excluded in the basic models (solid line),
 794 but included in the extended TF models (dashed line). R² value tells the fit of the basic
 795 model. Model tables for response curves in Supplement Table S.4a,b.

796

Figure 5. Accumulation curves with 95 % confidence intervals for a) NH_4^+-N and b) N_{tot} ($\mu g g^{-1}$) in moss tissues (mean of *P. schreberi* and *H. splendens*, years 2002 or 2003) vs. $TF N_{tot}$ deposition.

804 Figure 6. Response curves for the total N deposition (y) in the background forests of Finland
805 using mossN% as a predictor (x). Left side column: bulk N_{tot} deposition vs. moss samples
806 from forest openings and right side column: TF N_{tot} deposition vs. moss samples inside
807 forest. Mean mossN% values of the two feather mosses is used as a predictor in models in
808 the upper row and separate models are given for *P. schreberi* and *H. splendens* in the
809 middle.

812 TABLES

813

Sub-sample	Habitat	Sample years of mosses	N forms in mosses	Sample years of deposition, prec.	N forms in deposition
1	Forest n = 16	2009	N _{tot}	TF: 2007 - 2009	N _{tot} , NH ₄ ⁺ -N, NO ₃ ⁻ -N, DON
2	Opening n = 11	2009 (2010)	N _{tot}	BD: 2007 - 2009	N _{tot} , NH ₄ ⁺ -N, NO ₃ ⁻ -N, DON
3	Forest n = 10	2002 (2003)	N _{tot} , NH ₄ ⁺ -N, NO ₃ ⁻ -N, DON	TF: 2000 – 2002, (2001 - 2003)	N _{tot} , NH ₄ ⁺ -N, NO ₃ ⁻ -N, DON

814

815 Table 1. Overview of measurements in the three subsamples of mosses, N deposition and
816 precipitation (prec.) in different years. n = total number of sites. Years in parenthesis include
817 2 - 3 sites. TF = throughfall, BD = bulk deposition. We calculated three-year averages of the
818 deposition values corresponding the growth period of moss shoots used in chemical
819 analyses.

820

ICP Forests			Forest					Forest			Opening	
plot name	Plot abbr.	N latitude	Tree species	Basal area m ² ha ⁻¹	Canopy cover%	Org. soil C:N	Mosses cover %	Hylo sple mossN%	Pleu schr mossN%	Dicr spp. mossN%	Hylo sple mossN%	Pleu schr mossN%
Sevettijärvi1P	Sev1P	69°34'	pine	13.5	35	50.2	17		0.8	0.8		0.5 ^m
Pallasjärvi3S	Pal3S	67°59'	spruce	15.4	40	46.3	93	1.0	0.8	1.1	0.6	0.5 ^m
Kivalo6P	Kiv6P	66°21'	pine	18.9	23	46.8	89	0.8	0.8	1.1	0.6	0.6
Kivalo5S	Kiv5S	66°19'	spruce	18.0	33	41.3	90	1.1	1.0	1.3	0.7	0.6
Oulanka21S	Oul21S	66°18'	spruce	21.0	48	53.4	90	1.0	0.9	1.1	0.6 ^m	0.5 ^m
Lieksa20P	Lie20P	63°09'	pine	22.3	28	52.6	93	0.9	1.0	1.2	0.5 ^m	0.6 ^m
Uusikaarlepyy23S*	Uus23S	63°33'	spruce	32.7	51	21.7	40	1.9	2.2	1.9	1.7	1.4
Juupajoki10P	Juu10P	61°52'	pine	23.6	29	35.4	90	1	1.1	1.2	0.7	0.7
Juupajoki11S	Juu11S	61°51'	spruce	30.4	35	34.9	57	1.2	1.2	1.3	0.9	0.7
Punkaharju16P	Pun16P	61°46'	pine	32.0	60	35.1	96	1.2	1.2	1.3	0.7	0.7 ^m
Punkaharju17S	Pun17S	61°48'	spruce	30.8	42	31.7	75	1.4	1.7	1.9	1.2	0.9
Evo19S	Evo19S	61°14'	spruce	49.7	73	30.8	43	1.7	1.6	2.1	1 ^m	0.9 ^m
Luumäki34P	Luu34P	60°55'	pine	13.6	33	40.0	38		1.2	1.2		0.8 ^m
Luumäki35S	Luu35S	60°55'	spruce	28.2	65	26.7	84	1.5	1.4	1.6	0.9 ^m	0.8 ^m
Tammela13P	Tam13P	60°36'	pine	29.3	34	34.0	60	1.6	1.4	1.4	0.9 ^m	0.8 ^m
Tammela12S	Tam12S	60°38'	spruce	33.1	69	30.5	72	1.5	1.7	1.8	1.1	1.0

Table 2. Stand characteristics of the forest plots and mossN% of *Hylocomium splendens* (Hylo sple), *Pleurozium schreberi* (Pleu schr) and *Dicranum* spp. (Dicr spp.) collected inside (2009) and outside (2009-2010) forests. Missing data in openings modelled (marked ^m). Plots ordered along the north-south gradient (N latitude). The first six plots in the list belong to “north” and the remainder ten to “south”. Location of the plots marked in Fig. 1. Stand basal area and cover % mosses from the year 2009, C:N ratio of the organic soil layer from 2006 and cover% of tree canopy from 2014. Pine = *Pinus sylvestris*, spruce = *Picea abies*. *: Uus23S is an outlier plot owing to high N emissions from a nearby fox farm.

Mode I	Predictor	Bulk deposition			TF deposition		
		R ²	P values		R ²	P values	
			N form, stand	Interact		N form, stand	Interact.
a) <i>Pleurozium schreberi</i>							
1	N _{tot}	80.8	0.001		73.4	0.001	
2	N _{tot} +Tree+N _{tot} :Tree	89.8	0.001, ns ^T	ns	82.7	0.001, ns ^T	0.08
3	NH ₄ -N	74.4	0.001		39.6	0.007	
4	NO ₃ -N	80.0	0.001		35.6	0.011	
5	DON	39.2	0.007		89.1	0.001	
6	N _{inorg}	81.5	0.001		39.3	0.007	
7	N _{inorg} +DON	80.0	0.001 ⁱ ,ns ^D		88.2	ns ⁱ , 0.01 ^D	
8	N _{inorg} +DON:BA	81.9	0.001	ns	84.9	0.106	0.001
b) <i>Hylocomium splendens</i>							
1	N _{tot}	52.5	0.003		59.7	0.001	
2	N _{tot} +Tree+N _{tot} :Tree	83.1	0.03, ns ^T	ns	66.8	0.03, ns ^T	ns
3	NH ₄ -N	46.9	0.006		29.3	0.03	
4	NO ₃ -N	55.0	0.002		27.3	0.04	
5	DON	33.4	0.02		71.2	0.001	
6	N _{inorg}	51.8	0.01		30.2	0.03	
7	N _{inorg} +DON	47.4	0.08 ⁱ , ns ^D		69.1	ns ⁱ ,0.003 ^D	
8	N _{inorg} +DON:BA	47.4	0.01	ns	71.6	ns ⁱ	0.002
c) <i>Dicranum</i> spp.							
1	N _{tot}				53.9	0.001	
2	N _{tot} +Tree+N _{tot} :Tree				81.3	0.02,ns ^T ,	0.06
3	NH ₄ -N				21.5	0.05	
4	NO ₃ -N				18.1	0.06	
5	DON				78.5	0.001	
6	N _{inorg}				20.8	0.05	
7	N _{inorg} +DON				79.5	ns ⁱ ,0.001 ^D	
8	N _{inorg} +DON:BA				83.4	ns ⁱ	0.001

829

830 Table 3. Comparison of the models (no 1 – 8) explaining tissue N% of three moss species
831 using different N forms and stand variables as predictors. Moss samples collected from open
832 sites were modelled vs. bulk (BD) and those from forests vs. throughfall (TF) deposition. For
833 *Dicranum* spp. only forest data was available. Adjusted R² for models and P values for
834 predictors are given (the 1st P value is always for the 1st predictor in a model). Abbreviations:
835 interact. = interaction term, BA = stand basal area, i = N_{inorg}, D = DON, T = Tree species
836 (pine, spruce), ns = not significant. The outlier plot Uus23S omitted from the models. The
837 model tables are given in Supplement Table S.4a.

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839