

# The fate of $^{15}\text{N}$ -nitrate in a northern peatland impacted by long term experimental nitrogen, phosphorus and potassium fertilization

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**Abstract** Information about the impact of nitrogen (N) deposition on the fate of deposited N in peatland ecosystems is lacking. Thus we investigated the fate of experimentally added  $^{15}\text{N}$  in long-term N-fertilized treatments in a *Sphagnum*-dominated ombrotrophic bog. Fertilization significantly stimulated vascular plant and suppressed *Sphagnum* and *Polytrichum* moss growth. N content in peat, mosses, and vascular plants was raised by the fertilizer addition and reached a maximum at  $3.2 \text{ g m}^{-2}$  N input level with phosphorus (P) and potassium (K) addition. Most of N was retained in the vegetation and upper 10 cm of the peat. When N deposition equalled  $1.6 \text{ g m}^{-2}$  and less, or  $3.2 \text{ g m}^{-2}$  N with P and K addition, no inorganic N leaching was observed on the plots. This result indicates that co-fertilization with P and K raised the N retention capacity and that critical N loads with respect to N saturation depend on P and K

availability. Most of the deposited  $^{15}\text{N}$  was recovered in the bulk peat, which may be related to a rapid immobilization of inorganic N by microorganisms and mycorrhizal assimilation. Increase of N, P, and K fertilization increased the contribution of vascular plants to N retention significantly and reduced those of mosses. The increase was mainly related to enhanced productivity, vascular biomass and N content in tissues; the reduced retention by mosses resulted from both reduced moss biomass and assimilation. The study shows that the N filter function of ombrotrophic bogs will be influenced by interactions with other nutrients and shifts in plant community structure.

**Keywords** Peatland · Bog · Nitrogen deposition · Nitrogen saturation · Nitrogen retention

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## Introduction

Rates of atmospheric nitrogen (N) deposition are two to seven times the pre-industrial levels in many developed nations because of combustion of fossil fuels and agricultural fertilization (Vitousek et al. 1997; Galloway et al. 2004; Lamers et al. 2000; Moore et al. 2004). The increasing N deposition from the atmosphere has important implications for peatland ecosystems where N is a limiting nutrient for primary production. *Sphagnum*-dominated ombrotrophic peatlands are exclusively fed by wet and dry atmospheric deposition, which makes them sensitive to increased atmospheric N input (Bragazza et al. 2005). Concerns over the effects of increasing atmospheric N deposition on forest ecosystems have prompted many ecosystem-level N addition experiments over the last two decades (Kahl et al. 1993; Magill et al. 1997; Pregitzer et al. 2004). In comparison, we know less about the fate of N in peatland ecosystem and how the ecosystem structure and functioning are altered over longer periods of time.

Several experimental studies have suggested that both *Sphagnum* mosses and peat play a crucial role in retaining N and that high chronic N pollution leads to a decrease in N retention. Lamers et al. (2000) proposed that the natural nitrogen filter of ombrotrophic *Sphagnum* mosses would fail in raised bogs when N deposition is larger than 12–18 kg ha<sup>-1</sup> year<sup>-1</sup>. Li and Vitt (1997) used <sup>15</sup>N as a mean of elucidating N dynamics in a bog and a rich fen in continental western Canada for 2 years, and found that nearly all N was immediately sequestered by the moss layer, while shrubs were little affected. From their study, 29% <sup>15</sup>N was recovered from the top 0–5 cm of *Sphagnum* mosses in the bog. A study by Williams et al. (1999) showed that mosses absorbed on average 72% of added <sup>15</sup>N in 2 weeks and that little or no <sup>15</sup>N was detected in the underlying peat. Curtis et al. (2005) reported that *Sphagnum* moss and lichens showed far greater <sup>15</sup>N recovery per unit biomass than grasses and shrubs in a tracer study in moorland catchments, but the recovery in moss declined as N deposition increased. Blodau et al. (2006) reported that on average 48% of applied <sup>15</sup>N was obtained from the *Sphagnum* moss cover in mesocosms of two Canadian peatlands that had received low to moderate long-term N deposition,

were extracted from hollows, and were mostly devoid of vascular plants. While such findings are valuable, we lack comprehensive information about the fate of N under different long-term N deposition levels, and its distribution and transfer rates between vascular plants, mosses, peat, microbial biomass, and leachate in situ.

A further important finding of previous studies has been that the vegetation composition in peatlands appears to change with increasing N input (Bobbink et al. 1998; Berendse et al. 2001). *Sphagnum* moss growth was significantly reduced while vascular plant biomass increased when N was added to peatlands over longer periods of time (Heijmans et al. 2001; Bubier et al. 2007). These vegetation structure changes may affect many aspects of ecosystem functioning in peatlands, including N retention. A study conducted by Bragazza et al. (2005) regarding N retention ability of *Sphagnum* at different N deposition levels in Europe indicated the filter capacity decreased exponentially along N depositional gradient. Some evidence showed decreased nitrogen use efficiency with increased N availability for wetland vascular plants (Small 1972; Shaver and Melillo 1984; Bridgham et al. 1995). Aerts et al. (1992) and Hoosbeek et al. (2002) suggested that under elevated N deposition, potassium (K) and phosphorus (P) restrict *Sphagnum* moss growth and the filter function for N. These short- and long-term isotope tracer studies provided thus quite different results and indicate a need to understand the fate of N in these ecosystems in more detail, taking different levels of N deposition, the effects of P and K availability, and changes in the vegetation structure into account. We hypothesized that experimental long-term deposition of N, in addition to background deposition, would lead to a higher mobility and lower retention of N in vegetation and near-surface peat. This should be the case particularly at deposition levels well above 1.5–2.0 g m<sup>-2</sup> year<sup>-1</sup>, which have previously been identified as a threshold for N saturation in *Sphagnum* mosses representing a most important N filter in ombrotrophic bogs (Lamers et al. 2000; Vitt et al. 2003; Nordbakken et al. 2003; Bragazza et al. 2005). We further expected that concurrent fertilization with P and K, and shifts in the vegetation structure, would decrease N mobility and increase N retention by the vegetation by mitigating nutrient constraints.

To test these hypotheses, we carried out a  $^{15}\text{N}$  tracer study in a northern ombrotrophic bog in Canada that had experimentally been fertilized at N deposition levels of 0, 1.6, 3.2, and 6.4  $\text{g m}^{-2} \text{ year}^{-1}$  with and without addition of P and K for a period of 7 years, prior to the tracer experiment (Bubier et al. 2007). As treatments of exclusive N deposition of 3.2 and 6.4  $\text{g m}^{-2} \text{ year}^{-1}$  were only established 3 years into the experiment, we only analyzed and report the fate of  $^{15}\text{N}$ -nitrate in NPK fertilized plots and plots fertilized exclusively with N at a level of 1.6  $\text{g m}^{-2} \text{ year}^{-1}$ , and plots fertilized exclusively with P and K. Our specific objectives were to understand the fate of fertilized N in each ecosystem pool along with increasing N input and availability of P and K.

## Materials and methods

### Study sites

The Mer Bleue peatland is a large ombrotrophic bog located in the Ottawa River Valley, 10 km east of Ottawa, Ontario, Canada (45.40° N latitude, 75.50° W longitude). Mean annual temperature is 6.0°C, ranging from -10.8°C in January to 20.9°C in July, and annual precipitation 944 mm (Environment Canada; 1971–2000 climate normal). More detailed descriptions of the site can be found in Bubier et al. (2006, 2007) and Moore et al. (2004). Plant communities are dominated by the ericaceous shrubs *Chamaedaphne calyculata* (L.) Moench, *Ledum groenlandicum* Oeder and *Kalmia angustifolia* L. Clusters of the deciduous shrub *Vaccinium myrtilloides* Michx. The ground layer is dominated by mosses, mainly *S. magellanicum* Brid., *S. capillifolium* (Ehrh.) Hedw. and *Polytrichum strictum* Brid. (Bubier et al. 2006). The regional wet deposition rate of inorganic N from 1990 to 1996 was 0.81–1.2  $\text{g m}^{-2} \text{ year}^{-1}$  of which ca. 60% was deposited as  $\text{NO}_3^-$  (Bubier et al. 2007); including dry N deposition this value was likely about 1.5  $\text{g m}^{-2} \text{ year}^{-1}$ , representing the highest N deposition levels in North America (Moore et al. 2004).

### Experimental design and field sampling

We established triplicate 3 m × 3 m plots in fairly homogeneous appearing areas of hummock vegetation

for each of six treatments. Relative to the lowest peat surface in the vicinity of the experimental plots, average elevation ( $n = 9$ ) of plots as determined by leveling ranged from 26.3 to 37.4 cm (average 30.8 cm), with averages of treatments being different from each other by less than 2 cm. *Sphagnum capillifolium* homogeneously dominated the plots with about 90% coverage and some additional *S. magellanicum* in wetter locations, which were somewhat more abundant on the PK treatment, see below. Nutrients were added in the equivalent of 2 mm of water, seven times from early May to early September. The six treatments, separated by at least a one meter buffer zone, encompassed triplicate plots (Table 1). These consisted of a control treatment with no nutrient but distilled water addition; a PK treatment with P and K addition, a 5 N treatment with 5 times the wet ambient summer N deposition, which was assumed as 0.32  $\text{g N m}^{-2}$ ; and 5NPK, 10NPK and 20NPK treatments, representing 5, 10, and 20 times ambient wet summer N deposition, as well P and K addition. Nitrogen was added in 2 mm of irrigate in 7 doses per year as  $\text{NH}_4\text{NO}_3$  and P and K as  $\text{KH}_2\text{PO}_4$  from 2000 to 2007; the 10NPK and 20NPK applications started in 2001. Solute concentrations in irrigate were 4.12, 8.24, and 16.49  $\text{mmol l}^{-1}$  (5N, 10N, 20N as  $\text{NH}_4\text{NO}_3$ ) and 11.54  $\text{mmol l}^{-1}$  ( $\text{KH}_2\text{PO}_4$ ) in PK treatments. Much of the solute was intercepted by shrubs and washed down with subsequent rain. The rationale for adding P and K was to study the impact of

**Table 1** Fertilization treatments and  $^{15}\text{N}$  application levels at the Mer Bleue bog

Treatments ( $\text{g m}^{-2} \text{ year}^{-1}$ )	N ( $\text{NH}_4\text{NO}_3$ )	P $\text{KH}_2\text{PO}_4$	K $\text{KH}_2\text{PO}_4$	$^{15}\text{N}$ application ( $\text{mg m}^{-2}$ )
Control	0	0	0	91
PK	0	5.0	6.3	91
5N	1.6	0	0	229
5NPK	1.6	5.0	6.3	229
10NPK	3.2	5.0	6.3	457
20NPK	6.4	5.0	6.3	914

‘Treatment’ refers to the following experimental fertilizer load: Control without nutrient but distilled water addition; PK with only P and K addition, 5N with only N addition as 5 times of the ambient summer N deposition, which assumed as 0.32  $\text{g m}^{-2}$ ; 5NPK, 10NPK and 20NPK as 5, 10, 20 times of ambient summer N deposition and P and K input

growing N deposition independently of other potential nutrient constraints, and to study effects of interactions at a lower nutrient load only. This was done in recognition of limited resources; additional N treatments were added 3 years later but have not been analyzed in this study.

Peat cores, plants, roots, and pore water were sampled three times from July to October 2007, while recording hydrological and meteorological conditions. On, 6 August, 2007, samples for background values of  $^{15}\text{N}$  in peat, plants, roots, and microbial biomass were taken, two peat cores of  $10 \times 10$  cm from each plot. Four common species of vascular plants were sampled from each plot including *Chamaedaphne calyculata* (L.) Moench, *Ledum groenlandicum* Oeder and *Kalmia angustifolia* L and *Vaccinium myrtilloides* Michx. The dominant mosses, *S. magellanicum* Brid., *S. capillifolium* (Ehrh.) Hedw. and *P. strictum* Brid were collected and subsequently treated as one functional group in the context of this work. The stems and leaves of each vascular plant species were clipped and separated. Rhizon samplers (Eijkelkamp Agrisearch Equipment) and suction cups were installed to collect the pore water from three depths of 5–10, 15–25 and 30–40 cm. Samples were cooled, transported to the laboratory and frozen within 1 day.

Subsequently, the  $^{15}\text{N}$  tracer was applied as  $\text{NH}_4^{15}\text{NO}_3$  (10%) by substituting one regular fertilization application of  $\text{NH}_4\text{NO}_3$ , or 1/7 of the total N load per season. On the Control and PK treatments we applied only 1/7 of twice the ambient N deposition to avoid a fertilization effect of the tracer (Table 1). After 4 weeks (“2nd sampling”) and 8 weeks (“3rd sampling”) the plots were sampled again to recover the tracer. During this period, several precipitation events of some millimeter each occurred, such as on August 25th and 28th, and September 7th and 10th. As two of three plots per treatment had been labeled before in 2001, and remaining background  $^{15}\text{N}$  signals strongly varied on these plots, we were only able to analyze one plot per treatment with respect to the fate of the  $^{15}\text{NO}_3^-$  applied by us in 2007.

Peat samples were collected in 5 cm segments from 5, 10, 20, 30, and 40 cm depths. Fine roots, diameter  $< 1$  mm, and larger roots, diameter  $> 1$  mm, were hand-picked out with tweezers, and rinsed with deionised water. Bulk density of peat was calculated from the dry mass of segments and their

volume. A  $20 \times 20$  cm peat core with all above-ground plants was collected from each plot for vascular plant biomass counting (20 cm height was counted from surface of peat core). Total above- and belowground biomass, including visibly intact plant detritus, was quantified from these cores and extrapolated to unit area. Moss biomass was estimated from moss percent cover and growth rate and converted to gram per  $\text{m}^2$ . Subsamples of peat were sealed in plastic bags and stored at  $4^\circ\text{C}$  for up to 5 days before the extraction of microbial N.

All organic samples were dried at  $60^\circ\text{C}$ , ground with a ball mill to fine powder, and analyzed for  $\delta^{15}\text{N}$ , beginning with the lowermost segments to avoid contamination from the upper layers. Samples were stored in an evacuated desiccator prior to analysis.

#### Chemical analyses

The concentration of  $\text{NH}_4^+$  was measured by a salicylate colorimetric method on a photometer at 690 nm.  $\text{NO}_3^-$  was measured by ion chromatography, total N (TN) by Multi N/C 2100S analyzer (Analytik Jena) and dissolved organic N (DON) calculated by the difference between TN and the sum of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations. Peat and root samples from the upper 10 cm of peat were analyzed for content and isotopic ratio ( $\delta^{15}\text{N}$ ) of nitrogen using a Carlo Erba NA 1500 coupled to a Finnigan MAT delta D mass spectrometer.  $\delta^{15}\text{N}$  of peat was analyzed in treatments PK and 20NPK in all depths for assessing mobility of  $^{15}\text{N}$  in the peat. These treatments were assumed to represent end members in terms of N mobility.

To determine N isotope ratios in the N pools of the collected pore water, a sequential diffusion procedure was used, separately trapping individually  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and TN (Brooks et al. 1989), as modified and described in detailed in Blodau et al. (2006). In brief, the steps included volatilization and trapping of  $\text{NH}_4^+$  under basic conditions, conversion of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  by metals, repeating volatilization, and a Kjeldahl digestion of the remainder, repeating volatilization once again. The recovery rates of nitrogen after distillation for  $\text{NH}_4\text{Cl}$  and  $\text{NaNO}_3$  reached more than  $86 \pm 12$  and  $83 \pm 15\%$  in most of tests to validate the method.

Microbial biomass C and N was analyzed by a fumigation-extraction technique that has been detailed

in Basiliiko et al. (2006). Briefly, the peat samples were divided into two subsamples that were treated with or without chloroform ( $\text{CHCl}_3$ ) fumigation to measure microbial C and N. To quantify the  $^{15}\text{N}$  content of microbial biomass, the fumigated and non-fumigated samples were subjected to Kjeldahl digestion, and processed as the  $^{15}\text{N}$ -DON in pore water.

The  $^{15}\text{N}$  results were expressed in  $\delta$ -notation as the deviation from the international standards

$$(\text{AIR}) : \delta^{15}\text{N}[\text{‰}] = \left[ \frac{^{15}\text{N}/^{14}\text{N}_{\text{sample}}}{^{15}\text{N}/^{14}\text{N}_{\text{reference}}} - 1 \right] \cdot 1000 \quad (1)$$

The primary reference for N isotope abundance measurements is atmospheric  $\text{N}_2$ . For these analyses the reference material acetanilide were repeatedly analyzed for calibration and measurement control. The analytical uncertainty for measurement of isotopically labeled N, determined by comparison with reference materials, was  $\pm 2\%$ .

#### Calculations and statistics

We calculated biomass and N content of the ecosystem pools, as well as  $\delta^{15}\text{N}$  ratios and  $^{15}\text{N}$  content and  $^{15}\text{N}$  recovery from the pools. The N content ( $\text{g N m}^{-2}$ ) of each N pool was calculated as the product of its mass ( $\text{g m}^{-2}$ ) and N content ( $\text{mg N g}^{-1}$  dw (dry weight)). Area based dissolved N was calculated as the product of water content in the peat and N concentrations in pore water. Water content was measured in situ by FDR (Function Domain Reflectory)  $\text{ECH}_2\text{O}$  EC-5 probes calibrated for the peat. Similarly, we estimated the mass of  $^{15}\text{N}$  in each pool as the product of its  $^{15}\text{N}$  abundance ( $\text{atom}\% \text{ }^{15}\text{N}$  excess =  $\text{atom}\% \text{ }^{15}\text{N}$  in pool after labelling –  $\text{atom}\% \text{ }^{15}\text{N}$  in pool before labelling) and N content ( $\text{g N m}^{-2}$ ). The results were expressed as g or  $\text{mg N m}^{-2}$  per 10 cm thick layer.

Total recovery was calculated relative to the tracer added to plot as shown in Eq. 2.

$$\text{Recovery} = \frac{\left[ \frac{^{15}\text{N}}{^{15}\text{N}+^{14}\text{N}} \cdot (^{15}\text{N} + ^{14}\text{N}) \right]_{\text{measured}}}{\left[ \frac{^{15}\text{N}}{^{15}\text{N}+^{14}\text{N}} \cdot (^{15}\text{N} + ^{14}\text{N}) \right]_{\text{added}}} * 100\% \quad (2)$$

To compare the relative distribution of  $^{15}\text{N}$  among the ecosystem pools their relative share of

the total  $^{15}\text{N}$  recovered was calculated, standardized on 100%, and also expressed in percent recovery. To compare the relative recovery of  $^{15}\text{N}$  among treatments, recovery had to be standardized to the one-time  $^{15}\text{N}$  tracer input, which differed among treatments as outlined. The  $^{15}\text{N}$  recovered from a treatment plot was standardized to the lowest added  $^{15}\text{N}$  tracer load applied to the Control treatment (Eq. 3).

$$^{15}\text{N}_{\text{recovered,standardized}} = \frac{^{15}\text{N}_{\text{recovered,treatment}}}{^{15}\text{N}_{\text{added,treatment}}} \cdot ^{15}\text{N}_{\text{added,control}} \quad (3)$$

In terms of N contained in peat, roots and microbial biomass, only data from the upper 10 cm were analyzed statistically, as this was the only layer where information was available for all treatments. Microbial  $^{15}\text{N}$  was quantified as the difference of recoveries between fumigated and nonfumigated peat extracts. Microbial  $^{15}\text{N}$  was subtracted from soil  $^{15}\text{N}$  to provide the  $^{15}\text{N}$  recovery from peat itself. For a comparison of  $^{15}\text{N}$  retention among ecosystem pools, a relative retention was calculated based on a hypothetical 100% recovery from each treatment. Because of the previous  $^{15}\text{N}$  labeling at the beginning of fertilization in 2001, we only present data from the previously unlabelled plots. For pore water, no  $^{15}\text{N}$  background was obtained and  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in precipitation used instead (Peterson and Fry 1987). Given the  $\delta^{15}\text{N}$  variation in background samples was  $\pm 20\%$ , equal to  $\pm 0.008$  at.%, the influence of this approximation on the results was limited.

All statistical analyses were performed with the SPSS 11.5 package (SPSS Inc 2003, Chicago, Illinois, USA). Prior to statistical analysis, data were examined for homogeneity of variance and log transformed when required; however, untransformed means and standard errors are presented in figures and tables. Comparisons of treatment effects on N pools were analyzed by one-way ANOVA. The data of N content and total N stock, and  $^{15}\text{N}$  retention were not replicated, so a two-way ANOVA without replication was used. Tukey's honestly significant difference (HSD) test for multiple comparisons among means was employed to test for differences among treatments. Significance was set at  $p \leq 0.05$ .

## Results

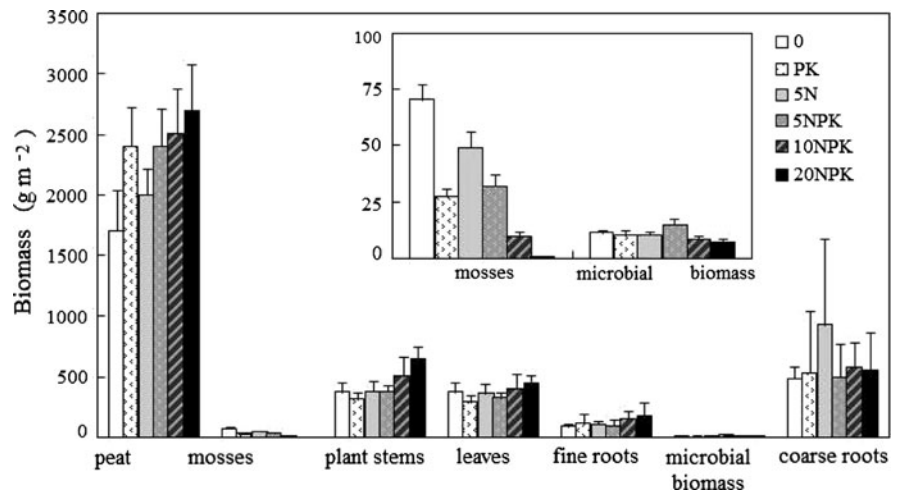
### Effects of fertilization on ecosystem structure and biomass

The effects of nutrient load on mass contained in the ecosystem pools are illustrated in Fig. 1. Addition of the nutrients affected peat mass, moss and vascular plant stem and root biomass significantly, but not vascular plant leaf biomass ( $p = 0.19$ ) and microbial C biomass ( $p = 0.42$ ). Aboveground, moss biomass decreased from  $70.5 \pm 8.0 \text{ g m}^{-2}$  (Control) to  $0.42 \text{ g m}^{-2}$  (20NPK). This estimate was based on a *Sphagnum* growth of  $106 \text{ g m}^{-2}$  and a *Polytrichum* growth of  $19 \text{ g m}^{-2}$  during the growing season and a decrease in surface coverage from  $91.8 \pm 6.6\%$  in Control to  $2.2 \pm 0.9\%$  in 20NPK, where only some *Polytrichum* remained. Vascular plants contributed most to aboveground biomass with  $597 \pm 116$  to  $1108 \pm 154 \text{ g m}^{-2}$ , including only stems and leaves; their biomass was highest in treatment 10NPK and

20NPK (Table 2). Fertilization raised the stem to leaf ratio and the aboveground contribution to total vascular biomass from 49.8% in Control to 59.2% in the 20NPK treatments. Fertilization significantly affected stem ( $p = 0.029$ ) but not leaf biomass. Multiple comparisons indicated that the difference between treatments was caused by the separation of PK and 20NPK from the other treatments. Vascular plant biomass did not compensate for the loss of moss growth in treatments 5NPK and PK, which had a lower vascular plant biomass than the Control.

Belowground, peat mass in the upper 10 cm layer varied from 1700 to  $2700 \text{ g (dw) m}^{-2}$  (Fig. 1). The least peat was contained in upper 10 cm of the Control, and the most in 20NPK plots. Differences were only significant between the Control and other treatments. Larger roots accounted for 3–10 times the mass of fine roots. The ratio of large to fine roots generally decreased with fertilization level suggesting enhanced fine root growth with nutrient load. The highest ratio of large to fine roots was observed in the

**Fig. 1** Mass of ecosystem pools (0–10 cm depth). Moss and microbial biomass are shown in the inset figure with different Y-axis scale



**Table 2** Biomass of vascular plant stems, leaves and roots in all treatments

Biomass ( $\text{g m}^{-2}$ )	Plant stems	Plant leaves	Fine roots	Coarse roots
Control	$373 \pm 42$	$373 \pm 42$	$90 \pm 60$	$482 \pm 417$
PK	$310 \pm 60$	$286 \pm 55$	$111 \pm 80$	$533 \pm 510$
5N	$380 \pm 88$	$360 \pm 83$	$105 \pm 21$	$930 \pm 706$
5NPK	$378 \pm 51$	$323 \pm 43$	$95 \pm 46$	$497 \pm 270$
10NPK	$503 \pm 157$	$396 \pm 124$	$154 \pm 54$	$578 \pm 201$
20NPK	$654 \pm 91$	$452 \pm 62$	$173 \pm 113$	$550 \pm 314$

Biomass was calculated for standard units from 20 cm  $\times$  20 cm peat cores with intact plants extracted from each treatment plot

5N treatment, where the biomass of large roots reached  $930 \pm 706 \text{ g m}^{-2}$ , 1.68 times the value in treatment 20NPK. Microbial biomass C ranged from  $7.2 \pm 0.85$  to  $15.2 \pm 4.1 \text{ g m}^{-2}$ . In contrast to the other pools, microbial biomass C fluctuated more and tended to decrease with the fertilization level (Fig. 1). Microbial biomass was largest in 5NPK and smallest in 20NPK plots, but differences were not significant among the treatments. Of the quantified pools, peat always contained the largest mass, with roots accounting for around 30% to the total peat mass. Aboveground vascular plants represented the second largest pool, exceeding the biomass of mosses, including *Polytrichum* and *Sphagnum*, by an order of magnitude (Fig. 1). The contribution of microbial biomass to the C pools was negligible.

#### Nitrogen contents and concentrations

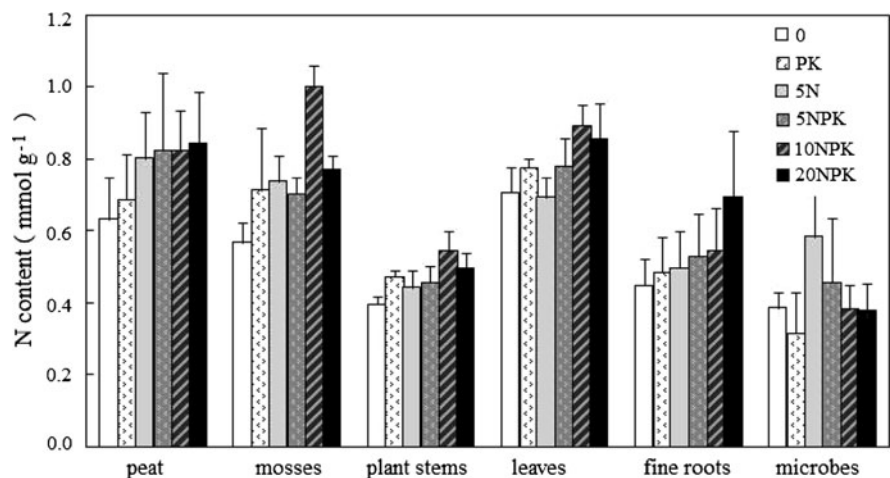
The treatments significantly affected N pools ( $p < 0.01$ ). Average nitrogen content increased generally with fertilization, rising in the sequence Control < PK < 5N < 5NPK < 20NPK < 10NPK, and ranged from  $0.54 \pm 0.08$  to  $0.70 \pm 0.06 \text{ mmol g}^{-1}$  (Fig. 2). Differences were only significant between Control and treatments 10NPK and 20NPK. Nitrogen content did not increase consistently in all pools, though. In plant stems, leaves and moss, the N content was highest in treatment 10NPK, whereas in peat and fine roots, N content peaked in 20NPK. The average N content in the ecosystem pools differed significantly from each other as well ( $p < 0.01$ ). The N contents of

peat, moss and vascular leaves were in same range from 0.71 to  $1.1 \text{ mmol g}^{-1}$ , double the content in vascular stems and roots. C:N ratios decreased with nutrient load in all biomass N pools. The lowest C:N ratios of 7–17 were found in microbial biomass, followed by moss, vascular leaves, root biomass and peat.

Dissolved concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  ranged from  $1.3 \pm 0.6$  to  $29.9 \pm 12.4 \text{ } \mu\text{mol l}^{-1}$  and  $0.74 \pm 0.5$  to  $46.8 \pm 25.5 \text{ } \mu\text{mol l}^{-1}$  in the upper 10 cm of peat pore water (Table 3). DON concentration was much higher and varied from  $90.1 \pm 11.6$  to  $506 \pm 136 \text{ } \mu\text{mol l}^{-1}$ . Treatments significantly affected N concentrations in pore water with highest  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations in treatment 20NPK, and highest DON concentration in treatment 5NPK, where  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations were also lowest.

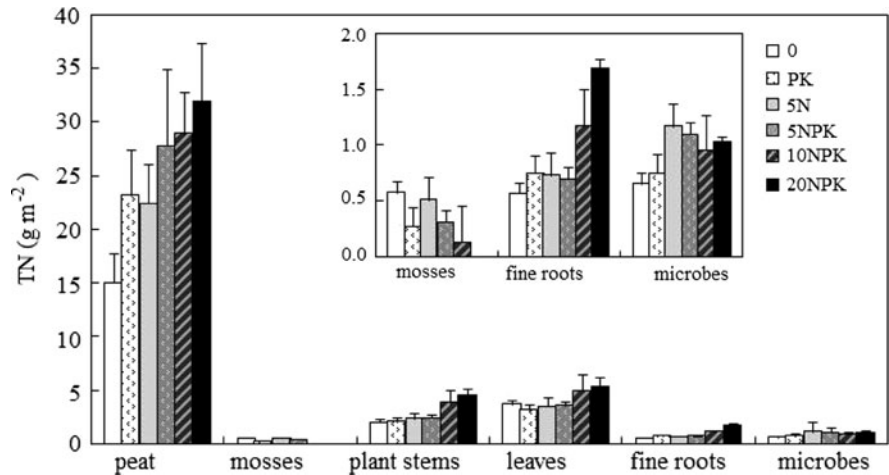
The total N reservoir on the plots, obtained by multiplying biomass with N content, was significantly elevated in the fertilized plots and increased in the order Control, PK, 5N, 5NPK, 10NPK and 20NPK, similar to the order of N content in treatments (Fig. 3). Difference were significant between treatments Control, PK, 5N, and treatments 5NPK, 10NPK, 20NPK ( $p < 0.01$ ), but not between Control and PK, and among 5NPK, 10NPK, 20NPK ( $p = 0.385$  and  $p = 0.096$ , respectively). In total, peat contained by far the largest N pool and microbial biomass the smallest. The vascular plants stored ten to hundreds times more N than the moss. The  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N and DON content ranged from 0.006 to  $0.87 \text{ mg m}^{-2}$ , and was thus negligible in comparison (Table 3).

**Fig. 2** Content of N in the ecosystem pools investigated



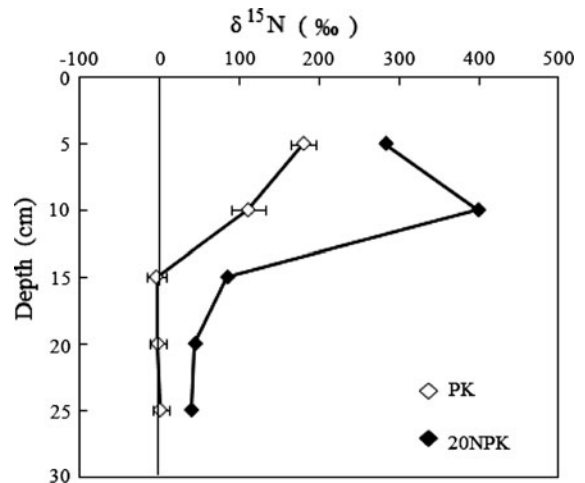
**Table 3** Concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and dissolved organic N (DON) in the pore water of the upper 10 cm of peat averaged over 3 sampling dates, and corresponding N pools of dissolved N ( $\pm$ standard deviation; for insignificant quantities values are not shown)

Treatments	$\text{NH}_4^+$ ( $\mu\text{mol l}^{-1}$ )	$\text{NO}_3^-$ ( $\mu\text{mol l}^{-1}$ )	DON ( $\mu\text{mol l}^{-1}$ )	$\text{NH}_4^+\text{-N}$ ( $\text{mg m}^{-2}$ )	$\text{NO}_3^-\text{-N}$ ( $\text{mg m}^{-2}$ )	TN ( $\text{mg m}^{-2}$ )
Control	$3.1 \pm 1.5$	$6.0 \pm 2.5$	$195.1 \pm 61.0$	0.02	0.02	$0.79 \pm 0.07$
PK	$2.7 \pm 1.9$	$3.5 \pm 1.6$	$140.7 \pm 30.9$	0.009	0.008	$0.73 \pm 0.31$
5N	$4.5 \pm 1.3$	$7.2 \pm 1.6$	$168.5 \pm 21.1$	0.009	0.017	$0.71 \pm 0.20$
5NPK	$2.2 \pm 0.9$	$1.7 \pm 0.5$	$155.7 \pm 23.3$	0.006	0.003	$0.72 \pm 0.16$
10NPK	$2.4 \pm 1.7$	$6.2 \pm 3.6$	$191.4 \pm 36.2$	0.013	0.029	$0.95 \pm 0.17$
20NPK	$29.9 \pm 2.8$	$46.8 \pm 9.1$	$211.0 \pm 60.8$	$0.146 \pm 0.006$	$0.228 \pm 0.007$	$1.15 \pm 0.27$

**Fig. 3** Total N content in the ecosystem pools investigatedRetention of  $^{15}\text{N}$ 

Increases in  $^{15}\text{N}$  content were detected in all N pools after labelling. In the bulk peat  $^{15}\text{N}$  ranged from 0.403 to 0.519 at.% and increased most in treatment 10NPK and 20NPK, whereas in Control, PK, 5N, and 5NPK the increase was smaller. Recovery of  $^{15}\text{N}$  after 4 weeks ranged from  $0.55 \pm 0.10 \text{ g m}^{-2}$  in Control to  $4.42 \pm 0.59 \text{ g m}^{-2}$  in 10NPK, excluding microbial biomass N. Depth profiles in treatment PK and 20NPK indicated a sharp decrease of the  $\delta^{15}\text{N}$  signal below 10 cm (Fig. 4).

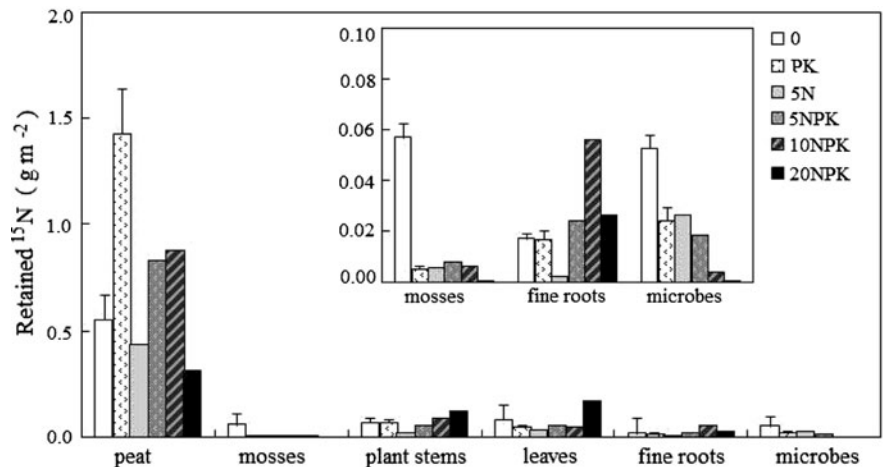
To account for the different load of tracer added in the treatments, we standardized  $^{15}\text{N}$  retention to the  $^{15}\text{N}$  input according to Eq. 3, which makes the retention of  $^{15}\text{N}$  in nitrogen pools comparable among treatments over the sampling period (Fig. 5). The following presentation of results is based on this procedure. Peat was the main sink for the added  $^{15}\text{N}$  but large differences between treatments occurred. In treatment PK, peat retained  $1.43 \pm 0.26 \text{ g m}^{-2}$  but in 20NPK only  $0.31 \pm 0.05 \text{ g m}^{-2}$ . In the moss,

**Fig. 4** Vertical profiles of  $\delta^{15}\text{N}$  in treatment PK and 20NPK

$^{15}\text{N}$  at.% was raised from 0.386% to a maximum of 0.602%. Retention in moss biomass was one to three orders of magnitude lower than in peat and ranged from  $0.001 \text{ g m}^{-2}$  in 20NPK to  $0.06 \text{ g m}^{-2}$  in the Control. In the vascular plants, the enrichment of  $^{15}\text{N}$



**Fig. 5** Standardized retention of  $^{15}\text{N}$  in the N pools and treatments. For calculations see text



varied from 0.390 to 0.640% in plant stems, and from 0.383 to 0.679% in plant leaves and reached highest levels in treatment 20NPK. Retention in plant stems was also lower than in peat by one order of the magnitude and ranged from  $0.02 \pm 0.005 \text{ g m}^{-2}$  in treatment 5N to  $0.12 \pm 0.02 \text{ g m}^{-2}$  in treatment 20NPK. Leaves retained slightly more, from  $0.03 \pm 0.008 \text{ g m}^{-2}$  (5N) to  $0.17 \pm 0.02 \text{ g m}^{-2}$  (20NPK). Enrichment in fine roots was similar as in above-ground parts of the plants, ranging from 0.374 to 0.604 at.%. However, due to their small biomass, much less  $^{15}\text{N}$  was retained in fine roots compared to above ground biomass, only  $0.002 \text{ g m}^{-2}$  compared to  $0.056 \text{ g m}^{-2}$ . Among the treatments, 5 N retained least and 10NPK most.

Regarding microbial biomass the strongest enrichment with  $^{15}\text{N}$ , 0.447 at.%, occurred in the Control plots, although the  $^{15}\text{N}$  tracer input was low, while the biomass was less enriched (0.373 at.%) in treatment 20NPK, despite with a 10-fold higher  $^{15}\text{N}$  tracer load. The  $^{15}\text{N}$  content in microbial biomass was the same magnitude as in mosses and fine roots, ranging from  $0.007 \pm 0.001 \text{ g m}^{-2}$  to  $0.066 \pm 0.047 \text{ g m}^{-2}$ . Standardized on  $^{15}\text{N}$  input, the  $^{15}\text{N}$  retention in microbial biomass ranged from  $0.001 \text{ g m}^{-2}$  to  $0.053 \pm 0.006 \text{ g m}^{-2}$ , and was highest in the Control and lowest in treatment 20NPK as well.

The  $^{15}\text{N}$  signal in dissolved inorganic and organic N was only analyzed on treatments Control, 5NPK and 20NPK. The  $\text{NO}_3^-$  in treatment 20NPK was most strongly enriched in the isotope, at up to 0.901 at.%, but owing to the low  $\text{NO}_3^-$  concentration,

it still accounted for a negligible quantity of the  $^{15}\text{N}$  applied. The overall recovery was only  $0.0002 \text{ mg m}^{-2} \text{ }^{15}\text{N-NH}_4^+$  and  $0.001 \text{ mg m}^{-2} \text{ }^{15}\text{N-NO}_3^-$  in Control and 5 NPK, and even in treatment 20NPK just  $0.063 \text{ mg m}^{-2} \text{ }^{15}\text{N}$  was recovered as  $\text{NO}_3^-$ . In general, recovered  $^{15}\text{N}$  in soil solution was very low and negligible compared other N pools.

#### $^{15}\text{N}$ balance and $^{15}\text{N}$ retention efficiency

After 4 weeks we recovered an estimated 53.8 to 176.6% of the applied  $^{15}\text{N}$ , including all N pools (Table 4). Recoveries of more than 100 % of the applied  $^{15}\text{N}$  are likely caused from uncertainty in the mass data of pools. This is especially relevant for the peat mass because bulk density and  $^{15}\text{N}$  recovery were determined from peat samples taken separately on the plots. The peat accounted for 49.1 to 81.6% of the total  $^{15}\text{N}$  recovery and uncertainty in the  $^{15}\text{N}$  content in this pool had thus a large impact on the recovery of  $^{15}\text{N}$  from the entire system. Vascular plants whose mass could be estimated more accurately, contributed 11.4 to 50.8% to the recovered  $^{15}\text{N}$  and were similarly important as the peat as a  $^{15}\text{N}$  sink on the 20NPK plots. The stems significantly retained more  $^{15}\text{N}$  than the leaves by a factor of two and more, despite a similar biomass, and dissolved  $^{15}\text{N}$  was negligible. Compared to the contribution of peat and vascular plants, moss and microbial biomass constituted a smaller pool, and the proportion declined with the increase of N load, accounting only for 0.1–0.15% in treatment 20NPK.

**Table 4**  $^{15}\text{N}$  recovery in all treatments and contribution of N pools to the total recovery in each treatment based on the second (4 weeks after tracer application) and third sampling campaigns (8 weeks after tracer application)

Treatment	Peat	Mosses	Plant stems	Plant leaves	Fine roots	Microbial biomass	Vascular plants	Total recovery
Between sampling 1 and sampling 2 (4 weeks)								
Control	64.3 ± 11.7	7.3 ± 1.2	8.9 ± 1.0	10.5 ± 1.2	2.2 ± 0.2	6.7 ± 0.8	21.6 ± 2.4	86.5 ± 16.1
PK	81.0 ± 16.3	3.5 ± 0.5	9.0 ± 0.9	2.6 ± 0.6	1.9 ± 0.2	2.8 ± 0.5	13.5 ± 1.7	176.6 ± 20.1
5N	81.6	1.3	4.4	6.5	0.5	5.3	11.4	53.8
5NPK	83.1	0.8	5.8	5.8	2.5	1.9	14.2	106.3
10NPK	81.1	0.6	8.1	4.7	5.2	0.4	17.9	117.1
20NPK	49.1	0.15	19.7	26.9	4.2	0.1	50.8	70.2
Between sampling 2 and sampling 3 (8 weeks)								
Control	76.5 ± 13.8	6.4 ± 1.4	2.5 ± 0.3	4.8 ± 0.2	1.8 ± 0.1	7.2 ± 0.8	9.1 ± 0.6	81.1 ± 16.6
PK	90.7 ± 16.3	1.9 ± 0.7	2.2 ± 0.4	1.9 ± 0.05	1.9 ± 0.3	1.6 ± 0.6	6.0 ± 0.75	167 ± 18.3
5N	83.7	5.3	0.3	0.2	1.6	4.8	2.1	61.5
5NPK	78.4	6.5	3.3	2.2	5.2	2.9	10.7	70.5
10NPK	33.1	11.2	35.9	25.1	2.7	1.6	63.7	29.2
20NPK	14.2	0.04	36.8	42.9	5.8	0.2	85.5	44.4

'Total recovery' represents the  $^{15}\text{N}$  recovery from each treatment plot according to Eq. 2 in the text which was based on the comparison between  $^{15}\text{N}$  recovery to experimental  $^{15}\text{N}$  input. The figures in the remainder of the table represent the percentage contribution of the respective N pool to total N recovery, which has been standardized to 100% in order to compare the relative contributions of ecosystem pools. Standard deviation was only available for treatment control and PK. Note that mosses on 20NPK plots consisted of *Polytrichum* only

The recovery of  $^{15}\text{N}$  4 weeks and 8 weeks after labeling suggested some differences in temporal dynamics of  $^{15}\text{N}$  translocation and transformations. In general, changes in tracer recovery with time were not significant ( $p = 0.091$ ), but in treatments 10NPK and 20NPK recovery declined from 117.1 to 29.2%, and from 70.2 to 44.4%, respectively (Table 4), mostly due to less tracer being retained in the peat. In treatment 10NPK peat initially retained  $0.88 \text{ g m}^{-2}$   $^{15}\text{N}$ , accounting for 81.1% of the recovered tracer, while after 4 weeks only  $0.09 \text{ g m}^{-2}$   $^{15}\text{N}$  was left. The pattern was similar in treatment 20NPK. This change was in part compensated for by increased  $^{15}\text{N}$  uptake by vascular plants. This pool increased its share of  $^{15}\text{N}$  recovered from 17.9 to 63.7% (10NPK) and from 50.8 to 85.5% (20NPK). In the other treatments, where tracer recovery varied less with time, peat remained the most important sink for the added tracer.

To eliminate the influence of bio- and peat mass, which differed across the treatments due to the impact of fertilization, we calculated a 'retention efficiency' per gram of dry weight of each pool

(Table 5). The effect of treatments on  $^{15}\text{N}$  retention efficiency was substantial, as the table illustrates, and varied by a factor of more than three, when all pools were accounted for. Statistically significant differences were only observed between treatment 5N and all other treatments ( $p = 0.018$ ), whilst no difference was detected among other treatments ( $p = 0.251$ ). In treatment 5N the retention efficiency of mosses and vascular plants was remarkably low.

The retention efficiency of all ecosystem pools was similar in magnitude, with the exception of mosses and microbial biomass, that were significantly greater. The N retention efficiency of mosses varied with treatments and the lower values were observed in treatments without P and K addition (5N), PK fertilization only, or in the high-N fertilized (20NPK) plots. Averaged over all the treatments, the mean retention efficiency was 0.29 (peat), 0.67 (moss), 0.13 (vascular stems), 0.19 (vascular leaves) and 0.18 (roots)  $\text{mg } ^{15}\text{N g (dw)}^{-1}$ . The retention efficiency of microbial biomass was calculated from measured C content, assuming that carbon accounted for 50% of microbial biomass, and averaged  $0.95 \text{ mg } ^{15}\text{N g (dw)}^{-1}$ .

**Table 5** Retention efficiency of N pools (averages based on the second and third sampling campaigns)

Treatments unit (mg g <sup>-1</sup> dry mass)	Peat	Mosses	Plant stems	Plant leaves	Fine roots	Microbial biomass	Average retention efficiency
Control	0.34	0.74	0.12	0.16	0.17	2.35	0.35 <sup>a</sup>
PK	0.59	0.28	0.17	0.14	0.21	1.15	0.27 <sup>a</sup>
5N	0.23	0.09	0.03	0.05	0.05	1.26	0.10 <sup>b</sup>
5NPK	0.29	0.77	0.10	0.11	0.30	0.61	0.24 <sup>a</sup>
10NPK	0.20	1.78	0.18	0.15	0.21	0.25	0.33 <sup>a</sup>
20NPK	0.07	0.34	0.21	0.38	0.14	0.05	0.21 <sup>a</sup>
Average	0.29 <sup>b</sup>	0.67 <sup>a</sup>	0.13 <sup>b</sup>	0.19 <sup>b</sup>	0.18 <sup>b</sup>	0.95 <sup>a</sup>	0.25

Numbers were calculated by dividing retained <sup>15</sup>N (in mg of N) in each pool by biomass and peat mass (in gram of dry mass), respectively. Superscript letters <sup>a</sup>, <sup>b</sup> in the last column and last row indicate the result of post hoc tests for average retention efficiency among treatments. Treatments with different superscript letter were significantly different from each other. Note that mosses on 20NPK plots consisted of *Polytrichum* only

In general, the <sup>15</sup>N retention efficiency of peat and vascular plants tended to decline between the second and third sampling, however differences were not significant ( $p = 0.175$ ).

## Discussion

We hypothesized that deposition of N would lead to lower retention of <sup>15</sup>N in vegetation and near-surface peat, particularly at a deposition level above 1.5–2.0 g m<sup>-2</sup> year<sup>-1</sup>, which has previously been identified as a threshold for N saturation in *Sphagnum* mosses based on observations along N deposition gradients and additional ecosystem experiments that added N only (Lamers et al. 2000; Berendse et al. 2001; Vitt et al. 2003). We also expected increasing dissolved inorganic N concentration in treatments fertilized at these levels and higher. However, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations were not elevated compared to controls, with exception of the 20 NPK treatment, which received 6.4 g N m<sup>-2</sup> year<sup>-1</sup>. This finding suggests that the filter function of the system remained intact up to an experimental deposition of 1.6 g N m<sup>-2</sup> year<sup>-1</sup> (5 N treatment), and beyond such loads when P and K were abundant. It also has to be seen that moss biomass had decreased and abundance of shrubs increased over the fertilization period with nutrient load (Bubier et al. 2007). Such a shift in the ecosystem structure should further reduce the system's ability to retain N because the *Sphagnum* moss layer has long been considered as the main filter for N in ombrotrophic bogs (Rudolph et al. 1993; Li

and Vitt 1997; Aldous 2002). Both findings raise questions about the mechanisms for N retention in the system and the way these mechanisms are sustained under chronic N deposition.

Based on previous work and our data, a number of mechanisms may have contributed to the relative robustness of N cycling despite considerable change in the ecosystem's structure. These mechanism include (I) increasing N contents in moss and vascular plant tissues, (II) increasing vascular plant biomass, (III) immobilization of inorganic N in the peat itself by microbial transformation and immobilization, and (IV) interactions with the availability of P and K, as indicated by the differential response of the 5 N, 5 NPK and PK treatments. Before discussing these mechanisms further, a few words on uncertainties in the data are required. First of all, we 'recovered' considerably more <sup>15</sup>N than applied in part of our plots. We believe that at least two factors contributed to this finding, namely inhomogeneous tracer application and high variability in bulk density, which had been determined on separate peat cores and was used to calculate the <sup>15</sup>N contained in the peat. The <sup>15</sup>N was applied the same way as the fertilizer in the 7 years before by using a hand held sprayer and attempting to homogeneously apply the solution. While over dozens of applications uneven spraying averages out, this was not the case for the <sup>15</sup>N application and we assume that this added variability in the recovery. Differences in interception by shrubs may have compounded variability in <sup>15</sup>N recovery; however <sup>15</sup>N signals in the vascular plants were fairly homogeneous suggesting that the tracer

was washed out with rainfall during this period. As a consequence of the methodological limitations, even large differences in  $^{15}\text{N}$  recoveries have to be treated with some caution. Second, N pools were not quantified below a depth of 10 cm, with the exception of the PK and 20NPK treatments. In the PK treatment, we detected no transfer of  $^{15}\text{N}$  label into larger depths. This is in agreement with Mer Bleue mesocosm experiments by Blodau et al. (2006) who reported little transfer of  $^{15}\text{N}$  beyond such depths at a N load of  $4.7 \text{ g N m}^{-2} \text{ year}^{-1}$  and with field results by Providoli et al. (2006) who recovered almost no applied  $^{15}\text{N}$  within 1 year in an alpine meadow. In the 20NPK treatment, however, about 27% of applied  $^{15}\text{N}$  was found below this depth, which adds some uncertainty about such a transfer in plots receiving an intermediate N load. Loss of  $^{15}\text{N}$  in form of  $\text{N}_2\text{O}$  was negligible (pers. comm. Sami Ullah) but rates of N fixation were not determined. Denitrification has generally been found to be a negligible to minor component of N cycling in peatbogs (Urban and Eisenreich 1988; Hemond 1983) but N fixation was found to be a significant input at an estimated rate of 0.1 to  $1.0 \text{ g N m}^{-2} \text{ year}^{-1}$ . The response of this process to N fertilization at high levels is unknown (Moore et al. 2004).

#### N retention by mosses

The response of N uptake of *Sphagnum* mosses to N loading has been conceived as a three phase process (Berendse et al. 2001) where (1) *Sphagnum* growth is initially stimulated at low levels of N ( $<1 \text{ g N m}^{-2} \text{ year}^{-1}$ ; Jauhiainen et al. 1994; Williams and Silcock 1997); (2) N no longer limits *Sphagnum* growth, but the *Sphagnum* layer has not yet reached its maximum organic N content, and (3) this content is reached and inorganic N leaches into the soil solution and becomes available for the roots of vascular plants (Bragazza et al. 2005). This three phase concept is broadly in agreement with our data, although we need to stress that we only considered plant functional types and did not separate *Sphagnum* species from *Polytrichum*. Nitrogen content rose approximately proportionally with N load and reached a maximum of  $15 \text{ mg N g}^{-1}$  in treatment 10NPK; in the 20NPK treatment, which was almost devoid of mosses, the content was lower. However, even the highest N content in the 10NPK treatment

remained well below N enrichment that have been described previously. Heijmans et al. (2001) found that N content in *Sphagnum magellanicum* tissue in monoliths of a Dutch bog reached a maximum of  $24.15 \text{ mg N g}^{-1}$  after fertilization with  $5 \text{ g N m}^{-2} \text{ year}^{-1}$  as  $\text{NO}_3\text{NH}_4$  in addition to atmospheric deposition on the same order of magnitude. With N content remaining moderate in comparison, mosses at Mer Bleue contributed only 11% to the total  $^{15}\text{N}$  retention under such conditions, which is much lower than reported by Heijmans et al. (2001), who found a retention of about 60% the in *Sphagnum*-dominated bog monoliths after a period of 15 months. Li and Vitt (1997) reported retention of 25–29% of deposited  $^{15}\text{N}$  in the moss layer of a boreal bog in Alberta, Canada. The main reason for the low N retention at our site was the decrease in moss biomass under the 10 and 20NPK fertilization regimes, which could not be compensated for by increased N content in the tissue. Previous adaptation to a legacy of N deposition and generally wetter conditions in the Dutch bog may have contributed to the greater resilience of *Sphagnum magellanicum* against N fertilization in comparison to the Mer Bleue experiment. This is reflected in the lower background N content of  $8.3\text{--}8.6 \text{ mg N g}^{-1}$  of the mosses, mainly *Sphagnum capillifolium*, at Mer Bleue compared to  $11 \text{ mg N g}^{-1}$  in *Sphagnum magellanicum* in Heijmans et al.'s (2001) experimental site.

Mosses did not retain much  $^{15}\text{N}$  overall, but more effectively retained N compared to other biomass pools and the bulk peat. Standardized to biomass, the *Sphagnum* moss capitulum and upper stem contained on average over 3 times more  $^{15}\text{N}$  than the vascular plants, which is in agreement with findings by Curtis et al. (2005) in UK moorlands. Also their retention efficiency of mosses, ranging from 0.05 to  $1.5 \text{ mg g (dw)}^{-1}$  of biomass, was similar as the range of 0.09 to  $1.78 \text{ mg g (dw)}^{-1}$  at Mer Bleue (Table 5). The N retention efficiency of moss varied with treatments and was lowest under fertilization with N alone (5N), suggesting that P and K are crucial for the mosses ability to retain N, which is in agreement with findings of Aerts et al. (1992) and Hoosbeek et al. (2002).

The decline in total N recovery on highly fertilized plots from the first to the second sampling campaign (Table 4) indicated some elevated N mobility. Given the lack of replication and the variability in tracer

application and bulk density, this trend is not quantitative; but it is consistent with soil solution data. Previously, a decline in  $^{15}\text{N}$  recovery and inorganic N leaching has been attributed to impairment of N uptake by mosses (e.g. Curtis et al. 2005). Such an explanation does not hold in this study due to the low retention of  $^{15}\text{N}$  in mosses, which remained below 11% of the total. Hence N mobility must have been triggered by another mechanism.

#### N retention in peat

Peat was the largest N pool and retained most of the  $^{15}\text{N}$ , which is in agreement with a previous controlled mesocosm study of the Mer Bleue bog and a peatland in the Canadian Experimental Lake Area (Blodau et al. 2006). The mechanisms responsible for the mass transfer is still poorly understood and also debated with respect to forest ecosystems (Davidson et al. 2003; Colman et al. 2008). Blodau et al. (2006) speculated that transport of particulate organic N plays a role, as the N transfer into the peat could not be explained by solute transport; but we are lacking data to verify this hypothesis. Some additional insight may be gained from an examination of  $^{15}\text{N}$  retention in microbial biomass and peat. Retention decreased with N load in both pools simultaneously, and in microbial biomass dramatically. One may infer that microbial immobilization played a role for  $^{15}\text{N}$  retention in the peat, which is supported by the fact that microorganisms appeared to be highly efficient scavengers for  $^{15}\text{N}$  in Control, PK and 5N treatment. The small mass and N pool size of this pool, in comparison with the peat, is not an argument per se against such a role. It has been shown in the past that microorganisms can turn over rapidly in soils. At least part of their detritus is also likely to enter the pool of organic matter. Fisk et al. (1998) and Staddon et al. (2003) estimated a mean residence time for N in soil microbial biomass ranging from 5 to 18 days and Schmidt et al. (2007) assumed an even faster microbial turnover in the rhizosphere. Given our biomass numbers, assuming a residence time of 7 days in the microbial biomass in controls, and the first 4 weeks past labeling, an estimated 26.8% of the  $^{15}\text{N}$  in the peat may have passed through microbial biomass, whereas in the 20NPK treatment this number was only 0.5%, potentially explaining part of the low retention of  $^{15}\text{N}$  in the peat of this

treatment. While this comparison provides a hypothesis it does not hold for trends in microbial biomass and retention of  $^{15}\text{N}$  across all treatments. The treatments generally showed a decrease in microbial biomass but no systematic decrease in the retention of N.

Abiotic incorporation should be mentioned as a potential retention mechanism, and may have played a role in analogy to results obtained in forest soils (Aber et al. 1998; Johnson et al. 2000; Dail et al. 2001; Micks et al. 2004). The abiotic mechanism was presented to proceed through an electron transfer from ferrous iron to  $\text{NO}_3^-$ , and produced  $\text{NO}_2^-$  being bound immediately to soil organic matter (Davidson et al. 2003). The significance of this mechanism has, however, recently been contested and attributed to experimental artefacts (Colman et al. 2007, 2008).

#### N retention in vascular plants

Vascular plants were partly able to compensate for the reduced N retention in other ecosystem pools through growth and increasing N content, which has been observed previously in this and other systems Northern America and Europe (Jonasson 1992; Graglia et al. 2001; Van Wijk et al. 2003; Bubier et al. 2007). It is interesting in this respect that vascular plants have not been identified as a very important N sink in peatlands. Li and Vitt (1997), for example, recovered less than 2% of applied  $^{15}\text{N}$  in aboveground shrub biomass in their field experiments. Some studies also suggested a decrease in N retention efficiency with increased N availability in wetland vascular plants (Small 1972; Shaver and Melillo 1984; Bridgham et al. 1995). We recorded an increase of relative N recovery with fertilization levels, from 21.6% in the Control treatment to 50.8% in treatment 20NPK, a sustained retention efficiency of leaves under a 10NPK load, and an even increased efficiency under the highest N load. In contrast, fertilization with 5N only appeared to reduce the retention efficiency, and in both 5N and PK treatments vascular plants retained little of the applied  $^{15}\text{N}$ . Similarly as seen in mosses at low to moderate fertilization levels, vascular plants thus retained additional N by increasing N content at highest fertilization levels, when combined with P and K input. This response somewhat differed among the

functional parts of the plants; plant stems and roots increased in biomass and N content, whereas leaves only increased their N content significantly, and also changed morphology to some extent (R. Smith, pers. comm.). Vascular plants, with multistratose leaves and thick cuticles, rely on their root system located several centimeters below the moss surface for N uptake (Li and Vitt 1997). Elevated N retention in vascular plants following elimination of the moss layer is thus not surprising.

#### N saturation and importance of P and K

Previous studies have mainly focused on the decay of *Sphagnum* under high N load and the inability of *Sphagnum* to retain deposited N in ombrotrophic bogs under such conditions (Malmer et al. 2003; Bubier et al. 2007). Our results suggest that the response of N retention to sustained N pollution in bogs is more complex and controlled by shifts in the ecosystem structure and specific responses of the major N pools in the system, which can partly compensate each other. To illustrate this point, a decreased recovery of the applied  $^{15}\text{N}$  tracer occurred primarily in two treatments, but likely for different reasons. The lowest  $^{15}\text{N}$  recoveries, 4 weeks after tracer application, were observed in treatment 5N with a moderate sole N load of  $1.6 \text{ gm}^{-2} \text{ year}^{-1}$  and under the highest N(PK) load of  $6.4 \text{ gm}^{-2} \text{ year}^{-1}$ . In treatment 5N, the retained  $^{15}\text{N}$  mostly stayed in the peat, and plants did not assimilate N as readily, likely due to low P or K availability constraining primary production. It is possible that some inorganic N was leached and retained at depths below 10 cm, because in this treatment the concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were somewhat elevated compared to the Control (Table 3). In treatment 20NPK, the adaptation of the system to the high N load over a period of 7 years was not sufficient to avoid breakthrough of inorganic N and a strongly reduced total retention of  $^{15}\text{N}$  in the system. However, although some inorganic N was leached, more of the  $^{15}\text{N}$  applied was also assimilated by vascular plants than in any other treatment. This effect was partly driven by changes in tissue composition; the C:N ratio of vascular plant stems and leaves was significantly lower than in the treatment 5N. The increase in N mobility appeared to develop because the other important N pools, peat, microbial biomass and

*Sphagnum* and *Polytrichum* mosses, failed in taking up as much N as in the other treatments. The results thus illustrate the importance of P and K availability for N retention. It is also noteworthy that P and K addition alone did not have such an effect and rather seemed to be detrimental for N retention. In particular, P and K addition appeared to impair N retention efficiency by mosses (Table 4). Bubier et al. (2007) earlier suggested a toxic effect of P and K addition to *Sphagnum* in our study, whereas it seemed to be beneficial to vascular plants.

#### Conclusions

Our findings suggest that P and K addition and shifts in the vegetation structure and biomass increased the retention of experimentally added N in the Mer Bleue bog. It is currently unclear what the time scale for further adaptation to N deposition would be and how future vegetation community changes may look like. However, under the current fertilization regime, with P and K being abundant, and after a period of 7 years, the systems' threshold value for inorganic N leaching was higher than the previously described range of  $1.5\text{--}2.0 \text{ g N m}^{-2} \text{ year}^{-1}$  that has been reported for *Sphagnum* mosses in studies that utilized nitrogen deposition gradients and additionally deposited N in ecosystem experiments (Lamers et al. 2000; Berendse et al. 2001; Bragazza et al. 2005). Given that the total background deposition at the site was about  $1.5 \text{ g N m}^{-2} \text{ year}^{-1}$ , the results of the 5N treatment with experimental N loading of  $1.6 \text{ g m}^{-2} \text{ year}^{-1}$  support this view even in absence of P and K fertilization. A decrease in recovery of  $^{15}\text{N}$  with fertilization of  $3.2 \text{ g m}^{-2} \text{ year}^{-1}$  N combined with P and K occurred, but neither  $\text{NH}_4^+$  nor  $\text{NO}_3^-$  concentrations in pore water were elevated compared to the Control treatment. Equally important, these results illustrate that the concept of N saturation in ombrotrophic bogs is currently poorly conceived and not very meaningful when defined by inorganic N leaching. The different N retention processes in *Sphagnum* and *Polytrichum* mosses, shrubs and herbs, and the peat, including microbial biomass, reacted quite differently to N and NPK fertilization. Moreover, the plant community structure adapted quite rapidly in response, which may constitute an important difference to forest ecosystems, whose

structure is probably more stable on shorter time scales.

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