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Fate of airborne nitrogen in heathland ecosystems: a ^{15}N tracer study

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Abstract

In the present study, we analyze the fate of airborne nitrogen in heathland ecosystems (NW Germany) by means of a ^{15}N tracer experiment. Our objective was to quantify N sequestration and N allocation patterns in an ecosystem that is naturally limited by N, but that has been exposed to airborne N inputs exceeding critical loads for more than 3 decades. We hypothesized that the system has a tendency towards N saturation, which should be indicated by low N sequestration and high N leaching. We analyzed ^{15}N partitioning (aboveground biomass and soil horizons) and investigated ^{15}N leaching over 2 years following a ^{15}N tracer pulse addition. ^{15}N tracer recovery was 90% and 76% in the first and second year, respectively. Contrary to our expectations, more than 99% of the tracer recovered was sequestered in the biomass and soil, while leaching losses were $<0.05\%$ after 2 years. Mosses were the most important short-term sink for ^{15}N (64% recovery in the first year), followed by the organic layer. In the second year, the moss layer developed from a sink to a source (23% losses), and soil compartments were the most important sink (gains of 11.2% in the second year). Low ^{15}N recovery in the current year's shoots of *Calluna vulgaris* ($<2\%$) indicated minor availability of ^{15}N tracer sequestered in the organic layer. N partitioning patterns showed that the investigated heaths still have conservative N cycling, even after several decades of high N loads. This finding is mainly attributable to the high immobilization capacities for N of podzols in soil compartments. In the long term, the podzol A- and B-horizons in particular may immobilize considerable amounts of incoming N. Since N compounds of these horizons are not readily bio-available, podzols have a high potential to withdraw airborne N from the system's N cycle.

Keywords: *Calluna vulgaris*, dry lowland heath, N cycling, N deposition, N retention, N saturation

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Introduction

Since the beginning of the industrialization in the 19th century the deposition of reactive nitrogen compounds has tripled on a global scale (Galloway *et al.*, 2004). Airborne nitrogen loads have increased the availability of nitrogen to plants which has been observed to result in changes in species composition and losses of species diversity in many terrestrial ecosystems (Bobbink *et al.*, 1998; van Diggelen & Marrs, 2003). Heaths in particular are characterized by nutrient-poor conditions, and species typical of heaths such as *Calluna vulgaris* (henceforth referred to as *Calluna*) are well adapted to low nitrogen availability (Gimingham, 1972). Therefore, heaths are highly susceptible to airborne nitrogen inputs. Several studies found an increase in biomass production for *Calluna* and an increase in shoot nitrogen concentrations either over deposition gradients or as a

result of fertilization (Lee *et al.*, 1992; Uren *et al.*, 1997; Power *et al.*, 1998; Carroll *et al.*, 1999; Kirkham, 2001; Pilkington *et al.*, 2005). Increasing susceptibility to secondary stress factors such as frost and drought as well as increasing herbivory by insects (heather beetle) were further impacts observed (Bobbink *et al.*, 2002). The long-term effects are decreasing lichen and moss diversity, increasing cover of herbaceous species and finally a shift from dwarf shrub- to grass-dominated systems (Heil & Diemont, 1983; Carroll *et al.*, 1999; Brys *et al.*, 2005; Calvo *et al.*, 2005).

Severe shifts in the functioning of heaths were expected to occur beyond critical loads of 10–20 $\text{kg N ha}^{-1} \text{yr}^{-1}$ (Bobbink *et al.*, 2002), but underlying processes are not fully understood. For example, deposition rates in dry lowland heaths of NW Germany have exceeded critical load thresholds for 30 years (i.e. input rates $>20 \text{ kg N ha}^{-1} \text{yr}^{-1}$; Matzner, 1980; Steubing *et al.*, 1992; Niemeier *et al.*, 2005). Because of the high N loads these systems have received over 3 decades, some indications of progressing N saturation are to be

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expected. Aber *et al.* (1998) presented a hypothetical course of N saturation for forest ecosystems in which advanced stages of N saturation are characterized by reduced aboveground biomass production as well as N losses via leaching. This is in agreement with the observations reported by Verhoeven *et al.* (1996) according to which the absence of ongoing growth responses as a result of N fertilization coincides with a shift from N to P limitation. However, recent fertilization experiments have indicated that growth of *Calluna* in dry lowland heaths in NW Germany still seems to be limited by N, even after 5 years of supplemental additions of $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (von Oheimb *et al.*, 2010). Leaching losses, however, increased from $2.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the late 70s to $3.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in 10–15-year-old *Calluna* stands (Matzner, 1980; Härdtle *et al.*, 2007b). Thus, currently available data provide no clear indication as to the extent to which the heaths in our study might be N saturated. The question also remains as to the fate of the airborne nitrogen which (heathland) ecosystems in NW Europe have received over a long period and in quantities that exceeded critical load thresholds. Despite positive growth responses of *Calluna* to nitrogen additions it is hardly conceivable that the aboveground biomass serves as an important long-term sink for incoming N (Power *et al.*, 1998). N storage in the heath soil thus seems to be a plausible explanation; however, continuously increasing soil N stores should cause accelerated N cycling accompanied by improved N availability (Berendse, 1990; Tye *et al.*, 2005) and this in turn does not correspond to the N limitation which still seems to be present even after 30 years of high airborne loads.

Many recent studies have attempted to analyze and to predict heathland responses to past and present N loads by means of long-term fertilization experiments. These studies revealed an increased accumulation of litter and increasing N concentration in the organic soil layer (Power *et al.*, 1998; Carroll *et al.*, 1999; Pilkington *et al.*, 2005).

Heathlands, thus, might have a flexible storage capacity for N in different compounds and different soil horizons. Pilkington *et al.* (2005) found that the soil N pool, especially of the organic layer, of an upland heath increased with the amount of experimentally added N after 11 years of N fertilization, and only under low N treatments added N was stored in the biomass. Power *et al.* (1998) performed budget calculations for a lowland heath which received 7.7 and $15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ during 7 years of experimental fertilizations. In the high N treatment, 18% of the N added was found in the aboveground biomass and 14% in the litter layer. Since leaching and denitrification losses were minimal, the authors concluded that the bulk of the experimentally added N has been accumulated in the remaining soil compartments (e.g. O_F , O_h - and A-horizons).

Budget calculations from fertilization experiments thus might be a helpful approach to analyze allocation patterns of airborne N in heathland ecosystems. However, the quantification of total N stores cannot explain the origin of gains and losses or retention times in a focal ecosystem compartment. ^{15}N tracer studies; however, overcome these problems, since they allow for quantifications of ecosystem N flows, as has been demonstrated in the case of forest ecosystems (Nadelhoffer & Fry, 1994; Buchmann *et al.*, 1996; Tietema *et al.*, 1998; Schleppei *et al.*, 1999; Providoli *et al.*, 2006). The amount of added ^{15}N tracer can be small enough to avoid additional disturbances of the ecosystem's N cycle, but N pathways can be traced and quantified through ecosystem compartments over time.

Quantitative analyses of the fate of airborne N loads with regard to different ecosystem compartments are important to understand ecosystem responses to long-term N inputs at the individual plant and community level. Such analyses may also allow for a better understanding of mechanisms underlying shifts in the species composition of a focal ecosystem. Moreover, information about allocation patterns of deposited N is needed to develop appropriate management strategies, which, in turn, are a prerequisite for the long-term protection of heaths and the huge amount of biodiversity they host in Europe (Maskell *et al.*, 2010). The present study aims to contribute to a better understanding of the fate of airborne N in low-N ecosystems, taking dry heathlands as an example. Our objective was to quantify N sequestration and N allocation patterns in an ecosystem that is naturally limited by N, but that has been exposed to N inputs exceeding critical load thresholds for more than 3 decades. To this end, a ^{15}N tracer experiment was carried out (by means of a tracer pulse addition in early summer) in dry lowland heaths in NW Germany. We analyzed ^{15}N allocation patterns in the aboveground biomass (current year's shoots, 1–2-year-old shoots) and in soil compartments (organic layer, A- and B-horizons) as well as ^{15}N leaching losses during two growing seasons. We hypothesized that the system has a tendency towards N saturation which is expressed in the form of low N sequestration and high N leaching rates. These expectations are based on the nitrogen saturation hypothesis of Aber *et al.* (1998) and Bobbink's Critical Loads Concept (Bobbink *et al.*, 2002). Our questions were: (i) What is the fate of airborne N within 2 years after ^{15}N tracer addition and which ecosystem compartments (biomass, soil) are the most important sinks for sequestered N? (ii) Are there indications of a beginning N saturation? (iii) Are there any indications of long-term sinks in the soil that may diminish the quantity of N in the system's N cycle?

Materials and methods

Study site

Our study area is located in Lower Saxony (NW Germany) and belongs to the Lüneburger Heide nature reserve, which comprises a heathland area 5000 ha in size. This area is characterized by Pleistocene sandy deposits, and the prevailing soil types are nutrient-poor podzols. The climate is of a humid suboceanic type with a mean precipitation of 811 mm yr⁻¹ and a mean temperature of 8.4 °C (Niemeyer *et al.*, 2005). The background deposition in the study area was determined to be in the range of 20.5–25.0 kg N ha⁻¹ yr⁻¹ (Härdtle *et al.*, 2007b).

Study design

In the study area, a series of seven replicated plots was selected at random. *Calluna* was monodominant (cover > 80%) in all plots. The age of *Calluna* ranged between 10 and 12 years and all plots had a well developed moss layer (mean cover 80–100%) dominated by *Hypnum cupressiforme* or *Pleurozium schreberi*. Plots comprised two subplots, each of which was 8 m² in size (2 m × 4 m). One subplot received ¹⁵N tracer (henceforth referred to as 'labeled subplot') and the other was used for the determination of ¹⁵N natural abundance (henceforth referred to as 'nonlabeled subplot'). Labeled and nonlabeled subplots were separated by a buffer zone 1 m in width to avoid cross contamination after ¹⁵N tracer addition.

For the calculation of leaching losses, two lysimeters per plot were installed at a distance of 2 m from the plots. One lysimeter received ¹⁵N tracer (henceforth referred to as 'labeled lysimeter') and the other was used for the determination of ¹⁵N natural abundance (henceforth referred to as 'nonlabeled lysimeter'). Only five of seven plots were equipped with lysimeters, as sampling and checking these devices is a time-consuming process. PVC pipes (90 cm length and 50 cm in diameter) were slowly hammered into the soil. The surrounding soil was removed consecutively, so that the pipe finally contained an undisturbed soil core covered by *Calluna* (DVWK, 1980). The bottom end of the pipe was then sealed and made air-tight with a PVC lid (with outlets for the seepage water connected to a pump), and then buried at the same location. A porous disc (PE-sinter; ecoTech, Bonn, Germany) covered by a nylon membrane (pore diameter 0.45 µm; Whatman Ltd., Maidstone, UK) was installed at the bottom of each lysimeter. All the seepage water leached through the lysimeter was sampled by means of a tension-controlled pump (-200 mbar) and collected continuously in glass bottles. Plots and lysimeters were fenced in to prevent grazing.

¹⁵N tracer addition

In the second week of June 2007, pulse labeling (Turner & Henry, 2009) with ¹⁵NH₄¹⁵NO₃ (98 at.%) was performed in all labeled subplots and labeled lysimeters. ¹⁵N addition was carried out with a special spray bottle equipped with a nozzle that allowed for an evenly distributed addition of the ¹⁵N tracer to the subplots and lysimeter surfaces. During this

procedure, *Calluna* twigs were lifted to avoid foliar contact and thus direct uptake of ¹⁵N by leaves. Labeled subplots and lysimeters received 100 mg ¹⁵NH₄¹⁵NO₃ m⁻² dissolved in 0.5 L deionized water. This quantity aimed at a target δ¹⁵N of 500‰ in *Calluna* and corresponded to 1.6% of the current annual airborne N loads. Therefore, no fertilization effect was expected. After tracer addition, the same amounts of deionized water were sprayed a second time to rinse vegetation surfaces and to disperse tracer solution. Nonlabeled subplots and nonlabeled lysimeters received (area-related) the same amounts of water without ¹⁵N tracer.

Biomass and soil sampling

From June 2007 to November 2008, we sampled the following compartments of the ecosystem: mosses, the current year's and 1–2-year-old shoots of *Calluna*, the organic layer, the albic, and the spodic horizon (i.e. O-, A- and B-horizon of podzols; FAO, 2006). Samples were collected on 11 occasions during the growing season (i.e. 2, 3, 4, 5, 10, 14, 19, 40, 51, 62, 73 weeks after ¹⁵N tracer addition; for exact sampling dates; see Table 1). Samples were taken from labeled and nonlabeled subplots on each occasion.

Twenty randomly chosen current year's shoots from the top of randomly chosen *Calluna* plants were cut with scissors from the whole subplot area and bulked to one sample. In March 2008, no current year's *Calluna* shoots were sampled, because *Calluna* does not start annual shoot growth until the end of May at the earliest. In addition, 1–2-year-old shoots were collected in the same way on each occasion. Moss samples (squares, 2 cm × 2 cm in size) consisted of six randomly chosen samples of the moss layer per subplot. Soil samples were collected from six randomly chosen locations per subplot. Squares of 2 cm × 2 cm were cut with a knife and comprised the entire depth of the organic horizon. Fresh fallen litter was excluded. A- and B-horizons were sampled from soil cores drilled with a soil auger (Pürckhauer, eco Tech).

Leachate sampling

Leachate from lysimeters was collected continuously over the growing season. Samples from labeled and nonlabeled lysimeters were taken at intervals depending on rain events (*n* of sampling occasions was 21). Total amounts of leachate were recorded for each sampling location. Simultaneously, precipitation data (mean monthly precipitation) were obtained from the German Weather Service (DWD, Hamburg, Germany) in order to assess precipitation effects on leaching patterns. During the winter months no sampling took place, because leachate was frozen in collecting flasks.

N contents and ¹⁵N analysis in biomass, soil and leachate

Plant samples were air-dried and sheared with an ultracentrifugal mill (ZM 200, Retsch, Haan, Germany). Soil samples were stored in a freezer (-18 °C) until analysis. Before analysis, samples were air-dried, sieved (2 mm) and ground with a mixer mill (MM 400, Retsch). Milled plant and soil samples

1552 U. FRIEDRICH *et al.***Table 1.** ^{15}N abundances in nonlabeled (Ref) and labeled subplots (^{15}N) of the heath compartments analyzed after ^{15}N tracer addition

Date of sampling	Time (weeks after ^{15}N addition)	Moss		Current year's shoots		1–2 year shoots		O-horizon		A-horizon		B-horizon	
		Ref	^{15}N	Ref	^{15}N	Ref	^{15}N	Ref	^{15}N	Ref	^{15}N	Ref	^{15}N
2007													
06/25	2	–5.7 (0.1)	788.3 (140.5)	–5.6 (0.5)	105.4 (30.0)	–6.5 (0.4)	91.5 (34.5)	–4.4 (0.3)	18.8 (6.3)	5.8 (0.2)	9.8 (0.5)	8.6 (0.5)	9.9 (0.2)
07/02	3	–5.7 (0.1)	836.1 (120.7)	–5.6 (0.4)	75.4 (13.6)	–6.3 (0.6)	60.3 (9.5)	–3.8 (0.4)	12.4 (5.1)	5.5 (0.3)	8.4 (0.6)	8.3 (0.5)	8.9 (0.5)
07/09	4	–5.9 (0.3)	918.2 (136.0)	–5.4 (0.5)	129.9 (30.2)	–6.3 (0.4)	103.7 (27.5)	–4.0 (0.3)	30.7 (7.8)	5.2 (0.2)	10.0 (1.1)	8.6 (0.5)	9.7 (0.6)
07/16	5	–5.8 (0.2)	852.5 (104.3)	–5.1 (0.4)	166.6 (33.5)	–6.6 (0.3)	117.4 (27.9)	–4.2 (0.4)	11.4 (4.6)	6.5 (0.2)	11.3 (1.9)	8.7 (0.4)	10.4 (0.4)
08/22	10	–6.1 (0.2)	719.9 (91.1)	–5.4 (0.5)	122.7 (22.6)	–5.7 (0.5)	110.9 (19.7)	–4.5 (0.3)	17.7 (4.1)	5.3 (0.3)	7.8 (0.6)	8.5 (0.4)	8.4 (0.7)
09/22	14	–6.0 (0.2)	602.1 (44.3)	–5.7 (0.4)	160.9 (27.2)	–5.6 (0.6)	128.6 (22.1)	–4.4 (0.4)	15.7 (4.6)	4.7 (0.6)	10.7 (1.8)	8.3 (0.5)	8.8 (0.5)
10/26	19	–6.1 (0.2)	877.5 (123.2)	–5.1 (0.5)	122.9 (25.6)	–5.8 (0.5)	85.9 (12.9)	–4.2 (0.3)	25.0 (5.6)	5.4 (0.6)	8.1 (1.1)	8.3 (0.4)	8.7 (0.6)
2008													
03/21	40	–5.2 (0.2)	577.8 (54.8)	ND ND	ND ND	–6.5 (0.3)	62.8 (15.8)	–4.1 (0.3)	26.2 (8.0)	5.5 (0.3)	8.2 (0.8)	8.6 (0.3)	9.0 (0.8)
06/05	51	–5.5 (0.1)	615.4 (85.3)	–5.8 (0.5)	60.1 (12.6)	–6.8 (0.4)	59.5 (17.0)	–4.0 (0.4)	27.7 (7.3)	4.9 (0.2)	10.7 (1.2)	8.4 (0.3)	9.7 (0.9)
08/25	62	–5.5 (0.2)	529.3 (53.6)	–5.6 (0.5)	58.9 (12.2)	–6.8 (0.4)	50.7 (12.8)	–4.4 (0.4)	28.0 (5.9)	5.8 (0.2)	9.2 (0.9)	8.2 (0.6)	9.2 (0.6)
11/04	73	–5.2 (0.2)	457.4 (44.0)	–5.9 (0.5)	66.6 (9.7)	–6.6 (0.5)	55.4 (10.1)	–4.1 (0.4)	26.0 (5.4)	4.9 (0.6)	8.3 (0.9)	8.4 (0.4)	9.1 (0.5)

Data are means of $\delta^{15}\text{N}$ (‰) with 1 SE in parentheses ($n = 7$; time in weeks after ^{15}N tracer addition; current year's shoots in March 2008 not determined (ND) due to the annual start of shoot growth in April/May; negative values indicate a depletion, positive values an enrichment of ^{15}N in the $^{15}\text{N}/^{14}\text{N}$ ratio in the sample compared with atmospheric N_2).

were stored at room temperature and redried at 105 °C before weighing. Total N and $\delta^{15}\text{N}$ were determined using a continuous flow elemental analyzer-isotopic ratio mass spectrometer (vario El cube, Elementar, Hanau, Germany, coupled to an Isoprime IRMS, Isoprime Ltd., Cheadle Hulme, UK).

Leachate was filtered, stored in a freezer (–18 °C) and defrosted at a temperature below 4 °C before analysis. NO_3^- -N contents of leachate samples were analyzed using an ion exchange chromatograph (DX-120, Dionex, Idstein, Germany; prestudies revealed negligible low NH_4^+ -N leaching). For determination of $\delta^{15}\text{N}$: leachate samples were prepared following a modified diffusion method (Sebilo *et al.*, 2004), and subsequently analyzed as described above.

Calculation of N pools and N leaching losses

Two of the 11 sampling occasions (August 2007 and 2008) were chosen as reference dates in order to compare ^{15}N tracer recovery for both years. A prerequisite for the calculation of the ^{15}N tracer recovery is the knowledge of the N pool sizes (total amount of N in a particular compartment) of labeled

subplots for all the compartments analyzed. N pools were calculated by means of the pool masses multiplied by their N contents. If compartment N contents between the two reference dates differed significantly (paired *t*-test, $P < 0.05$), N pool sizes were calculated separately for each date, based on the corresponding result for the N content. If differences were nonsignificant, means of N contents were used, resulting in the same N pool size for both reference dates (cf. Table 2).

Total pool mass of the moss compartment was calculated using the mean dry weight of mosses (per unit area) and the individual moss cover from each labeled subplot. In September 2007, *Calluna* aboveground biomass was harvested at sites 0.25 m² in size and situated near the plots. Biomass was separated into current year's shoots, 1–2-year-old shoots and remaining older biomass. For the current year's shoots as well as for 1–2-year-old shoots dry weights were determined after drying at 80 °C. Means of dry weights were used as the total pool mass of current year's shoots and 1–2-year-old shoots. Total soil masses of the O-, A- and B-horizon were calculated by means from the thickness of a soil horizon and its bulk density. Bulk densities of soil horizons of the podzols typical of the study area were taken from former analyses (Niemeyer

Table 2 Soil depth, mass, N content and ¹⁵N tracer recovery of the heath compartments analyzed for two late-summer dates (August 2007 and 2008)

Compartment	Soil depth (cm)	2007				2008				
		Mass (kg m ⁻²)	N content (%)	N pool (g N m ⁻²)	¹⁵ N _{rec} (mg N m ⁻²)	% ¹⁵ N _{rec}	N content (%)	N pool (g N m ⁻²)	¹⁵ N _{rec} (mg N m ⁻²)	% ¹⁵ N _{rec}
Moss		0.53 (0.01)	1.60 (0.09)	8.40 (0.50)	22.71 (3.18)	64.21 (8.99)	1.36* (0.06)	7.17 (0.35)	14.48 (2.00)	40.93* (5.65)
Current year's shoots		0.08 (0.01)	1.48 (0.07)	1.15 (0.05)	0.57 (0.13)	1.62 (0.35)	1.24* (0.03)	0.23 (0.02)	0.23 (0.04)	0.65* (0.12)
1-2 yr shoots		0.07 (0.00)	1.01 (0.05)	0.71 (0.03)	0.32 (0.06)	0.90 (0.18)	0.82* (0.03)	0.57 (0.02)	0.12 (0.03)	0.35* (0.08)
O-horizon	2.73 (0.20)	4.37 (0.44)	1.88 (0.07)	83.32 (10.39)	6.46 (1.10)	18.26 (3.10)	1.88 (0.08)	83.32 (10.39)	9.25 (1.55)	26.16 (4.39)
A-horizon	7.06 (0.97)	165.05 (27.13)	0.11 (0.02)	189.72 (42.13)	1.47 (0.44)	4.16 (1.25)	0.11 (0.01)	189.72 (42.13)	2.48 (0.44)	7.02* (1.25)
B-horizon	7.64 (0.94)	89.42 (14.91)	0.10 (0.01)	98.78 (33.11)	0.15 (0.08)	0.41 (0.23)	0.10 (0.03)	98.78 (33.11)	0.30 (0.09)	0.84* (0.26)
Leaching losses					0.05	0.03			0.09	0.05
Total recovery (%)						89.58				76.00

Data are means with 1 SE in parentheses. ¹⁵N tracer recovery is expressed as total mass of ¹⁵N tracer recovered (¹⁵N_{rec}) and as percent of total ¹⁵N tracer masses (% ¹⁵N_{rec}). Leaching losses are given as sum of ¹⁵N leaching losses since ¹⁵N tracer addition.

*Significant differences of ¹⁵N tracer recovery between August 2007 and 2008 ($P < 0.05$, paired t -test).

et al., 2005). The thickness of soil horizons was recorded using a soil corer. Averages from three measurements per plot were used.

N leaching losses were calculated from total amounts of leachate multiplied by NO₃-N contents from labeled lysimeters.

Calculation of ¹⁵N abundance, ¹⁵N enrichment and ¹⁵N tracer recovery

¹⁵N contents from labeled and nonlabeled subplots are referred to as ¹⁵N abundances and ¹⁵N natural abundances, respectively, and are presented in the δ notation:

$$\delta^{15}\text{N}(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1]1000, \quad (1)$$

where R_{sample} and R_{standard} are the ratios between ¹⁵N and ¹⁴N of the sample and the standard, respectively. By convention, atmospheric N₂ is used as standard ($\delta^{15}\text{N} = 0$ according to 0.3663 at.%; Coplen et al., 1992).

¹⁵N enrichment expresses the per mille isotope enrichment in a sample from a labeled subplot ($\delta^{15}\text{N}_{\text{sample}}$) vs. a reference sample from a nonlabeled subplot ($\delta^{15}\text{N}_{\text{ref}}$) (Fry, 2006):

$$^{15}\text{N enrichment (‰)} = \frac{\delta^{15}\text{N}_{\text{sample}} - \delta^{15}\text{N}_{\text{ref}}}{\delta^{15}\text{N}_{\text{ref}} + 1000} 1000. \quad (2)$$

¹⁵N tracer recovery in compartment N pools and ¹⁵N leaching losses were calculated as follows:

$$^{15}\text{N}_{\text{rec}} = m_{\text{pool}} \frac{\text{at.}\%^{15}\text{N}_{\text{pool}} - \text{at.}\%^{15}\text{N}_{\text{ref}}}{\text{at.}\%^{15}\text{N}_{\text{tracer}} - \text{at.}\%^{15}\text{N}_{\text{ref}}}, \quad (3)$$

where ¹⁵N_{rec} is the mass of ¹⁵N tracer recovered in the N pool of labeled subplots or in leachate losses from labeled lysimeters (g N m⁻²), m_{pool} is the mass of the N pool of labeled subplots or the amount of total N leaching losses from labeled lysimeters (g N m⁻²), at.%¹⁵N_{pool} is the at.%¹⁵N in the N pool of labeled subplots or in leachate losses from labeled lysimeters, at.%¹⁵N_{ref} is the at.%¹⁵N in the N pool of nonlabeled subplots or in leachate from nonlabeled lysimeters, and at.%¹⁵N_{tracer} is the at.%¹⁵N of the added ¹⁵N tracer (Nadelhoffer et al., 2004). Means of at.% ¹⁵N_{ref} from each compartment analyzed were tested for differences between the two reference dates in August 2007 and 2008 using paired t -tests ($P < 0.05$). If differences were nonsignificant, means of at.% ¹⁵N_{ref} were used for the calculation of the compartment ¹⁵N tracer recovery. ¹⁵N tracer recoveries in percent (%¹⁵N_{rec}) represent masses of ¹⁵N tracer recovered as percent of total ¹⁵N tracer masses added to the labeled subplots or to labeled lysimeters.

Statistical analyses

Differences between ¹⁵N abundances of labeled and ¹⁵N natural abundances from nonlabeled subplots were tested by means of $\delta^{15}\text{N}$ for each sampling occasion using independent t -tests ($P < 0.05$). Outliers defined by boxplots were omitted (<3% of the total data set). ¹⁵N leaching losses were determined by ¹⁵N tracer recovery in leachate from labeled lysimeters and presented as a cumulative curve for the 2-year period. Differences between ¹⁵N tracer recovery of the two reference dates in August 2007 and 2008 were tested using paired t -tests ($P < 0.05$). Shifts of ¹⁵N tracer recovery patterns

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were calculated as differences between ^{15}N tracer recovery of the two reference dates. All statistical analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

^{15}N abundances in biomass and soil

Means of $\delta^{15}\text{N}$ in the nonlabeled subplots ranged from -6.8‰ in 1–2-year-old *Calluna* shoots to 8.7‰ in the B-horizon (Table 1). ^{15}N natural abundances of soil compartments increased with depth from the O- to the B-horizon, whereas ^{15}N natural abundances in the biomass were ^{15}N depleted in comparison to the soil.

$\delta^{15}\text{N}$ values increased in all compartments of the labeled subplots (with the exception of the B-horizon) only 2 weeks after ^{15}N addition (end of June). The increase was highest in the moss layer with a peak 4 weeks after ^{15}N tracer addition (i.e. beginning of July 2007; $\delta^{15}\text{N} = 918.2\text{‰}$). Differences between corresponding compartments of non-labeled and labeled subplots were significant ($P < 0.05$) for biomass compartments and the O-horizon on all sampling occasions. For the A-horizon, differences were significant with the exception of one sampling occasion (October 2007). For the B-horizon, differences were significant for two of the 11 sampling occasions (June 25 and July 16; results not shown in Table 1).

^{15}N enrichment in biomass and soil

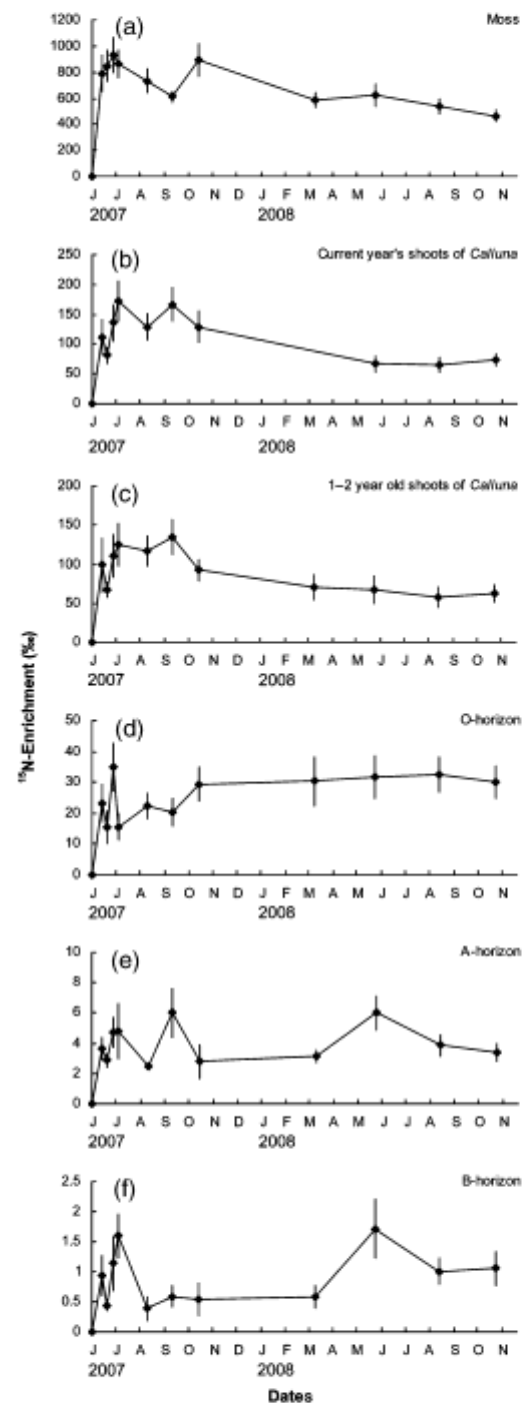
^{15}N enrichments showed a typical enrichment pattern for most of the compartments in the course of the experiment (Fig. 1). Mosses achieved the highest values, ranging between 465.0‰ and 929.5‰ . ^{15}N enrichment in the current year's shoots of *Calluna* was slightly higher than in 1–2-year-old shoots, altogether ranging between 57.9‰ and 172.3‰ . While the O-horizon achieved ^{15}N enrichments from 15.4‰ to 34.8‰ values were lower for the A- and B-horizon (0.4‰ – 6.0‰).

^{15}N enrichments showed a first maximum in all compartments 4–5 weeks after tracer addition (until July 16, 2007). After 3–4 months, ^{15}N enrichments continuously declined in the aboveground biomass (i.e. in the moss layer and the *Calluna* shoots), whilst values of all soil horizons showed a slight, but continuous tendency to increase in 2008.

Fig. 1 ^{15}N enrichment (‰) of the heath compartments analyzed for 11 sampling occasions following ^{15}N tracer addition. (a, moss layer; b, current year's shoots; c, 1–2-year-old shoots; d, O-horizon; e, A-horizon; f, B-horizon). It has to be noticed, that N pool sizes are not taken into account when calculating ^{15}N enrichments. Therefore, ^{15}N enrichments are not reflecting absolute ^{15}N tracer retentions or absolute fluxes between different compartment N pools.

^{15}N leaching losses

Total ^{15}N losses via leaching were negligible compared with the sequestration of ^{15}N in the aboveground



biomass and soil (about 0.05%; expressed as a cumulative curve in Fig. 2). Slight losses appeared within a period of 4–5 weeks (until the middle of July) immediately after tracer addition (with a perceivable lag phase during the first 2 weeks), but then remained very low for the remainder of the experiment. Notably, highest leaching losses during the first 4–5 weeks corresponded with maximum values for ¹⁵N enrichments in all compartments. Since the courses of monthly precipitation rates were well comparable for both years, there was no annual effect of precipitation on leaching rates.

¹⁵N tracer recovery

Recovery of ¹⁵N was highest for the moss layer, but there was a distinct decrease in ¹⁵N recovery in this compartment from 64% in 2007 to 41% in 2008 (Table 2,

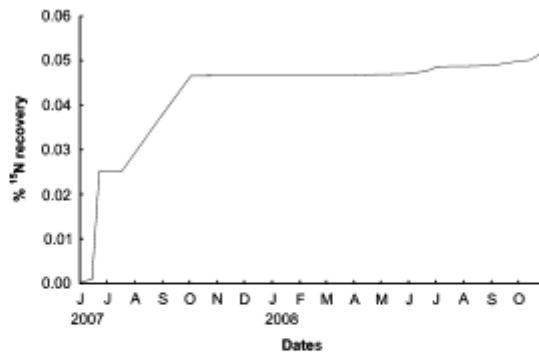


Fig. 2 ¹⁵N leaching losses expressed as a cumulative curve of ¹⁵N recovery (%).

Fig. 3). Recovery in current year’s shoots was higher than in 1–2-year-old shoots and ranged between 0.35% and 1.62% for both years. Decreases in the current year’s and 1–2-year-old shoots of *Calluna* were 1.0% and 0.6% from 2007 to 2008, respectively. A decrease in ¹⁵N recovery in biomass compartments (about 25%, Fig. 3) corresponded with an increase in soil compartments, but total losses from the aboveground biomass were higher than gains in soil compartments (leaching losses included; Fig. 3). ¹⁵N recovery in the O-horizon was 23.3% in 2007 and increased by 7.9% in 2008 (Fig. 3). In tendency, recovery in soil compartments decreased with depth, but recovery rates for all soil compartments significantly increased in 2008 (recovery in the A- and B-horizons approximately doubled in 2008). Total ¹⁵N tracer recovery for all compartments (including leaching losses) was 90% in 2007 and 76% in 2008.

Discussion

¹⁵N partitioning and recovery

Contrary to our expectations, the heaths studied still showed high sequestration and retention capacities for incoming N. This was indicated by both a high recovery in the biomass and soil compartments and negligible leaching losses. Thus, the focal heaths still exhibit characteristics of conservative N cycling (Tye *et al.*, 2005).

The bryophyte layer proved to be the major short-term sink for N, but became a source in 2008 (Fig. 3). This result indicates that mosses function as an important compartment responsible for the sequestration of

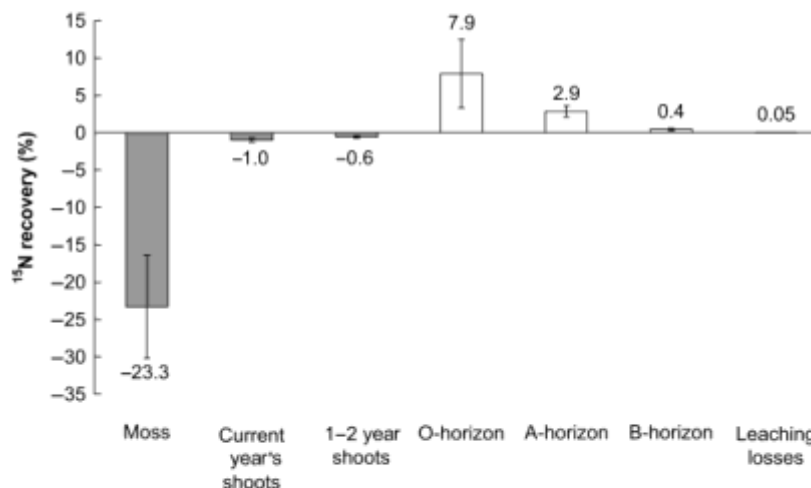


Fig. 3 Shifts in ¹⁵N tracer recovery patterns of heath compartments from August 2007 to 2008. Data represent differences in ¹⁵N tracer recovery (%) between the two dates. Leaching losses are the sum of total ¹⁵N tracer losses since ¹⁵N tracer addition in percent of added ¹⁵N tracer.

airborne N into the heath's N cycle, because incoming N is rapidly immobilized, but may be successively released in the following weeks or months. Since bryophytes are ectohydric plants (i.e. the cell surface absorbs dissolved nutrients; Proctor, 2008), enrichments for the moss layer comprised both nitrogen adhered to the moss plant's surface and assimilated nitrogen. In addition, it is conceivable that the microbial communities associated with the moss phytomass were also responsible for the immobilization of N (Tye *et al.*, 2005). These processes contribute to an immediate sequestration of N. The importance of cryptogams (including lichens) in the sequestration and partitioning of airborne N loads has also been shown for other ecosystems such as arctic tundra or arctic heaths (Gordon *et al.*, 2001; Tye *et al.*, 2005). Since airborne N inputs are the main N source for bryophytes (Bates, 2008), and airborne N has to pass the moss compartment before entering the soil, we suppose that ^{15}N tracer retention found for the moss compartment mirrors the natural partitioning processes. In this context it is important to note that high uptake capacities of the moss compartment are indicative of a low N saturation level (Curtis *et al.*, 2005).

In addition to the moss layer, tracer recovery was high for the O-horizon, but with distinctly increasing values in the second year. It is likely that the rapid sequestration of applied inorganic N in the O-horizon is mainly attributable to the microbial biomass present in this horizon. Jonasson *et al.* (1996) and Nordin *et al.* (2004) highlighted the importance of soil microbes with regard to a rapid and substantial immobilization of both inorganic and organic N compounds in soils. It is also presumed that proteins from microbial biomass act as precursors of recalcitrant soil organic matter and constitute the stable organic N pool of soils (Högberg, 1997; Hagedorn *et al.*, 2005; Horwath, 2007). High N retention in the O-horizon in heaths was also reported in other studies (Kristensen & McCarty, 1999; Kristensen, 2001; Pilkington *et al.*, 2005), but has never been quantified by means of ^{15}N tracer field experiments. In forest ecosystems, however, where several ^{15}N tracer studies took place, comparable N allocation patterns were found. In all studies the organic soil was the most important sink for added ^{15}N (Buchmann *et al.*, 1996; Tietema *et al.*, 1998; Nadelhoffer *et al.*, 2004; Providoli *et al.*, 2006).

Regarding the amounts of ^{15}N tracer recovered in the O-horizon, high ^{15}N tracer uptake by *Calluna* was expected, since its roots are mainly located in the O-horizon (Gimingham, 1972), and *Calluna* can utilize both inorganic and organic N compounds (due to its ericoid mycorrhiza; Read, 1991). However, comparison of ^{15}N recovery in *Calluna* shoots and in the O-horizon does not confirm this assumption. The current year's shoots of *Calluna* incorporated 0.23 mg N m^{-2} of ^{15}N until August 2008,

while 9.25 mg N m^{-2} remained in the O-horizon (Table 2). Thus, only a minor proportion of ^{15}N tracer from the O-horizon was available to plants. There may be two reasons for this: ^{15}N recovered in the O-horizon mainly belonged to a stable N pool not available to *Calluna* [i.e. slow cycling fractions of dissolved organic nitrogen (DON); Compton & Boone, 2002; Currie *et al.*, 2004; Jones *et al.*, 2004], or *Calluna* was the weaker competitor for N in comparison to the soil microbial communities (Schimel & Chapin, 1996; Schimel & Bennett, 2004). This may also indicate that a large proportion of the O-horizon's ^{15}N was immobilized by the microbial biomass (Kristensen & McCarty, 1999).

In our experiment, it is likely that increasing ^{15}N recovery in the O-horizon in 2008 was attributable to influxes of the moss compartment, but the chemical form of translocated N remains unclear. Since decomposition processes of the moss biomass are too slow, N losses from the moss compartment cannot be explained by this process alone. Other processes such as leaking cells (as a result of desiccation–rehydration events; Bates, 2008) could also contribute to ^{15}N tracer losses.

In addition to the O-horizon, ^{15}N recovery significantly increased in the A- and B-horizon during the second year. These shifts in recovery rates may indicate that an imbalance of ^{15}N fluxes from the aboveground biomass to the soil still exists, in which the importance of the A- and B-horizons as long-term sinks for N may increase with time. Considering both the doubling of ^{15}N recoveries in the second year and the huge N stores in the A- and B-horizons (about 190 and 99 g N m^{-2} , respectively; Table 2), it is likely that the podzol A- and B-horizons in particular can accumulate considerable amounts of N (Nielsen *et al.*, 2000). In addition, the formation and downward translocation of fulvic and humic acids (as organometallic compounds including considerable amounts of N) is a typical process that takes place in podzols (Brady & Weil, 2001; FAO, 2006). Since these compounds are insoluble to a great extent and not readily bio-available (Hagedorn *et al.*, 2005), N located in the A- and B-horizons may be partly withdrawn from the system's N cycle. Podzols thus have the potential to immobilize airborne N due to the long-term sequestration in their A- and B-horizons. This process may contribute to the phenomenon that dry heaths still appear to be N limited, and thus to immobilize high amounts of airborne N, even after decades of atmospheric inputs above critical loads. Thus, the soil horizons typical of podzols (i.e. the organic layer and the albic horizon, both forming the so-called raw humus, and spodic horizons) may serve as the most important long-term sinks for N in dry heaths.

Our findings in principle support Aber's revised nitrogen saturation hypothesis (Aber *et al.*, 1998), according to which high N leaching proves to be a final

response mechanism of an ecosystem to high and chronic inputs of N (so-called 'stage 3' of Aber's revised hypothesis; in contrast to his initial model, in which significant N leaching already appeared at the second stage of 'integrated responses of nitrogen-limited forests to chronic nitrogen additions'; Aber *et al.*, 1989). Thus, ecosystem responses to N inputs occur at different time scales (Bobbink *et al.*, 2002). Our findings, however, indicate that response patterns are strongly mediated by ecosystem properties such as soil types and characteristic soil horizons (e.g. thickness of the humus-horizons) as well as the species composition (e.g. the presence of a moss cover, successional stage of a heath). This may lead to an underestimation of the ecosystem's resilience towards long-term inputs of N, because the extent to which airborne N may be immobilized in an ecosystem compartment (e.g. podzol humus-horizons) is difficult to predict. This may also hamper the practical applicability of the Critical Load Concept, because the susceptibility of heathlands to chronic N inputs may vary in a wide range corresponding to the variability of soil conditions (which is currently mirrored by the wide range of critical loads between 10 and 20 kg N ha⁻¹ yr⁻¹ given for dry heaths; Bobbink *et al.*, 2002).

¹⁵N leaching losses and not quantified losses

Contrary to our expectations, ¹⁵N leaching losses were negligible in the course of the experiment. After a lag phase of 2 weeks (corresponding with the downward transport of applied tracer to the lower end of the lysimeter), highest leaching losses appeared within the first 5 weeks of the experiment (until the middle of July). These losses were due to the immediate leaching of small amounts of ¹⁵N tracer that had not been immobilized in the moss layer, by soil microbes or due to plant uptake. In our experiment, low leaching losses of N corresponded with high immobilization rates found for the moss layer and the O-horizon. In heaths, leaching losses are low as long as sites are undisturbed, but may increase as a result of disturbance such as heather beetle infestations or high-intensity management measures (Nielsen *et al.*, 1999, 2000; Härdtle *et al.*, 2007b). In the Netherlands, however, high leaching losses were found as a result of high N deposition rates and interpreted as an indication of N saturation (Schmidt *et al.*, 2004).

We assume that the highest proportion of not recovered ¹⁵N was accumulated in compartments that were not sampled in this study (i.e. aboveground biomass of *Calluna* older than 2 years or roots). In addition, N losses attributable to denitrification, volatilization and DON leaching were not quantified. However, we expect

denitrification losses to be of minor importance in dry heaths (Power *et al.*, 1998; Kristensen & McCarty, 1999; Härdtle *et al.*, 2007a), and losses by volatilization were also expected to be low in strongly acid soils. We also assume leaching of DON to be small (Nielsen *et al.*, 1999, 2000). According to Aber *et al.* (1998), N losses due to leaching of DON are in the range between 0.3 and 0.5 g m⁻² yr⁻¹ (in forest ecosystems under low and high N input rates). Owing to ground frost events lysimeters were not operated in winter, but winter losses should be low, since the downward movement of seepage water during frost events is limited and winter concentrations of dissolved inorganic N are lower than values found for the growing season (Härdtle *et al.*, 2007b).

Conclusions

The present study indicates that the dry lowland heath investigated is still limited by N, despite 30 years of ongoing high nitrogen deposition. This was indicated by high immobilization rates and negligible ¹⁵N leaching losses (about <0.05% of the total ¹⁵N tracer recovery after 2 years). The moss compartment served as the major short-term sink, but became a source in the second year. Bryophytes are, thus, an important ecosystem component responsible for the sequestration of airborne N into the heath's N cycle (rapid immobilization of incoming N, but successive N release in the following weeks or months). In the course of the experiment ¹⁵N recovery decreased in the aboveground biomass, but increased in all soil compartments. This indicates that an imbalance of ¹⁵N fluxes from the aboveground biomass to the soil continued to exist throughout the experiment. It is likely that the formation and downward translocation of ¹⁵N (e.g. as organometallic compounds) will continue in the future. Ecosystems associated with podzols may thus have the potential to immobilize airborne N loads due to their long-term sequestration in the podzol A- and B-horizons. This process may also explain why the heaths studied still exhibit conservative N cycling (high sequestration rates, no leaching losses), even after a long-term history of airborne N loads above critical loads.

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References

- Aber J, McDowell W, Nadelhoffer K *et al.* (1998) Nitrogen saturation in temperate forest ecosystems – hypotheses revisited. *BioScience*, **48**, 921–934.
- Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. *BioScience*, **39**, 378–386.

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- Bates JW (2008) Mineral nutrition and substratum ecology. In: *Bryophyte Biology* (eds Goffinet B, Shaw AJ), pp. 299–356. Cambridge University Press, Cambridge, UK.
- Berendse F (1990) Organic matter accumulation and nitrogen mineralization during secondary succession in heathland ecosystems. *Journal of Ecology*, **78**, 413–427.
- Bobink R, Ashmore M, Braun S, Flückiger W, van den Wyngaert JJJ (2002) *Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update*. Background document of the expert workshop on empirical critical loads for nitrogen. UN ECE Convention on long-range transboundary air pollution, Swiss agency for the Environment, Forest and Landscape, Bern, Switzerland, 128 pp.
- Bobink R, Hornung M, Roelofs JGM (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, **86**, 717–738.
- Brady NC, Weil RR (2001) *The Nature and Properties of Soils*. Prentice-Hall, London.
- Brys R, Jacquemyn H, de Blust G (2005) Fire increases aboveground biomass, seed production and recruitment success of *Molinia caerulea* in dry heathland. *Acta Oecologica*, **28**, 299–305.
- Buchmann N, Gebauer G, Schulze ED (1996) Partitioning of ^{15}N -labeled ammonium and nitrate among soil, litter, below- and above-ground biomass of trees and understory in a 15-year-old *Picea abies* plantation. *Biogeochemistry*, **33**, 1–23.
- Calvo L, Alonso I, Fernández AJ, De Luis E (2005) Short-term study of effects of fertilisation and cutting treatments on the vegetation dynamics of mountain heathlands in Spain. *Plant Ecology*, **179**, 181–191.
- Carroll JA, Caporn SJM, Cawley L, Read DJ, Lee LA (1999) The effect of increased deposition of atmospheric nitrogen on *Calluna vulgaris* in upland Britain. *New Phytologist*, **141**, 423–431.
- Compton JE, Boone RD (2002) Soil nitrogen transformations and the role of light fraction organic matter in forest soils. *Soil Biology and Biochemistry*, **34**, 933–943.
- Coplen TB, Krouse HR, Böhlke JK (1992) Reporting of nitrogen-isotope abundances. *Pure and Applied Chemistry*, **64**, 907–908.
- Currie WS, Nadelhoffer KJ, Aber JD (2004) Redistributions of ^{15}N highlight turnover and replenishment of mineral soil organic N as a long-term control on forest C balance. *Forest Ecology and Management*, **196**, 109–127.
- Curtis CJ, Emmett BA, Grant H, Kernan M, Reynolds B, Shilland E (2005) Nitrogen saturation in UK moorlands: the critical role of bryophytes and lichens in determining retention of atmospheric N deposition. *Journal of Applied Ecology*, **42**, 507–517.
- Deutscher Verband für Wasserwirtschaft und Kulturbau (e.V.) (DVWK) (ed.) (1980). *Empfehlungen zum Bau und Betrieb von Lysimetern*. Parey, Hamburg.
- Food and Agriculture Organisation of the United Nations (FAO) (2006) *World reference base for soil resources. A framework for international classification, correlation and communication*. World Soil Resources Reports 103, FAO, Rome, Italy.
- Fry B (2006) *Stable Isotope Ecology*. Springer, New York.
- Galloway NJ, Dentener FJ, Capone DG *et al.* (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153–226.
- Gimingham CH (1972) *Ecology of Heathlands*. Chapman & Hall, London.
- Gordon C, Wynn JM, Woodin SJ (2001) Impacts of increased nitrogen supply on high Arctic heath: the importance of bryophytes and phosphorus availability. *New Phytologist*, **149**, 461–471.
- Hardtke W, Niemeyer T, Assmann T, Meyer H, von Oheimb G (2007a) Can prescribed burning compensate for atmospheric nutrient loads in wet heathlands? *Phytocoenosis*, **37**, 161–174.
- Hardtke W, von Oheimb G, Niemeyer M, Niemeyer T, Assmann T, Meyer H (2007b) Nutrient leaching in dry heathland ecosystems: effects of atmospheric deposition and management. *Biogeochemistry*, **86**, 201–215.
- Hagedorn F, Maurer S, Bucher JB, Siegwolf RTW (2005) Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO_2 : a ^{15}N and ^{13}C tracer study. *Global Change Biology*, **11**, 1816–1827.
- Heil GW, Diemont WH (1983) Raised nutrient levels change heathland into grassland. *Vegetatio*, **53**, 113–120.
- Högberg P (1997) Tansley review No 95 – ^{15}N natural abundance in soil–plant systems. *New Phytologist*, **137**, 179–203.
- Hoerwath W (2007) Carbon cycling and formation of soil organic matter. In: *Soil Microbiology, Ecology, and Biochemistry* (ed. Paul EA), pp. 303–339. Academic Press, Amsterdam.
- Jonasson S, Michelsen A, Schmidt IK, Nielsen EV, Callaghan TV (1996) Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake. *Oecologia*, **106**, 507–515.
- Jones DL, Shannon D, Murphy DV, Farrar J (2004) Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. *Soil Biology and Biochemistry*, **36**, 749–756.
- Kirkham FW (2001) Nitrogen uptake and nutrient limitation in six hill moorland species in relation to atmospheric nitrogen deposition in England and Wales. *Journal of Ecology*, **89**, 1041–1053.
- Kristensen HL (2001) High immobilization of NH_4^+ in Danish heath soil related to succession, soil and nutrients: implications for critical loads of N. *Water, Air and Soil Pollution: Focus*, **1**, 211–230.
- Kristensen HL, McCarty GW (1999) Mineralization and immobilization of nitrogen in heath soil under intact *Calluna*, after heather beetle infestation and nitrogen fertilization. *Applied Soil Ecology*, **13**, 187–198.
- Lee JA, Caporn SJM, Read DJ (1992) Effects of increasing nitrogen deposition and acidification on heathlands. In: *Acidification Research: Evaluation and Policy Applications* (ed. Schneider T), pp. 97–106. Elsevier, Amsterdam.
- Maskell LC, Smart SM, Bullock JM, Thompson K, Stevens CJ (2010) Nitrogen deposition causes widespread loss of species richness in British habitats. *Global Change Biology*, **16**, 671–679.
- Matzner E (1980) Untersuchungen zum Elementhaushalt eines Heide-Ökosystems (*Calluna vulgaris*) in Nordwestdeutschland. *Göttinger Bodenkundliche Berichte*, **63**, 1–120.
- Nadelhoffer KJ, Colman BP, Currie WS, Magill A, Aber JD (2004) Decadal-scale fates of ^{15}N tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA). *Forest Ecology and Management*, **196**, 89–107.
- Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. In: *Stable Isotopes in Ecology and Environmental Science* (eds Lajtha K, Michener RH), pp. 22–44. Blackwell, London.
- Nielsen KE, Hansen B, Ladekar UL, Nornberg P (2000) Effects of N-deposition on ion trapping by B-horizons of Danish heathlands. *Plant and Soil*, **223**, 265–276.
- Nielsen KE, Ladekar UL, Nornberg P (1999) Dynamic soil processes on heathland due to changes in vegetation to oak and Sitka spruce. *Forest Ecology and Management*, **114**, 107–116.
- Niemeyer T, Niemeyer M, Mohamed A, Fottner S, Hardtle W (2005) Impact of prescribed burning on the nutrient balance of heathlands with particular reference to nitrogen and phosphorus. *Applied Vegetation Science*, **8**, 183–192.
- Nordin A, Schmidt IK, Shaver GR (2004) Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply. *Ecology*, **85**, 955–962.
- Pilkington MG, Caporn SJM, Carroll JA, Cresswell N, Lee JA, Reynolds B, Emmett BA (2005) Effects of increased deposition of atmospheric nitrogen on an upland moor: nitrogen budgets and nutrient accumulation. *Environmental Pollution*, **138**, 473–484.
- Power SA, Ashmore MR, Cousins DA (1998) Impacts and fate of experimentally enhanced nitrogen deposition on a British lowland heath. *Environmental Pollution*, **102** (S1), 27–34.
- Proctor MCF (2008) Physiological ecology. In: *Bryophyte Biology* (eds Goffinet B, Shaw AJ), pp. 237–268. Cambridge University Press, Cambridge.
- Providoli I, Bugmann H, Siegwolf R, Buchmann N, Schleppl P (2006) Pathways and dynamics of $^{15}\text{NO}_2$ and $^{15}\text{NH}_4^+$ applied in a mountain *Picea abies* forest and in a nearby meadow in central Switzerland. *Soil Biology and Biochemistry*, **38**, 1645–1657.
- Read DJ (1991) Mycorrhizas in ecosystems. *Experientia*, **47**, 376–391.
- Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, **85**, 591–602.
- Schimel JP, Chapin FS (1996) Tundra plant uptake of amino acid and NH_4^+ nitrogen in situ: plants compete well for amino acid N. *Ecology*, **77**, 2142–2147.
- Schleppl P, Bucher-Wallin L, Siegwolf R, Saurer M, Müller N, Bucher JB (1999) Simulation of increased nitrogen deposition to a montane forest ecosystem: partitioning of the added ^{15}N . *Water, Air and Soil Pollution*, **116**, 129–134.
- Schmidt IK, Tietema A, Williams D, Gundersen P, Beier C, Emmett BA, Estiarte M (2004) Soil solution chemistry and element fluxes in three European heathlands and their responses to warming and drought. *Ecosystems*, **7**, 638–649.
- Sebilo M, Mayer B, Grably M, Billiou D, Mariotti A (2004) The use of the 'ammonium diffusion' method for $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$ measurements: comparison with other techniques. *Environmental Chemistry*, **1**, 99–103.
- Steubing L, Fangmeier A, Lindemann K-O, Mück D (1992) *Populationsökologische Veränderungen in Heidegesellschaften durch Stickstoffeinträge aus der Luft*. FE-Vorhaben durchgeführt am Institut für Pflanzenökologie der Justus-Liebig-Universität Gießen, UBA, Berlin.
- Tietema A, Emmett BA, Gundersen P, Kjønaas OJ, Koopmans CJ (1998) The fate of ^{15}N -labelled nitrogen deposition in coniferous forest ecosystems. *Forest Ecology and Management*, **101**, 19–27.
- Turner MM, Henry HAL (2009) Interactive effects of warming and increased nitrogen deposition on ^{15}N tracer retention in a temperate old field: seasonal trends. *Global Change Biology*, **15**, 2885–2893.
- Tye AM, Young SD, Crout NMJ, West HM, Stapleton LM, Poulton PR, Laybourn-Parry J (2005) The fate of ^{15}N added to high Arctic tundra to mimic increased inputs of atmospheric nitrogen released from a melting snowpack. *Global Change Biology*, **11**, 1640–1654.

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- Uren SC, Ainsworth N, Power SA, Cousins DA, Huxedup LM, Ashmore MR (1997) Long-term effects of ammonium sulphate on *Calluna vulgaris*. *Journal of Applied Ecology*, **34**, 208–216.
- van Diggelen R, Marrs RH (2003) Restoring plant communities – introduction. *Applied Vegetation Science*, **6**, 106–110.
- Verhoeven JTA, Koerselman W, Meuleman APM (1996) Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. *Trends in Ecology and Evolution*, **11**, 494–497.
- von Oheimb G, Power SA, Falk K *et al.* (2010) N:P ratio and the nature of nutrient limitation in *Calluna*-dominated heathlands. *Ecosystems*, **13**, 317–327.