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Fate of airborne nitrogen in heathland ecosystems – a ¹⁵N tracer study



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Keywords:	Calluna vulgaris, dry lowland heath, N cycling, N deposition, N retention, N saturation
Abstract:	<p>In the present study we analyze the fate of airborne nitrogen in heathland ecosystems (NW Germany) by means of a ¹⁵N 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 ¹⁵N partitioning (aboveground biomass and soil horizons) and investigated ¹⁵N leaching over 2 years following a ¹⁵N tracer pulse addition. ¹⁵N 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</p>

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	<p>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 2008). Low ^{15}N tracer recovery in the current year's shoots of <i>Calluna vulgaris</i> (< 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.</p>
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3 **Fate of airborne nitrogen in heathland ecosystems – a ¹⁵N tracer study**
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7 Running title: Fate of airborne nitrogen in heathlands
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33 Keywords: *Calluna vulgaris*, dry lowland heath, N cycling, N deposition, N retention, N
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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 2008). Low ^{15}N tracer 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.

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 kg N ha⁻¹ 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 kg N ha⁻¹ yr⁻¹; Matzner, 1980; Steubing *et al.*, 1992; Niemeyer *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 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

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3 production as well as N losses via leaching. This is in agreement with the observations
4 reported by Verhoeven *et al.* (1996) according to which the absence of ongoing growth
5 responses as a result of N fertilization coincides with a shift from N to P limitation. However,
6 recent fertilization experiments have indicated that growth of *Calluna* in dry lowland heaths
7 in NW Germany still seems to be limited by N, even after 5 years of supplemental additions
8 of 50 kg N ha⁻¹ yr⁻¹ (von Oheimb *et al.*, 2010). Leaching losses, however, increased from 2.0
9 N ha⁻¹ yr⁻¹ in the late 70s to 3.7 kg N ha⁻¹ yr⁻¹ in 10-15 year old *Calluna* stands (Matzner,
10 1980; Härdtle *et al.*, 2007). Thus, currently available data provide no clear indication as to the
11 extent to which the heaths in our study might be N saturated. The question also remains as to
12 the fate of the airborne nitrogen which (heathland) ecosystems in NW Europe have received
13 over a long period and in quantities that exceeded critical load thresholds. Despite positive
14 growth responses of *Calluna* to nitrogen additions it is hardly conceivable that the
15 aboveground biomass serves as an important long-term sink for incoming N (Power *et al.*,
16 1998). N storage in the heath soil thus seems to be a plausible explanation; however,
17 continuously increasing soil N stores should cause accelerated N cycling accompanied by
18 improved N availability (Berendse, 1990; Tye *et al.*, 2005) and this in turn does not
19 correspond to the N limitation which still seems to be present even after 30 years of high
20 airborne loads.
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45 Many recent studies have attempted to analyze and to predict heathland responses to past and
46 present N loads by means of long-term fertilization experiments. These studies revealed an
47 increased accumulation of litter and increasing N concentration in the soil compartments
48 (Power *et al.*, 1998; Carroll *et al.*, 1999; Pilkington *et al.*, 2005).
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53 Heathlands, thus, might have a flexible storage capacity for N in different compounds and
54 different soil horizons. Pilkington *et al.* (2005) found that the soil N pool, especially of the
55 organic layer, of an upland heath increased with the amount of experimentally added N after
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3 11 years of N fertilization, and only under low N treatments added N was stored in the
4 biomass. Power *et al.* (1998) performed budget calculations for a lowland heath which
5 received 7.7 and 15.4 kg N ha⁻¹ yr⁻¹ during 7 years of experimental fertilizations. In the high
6 N treatment, 18% of the N added was found in the aboveground biomass and 14% in the litter
7 layer. As a consequence, there was a significant increase in N concentration in the organic
8 layer, but calculation of total N stores in the soil were not able to explain the remaining
9 amounts of experimentally added N.

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11 Budget calculations from fertilization experiments thus might be a helpful approach to
12 analyze allocation patterns of airborne N in heathland ecosystems. However, the
13 quantification of total N stores cannot explain the origin of gains and losses or retention times
14 in a focal ecosystem compartment. ¹⁵N tracer studies, however, overcome these problems,
15 since they allow for quantifications of ecosystem N flows, as has been demonstrated in the
16 case of forest ecosystems (Nadelhoffer & Fry, 1994; Buchmann *et al.*, 1996; Tietema *et al.*,
17 1998; Schleppei *et al.*, 1999; Providoli *et al.*, 2006). The amount of added ¹⁵N tracer can be
18 small enough to avoid additional disturbances of the ecosystem's N cycle, but N pathways
19 can be traced and quantified through ecosystem compartments over time.

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21 Quantitative analyses of the fate of airborne N loads with regard to different ecosystem
22 compartments are important to understand ecosystem responses to long-term N inputs at the
23 individual plant and community level. Such analyses may also allow for a better
24 understanding of mechanisms underlying shifts in the species composition of a focal
25 ecosystem. Moreover, information about allocation patterns of deposited N is needed to
26 develop appropriate management strategies, which, in turn, are a prerequisite for the long-
27 term protection of heaths and the huge amount of biodiversity they host in Europe (Maskell *et*
28 *al.*, 2010). The present study aims to contribute to a better understanding of the fate of
29 airborne N in low-N ecosystems, taking dry heathlands as an example. Our objective was to
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3 quantify N sequestration and N allocation patterns in an ecosystem that is naturally limited by
4 N, but that has been exposed to N inputs exceeding critical load thresholds for more than 3
5 decades. To this end, a ^{15}N tracer experiment was carried out (by means of a tracer pulse
6 addition in early summer) in dry lowland heaths in NW Germany. We analyzed ^{15}N allocation
7 patterns in the aboveground biomass (current year's shoots, 1-2 year old shoots) and in soil
8 compartments (organic layer, A- and B-horizons) as well as ^{15}N leaching losses during 2
9 growing seasons. We hypothesized that the system has a tendency towards N saturation which
10 is expressed in the form of low N sequestration and high N leaching rates. Our questions
11 were: (i) What is the fate of airborne N within 2 years after ^{15}N tracer addition and which
12 ecosystem compartments (biomass, soil) are the most important sinks for sequestered N? (ii)
13 Are there indications of a beginning N saturation? (iii) Are there any indications of long-term
14 sinks in the soil that may diminish the quantity of N in the system's N cycle?
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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 5,000 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 to 25.0 kg N ha⁻¹ yr⁻¹ (Härdtle *et al.*, 2007).

Study design

In the study area a series of 7 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% to 100%) dominated by *Hypnum cupressiforme* or *Pleurozium schreberi*. Plots comprised 2 subplots, each of which was square and 8 m² in size. 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 “non-labeled subplot”). Labeled and non-labeled 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 2 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 “non-labeled lysimeter”). Only 5 of 7 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

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3 removed consecutively, so that the pipe finally contained an undisturbed soil core covered by
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5 *Calluna* (DVWK, 1980). The bottom end of the pipe was then sealed and made air-tight with
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7 a PVC lid (with outlets for the seepage water connected to a pump, see below), and then
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9 buried at the same location. A porous disc (PE-sinter; ecoTech, Bonn, Germany) covered by a
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11 nylon membrane (pore diameter 0.45 μm ; Whatman Ltd., Maidstone, UK) was installed at the
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13 bottom of each lysimeter. All the seepage water leached through the lysimeter was sampled
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15 by means of a tension-controlled pump (-200 mbar) and collected continuously in glass
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17 bottles. Plots and lysimeters were fenced in to prevent grazing.
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24 *¹⁵N tracer addition*

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26 In June 2007 pulse labeling (Turner & Henry, 2009) with $^{15}\text{NH}_4^{15}\text{NO}_3$ (98 atom%) was
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28 performed in all labeled subplots and labeled lysimeters. ^{15}N addition was carried out with a
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30 special spray bottle equipped with a nozzle that allowed for an evenly distributed addition of
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32 the ^{15}N tracer to the subplots and lysimeter surfaces. During this procedure *Calluna* twigs
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34 were lifted to avoid foliar contact and thus direct uptake of ^{15}N by leaves. Labeled subplots
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36 and lysimeters received 100 mg $^{15}\text{NH}_4^{15}\text{NO}_3$ per m^2 dissolved in 0.5 l deionized water. This
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38 quantity aimed at a target $\delta^{15}\text{N}$ of 500‰ in *Calluna* and corresponded to 1.6% of the current
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40 annual airborne N loads. Therefore no fertilization effect was expected. After tracer addition,
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42 the same amounts of deionized water were sprayed a second time to rinse vegetation surfaces
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44 and to disperse tracer solution. Non-labeled subplots and non-labeled lysimeters received
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46 (area-related) the same amounts of water without ^{15}N tracer.
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55 *Biomass and soil sampling*

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57 From June 2007 to November 2008 we sampled the following compartments of the
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59 ecosystem: mosses, the current year's and 1-2 year old shoots of *Calluna*, the organic layer,
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3 the albic, and the spodic horizon (i.e. O-, A- and B-horizon of podzols; FAO, 2006). Samples
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5 were collected on 11 occasions during the growing season (i.e. 2, 3, 4, 5, 10, 14, 19, 40, 51,
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7 62, 73 weeks after ^{15}N tracer addition; for exact sampling dates see Table 1). Samples were
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9 taken from labeled and non-labeled subplots on each occasion.
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12 20 randomly chosen current year's shoots from the top of randomly chosen *Calluna* plants
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14 were cut with scissors from the whole subplot area and bulked to one sample. In March 2008
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16 no current year's *Calluna* shoots were sampled, because *Calluna* does not start annual shoot
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18 growth until the end of May at the earliest. In addition, 1-2 year old shoots were collected in
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20 the same way on each occasion. Moss samples (squares, 2 cm x 2 cm in size) consisted of 6
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22 randomly chosen samples of the moss layer per subplot. Soil samples were collected from 6
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24 randomly chosen locations per subplot. Squares of 2 cm x 2 cm were cut with a knife and
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26 comprised the entire depth of the organic horizon. Fresh fallen litter was excluded. A- and B-
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28 horizons were sampled from soil cores drilled with a soil auger (Pürckhauer, ecoTech Bonn,
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30 Germany).
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39 *Leachate sampling*

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41 Leachate from lysimeters was collected continuously over the growing season. Samples from
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43 labeled and non-labeled lysimeters were taken at intervals depending on rain events (n of
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45 sampling occasions was 21). Total amounts of leachate were recorded for each sampling
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47 location. During the winter months no sampling took place, because leachate was frozen in
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49 collecting flasks.
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55 *N contents and ^{15}N analysis in biomass, soil and leachate*

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57 Plant samples were air-dried and sheared with an ultracentrifugal mill (ZM 200, Retsch,
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59 Haan, Germany). Soil samples were stored in a freezer (-18°C) until analysis. Prior to
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3 analysis, samples were air-dried, sieved (2 mm) and ground with a mixer mill (MM 400,
4 Retsch, Haan, Germany). Milled plant and soil samples were stored at room temperature and
5 re-dried at 105°C before weighing. Total N and $\delta^{15}\text{N}$ were determined using a continuous
6 flow elemental analyzer-isotopic ratio mass spectrometer (vario El cube, Elementar, Hanau,
7 Germany, coupled to an Isoprime IRMS, Isoprime Ltd., Cheadle Hulme, UK).

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10 Leachate was filtered, stored in a freezer (-18°C) and defrosted at a temperature below 4°C
11 prior to analysis. $\text{NO}_3\text{-N}$ contents of leachate samples were analyzed using an ion exchange
12 chromatograph (DX-120, Dionex, Idstein, Germany; prestudies revealed negligible low $\text{NH}_4\text{-}$
13 N leaching). For determination of $\delta^{15}\text{N}$ leachate samples were prepared following a modified
14 diffusion method (Sebilo, 2004), and subsequently analyzed as described above.

25 26 27 28 29 *Calculation of N pools and N leaching losses*

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31 2 of the 11 sampling occasions (August 2007 and August 2008) were chosen as reference
32 dates in order to compare ^{15}N tracer recovery for both years. A prerequisite for the calculation
33 of the ^{15}N tracer recovery (see below) is the knowledge of the N pool sizes of labeled subplots
34 for all the compartments analyzed. N pools were calculated by means of the pool masses and
35 their N contents. If compartment N contents between the 2 reference dates differed
36 significantly (paired T-Test, $P < 0.05$), N pool sizes were calculated separately for each date,
37 based on the corresponding result for the N content. If differences were non-significant,
38 means of N contents were used, resulting in the same N pool size for both reference dates (cf.
39 Table 2).

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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

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3 older biomass. For the current year's shoots as well as for 1-2 year old shoots dry weights
4 were determined after drying at 80°C. Means of dry weights were used as the total pool mass
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6 of current year's shoots and 1-2 year old shoots. Total soil masses of the O-, A- and B-
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8 horizon were calculated by means from the thickness of a soil horizon and its bulk density.
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10 Bulk densities of soil horizons of the podzols typical of the study area were taken from former
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12 analyses (Niemeyer *et al.*, 2005). The thickness of soil horizons was recorded using a soil
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14 corer (see above). Averages from 3 measurements per plot were used.
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20 N leaching losses were calculated from total amounts of leachate multiplied by NO₃-N
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22 contents from labeled lysimeters.
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27 *Calculation of $\delta^{15}\text{N}$ enrichment and ^{15}N tracer recovery*

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29 ^{15}N contents from labeled and non-labeled subplots are referred to as ^{15}N abundances and ^{15}N
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31 natural abundances, respectively, and are presented in the δ notation:
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$$36 \quad (1) \quad \delta^{15}\text{N} (\text{‰}) = (R_{\text{sample}} / R_{\text{standard}} - 1) * 1000,$$

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40 where R_{sample} and R_{standard} are the ratios between ^{15}N and ^{14}N of the sample and the standard,
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42 respectively. By convention, atmospheric N₂ is used as standard ($\delta^{15}\text{N} = 0$ according to
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44 0.3663 atom‰; Coplen *et al.*, 1992).
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48 ^{15}N enrichment expresses the per mille isotope enrichment in a sample from a labeled subplot
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50 ($\delta^{15}\text{N}_{\text{sample}}$) vs. a reference sample from a non-labeled subplot ($\delta^{15}\text{N}_{\text{ref}}$) (Fry, 2006):
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$$55 \quad (2) \quad ^{15}\text{N enrichment (‰)} = ((\delta^{15}\text{N}_{\text{sample}} - \delta^{15}\text{N}_{\text{ref}}) / (\delta^{15}\text{N}_{\text{ref}} + 1000)) * 1000.$$

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

$$(3) \quad {}^{15}\text{N}_{\text{rec}} = m_{\text{pool}} * (\text{atom}\% {}^{15}\text{N}_{\text{pool}} - \text{atom}\% {}^{15}\text{N}_{\text{ref}}) / (\text{atom}\% {}^{15}\text{N}_{\text{tracer}} - \text{atom}\% {}^{15}\text{N}_{\text{ref}}),$$

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), atom% ¹⁵N_{pool} is the atom% ¹⁵N in the N pool of labeled subplots or in leachate losses from labeled lysimeters, atom% ¹⁵N_{ref} is the atom% ¹⁵N in the N pool of non-labeled subplots or in leachate from non-labeled lysimeters, and atom% ¹⁵N_{tracer} is the atom% ¹⁵N of the added ¹⁵N tracer (Nadelhoffer *et al.*, 2004). Means of atom% ¹⁵N_{ref} from each compartment analyzed were tested for differences between the 2 reference dates in August 2007 and August 2008 using paired T-Tests (*P* < 0.05). If differences were non-significant, means of atom% 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 non-labeled subplots were tested by means of δ¹⁵N 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 2 reference dates in August 2007 and August 2008 were tested using paired T-

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3 Tests ($P < 0.05$). Shifts of ^{15}N tracer recovery patterns were calculated as differences between
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5 ^{15}N tracer recovery of the 2 reference dates. All statistical analyses were carried out using
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8 SPSS 17.0 (SPSS Inc., Chicago, IL).
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Results

¹⁵N abundances in biomass and soil

Means of $\delta^{15}\text{N}$ in the non-labeled 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, while ^{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. 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 1 sampling occasion (October 2007). For the B-horizon, differences were significant for 2 of the 11 sampling occasions (June 25 and July 16; results not shown in Table 1).

¹⁵N 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‰ to 6.0‰).

^{15}N enrichments showed a first maximum in all compartments 4 to 5 weeks after tracer addition (until July 16, 2007). After 3-4 months, ^{15}N enrichments continuously declined in the

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3 aboveground biomass (i.e. in the moss layer and the *Calluna* shoots), whilst values of all soil
4 horizons showed a slight, but continuous tendency to increase in 2008.
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9 10 *¹⁵N leaching losses*

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12 Total ¹⁵N losses via leaching were negligible compared to the sequestration of ¹⁵N in the
13 aboveground biomass and soil (about 0.05%; expressed as a cumulative curve in Fig. 2).
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15 Slight losses appeared within a period of 4-5 weeks immediately after tracer addition (with a
16 perceivable lag phase during the first 2 weeks), but then remained very low for the remainder
17 of the experiment. Notably, highest leaching losses during the first 4-5 weeks corresponded
18 with maximum values for ¹⁵N enrichments in all compartments.
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28 29 *¹⁵N tracer recovery*

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31 Recovery of ¹⁵N was highest for the moss layer, but there was a distinct decrease in ¹⁵N
32 recovery in this compartment from 64% in 2007 to 41% in 2008 (Table 2, Fig. 3). Recovery
33 in current year's shoots was higher than in 1-2 year old shoots and ranged between 0.35% and
34 1.62% for both years. Decreases in the current year's and 1-2 year old shoots of *Calluna* were
35 1.0% and 0.6% from 2007 to 2008, respectively. A decrease in ¹⁵N recovery in biomass
36 compartments (about 25%, Fig. 3) corresponded with an increase in soil compartments, but
37 total losses from the aboveground biomass were higher than gains in soil compartments
38 (leaching losses included; Fig. 3). ¹⁵N recovery in the O-horizon was 23.3% in 2007, and
39 increased by 7.9% in 2008 (Fig. 3). In tendency, recovery in soil compartments decreased
40 with depth, but recovery rates for all soil compartments significantly increased in 2008
41 (recovery in the A- and B-horizons approximately doubled in 2008). Total ¹⁵N tracer recovery
42 for all compartments (including leaching losses) was 90% in 2007 and 76% in 2008.
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Discussion

¹⁵N abundances in biomass and soil

¹⁵N abundances in all compartments of the labeled subplots were significantly ¹⁵N enriched in comparison to ¹⁵N natural abundances in the non-labeled subplots. Only the B-horizon showed some non-significant differences. This may be caused by a dilution effect that took place when the tracer entered the large soil N pool of the B-horizon (see Table 2). ¹⁵N natural abundances found for the biomass were slightly higher compared to biomass values in ombrotrophic bogs (Skinner *et al.*, 2006) and upland moorlands (Curtis *et al.*, 2005), which may be attributable to differences in soil conditions and the higher background deposition in the study area.

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 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

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3 sequestration of N. The importance of cryptogams (including lichens) in the sequestration and
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5 partitioning of airborne N loads has also been shown for other ecosystems such as arctic
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7 tundra or arctic heaths (Gordon *et al.*, 2001; Tye *et al.*, 2005). Since airborne N inputs are the
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9 main N source for bryophytes (Bates, 2008), and airborne N has to pass the moss
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11 compartment before entering the soil, we suppose that ^{15}N tracer retention found for the moss
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13 compartment mirrors the natural partitioning processes. In this context it is important to note
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15 that high uptake capacities of the moss compartment are indicative of a low N saturation level
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21 (Curtis *et al.*, 2005).

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23 In addition to the moss layer, tracer recovery was high for the O-horizon, but with distinctly
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25 increasing values in the second year. It is likely that the rapid sequestration of applied
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27 inorganic N in the O-horizon is mainly attributable to the microbial biomass present in this
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29 horizon. Jonasson *et al.* (1996) and Nordin *et al.* (2004) highlighted the importance of soil
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31 microbes with regard to a rapid and substantial immobilization of both inorganic and organic
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33 N compounds in soils. It is also presumed that proteins from microbial biomass act as
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35 precursors of recalcitrant soil organic matter and constitute the stable organic N pool of soils
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37 (Högberg, 1997; Hagedorn *et al.*, 2005; Horwath, 2007). High N retention in the O-horizon in
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39 heaths was also reported in other studies (Kristensen & McCarty, 1999; Kristensen, 2001;
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41 Pilkington *et al.*, 2005), but has never been quantified by means of ^{15}N tracer field
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43 experiments. In forest ecosystems, however, where several ^{15}N tracer studies took place,
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45 comparable N allocation patterns were found. In all studies the organic soil was the most
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47 important sink for added ^{15}N (Buchmann *et al.*, 1996; Tietema *et al.*, 1998; Nadelhoffer *et al.*,
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49 2004; Providoli *et al.*, 2006).

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51 Regarding the amounts of ^{15}N tracer recovered in the O-horizon, high ^{15}N tracer uptake by
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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

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3 mycorrhiza; Read, 1991). However, comparison of ^{15}N recovery in *Calluna* shoots and in the
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5 O-horizon does not confirm this assumption. The current year's shoots of *Calluna*
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7 incorporated 0.23 mg N m^{-2} of ^{15}N until August 2008, while 9.25 mg N m^{-2} remained in the
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9 O-horizon (Table 2). Thus, only a minor proportion of ^{15}N tracer from the O-horizon was
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11 available to plants. There may be two reasons for this: ^{15}N recovered in the O-horizon mainly
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13 belonged to a stable N pool not available to *Calluna* (i.e. slow cycling fractions of dissolved
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15 organic nitrogen (DON); Compton & Boone, 2002; Currie *et al.*, 2004; Jones *et al.*, 2004), or
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17 *Calluna* was the weaker competitor for N in comparison to the soil microbial communities
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19 (Schimel & Chapin, 1996; Schimel & Bennett, 2004). This may also indicate that a large
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21 proportion of the O-horizon's ^{15}N was immobilized by the microbial biomass (Kristensen &
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23 McCarty, 1999).
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29 In our experiment it is likely that increasing ^{15}N recovery in the O-horizon in 2008 was
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31 attributable to influxes of the moss compartment, but the chemical form of translocated N
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33 remains unclear. Since decomposition processes of the moss biomass are too slow, N losses
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35 from the moss compartment cannot be explained by this process alone. Other processes such
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37 as leaking cells (as a result of desiccation-rehydration events; Bates, 2008) could also
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39 contribute to ^{15}N tracer losses.
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43 In addition to the O-horizon, ^{15}N recovery significantly increased in the A- and B-horizon
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45 during the second year. These shifts in recovery rates may indicate that an imbalance of ^{15}N
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47 fluxes from the aboveground biomass to the soil still exists, in which the importance of the A-
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49 and B-horizons as long-term sinks for N may increase with time. Considering both the
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51 doubling of ^{15}N recoveries in the second year and the huge N stores in the A- and B- horizons
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53 (about 1900 kg ha^{-1} and 990 kg ha^{-1} , respectively; Table 2), it is likely that the podzol A- and
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55 B-horizons in particular can accumulate considerable amounts of N (Nielsen *et al.*, 2000). In
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57 addition, the formation and downward translocation of fulvic and humic acids (as
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3 organometallic compounds including considerable amounts of N) is a typical process that
4 takes place in podzols (Brady & Weil, 2001; FAO, 2006). Since these compounds are
5 insoluble to a great extent and not readily bio-available (Hagedorn *et al.*, 2005), N located in
6 the A- and B-horizons may be partly withdrawn from the system's N cycle. Podzols thus have
7 the potential to immobilize airborne N due to the long-term sequestration in their A- and B-
8 horizons. This process may contribute to the phenomenon that dry heaths still appear to be N
9 limited, and thus to immobilize high amounts of airborne N, even after decades of
10 atmospheric inputs above critical loads. Thus, the soil horizons typical of podzols (i.e. albic
11 and spodic horizons) may serve as the most important long-term sinks for N in dry heaths.
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27 *¹⁵N leaching losses and not quantified losses*

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29 Contrary to our expectations, ¹⁵N leaching losses were negligible in the course of the
30 experiment. After a lag phase of 2 weeks (corresponding with the downward transport of
31 applied tracer to the lower end of the lysimeter), highest leaching losses appeared within the
32 first 5 weeks of the experiment. These losses were due to the immediate leaching of small
33 amounts of ¹⁵N tracer that had not been immobilized in the moss layer, by soil microbes or
34 due to plant uptake. In our experiment low leaching losses of N corresponded with high
35 immobilization rates found for the moss layer and the O-horizon. In heaths, leaching losses
36 are low as long as sites are undisturbed, but may increase as a result of disturbance such as
37 heather beetle infestations or high-intensity management measures (Nielsen *et al.*, 1999,
38 2000; Härdtle *et al.*, 2007).
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53 We assume that the highest proportion of not recovered ¹⁵N was accumulated in
54 compartments that were not sampled in this study (i.e. aboveground biomass of *Calluna* older
55 than 2 years, roots as well as soil compartments below the B-horizon). In addition, N losses
56 attributable to denitrification, volatilization and DON leaching were not quantified. However,
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3 we expect denitrification losses to be of minor importance in dry heaths (Power *et al.*, 1998;
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5 Kristensen & McCarty, 1999), and losses by volatilization were also expected to be low in
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7 strongly acid soils. We also assume leaching of DON to be small (Nielsen *et al.*, 1999, 2000).
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10 Due to ground frost events lysimeters were not operated in winter, but winter losses should be
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12 low, since the downward movement of seepage water during frost events is limited and winter
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14 concentrations of dissolved inorganic N are lower than values found for the growing season
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16 (Härdtle *et al.*, 2007).
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19 20 21 22 **Conclusions**

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24 The present study indicates that the dry lowland heath investigated is still limited by N,
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26 despite 30 years of ongoing high nitrogen deposition. This was indicated by high
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28 immobilization rates and negligible ^{15}N leaching losses (about < 0.05% of the total ^{15}N tracer
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30 recovery after 2 years). The moss compartment served as the major short-term sink, but
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32 became a source in the second year. Bryophytes are, thus, an important ecosystem component
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34 responsible for the sequestration of airborne N into the heath's N cycle (rapid immobilization
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36 of incoming N, but successive N release in the following weeks or months). In the course of
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38 the experiment ^{15}N recovery decreased in the aboveground biomass, but increased in all soil
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40 compartments. This indicates that an imbalance of ^{15}N fluxes from the aboveground biomass
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42 to the soil continued to exist throughout the experiment. It is likely that the formation and
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44 downward translocation of ^{15}N (e.g. as organometallic compounds) will continue in the
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46 future. Ecosystems associated with podzols may thus have the potential to immobilize
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48 airborne N loads due to their long-term sequestration in the podzol-A- and B-horizons. This
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50 process may also explain why the heaths studied still exhibit conservative N cycling (high
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52 sequestration rates, no leaching losses), even after a long-term history of airborne N loads
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54 above critical loads.
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3 Table 1. ^{15}N abundances in non-labeled (Ref) and labeled subplots (^{15}N) of the heath compartments analyzed after ^{15}N tracer addition. Data are
4 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
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8 (n.d.) due to the annual start of shoot growth in April/May).
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Date of sampling	Time (weeks after ¹⁵ N addition)	Moss		Current yr's shoots		1-2 yr 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)	n.d. n.d.	n.d. n.d.	-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)

Table 2. Mass, N content and ^{15}N tracer recovery of the heath compartments analyzed for 2 late-summer dates (August 2007 and 2008). Data are means with 1 SE in parentheses. ^{15}N tracer recovery is expressed as total mass of ^{15}N tracer recovered ($^{15}\text{N}_{\text{rec}}$) and as percent of total ^{15}N tracer masses ($\% ^{15}\text{N}_{\text{rec}}$). Leaching losses are given as sum of ^{15}N leaching losses since ^{15}N tracer addition. Asterisks indicate significant differences of ^{15}N tracer recovery between August 2007 and August 2008 ($P < 0.05$, paired T-Test).

Compartment	Mass (t ha ⁻¹)	2007				2008			
		N content (%)	N Pool (g N m ⁻²)	$^{15}\text{N}_{\text{rec}}$ (mg N m ⁻²)	$\% ^{15}\text{N}_{\text{rec}}$	N content (%)	N Pool (g N m ⁻²)	$^{15}\text{N}_{\text{rec}}$ (mg N m ⁻²)	$\% ^{15}\text{N}_{\text{rec}}$
Moss	5.28 (0.13)	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 yr's shoots	0.78 (0.06)	1.48 (0.07)	1.15 (0.05)	0.57 (0.13)	1.62 (0.35)	1.24 (0.03)*	0.97 (0.02)	0.23 (0.04)	0.65* (0.12)
1-2 yr shoots	0.70 (0.01)	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	43.73 (4.36)	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	1650.52 (271.35)	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	894.21 (149.14)	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

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3 Figure 1. ^{15}N enrichment (‰) of the heath compartments analyzed for 11 sampling occasions
4 following ^{15}N tracer addition. (a: moss layer, b: current year's shoots, c: 1-2 year old shoots,
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8 d: O-horizon, e: A-horizon, f: B-horizon).
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Figure 2. ^{15}N leaching losses expressed as a cumulative curve of ^{15}N recovery (%).

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3 Figure 3. Shifts in ^{15}N tracer recovery patterns of heath compartments from August 2007 to
4 August 2008. Data represent differences in ^{15}N tracer recovery (%) between the 2 dates.
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6 Leaching losses are the sum of total ^{15}N tracer losses since ^{15}N tracer addition in percent of
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8 added ^{15}N tracer.
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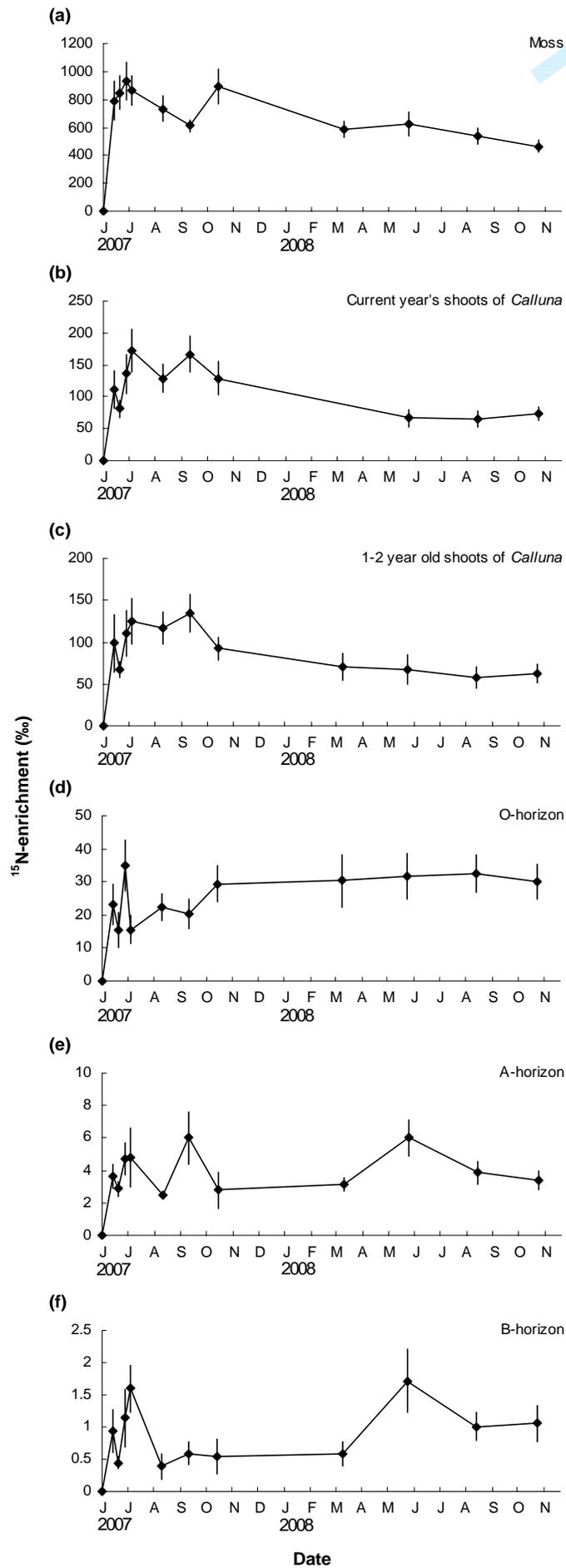
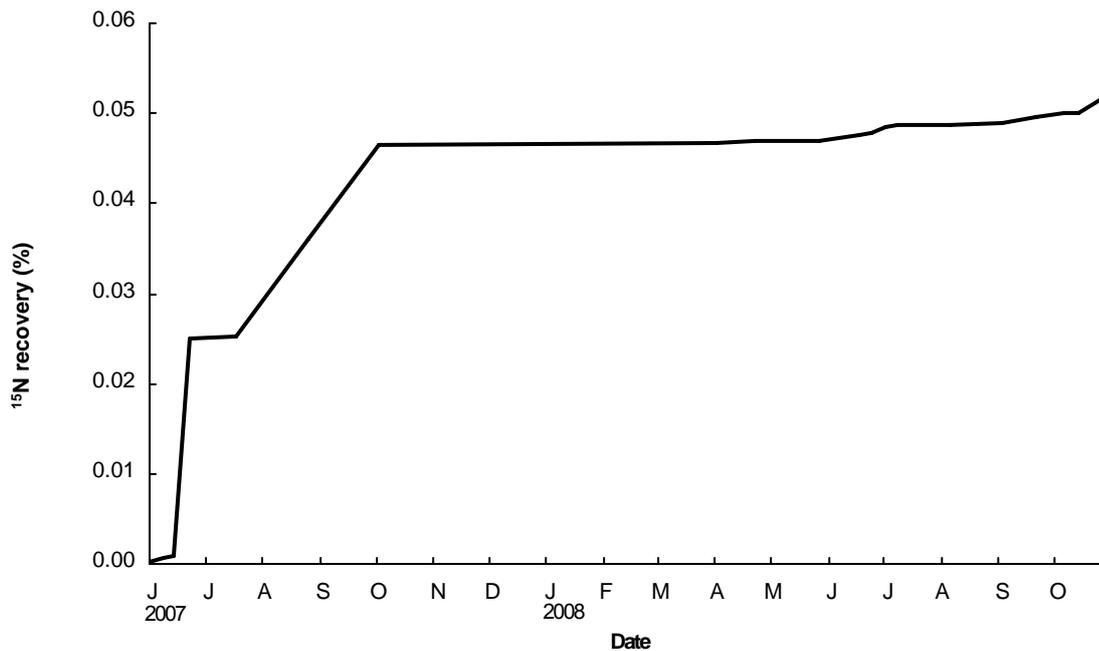


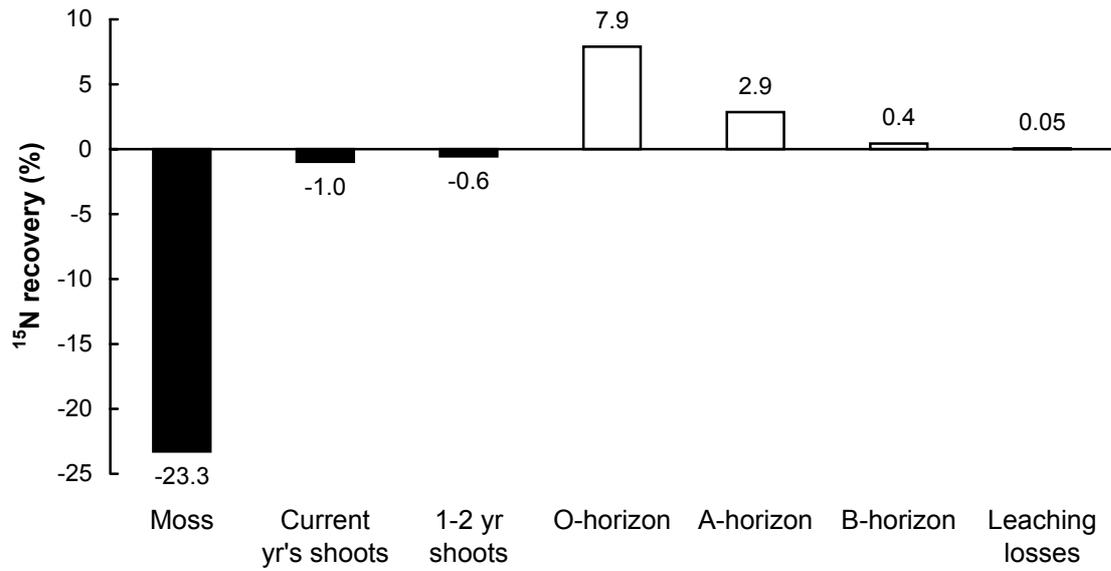
Figure 1



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Figure 2

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Figure 3