Natural abundance of ^{15}N of a spruce forest ecosystem under acid rain and manipulated clean rain field conditions

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ABSTRACT: We analysed stable isotopes of N in a spruce forest under ambient rainfall (no further manipulation of the atmospheric input) and clean rain application (10 years of reduced inorganic N- and acid-constituent input). The objectives of the study were to assess whether or not the natural ^{15}N abundance would function as an indicator for the N-status of our forest ecosystems. For this purpose, natural ^{15}N abundance values were measured in needles, litter fall, roots, soil, bulk precipitation, throughfall and soil water of both plots. In the bulk precipitation and throughfall the δ^{15}N values of NO_3-N were in the range reported by other studies (–16 to + 23‰). In both plots, the throughfall was greatly depleted of ^{15}N compared to the bulk precipitation and this was attributed to nitrification in the canopy leaves, leading to δ^{15}N-depleted nitrate production in the leaves that leaches down the soil surface. Nitrate in seepage water showed a general increase in δ^{15}N values when it passes through the upper mineral soil (10 cm soil depth) and infiltrates into deeper mineral soil horizons (100 cm soil depth), similar to the δ^{15}N enrichment of total nitrogen in the mineral soil. We observed ^{15}N depletion in both green needles and litter fall at the clean rain plot, compared to the N-saturated control plot. We assumed it to be due to increased mycorrhizal N-uptake under N limited, i.e. clean rain conditions which are indicated by relatively lower N concentrations of green needles. With respect to the vertical gradient of the ^{15}N abundance in the forest floor, both plots differ from each other, showing an untypical peak of δ^{15}N depletion in the humus layer, which is more pronounced at the control plot. In contrast to the mineral soil where mineralisation is a dominant process for fractionation we attribute the δ^{15}N pattern in the forest floor to additional processes like litter input and immobilisation. We conclude that the δ^{15}N natural abundance analysis is helpful for interpreting the N-status of forest ecosystems but further research is needed especially with respect to the soil-root interface.

Keywords: acid rain; clean rain; δ^{15}N; stable isotopes; nitrogen; precipitation; throughfall; seepage water; needles; litter; spruce forest

The use of natural abundance of stable isotopes to elucidate physiological processes in plants is one of the oldest applications of isotope analysis in ecology. The characteristics of the isotopic compositions of pollutants can provide useful information with regard to their source and quantity in an environment. Over decades, N deposition has affected forest areas of Europe and North America (van Egmond et al. 2002; Matson et al. 2002). Nitrogen saturation is associated with increased rates of N cycling and losses of NO_3^- with seepage water (Aber et al. 1989; Diese, Wright 1995; Lajtha et al. 1995; Matson et al. 2002). The natural abundance values of ecosystem pools act as an indicator of nitrogen losses from forest ecosystems (Nadelhoffer, Fry 1994). Nitrogen cycling through the ecosystem is associated with isotopic fractionation and thus it can be used to identify the importance of ecosystem processes (Nadelhoffer, Fry 1994) without the addition of tracers or other disturbances which might falsify the results. Several ecosystem researchers (Garten 1993; Nadelhoffer, Fry 1994) predicted that the

^{15}N natural abundance values would increase for systems approaching N-saturation. So, the natural ^{15}N abundance values might identify the position of forests along a gradient of N availability (Pardo et al. 2006). For example, relatively depleted δ^{15}N values in vegetation or in both vegetation and soil indicate low rates of N losses. In contrast, relatively high N-exports from a compartment (soil) lead to its enrichment of δ^{15}N values. The uptake of N from atmospheric deposition by aboveground parts of trees has been hypothesised to be involved in the complex phenomenon of forest decline (Nihlgard 1985; Schulze et al. 1987; Eilers et al. 1992). Furthermore, the ^{15}N natural abundance technique was used in forest ecosystem health studies, for example, needles from a healthy Norway spruce stand were more depleted of ^{15}N than those from a declining stand receiving increased N and S depositions (Gebauer, Schulze 1991; Gebauer et al. 1994).

Fractionation is particularly important during nitrification, denitrification and ammonia volatilisation processes (Mariotti et al. 1981) and can result in ^{15}N enrichment of the soil N pool (Shearer et al. 1974; Högberg 1990). As a consequence, ^{15}N enrichment of the foliage can occur as N supply increases following fertiliser applications as demonstrated in agricultural systems (Meints et al. 1975) and forest ecosystems (Högberg 1990), or chronic atmospheric N-pollution (Gebauer, Schulze 1991). A positive relationship between N supply and an increase in the relative ^{15}N enrichment of vegetation to soil has been observed for several vegetation types (Garten 1993; Johanisson, Högberg 1994). This has been attributed to accelerated nitrification in N rich sites leading to constant and increasing uptake of ^{15}N enriched NH_4-N by vegetation as ^{15}N depleted NO_3-N is leached from the system (Garten, Migroet 1995). However, ^{15}N enriched vegetation has also been associated with high nitrate reductase activity and utilisation of ^{15}N enriched nitrate by plants (Pate et al. 1993). On the soil surface, organic matter δ^{15}N are generally similar to or slightly greater than in plant litter (Fry 1991). Reports on the forest sites across North America (LTER project) and Europe (NITREX project) illustrate an overall pattern of ^{15}N enrichment resulting in the following order of increasing enrichment: plant < fresh litter < organic soil < mineral soil.

The impact of anthropogenic atmospheric deposition on the chronic destabilisation of forest ecosystems was well reported by Gordon et al. (1992), Ulrich (1994), and Puhe and Ulrich (2002). However, it was desirable to stimulate experimental scenarios of changes in atmospheric deposition as well as climate extremes under field conditions in order to test the hypothesis and to validate models of ecosystem reaction under such possible future environmental constraints. This type of experimentation was conducted in the Solling roof experiment, Germany, where clean rain manipulation experiments have been performed by means of a roof construction in a 74-years-old (2007) Norway spruce plantation forest since 1991. The goal of this roof experiment is to test how this ecosystem would react to a strong input reduction of N, S and acidity (Bredemeier et al. 1993, 1995).

Results of the roof project indicate a cascade of reactions after long-term application of "clean rain", starting with a fast and strong reduction of the sulphate, nitrate and aluminium concentration in the soil solution of the upper soil horizons (Bredemeier et al. 1995; Lamersdorf et al. 1999) and a recovery of the fine root growth (fine root biomass and deep rooting) (Murach, Parth 1999; Lamersdorf, Borken 2004). Simultaneously the needle nitrogen concentration decreased, and thus an increase in the C/N ratio of litter material was initiated (Dohrenbusch et al. 2002). More recent results indicate that the gross and net N-mineralisation rates increased while no net N-nitrification was detectable at the clean rain plot (Corre, Lamersdorf 2004). These findings are interpreted as indicators for a recovery of the internal N-cycling from N saturation towards a closed N-cycle. Other parameters like the microbial biomass, exchangeable base cations, soil and soil solution pH showed no or only slight changes towards recovery (Corre, Lamersdorf 2004).

However, there is still a general need to better understand the fate of anthropogenic nitrogen, its accumulation rate, and the consequences of short-term and long-term accumulation in ecosystems (Galloway et al. 2002). The present study is concerned with the natural abundance of the stable isotope ^{15}N in the Solling roof project. The specific objective of the study is to compare the natural abundance of ^{15}N between the ambient (no further manipulation of the atmospheric input) and clean rain scenario, i.e. to the status after about 10 years of reduced inorganic N- and acid-constituent input (clean rain application). We hypothesised that the signals of natural ^{15}N abundance would function as an indicator for the N-status of our forest ecosystem.

MATERIALS AND METHODS

Site description

The Solling roof project is located in the mountainous Solling area of Germany at 51°31'N, 9°34'E and is a part of the Weser river mountain range. The climate is a "mountain climate" with an average annual air temperature of 6.4°C and an annual precipitation of 1,090 mm. The research plots are located on a plateau at about 500 m a.s.l. The soil is classified as Typic Dystrochrept (USDA) or acidic Dystric Cambisols (FAO), developed from a loess solifluction layer overlaying weathered sandstone. The soil is silty loam and has a base saturation of 5–7% down to 80 cm. The pH ($CaCl_2$) value is 3.2 at 0–10 cm soil depth and increases to 4.2 at 40–80 cm soil depth. The forest floor is 5–10 cm thick and is classified as moder type humus.

The vegetation consists of a 74-years-old (2007) Norway spruce (*Picea abies* [L.] Karst.) plantation. Beech (*Fagus sylvatica*) trees were replaced by Norway spruce trees and this species now exists in the second generation. The forest was thinned prior to the start of the roof experiment in 1991 and reduced to about 830 trees/ha. In 1998 the forest had a mean basal area of 57 m^2. Fine roots are primarily concentrated in the forest floor. A detailed description of the sites was given by Bredemeier et al. (1993, 1995, 1998), Blanck et al. (1993), Murach et al. (1993), Murach and Parth (1999) and Lamersdorf and Borken (2004).

For the input manipulation scenario two roofs, each covering an area of 300 m^2, were built in 1991 underneath the canopy at about 3.5 m above the ground. The roof consists of a permanent timber frame construction, covered with highly transparent polycarbonate plates. Each roof plot was surrounded with a plastic sheet in the soil to prevent lateral water flow and root penetration to/from outside. Soil solution was sampled continuously with suction lysimeter cups (*P-80* material, CeramTec AG, Marktredwitz, Germany) installed with 5 replicates per depth in the centre of the roof plots at 0 cm (humus lysimeter), 10, 40, and 100 cm mineral soil depth. The following plots were selected for the ^{15}N natural abundance study:

I. **Clean rain plot:** At this plot pre-industrial throughfall is applied. Incoming throughfall water is filtered, deionised, chemically adjusted to a given "clean rain" concentration and redistributed to the ground by a sprinkling system underneath the roof cover whenever 1 mm of rainfall occurred. This application started in September 1991. Table 1 summarises the mean input fluxes of major elements for the period 1992 to 2001, in comparison with the control plot and the percent of reduction for the clean rain plot.

II. **Control plot:** The collected throughfall water from the roof is filtered and re-sprinkled immediately underneath the roof cover without any chemical manipulation.

In addition to the above-mentioned plots, we sampled bulk rainfall from an open field plot, located at a distance of only about 500 m from the roof plots (hereafter called "bulk precipitation").

Sampling

Samples from sprinkled throughfall water were collected biweekly underneath the roofs with two samplers, filtered to remove organic debris and combined to monthly samples for chemical analysis. Soil water samples from 10 and 100 cm soil depths were analysed for the ^{15}N natural abundance. All water samples were immediately stored after sampling at 4°C prior to bulking and chemical analysis.

Samples of current year green needles were collected in October 1992, 1994, and 1998 from the 7th whorl (n = 6 per plot and year) with the help of the crane. Fresh needle litter was collected monthly between 1992 and 1994 from a defined area of the roofs (17m^2, n = 14 per plot). Fine roots (≤ 2 mm) were sampled in October 2001. For the mineral soil the fine root samples were taken from in-growth cores (n = 4), for the forest floor roots were taken from stainless steel cores (n = 4). Fine roots were separated into a live and dead fraction according to the method described by Murach (1984). Fine root material was collected separately for the O_{L+F} (L = litter layer, F = fermentation layer), O_H (H = humus layer), 0–5, 5–10, 10–40 cm soil layers (for further details of the

Table 1. Mean input rates (kg/ha) for the clean rain and control plot in the Solling roof project, 1992 to 2001, and percent of reduction for the clean rain treatment (according to Lamersdorf, Borken 2004)

	Clean rain plot	Control plot	Reduction (%)
	(kg/ha)		
H^+	0.12	0.54	78
SO_4-S	12.2	26.0	53
NO_3-N	7.6	15.0	49
NH_4-N	2.1	15.0	86

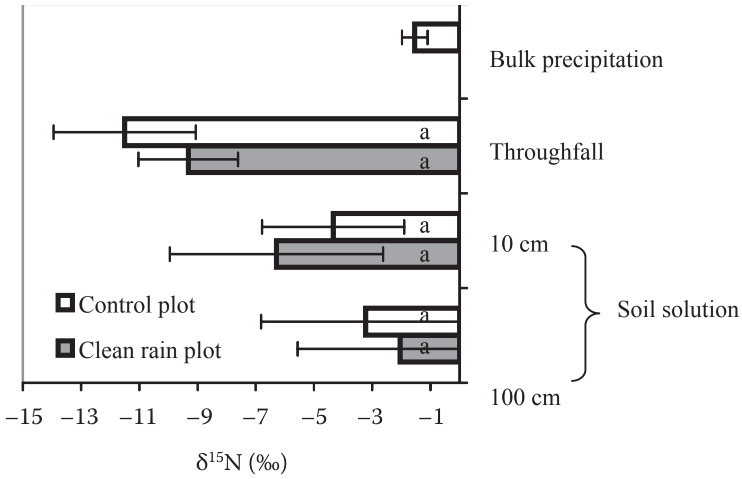

Fig. 1. Natural ^{15}N abundance ($\delta^{15}N$, ‰) of nitrate in bulk precipitation (n = 5), throughfall (sprinkling water underneath the clean rain and control roof, n = 2 per plot), and soil solution at 10 and 100 cm soil depth (n = 5 per plot), measured in monthly samples throughout the year 2000 (± SE) (the same letters indicate no statistical differences)

sampling methods applied in 2001 see Lamersdorf, Borken 2004).

In order to assess the vertical distribution of stable isotopes in the soil, undisturbed soil cores (n = 4) were taken with a steel soil core sampler (30 × 8 cm) up to the soil depth of 20–25 cm at random. The soil cores were cut into 1–2 cm slices for the laboratory analysis.

Analytical methods

All solid samples were dried (plant material at 40°C, soil samples at 60°C) and ground into a fine powder in a planetary mill. Total organic C and N in the plant tissue and in organic and mineral soil layers were measured using a C-N-analyser (CHN-O-Rapide, VarioEL, Elementar, Germany). ^{15}N was measured with a Finnigan MAT Delta plus stable isotopic ratio mass spectrometer (IRMS), equipped with an elemental analyser. For determination of ^{15}N in water samples the diffusion method were used according to Corre et al. (2003). Results of the IRMS measurements are given in the δ-notation. The δ-values of the isotopes are expressed as parts per 1,000 differences from standard atmospheric isotopes (Shearer, Kohl 1993).

$$\delta^{15}N = \{[(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{air})] - 1\} \times 1{,}000\ (‰)$$

where:
air – reference standard gas.

Statistical analysis

A non-parametric test (Mann-Whitney U-test) was used to find out differences between treatments on the 5% level. For correlation analysis, Spearman's rank method was applied (SPSS, version 10).

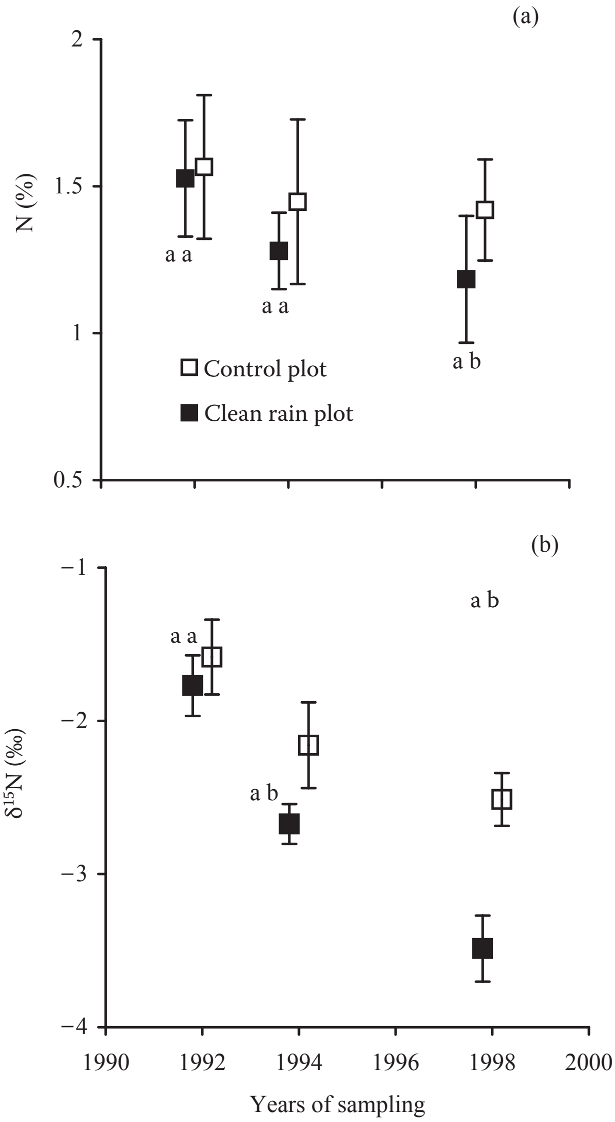

Fig. 2. Total N concentration (a) and natural ^{15}N abundance ($\delta^{15}N$‰) (b) of current year needles for the clean rain and control plot, sampled in 1992, 1994 and 1998 (n = 6, ± SE) (the same letters indicate no statistical differences)

RESULTS

Natural ^{15}N abundance of nitrate and ammonium in forest waters

Fig. 1 presents the natural $\delta^{15}N$ abundance of nitrate in bulk precipitation (taken from Sah, Brumme 2003), throughfall (sprinkling water), and soil water samples. The mean $\delta^{15}N$ values of nitrate in the forest ranged from –11.5‰ to –2.0‰. Bulk precipitation (–1.5 ± 0.4‰, where error bars indicate the standard error of means) was considerably depleted of $\delta^{15}N$-nitrate when it passed through the forest canopy (–11.5 ± 2.4‰), i.e. when it was re-sprinkled without further manipulation on the roof control plot. Bulk precipitation of ammonium (–0.6 ± 2.8‰) shows an opposite trend after passing through the tree canopy and was highly enriched in $\delta^{15}N$ values (+16.9 ± 2.2‰, not shown in Fig. 1) in the control plot. However, ^{15}N in ammonium was not detectable by the mass spectrometer analysis in the throughfall of the clean rain plot and in soil solution samples of both plots. The throughfall water after passing through the O-horizon and the upper mineral soil layers, the $\delta^{15}N$ abundance of nitrate increased at the 100 cm soil depth from –4.3‰ to –3.2‰ at the control plot and from –6.2‰ to –2.0‰ at the clean rain plot, i.e. the ^{15}N enrichment was significantly ($P \leq 0.05$) higher at the clean rain plot (Fig. 1).

Natural ^{15}N abundance of needles and fine roots

The total N content as well as the natural ^{15}N abundance of current year needles indicated a decreasing trend from the year 1992 to 1998 in both roof plots (Fig. 2). One year after the beginning of clear rain application in September 1991, the $\delta^{15}N$ values were not significantly different between the plots (control plot –1.6 ± 0.2‰, SE; clean rain plot –1.8 ± 0.2‰) (Fig. 2b). With prolonged application of clean rain, the decrease in needle $\delta^{15}N$ values was higher compared to the control plot. For the control plot, the depletion of $\delta^{15}N$ values in needles was very low and statistically insignificant between 1994 and 1998 (–2.5 ± 0.2‰, SE in 1998 and –2.2 ± 0.3‰ in 1994) in contrast to the clean rain plot, where significantly different $\delta^{15}N$ values were found between the plots in 1994 and 1998 (–3.5 ± 0.2‰ in October 1998 compared to –2.7 ± 0.3‰ in August 1994). Similarly, the decrease in total N concentration between 1992 and 1998 was higher for the clean rain plot and resulted in significant differences between the plots in 1998 (Fig. 2a). The N concentration decreased from 1.56 ± 0.24 to 1.39 ± 0.17% in the control plot and from 1.52 ± 0.20% to 1.16 ± 0.22% in the clean rain plot between 1992 and 1998, respectively, but was not significantly different.

A similar decrease in $\delta^{15}N$ values, as observed for green needles, was found for needle litter material (Fig. 3b). The $\delta^{15}N$ values of needle litter from both plots decreased significantly in 1998, compared to the previous years (1992, 1993 and 1994). However, significantly different ^{15}N values between the plots were only found for the years 1992, 1993 and 1994 (Fig. 3b), but not for 1998. On the contrary, the total N concentrations did not change significantly with time (Fig. 3a) but N concentrations in needle litter of the control plot were always (except 1992) significantly higher compared to the clean rain plot.

Fig. 4 shows the vertical gradients of $\delta^{15}N$ natural abundance in live and dead fine roots for the clean rain and control plot. Both live and dead fine roots of the clean rain plot show significantly higher ^{15}N enrichment almost in all soil horizons (except in O_{L+F} horizon of dead fine root), compared to the

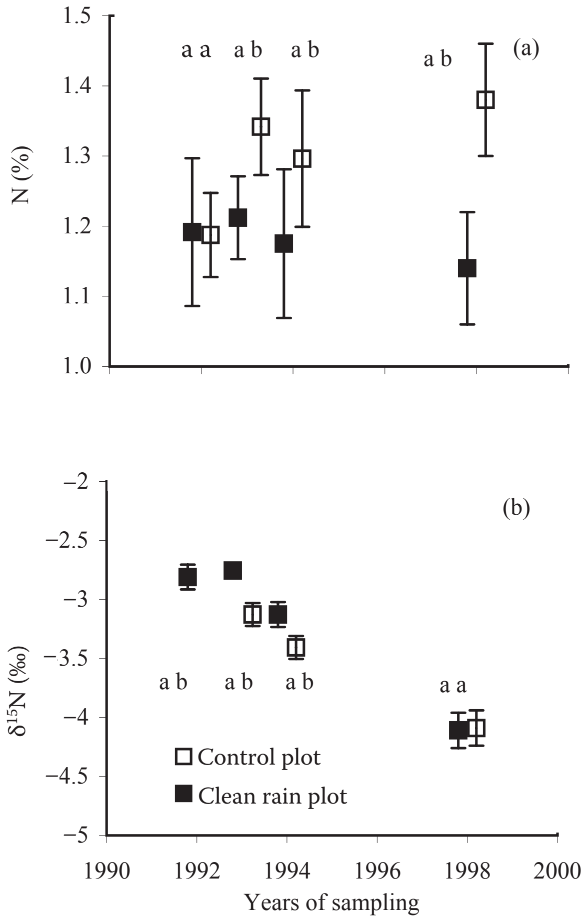

Fig. 3. Total N concentration (a) and natural ^{15}N abundance ($\delta^{15}N$, ‰) (b) of needle litter for the clean rain and control plot, sampled in 1992, 1993, 1994, and in 1998 (n = 4–9, ± SE) (the same letters indicate no statistical differences)

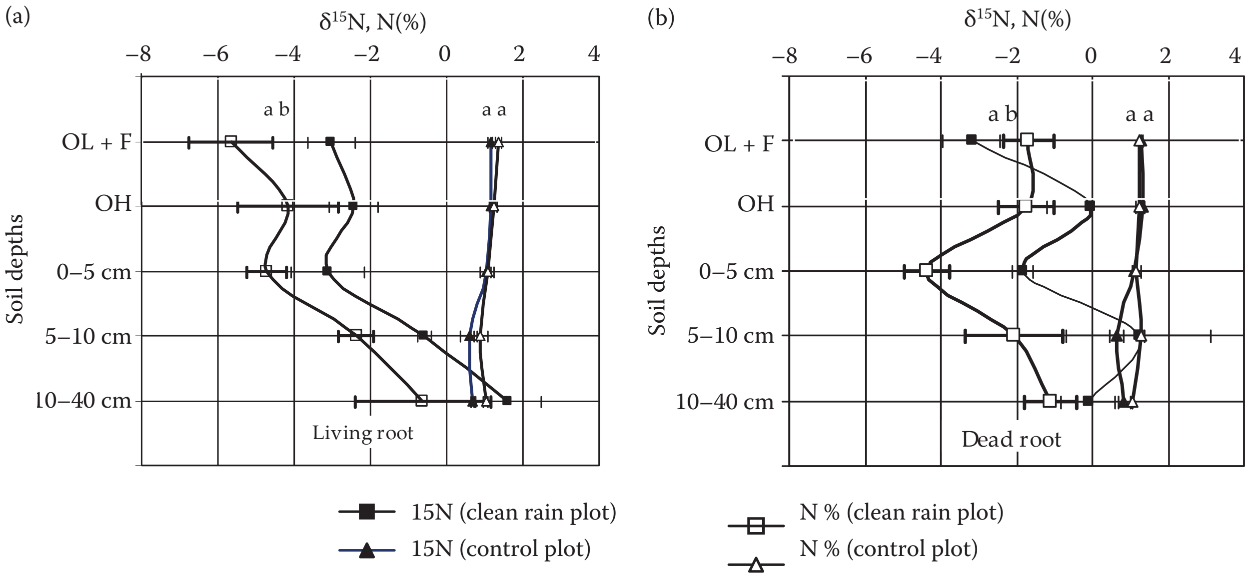

Fig. 4. N concentrations and ^{15}N natural abundance values in the living (a) and dead roots (b) both plots (n = 4/depth, error bars represent standard error of means)

control plot. Furthermore, $\delta^{15}N$ values of live and dead fine roots increased with the depth of mineral soil in both plots similarly like it was observed for the vertical gradient of $\delta^{15}N$ of nitrate in soil solution (Fig. 1) and total soil N (Fig. 5). Thus a positive correlation between $\delta^{15}N$ in total soil N and the live fine root fraction was found at both sites (clean rain plot $r = 0.72$ and control plot $r = 0.90$) but significant differences ($P \leq 0.05$) were only indicated for the control plot. No such trend was found for the dead fine root fraction in both roof plots. However, dead fine roots showed generally higher ^{15}N-enrichment than live fine roots in both plots but differences were significant ($P \leq 0.05$) only at the forest floor. For both plots, the N concentrations of the fine roots did not change significantly with the soil depths. The total N-content of live and dead fine roots of the clean rain plot tended to be lower but significant differences were found only for the live fine roots from the 0 to 10 cm mineral soil depth (Lamersdorf, Borken 2004).

High-resolution vertical gradient of ^{15}N, total N and C in the soil matrix

The $\delta^{15}N$ values of uppermost forest floor (clean rain plot: –2.7 ± 0.2‰; roof control plot: –4.0 ± 0.1‰, Fig. 5) correspond to the ^{15}N abundance of needle litter which ranged from –2.9‰ at the clean rain to –4.4‰ at the control plot. For the mineral soil layers, both plots showed the typical soil ^{15}N enrichment from about 0‰ to 9‰ with increasing soil depth. With regard to the forest floor (O_{L+F}, O_H) both plots showed a different pattern of $\delta^{15}N$ values within the upper 5 cm depth. The increase in ^{15}N abundance was interrupted by a decrease which was more pronounced at the control than at the clean rain plot. For both plots, the C content continuously decreased from 47% in the forest floor to 0.2% in 20 cm depth. In contrast, the N content increased slightly within the forest floor and decreased with increasing depth to 0.1% in 20 cm depth.

DISCUSSION

Natural abundance of ^{15}N in the bulk precipitation, throughfall and soil solution

Ammonium in bulk precipitation was slightly more enriched with ^{15}N than nitrate in the year 2000 at the Solling site (Sah, Brumme 2003). Similar results were reported from the other studies (Hoering 1957; Moore 1977; Heaton 1986; Koopmann et al. 1997). A higher enrichment of ^{15}N in ammonium (+10.8‰) compared to nitrate (–12.1‰) was found closer to the emission sources in the Netherlands (Koopmann et al. 1997). These differences were explained by the presence of dry deposition and by the high intensity of animal stock breeding in the immediate vicinity of the studied sites. Relatively far away from the emission source a lower enrichment was observed (–0.6‰ for NH_4-N and –3.3‰ for NO_3-N), which was in the same range as found at the Solling site. Moore (1977) reported $\delta^{15}N$ values for NH_3^- gas samples, collected close to the emission source in barnyards, of more than 20‰. However, several studies reported higher ^{15}N enrichment of nitrate in bulk precipitation than the co-existing am-

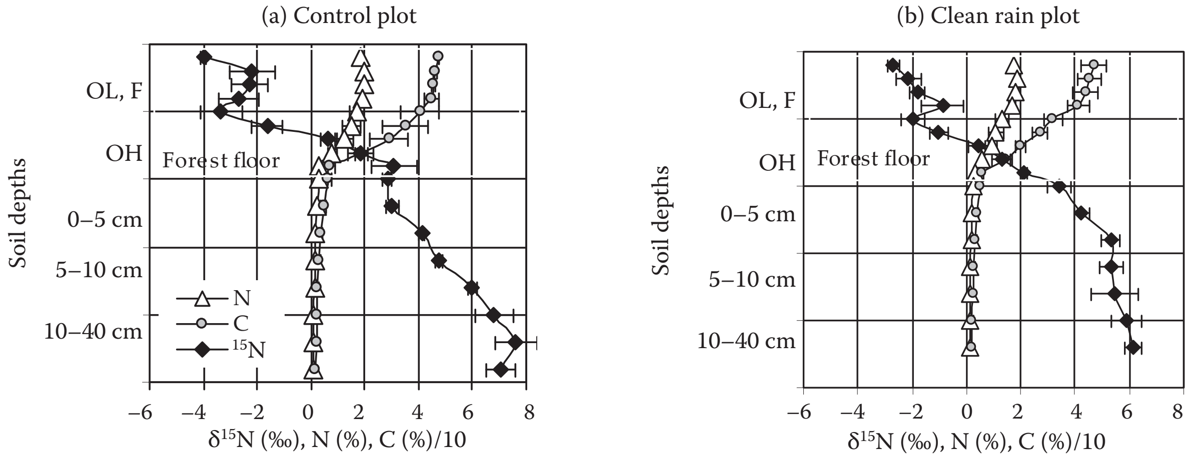

Fig. 5. Vertical gradient of total N (%), total C concentrations (%), and natural ^{15}N abundance ($\delta^{15}N$‰) in the forest floor and mineral soil at the clean rain and control plot (n = 5/depth, ± SE)

monium (see also a review by Kendall 1998) and this was explained by the washout of ^{15}N depleted atmospheric NH_3 (Freyer 1978).

The pathway of ambient rainfall through the canopy significantly influenced the $\delta^{15}N$ abundance of nitrogen. Depletion of ^{15}N-nitrate in throughfall in the Solling roof plots may be attributed to nitrification in the canopy leaves. Nitrification process results in the ^{15}N-enrichment of ammonium and ^{15}N depletion of nitrate (Högberg 1997). Papen et al. (2002) found evidence that autotrophic ammonia and nitrite oxidisers colonise in appreciable cell numbers the phyllosphere of spruce trees in a forest ecosystem exposed for decades to high levels of atmospheric nitrogen. The bacteria are predominantly located inside the spruce needles, most likely within the stomata cavity. The opposite result, a higher ^{15}N abundance of nitrate in throughfall relative to bulk precipitation, was reported from the NITREX sites and other studies (Garten 1992; Koopmann et al. 1997; Emmett et al. 1998) and was attributed to its higher rate of dry deposition.

For throughfall water after passing through the forest floor and the upper mineral soil layers, the $\delta^{15}N$ abundance of nitrate steadily increased. This finding is in agreement with most of the other studies (Koopmann et al. 1997; Emmett et al. 1998) and may indicate a higher proportion of mineralised nitrate from soil organic nitrogen at greater depths which is more enriched with ^{15}N (Fig. 5).

Forest floor and mineral soil

For the mineral soil, a typical vertical gradient of ^{15}N-enrichment was observed in both plots (Fig. 5). This pattern reflects an isotopic discrimination of $\delta^{15}N$ during the mineralisation of soil N. Similar values of ^{15}N enrichment of forest soils (+5 to +10‰) were reported in the majority of other studies (Shearer et al. 1974; Nadelhoffer, Fry 1988, 1994; Gebauer, Schulze 1991; Garten, Migroet 1994; Högberg et al. 1996; Koopmann 1996; Koopmann et al. 1997; Emmett et al. 1998; Nadelhoffer et al. 1999; Sah, Brumme 2003; Sah, Ilvesniemi 2006).

The roof control plot, however, differs from the clean rain plot with respect to the vertical gradient of the ^{15}N abundance of N in the forest floor. Except for the uppermost few cm, there is almost a trend of soil $\delta^{15}N$ depletion with increasing depth in the forest floor of the control plot. Data from the neighbouring old growth spruce and beech forest at the Solling site showed a constant or slightly decreased trend of $\delta^{15}N$ values within the forest floor (Sah, Brumme 2003). A decrease in $\delta^{15}N$ was also observed at the clean rain plot but was restricted to a depth of 4 cm to 5 cm.

The patterns in the forest floor are attributed to more than one process in contrast to the mineral soil where mineralisation is a dominant process for fractionation. Needle litter enters the upper forest floor and determines the starting point of $\delta^{15}N$ values in the top forest floor. After litter fall, the immobilisation of deposited ammonium and nitrate is a dominant process which may have changed the $\delta^{15}N$ values of the forest floor material. The immobilisation of ammonium, which was highly ^{15}N-enriched in the sprinkling water underneath the control plot, may be responsible for increasing $\delta^{15}N$ values in the top forest floor. Ammonium is less mobile than nitrate and is often preferentially immobilised in the forest floor (Tietema et al. 1998). With increasing soil depth, either mineralisation of soil organic nitrogen or immobilisation of depleted nitrate from atmospheric deposition becomes of higher importance. For the clean rain plot, much higher rates of gross mineralisation, immobilisation, and microbial turnover indicated a higher N cycling within the forest floor (Corre, Lamersdorf 2004) while nitrate losses are negligible compared to the control plot (Lamersdorf, Borken 2004). However, although there are very few evidences that the fractionation of N isotopes during the mineralisation of ammonium in soils is of significance (Nadelhoffer, Fry 1994; Högberg 1997), and the higher lighter N-uptake by the microbial biomass might have increased ^{15}N values of the soil at the clean rain plot and probably explain the ^{15}N increase in contrast to the control plot. The decrease in ^{15}N between 1 and 5 cm depths at the control plot may result from low mineralisation and immobilisation of depleted nitrate from atmospheric deposition. In the adjacent spruce and beech forests at the Solling site the forest floor was found to be a strong sink for atmospheric nitrogen. Annual rates of accumulation ranged from 21 kg to 42 kg N/ha in the forest floor of the beech and spruce forests during 35 years of repeated soil inventories (Meiwes et al. 2001).

Vegetation

The clean rain treatment caused a significant decrease in $\delta^{15}N$ values of needles and needle litter between 1992 and 1998 (Fig. 2) and is attributed to the reduction of N deposition. Whether the observed decrease in N content and ^{15}N values of green needles in both plots results from the emission control in Germany at the end of the eighties or just displays the temporal variation of values may not be clear from our data, since the data on the values for N content in needles are not available prior to 1992. However, a close correlation between N availability and $\delta^{15}N$ values is consistent with the literature studies on a local scale (such as Aber et al. 1989) as well as on a regional scale (such as Pardo et al. 2006). Additionally, in a regional scale study Pardo et al. (2006) reported that foliar $\delta^{15}N$ was more closely related to local drivers (net nitrification and mineralisation, and forest floor and soil C:N) of N cycling than the regional driver of deposition. Furthermore, they stated that foliar $\delta^{15}N$ was more strongly related to nitrification rates than was the foliar N concentration and the N concentration were strongly correlated with N deposition. In our case study, among the local drivers of N cycling, only net nitrification and mineralisation rates differ between the plots and the forest floor and soil C/N ratio do not vary much. Hence we assume that the foliar $\delta^{15}N$ increase in N saturated site is due to its higher rate of net nitrification.

Live and dead fine roots of the N limited clean rain plot were enriched with ^{15}N compared to the N-rich control plot (Fig. 4), which is in contrast to the pattern observed in the needles. In general, a positive correlation between fine root $\delta^{15}N$ and N deposition was observed on the regional scale reported by Pardo et al. (2006). ^{15}N enriched roots in N-saturated sites are attributed to fractionation during nitrification, which is a dominant process for ^{15}N accumulation of ^{15}N enriched ammonium and leaching losses of ^{15}N depleted nitrate in N saturated systems. The ^{15}N enrichment in the clean rain plot may have another explanation. Högberg et al. (1996) found that mycorrhizal roots were roughly 2‰ enriched relative to non-mycorrhizal roots. The clean rain application increased fine root biomass (Lamersdorf, Borken 2004) and may also have increased mycorrhizal growth since N-fertilisation experiment (Egerton-Warburton, Allen 2000) and atmospheric N deposition gradients (Egerton-Warburton, Allen 2000; Lilleskov et al. 2001, 2002) reported an increase in the root infection with mycorrhiza with a decrease in atmospheric N deposition and soil N fertilisation.

CONCLUSIONS

The previous results of our studied sites documented that the clean rain plot recovered substantially from the effect of acid deposition and N saturation and showed no further N loss as nitrate with seepage water output but the control plot still suffers from enhanced N deposition and shows N leaching from the soil. Our results of ^{15}N patterns in needles and soils support this finding and show that the natural abundance of ^{15}N can be considered as an indicator of the forest N status. For example, a decrease in total N concentrations and $\delta^{15}N$ values of needles at the clean rain treatment gives indications of a low atmospheric N input. The typical increase in $\delta^{15}N$ values with increasing soil depth was modified by atmospheric deposition in the forest floor. High N deposition decreased $\delta^{15}N$ values in the forest floor probably by immobilising depleted nitrate. However, further researches are needed on the C and N cycles related to mycorrhizal uptake to verify our hypothesis.

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Přirozené zastoupení izotopu dusíku ^{15}N v lesním smrkovém ekosystému vystavenému kyselým srážkám a srážkám bez kyselých příměsí

ABSTRAKT: V průběhu desetiletého pokusu byly zjišťován výskyt izotopů dusíku ve smrkovém porostu, vystavenému kyselým srážkám, a v kontrolním porostu s „čistými srážkami“ bez příměsí dusíku a kyselých složek. Cílem pokusu bylo vyhodnotit, zda by přirozený obsah izotopu dusíku ^{15}N mohl sloužit jako indikátor úrovně dusíku v ekosystémech horských lesů v Německu. Za tímto účelem bylo zjišťováno množství izotopu ^{15}N v jehličí, opadu, kořenech, půdě, srážkách na volné ploše, podkorunových srážkách a půdní vodě na obou lokalitách (testované a kontrolní). Poměrné hodnoty vypočtené z rozdílu poměrů izotopů ^{15}N a ^{14}N v testovaném vzorku a okolním vzduchu (výpočet podle vzorce: $\delta\,^{15}N = \{[(^{15}N/^{14}N_{vzorek})/\ (^{15}N/^{14}N_{vzduch})] - 1\} \times 1\,000$) se pohybovaly v rozpětí hodnot uváděných v dostupných publikacích (–16 až +23‰). Na obou sledovaných lokalitách byly hodnoty ^{15}N nižší v podkorunových srážkách než ve srážkách na volné ploše. To bylo přisuzováno nitrifikaci listů v korunách, vedoucí k odčerpání izotopu dusíku ^{15}N a vyplavování na půdní povrch. Nitráty v prosakující půdní vodě zvyšovaly hodnoty izotopu ^{15}N v horní minerální půdní vrstvě (zjišťováno v hloubce 10 cm) a pronikaly do hlubších půdních horizontů (zjišťováno v hloubce 100 cm). Podobně byly minerální vrstvy půdy obohacovány i celkovým dusíkem. Snížení hodnot izotopu ^{15}N na kontrolní lokalitě (v porovnání s lokalitou s kyselými srážkami) bylo zjištěno v obsahu jehličí i opadu. Předpokládáme, že to bylo vyvoláno zvyšující se mykorhizní absorpcí dusíku v podmínkách s jeho omezeným přísunem, tj. při „čistých srážkách“ bez kyselých příměsí. Omezený přísun dusíku byl rovněž patrný na nízkém obsahu N v jehličí. Ve vertikálním gradientu nahromadění izotopu ^{15}N v humusovém půdním horizontu byly mezi lokalitami rozdíly, výraznější snížení jeho hodnot bylo patrné na kontrolní lokalitě. Na rozdíl od minerálních půdních vrstev, kde je mineralizace dominantním procesem pro rozdělení na frakce, je hodnota ^{15}N ovlivněna dalšími procesy vyvolanými přísunem opadu aj. Na základě dosavadních poznatků lze konstatovat, že hodnota izotopu dusíku ^{15}N je vhodným indikátorem pro posouzení stavu dusíku v lesních ekosystémech. S pokračováním pokusu se počítá i v dalších letech.

Klíčová slova: kyselé srážky; filtrované srážky; izotop dusíku ^{15}N; stabilní izotopy; dusík; srážky; podkorunové srážky; půdní voda; jehličí; opad; smrkový porost

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