Oecologia (1994) 97:322–325

ORIGINAL PAPER

Christian Johannisson · Peter Högberg

^{15}N abundance of soils and plants along an experimentally induced forest nitrogen supply gradient

Received: 15 July 1993 / Accepted: 8 December 1993

Abstract ^{15}N abundances of soils and a grass species (*Deschampsia flexuosa* (L.) Trin.) were analysed in a forest fertilization experiment 10 years after the last fertilization. Nitrogen had been given as urea, at seven doses, ranging from 0 to 2400 kg N ha^{-1}. Previously, we have shown that plants in systems experiencing large losses of N become enriched with ^{15}N. This was explained by the fact that processes leading to loss of N, e.g. ammonia volatilization, nitrification followed by leaching or denitrification and denitrification itself, tend to fractionate against ^{15}N. In this experiment, ^{15}N abundance increased with dose of N applied in both grass and soil total-N, but more so in the grass. This was interpreted to be due to the grass sampling small but active pools of N subject to losses. In contrast, soil total-N largely consists of inactive N that does not immediately exchange with pools of N from which fractionating losses occur. Hence, soil total-N shows a large pretreatment ^{15}N memory effect, and is, therefore, an integrator of the long-term N balance. When short-term changes (years, decades) in N balances are monitored using variations in ^{15}N abundance, plants are more suitable indicators of such change than is soil total-N.

Key words ^{15}N · Forests · N cycling · Soil N pools

Introduction

Elevated levels of nitrogen deposition, and adverse effects thereof on terrestrial and aquatic ecosystems, are of increasing concern (Aber et al. 1989; Schulze 1989; Tamm 1991). Högberg (1990b, 1991) proposed studies of the abundance of the stable nitrogen isotope ^{15}N to monitor changes in the N balance of ecosystems. Processes leading to loss of N, i.e. NH_3 volatilization, nitrification followed by denitrification or leaching, and denitrification, discriminate against the heavier ^{15}N (Shearer and Kohl 1986). This leaves the remaining N enriched in ^{15}N as suggested by Vitousek et al. (1989) and experimentally verified by Högberg (1990b, 1991) and Högberg and Johannisson (1993) studying plants in a forest fertilization trial.

When fertilizer N has a ^{15}N abundance lower than that of soil-derived N, nitrogen fertilization should lower the ^{15}N abundance of N in the system (Kohl et al. 1973). However, as stated above, when inputs of N are large, this initial decrease is followed by an enrichment in ^{15}N (Högberg 1991) as a result of loss of N (Högberg and Johannisson 1993).

It has generally been concluded that the ^{15}N abundance of vegetation reflects the ^{15}N abundance of N sources available to the plants (Shearer and Kohl 1986). Commonly, plants have lower ^{15}N abundances than soil total-N (Gebauer and Schulze 1991; Handley and Raven 1992; Virginia and Delwiche 1982; Vitousek et al. 1989), but exceptions have been reported (Pate et al. 1993). Gebauer and Schulze (1991) showed that the ^{15}N abundance of both soil total-N and N in fine roots increased with depth in a spruce forest, but also that the ^{15}N abundance of roots was 0.5–4.2 δ ^{15}N (‰) lower than that of soil total-N.

The relation between the ^{15}N abundances of plants and soils is evidently crucial when evaluations of N balances and the development of N saturation are to be based on plant samples (Högberg 1990b, 1991). For example, Sutherland et al. (1993) found a greater variability in ^{15}N abundance of plants than of soils in durum wheat fields, and attributed this to the greater susceptibility of the small mineral-N pool to isotopic fractionation than the larger inert soil organic-N pool. In this paper we show how a wide range of defined N additions to a forest have affected ^{15}N abundances of both plants and soils, and based on these observations, make inferences about soil N pool dynamics.

C. Johannisson (✉) · P. Högberg
Section of Forest Soils, Department of Forest Ecology, Swedish University of Agricultural Sciences,
S-901 83, Umeå, Sweden

Materials and methods

The experimental site is located in a Norway spruce (*Picea abies* Karst.) forest on a gentle slope, at Nyby in northern Sweden (64°20'N, 18°27'E, 370 m a.s.l.). The soil is a podsolized sandy-silty till supporting a rather poor tree stand. In 1966, when the stand was 142 years old, the mean tree height was only 16 m. The field layer is dominated by *Vaccinium myrtillus* L., while the grass species *Deschampsia flexuosa* (L.) Trin. is sparsely distributed (Kellner 1993). Soil $pH_{(H2O)}$ varies between 4.0–4.5 and 4.0–4.2 in the mor layer and in the upper 5 cm of the mineral soil, respectively (H.-Ö. Nohrstedt, pers. comm.). The C/N ratio of the H-layer of the mor is around 32. Deposition of N is below 5 kg ha^{-1} yr^{-1}.

We studied the experiment D67 maintained by the Research Institute SkogForsk. Two blocks of plots, with seven 30 × 30 m plots each, had been fertilized with urea at seven levels (NO-N6) in the years of 1966, 1971, 1976 and 1981. Total additions of N varied between 0 and 2400 kg ha^{-1}. The experimental design is summarized in Table 1. We have not measured the ^{15}N abundance of fertilizer-N, but know that the fertilizer was supplied by a Swedish manufacturer, Supra, which has been reported to produce urea significantly depleted in ^{15}N (Högberg 1990b). Low annual additions of urea ($\bar{x}=36$ kg N ha^{-1}), supplied by Supra, caused a consistent decline in ^{15}N abundance in a nearby experiment during the period 1971–1989 (Högberg 1991).

Fertilized spruce grew consistently better than non-fertilized spruce, and responded positively to each fertilization without obvious signs of saturating responses to N. There was, however, no additional growth effect of N applications at levels greater than N4 (F. Pettersson, pers. comm.). In 1989, the annual increment of basal area on fertilized plots was 120–240% of that on control (NO) plots. Nitrogen fertilization also increased the coverage of *D. flexuosa* at the expense of *V. myrtillus*.

Ten cores (10 cm diameter) of the mor-layer were taken at random from the inner 20 × 20 m of each plot in late August 1991, i.e. 10 years after the last fertilization. The H-layer was used for analysis. A composite sample of a few grams of vegetative shoots of the grass *D. flexuosa* was taken from each of the plots (see Högberg et al. 1986). The samples were ovendried (70°C, 48 h). Subsamples of soils and grass were ground in a ball mill and analysed for total-N and ^{15}N abundance (Haystead, 1983). Results are reported in per cent N and in δ ^{15}N (‰): δ $^{15}N = 1000 \times (R_{sample}-R_{standard})/R_{standard}$(‰), where R = mass 29/mass 28, and the standard used was pure N_2 with a ^{15}N abundance of −2.6 δ ^{15}N (‰) relative to atmospheric N_2 (Mariotti 1983).

The dominating field layer species, *V. myrtillus*, assimilates little NO_3^- (Gebauer et al. 1988; Högberg et al. 1990; Lee and Stewart 1978; but see Ingestad 1973). In contrast, *D. flexuosa*, although preferentially utilizing NH_4^+ (Gigon and Rorison 1972), also readily assimilates NO_3^- (Högberg et al. 1986; Lee and Stewart 1978) and was, therefore, used as an indicator of ^{15}N of the pools of N available to plants. Whether NH_4^+ or NO_3^- is taken up also depends on their concentrations and mobility in the soil system (see e.g. Marschner et al. 1991).

Data were analysed by the GLM procedure (e.g. $^{15}N_{soil}$ = block; covariate N-dose) of MINITAB (PC Version 8.0, Microsoft Corp., 1991). Partial correlation between $^{15}N_{soil}$ and $^{15}N_{grass}$, and between $^{15}N_{soil}$ and N concentration in the soil, was calculated after elimination of treatment and block effects. It was tested whether the (theoretical) partial correlation was zero by means of the test statistic $t = r_1\,(n-4)^{1/2}/(1-r^2{}_1)^{1/2}$, where r_1 is the coefficient of correlation between residuals. The statistic t has approximately a t-distribution with $n-4$ degrees of freedom under the null-hypothesis (a modification of Quade 1989). In the case of the soil samples, means per plot were used in the analyses.

Table 1 Experimental design of the experiment D67 at Nyby

Number of blocks	2
Number of treatments	7
Number of fertilizations	4
Years of fertilization	1966, 1971, 1976, 1981

N applied (kg N ha^{-1})	Per occasion	Total
N0	0	0
N1	120	480
N2	180	720
N3	240	960
N4	360	1440
N5	480	1920
N6	600	2400

Results

Ten years after the last fertilization, treatment effects on ^{15}N abundance were still evident in the soil and in the grass. The ^{15}N abundance of the soil and of the grass increased with dose of N fertilizer applied ($P = 0.014$ and $P = 0.000$, respectively; Fig. 1). The grass had a lower ^{15}N abundance than the soil, but the increase in ^{15}N was larger in the grass than in the soil ($P = 0.000$). Nevertheless, when treatment effects on ^{15}N abundance were partialled out or accounted for, variations in ^{15}N of the grass were highly correlated with the ^{15}N abundance of the soil ($r_1 = 0.806$, $P = 0.002$). For example, relatively high ^{15}N abundance in the grass in treatment N1 in block 1 corresponded to a high ^{15}N abundance in the soil. The difference between the grass and the soil was significantly smaller in block 1 ($P = 0.004$). The standard deviation of the mean for each plot varied between 0.6–1.7 δ ^{15}N (‰) in the soil, i.e. it was large compared to the absolute values.

The concentration of N in the soil increased with level of fertilizer application (Fig. 2, $P = 0.000$). A block effect was found ($P = 0.039$); block 1 having a somewhat higher N concentration than block 2. There was no sig-

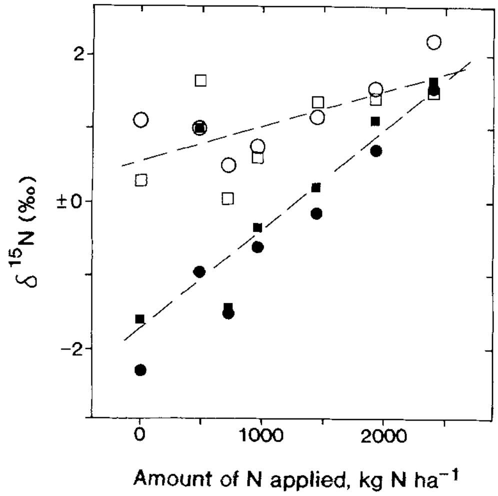

Fig. 1 ^{15}N abundances of soils (*open symbols*) and of the grass species *Deschampsia flexuosa* (*closed symbols*) in 1991, in relation to amount of N applied during the period 1966–1981 to plots at Nyby. *Squares*, block I; *circles*, block II

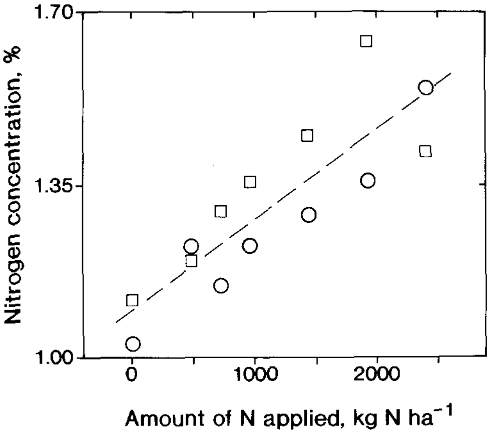

Fig. 2 Nitrogen concentrations of soils (H-layer) in 1991, in relation to amount of N applied during the period 1966–1981 to plots at Nyby. Symbols as in Fig. 1

nificant treatment effect on the N concentration in the grass. When treatment effects were partialled out, neither the ^{15}N abundance of the grass nor that of the soil were correlated with the N concentration of the soil ($r_1 = -0.031$ and $r_1 = 0.012$, respectively).

Discussion

Most N in soils is in the form of organic N (Tamm 1991), and the greater portion of soil organic N is fixed in stable forms (Jansson 1958; Paul and Clark 1988; Paul and Juma 1981). This humus N may constitute a passive organic phase existing more or less beside the pathways of the mineralization-immobilization turnover cycle (Jansson 1958). The active organic phase would then be more readily mineralizable N of plant and microbial origin plus N in the living microbial biomass. Nitrogen transformations, e.g. mineralization, nitrification and immobilization, within the biologically active pools of microbial N, NH_4^+ and NO_3^- are considerable although the actual concentrations of NH_4^+ and, especially, NO_3^- are low in forest soils (Davidson et al. 1992).

After fertilization urea is hydrolysed to NH_3, which readily reacts to form NH_4^+. Estimates of volatilization losses of NH_3-N during the first few weeks after fertilizer application vary from $<5\%$ to $>40\%$ of the N applied in urea fertilized forests (Nason et al. 1988). NH_3 is, furthermore, chemically incorporated into stable organic compounds in the mor layer (Foster et al. 1985). Volatilization of NH_3 fractionates against ^{15}N (Shearer and Kohl 1986), leading to ^{15}N enrichment of the soil NH_4^+ pool. Except for the first few weeks following fertilization, losses by NH_3 volatilization should be negligible from the acid soils at Nyby.

In this experiment, the last fertilization took place 10 years before the study. The ^{15}N abundance of plant N should primarily reflect the ^{15}N abundances of NH_4^+ and NO_3^- in the soil solution or on exchange sites plus active organic pools of N. Losses of soil-N in urea-fertilized forests should be from the NH_3/NH_4^+ pool directly following fertilization, and thereafter from the NO_3^- pool, and therefore affect the isotopic composition of these pools, as well as that of pools of N exchanging with them (see Davidson et al. 1992). In contrast, the ^{15}N abundance of soil total-N is an integrated measure of the ^{15}N abundance of all pools of N, which involves dilution of treatment effects on ^{15}N abundance by a large pool of old unreactive stable organic N. Hence, the change in ^{15}N abundance of the soil is less evident than that of the grass (Fig. 1).

Theoretically, the plants could acquire a higher ^{15}N abundance than soil total-N at high levels of N application (Fig. 1), as a result of increasingly higher ^{15}N enrichment of active pools of N. This has now been observed in a nearby experiment similar to the Nyby trial (L. Högbom & C. Johannisson, pers. comm.). Plants and soils have also been studied along a steep natural N supply gradient c. 50 km from our site in Nyby; the difference in ^{15}N abundance between plants and soils diminished with increasing supply of N there also (M. Högberg & P. Högberg, pers. comm.). Recently, Pate et al. (1993) reported that in Australian woodland disturbed by fire, resin extractable NO_3^- and NO_3^- in the xylem sap was enriched with ^{15}N relative to soil total-N. The ^{15}N abundance of plants increased with increasing utilization of NO_3^-, and some plants reached a higher ^{15}N abundance than soil total-N. This contradicts the simple view that one only has to consider that products should become progressively depleted (Högberg 1990a), which would be appropriate in a closed system without losses.

We have assumed that fertilizer-N had a lower ^{15}N abundance than soil total-N. The fact that the lowest ^{15}N abundance of soil total-N is found in N2, from which treatment the ^{15}N abundance progressively increases with amount of N applied, supports this assumption.

Exchange of N between active and passive pools of N, should, with time, reduce the magnitude of the ^{15}N enrichment of the active pools of N. As the experiment was sampled 10 years after the last treatment, this could explain why the difference in ^{15}N abundance in the grass between the lowest and the highest dose of N applied was only one-third of the difference found at Norrliden (Högberg 1990b), which was studied under conditions of experimental steady-state, i.e. during the 18th year of annual fertilizations. Estimates of the microbially available pool of N (Nordgren 1992) at Nyby showed no differences between treatments in 1993 (A. Nordgren, pers. comm.). Neither did we observe any increase in N concentration in the grass with increasing dose of N applied, which is in contrast to the large increase in soil N concentration (Fig. 2). Hence, the isotopic labelling of the pools of N available to plants is largely reflecting the N dynamics during and shortly after experimental treatments, and not the current N dynamics.

In conclusion, applications of unlabelled N to a forest ecosystem induced increases in ^{15}N abundances of both soils and plants, which were detectable 10 years after the last treatment. Workers interested in monitoring short-term (years, decades) changes in N balances of forests by use of variations in ^{15}N abundance (Högberg 1990b) should observe the plants, which sample small, but active pools of soil N involved in losses from the system as well as other fractionating processes, rather than soil total-N, which mainly represents passive N carrying a large ^{15}N memory effect. Those interested in the long-term N balance should analyze the soils.

Acknowledgements We would like to thank L. Bondesson for advice on the statistics, H.-Ö. Nohrstedt and F. Pettersson at Skog-Forsk and G.R. Stewart, Brisbane, Australia, for valuable information. The work was financially supported by the Swedish Research Council for Forestry and Agriculture (SJFR), and is also a contribution to the EC project NiPhys.

References

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. BioScience 39:378–386

Davidson EA, Hart SC, Firestone MK (1992) Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73:1148–1156

Foster NW, Beauchamp EG, Corke CT (1985) Immobilization of nitrogen-15-labelled urea in a Jack pine forest floor. Soil Sci Soc Am J 49:448–452

Gebauer G, Schulze E-D (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. Oecologia 87:198–207

Gebauer G, Rehder H, Wollenweber B (1988) Nitrate, nitrate reduction and organic nitrogen in plants from different ecological and taxonomical groups of Europe. Oecologia 75:371–385

Gigon A, Rorison IH (1972) The response of some ecologically distinct plant species to nitrate- and to ammonium-nitrogen. J Ecol 60:93–102

Handley LL, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ 15:965–985

Haystead A (1983) Analysis of nitrogen isotope ratios by mass spectrometry. In: Smith KA (ed) Soil Analysis: Instrumental Techniques and Related Procedures. Marcel Dekker, New York, pp 377–406

Högberg P (1990a) ^{15}N natural abundance as a possible marker of the ectomycorrhizal habit of trees in mixed African woodlands. New Phytol 115:483–486

Högberg P (1990b) Forests losing large quantities of nitrogen have elevated ^{15}N:^{14}N ratios. Oecologia 84:229–231

Högberg P (1991) Development of ^{15}N enrichment in a nitrogen-fertilized forest soil-plant system. Soil Biol Biochem 23:335–338

Högberg P, Johannisson C (1993) ^{15}N abundance of forests is correlated with losses of nitrogen. Plant Soil 157:147–150

Högberg P, Granström A, Johansson T, Lundmark-Thelin A, Näsholm T (1986) Plant nitrate reductase activity as an indicator of availability of nitrate in forest soils. Can J For Res 16:1165–1169

Högberg P, Johannisson C, Nicklasson H, Högbom L (1990) Shoot nitrate activities of field-layer species in different forest types I. Scand J For Res 5:449–456

Ingestad I (1973) Mineral nutrient requirement of *Vaccinium vitis-idea* and *V. myrtillus*. Physiol Plant 29:239–246

Jansson SL (1958) Tracer studies on nitrogen transformations in soil with special attention to mineralization-immobilization relationships. Ann Roy Agr Coll Sweden 24:101–361

Kellner O (1993) Effects on associated flora of sylvicultural nitrogen fertilization repeated at long intervals. J Appl Ecol 30:563–574

Kohl DH, Shearer GB, Commoner B (1973) Variation of ^{15}N in corn and soil following application of fertilizer nitrogen. Soil Sci Soc Am Proc 37:888–892

Lee JA, Stewart GR (1978) Ecological aspects of nitrogen metabolism. Adv Bot Res 6:1–43

Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature 303:685–687

Marschner H, Häussling M, George E (1991) Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce (*Picea abies* (L.) Karst.). Trees 5:14–21

Nason GE, Pluth DJ, McGill WB (1988) Volatilization and foliar recapture of ammonia following spring and fall application of nitrogen-15 urea to a Douglas-fir ecosystem. Soil Sci Soc Am J 52:821–828

Nordgren A (1992) A method for determining microbially available N and P in an organic soil. Biol Fertil Soils 13:195–199

Pate JS, Stewart GR, Unkovich M (1993) ^{15}N natural abundance of plant and soil components of a *Banksia* woodland ecosystem in relation to nitrate utilization, life form, mycorrhizal status and N_2-fixing abilities of component species. Plant Cell Environ 16:365–373

Paul EA, Clark FE (1988) Soil Microbiology and Biochemistry. Academic Press, San Diego

Paul EA, Juma NG (1981) Mineralization and immobilization of soil nitrogen by microorganisms. Ecol Bull 33:179–195

Quade D (1989) Partial correlation. In: Kotz S, Johnson NL (eds) Encyclopedia of Statistical Sciences. John Wiley, New York, pp 117–120

Schulze E-D (1989) Air pollution and forest decline in a spruce (*Picea abies*) forest. Science 244:776–783

Shearer GB, Kohl DH (1986) N_2-fixation in field settings: estimations based on natural ^{15}N abundance. Aust J Plant Physiol 13:699–757

Sutherland RA, Kessel C van, Farrell RE, Pennock DJ (1993) Landscape-scale variations in plant and soil nitrogen-15 natural abundance. Soil Sci Soc Am J 57:169–178

Tamm C-O (1991) Nitrogen in Terrestrial Ecosystems (Ecological Studies 81). Springer, Berlin, Heidelberg, New York, p 15 and p 75

Virginia RA, Delwiche CC (1982) Natural ^{15}N abundance of presumed N_2-fixing and non-N_2-fixing plants from selected ecosystems. Oecologia 54:317–325

Vitousek PM, Shearer G, Kohl DH (1989) Foliar ^{15}N natural abundance in Hawaiian rainforest: patterns and possible mechanisms. Oecologia 78:383–388