

Nitrogen mineralization and potential nitrification at different depths in acid forest soils

T. Persson and A. Wirén

Dept of Ecology and Environmental Research, Swedish University of Agricultural Sciences, P.O. Box 7072, S-75007 Uppsala, Sweden

Key words: acidity, autotrophic nitrifiers, N mineralization, nitrification, pH, soil horizon

Abstract

Soil samples were collected from the litter, humus and five mineral soil layers to a depth of 50 cm in acid, but highly productive, Norway spruce forests in S Sweden and E Denmark for determination of net N mineralization and potential nitrification. The samples were sieved while still fresh and incubated at a constant temperature (15°C) and soil moisture for 74–117 days with periodic subsamplings. Net N mineralization rates, expressed per g organic matter or per g of total N, decreased with increasing depth. Net N mineralization in the ten soils studied ranged from 35 to 105 kg N ha⁻¹ yr⁻¹, of which the organic horizons contributed 32–74%. Nitrate formation patterns were variable. Almost no nitrification could be detected at pH (H₂O) values lower than 4.0. Nitrate was formed in humus layers with pH values of 4.0–4.5, but the nitrification was never complete. By contrast, the nitrification was almost complete at a depth of 10–50 cm, where the pH (H₂O) was 4.1–4.5. Addition of CaCO₃ stimulated nitrification in the humus layer, indicating the presence of acid-sensitive nitrifiers, while nitrification in the mineral soil was sometimes stimulated and sometimes inhibited by the addition of CaCO₃. Tests with the acetylene block method showed that nitrification, when it occurred, was autotrophic in the humus layer but less easy to characterize in the mineral soil. Thus, further studies are needed to characterize the nitrifier organisms and identify the factors regulating the activity of these organisms. Actual nitrification rates in the field could not be determined, but our findings showed that the B horizon in acid forest soils has a great potential for nitrification. Because N deposition does not appear to decrease in Scandinavian forests, we conclude that an ammonium surplus in the forest floor followed by a downward transport of ammonium to the nitrifying subsoil can increase the risk of nitrate leaching in the future.

Introduction

Acid coniferous forests in the Nordic countries have traditionally been considered to have a tight N cycle, with almost all mineralized N being taken up by roots and no NO₃⁻ being formed (Tamm, 1990). However, recent observations indicate that this is not necessarily the case. NO₃⁻ leaching has been recorded in some forest areas in Denmark and Sweden, most of which have been subjected to moderate to high levels of N deposition (Gundersen and Rasmussen, 1990; Hallgren Larsson and Westling, 1993; Nilsson and Grennfelt, 1988; Schulze et al., 1989). NO₃⁻ below the rooting zone may originate from deposited NO₃⁻ transported directly through the soil profile or from NO₃⁻ formed in the soil through nitrification.

Nitrification is reported to be performed by both acid-tolerant and acid-sensitive nitrifiers (De Boer et al., 1990). In acid coniferous forest soils, the pH often reaches a minimum value in the H layer (the lower O horizon) or in the upper E horizon, below which the pH increases with increasing depth (Skjyllberg, 1993). Thus, in soils considered as very acid based on measurements in the humus layer, acid-sensitive nitrifiers might, nevertheless, account for the nitrate formation in deeper soil layers where the pH is more favourable. In soils lacking acid-tolerant nitrifiers, nitrate formation should, consequently, be restricted to soil layers with pH higher than a certain critical level.

To test this hypothesis, but also to obtain information on the net N mineralization and potential nitrification in different soil layers in various types of conifer-

ous forest sites, seven soil layers from a total of ten sites in S Sweden and E Denmark were studied. For some of the sites, tests were made to characterize the types of nitrifiers present. The study was based on incubation experiments in the laboratory.

Materials and methods

Field sampling

Soil samples were taken from nine sites in S Sweden and one site in Sealand, E Denmark, from November 1990 to October 1992. The samples were taken from untreated (non-fertilized, non-limed) stands of Norway spruce, *Picea abies* (L.) Karst., or clear-cut plots earlier covered with Norway spruce. All soils were podzolized but to a variable extent. Site characteristics are given in Table 1. Estimates of bulk precipitation in the open field and canopy throughfall were not available from sites 4 and 7. The deposition of N ranged from about 10 (site 6) to 31 (site 9) kg N ha⁻¹ yr⁻¹ (Gundersen and Rasmussen, 1990; Hallgren Larsson and Westling, 1993; B. Bergkvist, pers. comm.; H.-Ö. Nohrstedt, pers. comm.; B. Popovic, pers. comm.) in the remaining sites. NO₃⁻ leaching, based on measurements of inorganic N in soil water collected by suction lysimeters (30 and 50 cm depth) and data on evapotranspiration, was calculated to be in the range of 0 to 30 kg N ha⁻¹ yr⁻¹ (Table 1). Some of these data are very approximate because of short observation periods.

Litter (L) and humus layers (FH) were quantitatively sampled with a 20 × 20 cm frame, while the mineral soil was sampled to a depth of 50 cm with a 28-cm² corer in the soil exposed in connection with the litter and humus sampling. The mineral soil was divided into five 10-cm thick sublayers. Within each plot, five samples were taken systematically, and samples from the same soil layer were pooled for each plot. Four localities, i.e., Hasslöv, Farabol 1, Farabol 2 and Skogaby (sites 5, 6, 7 and 8), had replicate plots, but all other sites lacked true replicates. The Farabol site was partly clear-cut in early 1992. Two plots in the remaining forest, "Farabol 1", served as controls for three plots in the clear-cut area, "Farabol 2". The litter layer was not included in the sampling at Åled and Harplinge (sites 9 and 10), and the samples from Hasslöv were only taken to a depth of 30 cm.

Laboratory treatment

The samples were immediately transported to the laboratory, where they were treated without being dried or frozen. Litter samples were separated from moss and twig fragments, so the remaining litter material mainly consisted of spruce needles. Humus samples were passed through a 5-mm sieve, and the mineral soil through a 2-mm sieve. We determined the fresh weight/dry weight ratios at 105°C and the ash content/loss on ignition at 600°C. Based on the former determination, portions corresponding to 6 g dw (dry weight) of litter, 16 g dw of humus material and 100 g dw of mineral soil were placed in plastic containers (50 cm² surface area). The containers had a lid with a 5-mm diameter aperture for gas exchange and were placed in a room with a constant temperature of 15°C. The water content of the samples was kept at 60% of water-holding capacity, WHC (100% WHC was defined as the water content of inundated soil allowed to drain for 12 h in a 5-cm high cylinder). Because no leaching could occur, it was assumed that the inorganic nitrogen accumulated in the system. A whole incubation period lasted from 95 to 200 days. To make the estimates as comparable as possible, only the first 74 to 117 days of the incubation period were used in this study. Destructive samplings were made at certain intervals to characterize the temporal pattern of mineralization. A minimum of three replicates were collected on each sampling occasion. The net N-mineralization rate in a given soil layer was calculated as the inorganic (NH₄⁺ + NO₃⁻) N accumulated during the period divided by the number of days. NO₂⁻ was never found in appreciable amounts. Nitrification was calculated in the same manner (daily mean accumulation of NO₃⁻-N). With this method of calculation, the nitrification rate can exceed the mineralization rate if the NH₄⁺ pool decreases during the incubation.

To get a rough estimate of the annual mineralization of N and annual nitrification, the rates (per g per day) obtained in the laboratory at 15°C and optimal moisture were multiplied by (1) the amount of sieved soil per soil layer (g ha⁻¹) estimated from the quantitative samplings described above and corrected for stoniness, (2) a temperature correction factor assuming a Q₁₀ of 3.0 (Persson, 1989) between 15°C and the annual mean air temperature on the site (assumed to be 6.5°C for all sites), and (3) a moisture correction factor (75% of the rates at 60% WHC for all sites) (Persson, 1989). Because the estimates of nitrification were based on root-free samples, in which NO₃⁻ accu-

Table 1. Characteristics of the study sites at the time of sampling. Mean air temperature ranged from 6.5 to 7.5°C for all localities. cc=clear-cutting 3–7 months before sampling. Data obtained from J. Bergholm, B. Bergkvist, L.-O. Nilsson, H.-Ö. Nohrstedt, B. Popovic, L. Rasmussen, E. Ring, G. Wiklander, E. Hallgren Larsson and O. Westling (pers. comm.)

Site	Latitude/ longitude	Altitude (m above mean sea level)	Mean annual precip. (mm)	Stand age (years)	Stem volume (m ³ ha ⁻¹)	Current growth (m ³ ha ⁻¹)	Leaching of inorg. N (kg ha ⁻¹ y ⁻¹) (Appr. data)	Sampl. date
1. Dalby	55° 41' N 13° 27' E	90	680	51	400	17	15	July 25 1991
2. Söderåsen	56° 07' N 13° 02' E	85	860	68	420	16	5	July 26 1991
3. Strødam	55° 58' N 12° 19' E	20	620	45+cc	470	7*	17*	July 24 1991
4. Vallåsen	56° 23' N 13° 00' E	180	1100	37	400	20	not est.	Dec. 10 1991
5. Hasslöv	56° 24' N 13° 00' E	190	1100	41	350	21	1	Nov. 8 1990
6. Farabol 1	56° 25' N 14° 35' E	140	680	71	440	10	0	Oct. 1 1992
7. Farabol 2	56° 25' N 14° 35' E	140	680	71+cc	440	10 *	0*	Oct. 7 1992
8. Skogaby	56° 33' N 13° 13' E	95–115	1150	28	220	16	0	Apr. 27 1992
9. Harplinge	56° 45' N 12° 45' E	70	800	42	390	15	30	July 12 1992
10. Åled	56° 46' N 12° 56' E	140	1040	60	490	15	7	June 17 1992

*Before clear-cutting.

mulation was not reduced by root uptake, we have called these estimates “potential nitrification” in the following text.

In some experimental series, acetylene (C₂H₂) gas was injected once a week into containers with gastight lids to inhibit autotrophic nitrification (De Boer et al., 1993; Hyman and Wood, 1985; Hynes and Knowles, 1982). The treatment resulted in a C₂H₂ concentration of 1% during 5 h, whereupon the lids were removed for 2 h to allow the C₂H₂ to disappear.

In other experimental series, CaCO₃ and H₂SO₄ were added to the soil samples to increase the pH to 6 or decrease the pH to 4, respectively, in order to check whether the nitrifiers were acid-sensitive. In the first case, CaCO₃ powder was mixed into the samples. In the second case, solutions of 0.05 M H₂SO₄ were added with a pipette.

Before starting the incubation and at each destructive sampling, the pH was determined with a glass

electrode in the supernatant after shaking for 2 h and sedimentation in an open flask for 24 h in both distilled water and in 1 M KCl. The fresh soil:extractant proportions were 1:1 by volume (about 1:10 by dry matter for litter and humus and 1:2.5 for mineral soil).

Inorganic N was extracted with a 1 M KCl solution for 1 h. The filtrate was analyzed for NH₄⁺ according to a gas diffusion method based on the flow injection technique (Svensson and Anfält, 1982). NO₃⁻ was reduced with amalgamated Cd to NO₂⁻, which was then complexed to form an azo dye and measured on an autoanalyzer (Anonymous, 1976). Total C and N were analyzed in a Carlo-Erba NA 1500 Analyzer.

Differences between soil layers were tested for the sites with replicate plots. For sites 6, 7 and 8, a three-way analysis of variance was used with “site” and “soil layer” as fixed factors and “block within site” as a random factor. Because of a significant (statistical) interaction between “site” and “soil layer”, pairwise

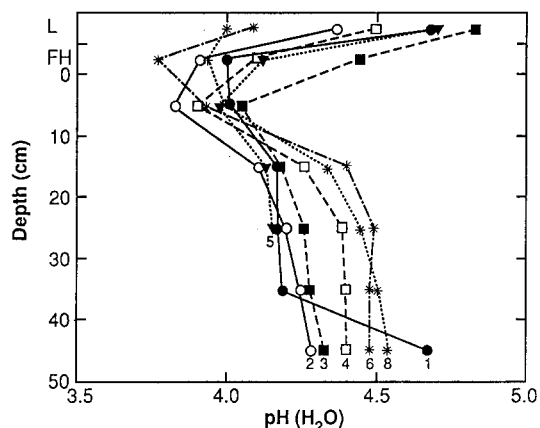


Fig. 1. Mean pH (H_2O) in different soil layers and sites. The pH profiles in sites 7 and 10 (not shown) resembled that in site 6, and the profile in site 9 resembled that in site 1. See Table 1 for site numbers.

comparisons of soil layers were made for individual sites using Student's *t*-test. For site 5, which was treated separately to avoid heteroscedasticity, a two-way analysis of variance was made with "soil layer" and "block" as factors. In the following text, means are given \pm one standard error.

Results

The sites studied were all acidic and had a pH minimum in the humus (FH) layer or at a depth of 0–10 cm below the humus layer in the mineral soil (Fig. 1). Detailed studies showed that the lowest pH was either in the O_a horizon (H layer) or in the upper E horizon (0–5 cm below the humus layer). The estimated pH (H_2O) varied between 3.7 and 4.5 in the humus layer and between 4.1 and 4.4 in the upper B horizon (10–20 cm depth). The pH (KCl) (not shown here) varied between 2.6 and 3.2 in the humus layer and from 3.4 to 4.2 in the upper B horizon.

The soil profile to a depth of 50 cm in the mineral soil contained from 88 to 148 t C ha⁻¹ and from 3.8 to 7.2 t N ha⁻¹ (Table 2). On average, 26% of the C pool and 22% of the N pool was found in the organic (LFH) layers, while as much as 74% and 78% of the C and N pool, respectively, was found in the mineral soil.

The C:N ratios, which can be calculated from the data in Table 2, decreased downwards from the litter layer (range: 26–38) to 10–20 cm depth in the mineral soil (range: 16–26). Below this depth, the C:N ratio did not change to any great extent.

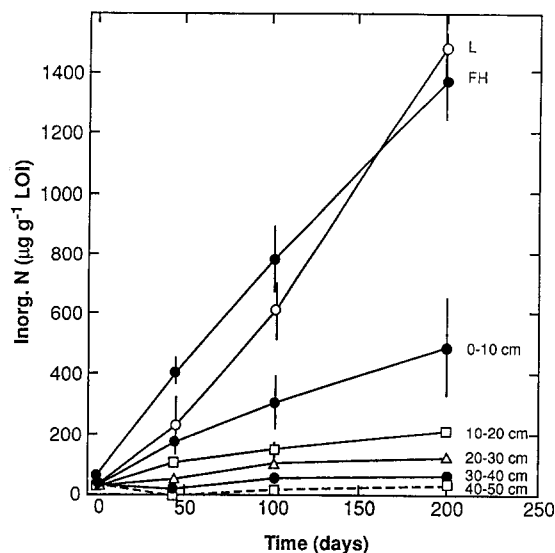


Fig. 2. Accumulation of inorganic N ($NH_4-N + NO_3-N$) in different soil layers from control plots ($n=4$) at Skogaby (site 8) after incubation at constant temperature (15°C) and moisture (60% of WHC). L=litter layer, FH=humus layer 0–10 cm etc.=depths below the humus layer, LOI=loss on ignition. Bars indicate \pm one S.E. Where bars are not visible, they are covered by the symbol.

The accumulation of inorganic N ($NH_4^+-N + NO_3^- -N$) differed between soil layers and sites. Figure 2 shows the accumulation (mineralization) pattern for the seven soil layers at Skogaby (site 8). The mineralization rates were distinctly higher for the litter and humus layers than for the mineral soil layers, where the mineralization rates decreased with increasing depth. Soil horizons from the other sites showed similar mineralization patterns with the exception of the litter layer, in which the net N mineralization varied considerably between sites (see below).

Rates of inorganic N accumulation differed between sites, but for a given site and soil layer, the rates changed only moderately over time (as in Fig. 2). Therefore, calculating a mean rate of net N mineralization was considered to be acceptable. High starting values of inorganic N were found in the humus material from site 7 (520 $\mu\text{g N g}^{-1}$ org. matter) and site 3 (270 $\mu\text{g N g}^{-1}$ org. matter), where the forests were cut 7 and 3 months, respectively, before sampling. The mineralization rates in these materials fell well within the range of those obtained for the other soils.

The mean rates of net N mineralization calculated for each site and horizon are given in Table 3. The statistical tests made for sites 5–8, which had replicate plots, showed significant differences between soil

Table 2. C and N pools estimated for each site (see Table 1) and soil layer studied. S.E. was about 10% of the mean in the upper soil layers in the sites with replicate plots (sites 5–8). Below 20 cm depth, S.E. was 10–20% of the mean because of variation in stoniness

Soil layer	Site no.										Mean
	1	2	3	4	5	6	7	8	9	10	
	C pool (t ha⁻¹)										
L	6	6	4	7	8	1	2	2	n.e.	n.e.	4
FH	27	33	22	32	19	23	27	18	19	27	25
0–10 cm	34	29	29	39	39	29	24	26	35	43	33
10–20 cm	18	11	15	14	24	18	17	18	35	26	20
20–30 cm	13	12	10	15	19	14	11	15	25	19	15
30–40 cm	13	8	7	14	n.e.	13	8	11	14	17	12
40–50 cm	9	5	5	9	n.e.	11	7	8	11	13	9
Total	119	108	88	129	129*	109	98	98	143*	148*	117
% in LFH	28	36	29	30	21*	22	30	21	17*	21*	26
	N pool (t ha⁻¹)										
L	0.2	0.2	0.2	0.3	0.3	0.03	0.1	0.1	n.e.	n.e.	0.16
FH	1.1	1.2	0.9	1.2	0.7	0.8	0.9	0.7	0.9	1.0	0.94
0–10 cm	1.8	1.0	1.2	1.6	2.1	1.0	0.9	1.1	1.8	1.8	1.43
10–20 cm	1.1	0.6	0.4	0.6	1.4	0.7	0.7	0.8	1.8	1.1	0.92
20–30 cm	0.8	0.5	0.5	0.6	1.1	0.6	0.5	0.6	1.3	0.7	0.71
30–40 cm	0.8	0.3	0.4	0.5	n.e.	0.6	0.4	0.5	0.7	0.7	0.54
40–50 cm	0.5	0.2	0.3	0.4	n.e.	0.5	0.3	0.4	0.6	0.6	0.41
Total	6.3	3.9	3.8	5.2	6.5*	4.2	3.8	4.0	7.2*	6.1*	5.1
% in LFH	21	35	28	27	15*	19	26	18	15*	20*	22

*Missing data (n.e.) replaced by the mean value for all other sites and included in the totals.

layers within sites (Table 3) and between sites for a given soil layer (not shown in the table). Based on the estimates of the components of variance in these four sites, it was concluded that significant differences should also occur between soil layers and sites for some of the sites that lacked replicate plots.

In the litter layer, net N mineralization rates differed greatly between sites (Table 3). Site 1, with a high net N mineralization rate, had a low C:N ratio (26) in the litter layer, and sites 6 and 7 had low rates and high C:N ratios (37–38). However, as a whole C:N ratios were not especially well correlated with mineralization rates. In the other soil layers, the net N mineralization rates showed less variation between sites. Still, mineralization rates differed between sites by a factor of 2.5 in the humus layers and by even more in the mineral soil layers. On most sites the net N mineralization rates decreased with increasing depth. However, high net N mineralization rates were found throughout the soil profile on site 3, which differed from other sites in terms of stand history in having several generations of

beech (*Fagus silvatica* L.) preceding the spruce plantation.

With the exception of site 3, and to some extent sites 1, 9 and 5, the nitrification potential was low in the litter and humus layers (Table 3). At greater depths, the nitrification potential increased, and below 20-cm depth the potential nitrification was high at all sites, i.e., most of the inorganic N formed was found as NO_3^- . The estimated rates of potential nitrification were sometimes higher than the net N mineralization rates owing to nitrification of NH_4^+ present at the start of the incubation. In deeper soil layers, low mineralization rates, comparatively high initial NH_4^+ concentrations and a high nitrification potential meant that the relation between potential nitrification and net N mineralization was not seldom estimated to be far more than 100% (Table 3).

In the present material, no nitrification occurred at pH levels lower than 3.95 in water extracts (Fig. 3) and pH 2.9 in KCl extracts (not shown). In the organic layers, nitrification was not found to be complete below

Table 3. Net N mineralization rate and potential nitrification rate for each site (see Table 1) and horizon, estimated from the laboratory incubations. Note that calculated nitrification rates are in many cases higher than net N mineralization rates owing to the nitrification of NH_4^+ present at the start of the incubation (see text). LOI=loss on ignition. Significant differences ($p < 0.05$) between soil layers for a given site with replicate plots are indicated by different letters

Soil layer	Site no.										Mean
	1	2	3	4	5	6	7	8	9	10	
Net N mineralization rate ($\mu\text{g N g}^{-1} \text{ LOI d}^{-1}$)											
L	20.1	10.4	9.4	11.6	11.0 ^a	1.0 ^b	0.0 ^b	6.6 ^a	n.e.	n.e.	8.8
FH	8.7	3.8	8.3	4.1	4.8 ^b	5.2 ^a	3.2 ^a	7.1 ^a	5.1	4.9	5.5
0–10 cm	1.8	2.6	2.7	1.6	4.7 ^b	0.8 ^b	1.2 ^b	2.7 ^b	3.1	1.9	2.3
10–20 cm	1.3	1.3	2.4	2.4	2.3 ^c	0.6 ^b	1.5 ^b	1.1 ^c	1.0	0.7	1.5
20–30 cm	0.9	0.5	2.0	1.5	1.4 ^c	0.3 ^b	1.0 ^b	0.7 ^c	0.7	0.5	1.0
30–40 cm	0.4	0.2	2.2	0.8	n.e.	0.4 ^b	0.6 ^b	0.3 ^c	0.8	0.4	0.7
40–50 cm	0.3	0.1	2.3	0.6	n.e.	0.3 ^b	0.4 ^b	0.1 ^c	0.9	0.4	0.6
Potential nitrification rate ($\mu\text{g N g}^{-1} \text{ LOI d}^{-1}$)											
L	12.7	0.0	10.4	0.0	4.2 ^a	0.0 ^a	0.0 ^b	0.0 ^c	n.e.	n.e.	3.4
FH	2.6	0.0	5.7	0.0	0.7 ^b	0.0 ^a	0.0 ^b	0.0 ^c	1.2	0.1	1.0
0–10 cm	2.3	0.0	2.6	0.0	1.1 ^b	0.0 ^a	0.2 ^b	0.0 ^c	2.3	0.4	0.9
10–20 cm	1.3	1.6	3.8	0.1	1.7 ^b	0.3 ^a	1.0 ^a	0.3 ^{bc}	1.1	0.9	1.2
20–30 cm	1.2	0.7	2.9	0.8	1.4 ^b	0.3 ^a	1.0 ^a	0.6 ^a	0.7	0.6	1.0
30–40 cm	0.6	0.4	3.0	1.0	n.e.	0.4 ^a	0.9 ^a	0.4 ^{ab}	0.8	0.5	0.9
40–50 cm	0.6	0.3	3.2	0.5	n.e.	0.4 ^a	0.7 ^a	0.2 ^{bc}	1.0	0.5	0.8
Potential nitrification rate (% of net N mineralization rate)											
L	63	0	110	0	38	0	0	0	n.e.	n.e.	
FH	30	0	69	0	14	0	0	0	23	2	
0–10 cm	130	0	95	0	24	0	13	1	76	20	
10–20 cm	100	120	160	5	76	53	68	24	110	140	
20–30 cm	120	130	150	52	100	100	100	90	100	120	
30–40 cm	140	160	140	130	n.e.	110	150	130	110	120	
40–50 cm	200	300	140	75	n.e.	130	140	200	110	130	

a pH (H_2O) of 4.7, while in the mineral soil, the ratio of nitrification to net N mineralization was often 100% down to a pH value of 4.0.

The calculated rates of annual net N mineralization per hectare corrected for mean annual temperature, soil moisture and soil pools are given in Table 4. With few exceptions, the annual mineralization of N was higher in the humus layer than in any of the other soil layers. N mineralization was also high in the litter layer and the 0–10 cm mineral soil layer in certain sites. The organic horizons accounted for 32–74% of the estimated annual mineralization, even though these layers contained only 15–35% of the N pool (Table 2). According to an analysis of the data in Tables 2 and 4, the relative contribution of the N pool in each soil layer to net N mineralization was found to decrease

with increasing depth (Table 5), indicating that organic matter N was more labile in the upper soil layers.

The potential nitrification was estimated to be relatively high in sites 1, 3, 5 and 9, low in sites 2, 4, 6, 7 and 8, and intermediate in site 10. In the sites with a low ability to form nitrate, practically all of it was formed in the mineral soil at a depth of 10 cm or more (Table 4). The potential nitrification calculated per hectare was larger in the mineral soil than in the organic horizons, even in the sites with pronounced nitrification in the litter and humus layers.

Annual net N mineralization and potential nitrification differed between acid and less acid sites when grouped according to their pH (H_2O) levels in the humus layer (Fig. 4). Four sites (nos. 2, 6, 8 and 10) had a pH below 4.0 in this layer, and the other six sites had a pH of 4.0 or higher. On average, the pH was

Table 4. Annual net N mineralization and potential nitrification for each site (see Table 1) and soil layer as calculated from laboratory incubations, temperature and moisture corrections, and organic pools in the field (see text). The relative contribution from the organic horizon (LFH) is also indicated. In some cases, potential nitrification exceeds mineralization for reasons given in Table 3

Soil layer	Site no.										Mean
	1	2	3	4	5	6	7	8	9	10	
Annual net N mineralization (kg N ha⁻¹ y⁻¹)											
L	22	12	8	20	16	0	0	3	n.e.	n.e.	10.0
FH	46	25	35	37	18	23	17	26	22	39	28.9
0–10 cm	13	16	16	17	47	5	7	19	25	20	18.3
10–20 cm	6	5	6	13	14	3	6	6	15	9	8.2
20–30 cm	3	2	5	7	7	1	4	3	5	3	4.0
30–40 cm	2	1	5	3	n.e.	1	1	1	4	2	2.2
40–50 cm	1	0	4	2	n.e.	1	1	0	3	1	1.5
Total	93	60	78	99	105*	35	36	58	84*	84*	73.2
% in LFH	74	61	55	57	32*	67	47	49	39*	58*	54
Potential nitrification (kg N ha⁻¹ y⁻¹)											
L	14	0	9	0	6	0	0	0	n.e.	n.e.	3.6
FH	14	0	24	0	2	0	0	0	5	1	4.6
0–10 cm	16	0	15	0	9	0	1	0	20	3	6.4
10–20 cm	6	6	9	1	11	1	4	1	19	7	6.5
20–30 cm	4	2	7	4	7	1	4	3	6	5	4.2
30–40 cm	2	1	7	5	n.e.	2	3	2	4	2	3.0
40–50 cm	2	0	5	2	n.e.	2	2	1	3	2	2.0
Total	58	10	76	10	40*	6	13	7	60*	23*	30.3

*Missing data (n.e.) replaced by the mean value for all other sites and included in the totals.

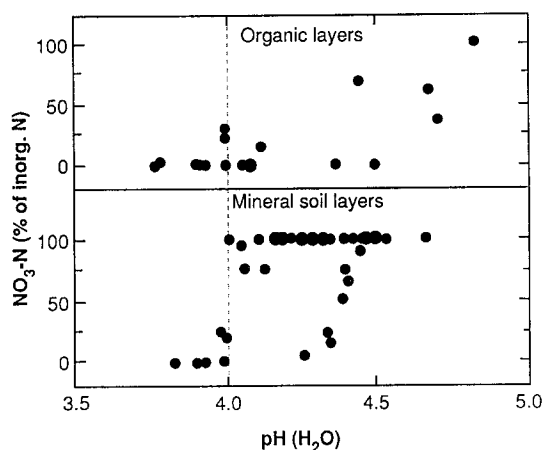


Fig. 3. Relationship between pH (H₂O) at the start of incubation and percent NO₃-N of mineralized N (NH₄-N + NO₃-N) formed during incubations for 74–117 days in the organic and mineral soil layers from ten forest sites.

3.89±0.04 in the first group and 4.14±0.07 in the second group. Annual net N mineralization in the humus

Table 5. Relationship (%) between annual net N mineralization (kg N ha⁻¹ y⁻¹) and the total N pool (kg N ha⁻¹) estimated for each soil layer in the ten sites studied

Soil layer	Range	Mean
L	0.02-10.14	4.81
FH	1.81- 4.18	2.98
0–10 cm	0.46- 2.21	1.23
10–20 cm	0.40- 2.06	0.95
20–30 cm	0.19- 1.09	0.59
30–40 cm	0.16- 1.32	0.45
40–50 cm	0.03- 1.50	0.41

layer did not differ between groups (Fig. 4), but in the deeper mineral soil, net N mineralization was significantly higher for the second group. However, the

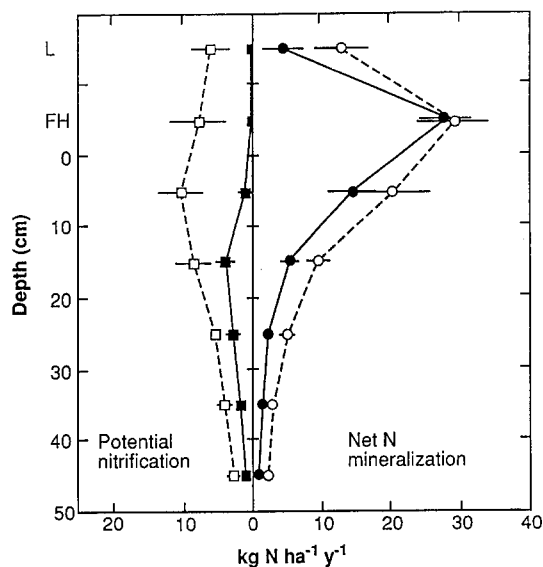


Fig. 4. Annual net N mineralization (right) and potential nitrification (left), in $\text{kg N ha}^{-1} \text{y}^{-1}$, in different soil layers for the sites with pH (H_2O) <4.0 (continuous line) and >4.0 (broken line) in the humus (FH) layer. Background data in Table 4 and Figure 1. Bars indicate \pm one S.E. Where bars are not visible, they are covered by the symbol.

higher value of the second group cannot be ascribed to its acidity since it actually had a lower pH than the first group in the mineral soil, for example, 4.29 ± 0.05 and 4.39 ± 0.07 , respectively, at 20–30 cm depth (see also Fig. 1). Instead, the higher net N mineralization in the mineral soil appeared to be correlated with the C:N ratio, which, on average, was lower for the sites in the second group.

The two groups of sites differed pronouncedly as regards potential nitrification, which was greater in the second group throughout the soil profile. The differences were especially obvious in the three uppermost soil layers, where practically no nitrification occurred in the first group (Fig. 4).

The addition of C_2H_2 to samples from sites 1 and 3 inhibited NO_3^- formation in the organic horizons, indicating that nitrification was autotrophic (Fig. 5). In the top 10 cm of the mineral soil from site 1, NO_3^- formation was not inhibited. The treatment with C_2H_2 caused some reduction in mineralized N as compared with the untreated soil layers. Possible explanations will be discussed below.

Addition of CaCO_3 increased the degree of nitrification in the humus layers from sites 1 and 3. No NO_3^- was formed in the humus from site 2 whether or not any lime was added (Fig. 6). In the 10–20 cm soil layer, both net N mineralization and nitrification were

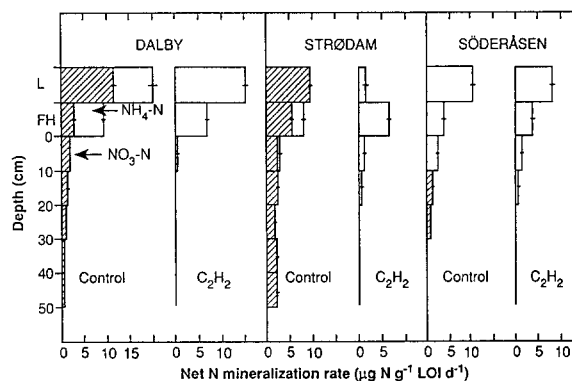


Fig. 5. Net N mineralization rate and nitrification rate ($\text{NH}_4\text{-N}$ unfilled, $\text{NO}_3\text{-N}$ hatched) in different soil layers from Dalby (site 1), Strødam (site 3) and Söderåsen (site 2) with and without treatment with acetylene (C_2H_2) during a 95-day incubation. LOI=loss on ignition. Bars indicate \pm one S.E. Where bars are not visible, they are covered by the drawing line.

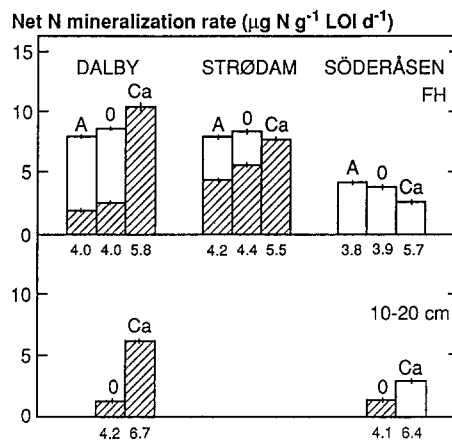


Fig. 6. Net N mineralization rate and nitrification rate ($\text{NH}_4\text{-N}$ unfilled, $\text{NO}_3\text{-N}$ hatched) in humus (FH) and 10–20 cm mineral soil layers from Dalby (site 1), Strødam (site 3) and Söderåsen (site 2) after treatment with H_2SO_4 (A) and CaCO_3 (Ca) in comparison with untreated soil (0). Results from a 95-day incubation. Initial pH (H_2O) indicated below the columns. LOI=loss on ignition. Bars indicate \pm one S.E.

stimulated by CaCO_3 additions at site 1 (Fig. 6). In the corresponding soil layer from site 2, CaCO_3 treatment stimulated net N mineralization but inhibited nitrification. No CaCO_3 was added to the 10–20 cm soil layer from site 3.

Addition of H_2SO_4 caused a slight reduction of pH in the humus layers from sites 2 and 3 (Fig. 6). The effect on net N mineralization rates was not significant, probably because the acid treatment was not strong enough. By contrast, the acid treatment resulted in a slight, but significant, decrease in nitrification rates in the humus layers from sites 1 and 3.

Discussion

Methodology

The study was based on the technique of incubating sieved soil samples in the laboratory in order to estimate net N mineralization and potential nitrification rates. Because the main aim was to compare soil layers within and between sites under equal temperature and moisture conditions, this technique was preferred over in-situ core methods (e.g., Raison et al., 1987; Tietema et al., 1990). Another reason for using the former technique was that the soils were too stony to obtain appropriate numbers of intact soil cores from greater soil depths.

Conclusions based on incubations of sifted soil need to be interpreted with great caution since sifting can both accelerate and retard the mineralization process. For example, incubated humus layers with high C:N ratios have often been reported to release inorganic N after a lag period (e.g., Popovic, 1980; Zöttl, 1960), which can sometimes be as long as several months (Persson and Wirén, 1993). For certain soils, this lag period can be prolonged by sifting (T. Persson, in prep.). To detect and correct for such disturbances, we incubated the samples for at least 95 days and made periodic subsamplings. However, the rates at which the soils in the present study accumulated inorganic N did not change too much with time except in the 30–40 and 40–50 cm layers from some sites (see Fig. 2), where N was immobilized for a period of time.

Another source of uncertainty was the scaling up from the laboratory rate at given temperature and moisture (Table 3) to the annual mean rate in the field (Table 4). Good literature data on temperature and moisture effects on net N mineralization and nitrification were scarce, especially at temperatures below zero. Therefore, we used a Q_{10} relation found for net N mineralization between 5 and 15°C and a simple correction factor for suboptimal moisture (see Materials and methods).

The soil samplings were carried out during different seasons (Table 1). This might have affected the outcome of the incubations because phenological patterns in microbial activity can play a role despite uniformity in temperature and soil moisture conditions. However, we could not find any pronounced difference in net N mineralization rates in site 5 between humus materials sampled in April and those sampled in October (T. Persson and A. Wirén, unpubl.). These results do not guarantee that seasonal differences can not occur under other circumstances. Consequently, the differ-

ences found between sites in the present study may, to some extent, be due to seasonal variation as well.

N mineralization and nitrification

On average, 78% of the net N mineralization occurred above 10 cm depth (i.e., the litter, humus, and 0–10 cm mineral soil layer), and 22% occurred in the 10–50 cm layer in the mineral soil. These results are in agreement with the common view that the uppermost soil layers account for most of the inorganic N supplied to plant roots in coniferous forests. Therefore, most mineralization and nitrification studies in forests have focussed on the forest floor alone (e.g., Kriebitzsch, 1978) or on the LFH layer plus the uppermost mineral soil (e.g., Tietema et al., 1992, 1993; Vitousek et al., 1982), whereas very few investigators (e.g., Beese, 1986; Tokuchi et al., 1993) have actually tried to estimate net N mineralization in deeper soil layers. This disinterest in the mineral soil might have biased our understanding of the nitrification process.

The depth of the soil had a strong influence on the nitrification potential, i.e., the ability to form NO_3^- in the absence of plant roots. Five of our ten soils completely lacked the ability to form NO_3^- in the humus layer, whereas NO_3^- could be formed in all ten soils at a depth of 10–20 cm in the mineral soil or deeper. This indicates that the mineral soil layers, especially the B horizon, might be an important source of NO_3^- and thereby contribute substantially to NO_3^- leaching in coniferous forests.

The absence of NO_3^- formation in the uppermost soil layers in some of the sites could not be explained by low N availability (e.g., Robertson, 1982; White and Gosz, 1987) since, with the exception of the litters from sites 6 and 7, much NH_4^+ was produced during the incubations. The pH seemed to be a more important factor: No nitrification was noted at a pH (H_2O) of 3.95 or lower in any of the sites. In the litter and humus layers, the degree of nitrification increased with increasing pH, whereas in the mineral soil, the degree of nitrification increased abruptly from 0% at pH 3.95 to 100% at pH 4.0 (see Fig. 3). Also, factors other than pH might have restricted NO_3^- formation as indicated by the fact that NO_3^- formation was sometimes low despite a favourable pH. However, our initial hypothesis that acid-sensitive nitrifiers were responsible for most of the nitrate formation could not be invalidated by the present data.

Responses to the additions of H_2SO_4 and CaCO_3 indicated that the humus samples from sites 1 and 3

contained acid-sensitive nitrifiers. Although the doses of acid were too small to reveal whether further acidification would inhibit nitrification, the addition of CaCO_3 clearly stimulated nitrification. Results with the humus samples from site 2 were less conclusive since the addition of CaCO_3 did not result in any nitrification. That this lack of NO_3^- formation could have been due to the absence of nitrifiers is indicated by results obtained in another experiment, with another soil, by the present authors. In that experiment $(\text{NH}_4)_2\text{SO}_4$ and CaCO_3 were added to humus samples with and without fragments from nitrifying humus. In the former case, almost all inorganic N was nitrified, whereas in the latter no NO_3^- was formed. The chemical conditions were conducive to nitrification in both treatments, but the process could only be started with an inoculum of nitrifiers.

Responses to the addition of CaCO_3 to the 10–20 cm mineral soil samples differed between sites 1 and 2. An increase in net N mineralization occurred in both soils, but in the former all inorganic N was transformed to NO_3^- , whereas in the latter, the addition of CaCO_3 inhibited all NO_3^- formation. The results indicate that sites 1 and 2 differed in their types of nitrifiers, which, in turn, had different pH optima in this soil layer. Further tests of the characteristics of the nitrifier population in site 2 have been started. The addition of C_2H_2 to soil samples from sites 1 and 3 inhibited all nitrification in the litter and humus layers, which indicated that the nitrifiers were autotrophic (e.g., De Boer et al., 1993). However, in the 0–10 cm mineral soil layer from site 1, the C_2H_2 treatment did not inhibit NO_3^- formation. Although this generally indicates the presence of heterotrophic nitrifiers, other circumstances suggest that this may not have been the case. The C_2H_2 treatment caused a pronounced reduction in net N mineralization in all mineral soil layers, possibly because C_2H_2 can be used as a C source by heterotrophic microorganisms (Klemmedtsson et al., 1990; Terry and Leavitt, 1992) which, then, assimilate inorganic N from the surrounding soil. It is possible that the 5-h pulse per week of C_2H_2 was enough to initiate microbial growth, especially if all C_2H_2 was not removed from the soil during the aeration. If C_2H_2 was consumed by microorganisms, its effect as a nitrification inhibitor could have been reduced. Although we believe that the doses of C_2H_2 were high enough to inhibit autotrophic nitrification, the question of whether heterotrophic or autotrophic nitrifiers were present in the mineral soil still remains to be answered.

Treatment with C_2H_2 strongly reduced net N mineralization in the litter layer from site 3, whereas the reduction was much weaker in the litter layers from sites 1 and 2. We do not have any good explanation for this discrepancy.

Field mineralization

The accuracy of the estimates of annual net N mineralization can be checked by comparing them with independently obtained data on N uptake by plants. At Skogaby (site 8), net N mineralization was estimated at $58 \text{ kg N ha}^{-1} \text{ y}^{-1}$ (Table 4) and throughfall at $17 \text{ kg inorganic N ha}^{-1}$ in 1990 (B. Bergkvist, pers. comm.), while N leaching was negligible (J. Bergholm, pers. comm.). The estimated supply of inorganic N to the plants was, thus, about $75 \text{ kg N ha}^{-1} \text{ y}^{-1}$. The mean annual uptake of N by the above-ground parts of the trees (accumulation plus litterfall) was estimated at $32 \text{ kg N ha}^{-1} \text{ y}^{-1}$ in the control plots in site 8 (Nilsson and Wiklund, 1994). The fine-root (<2 mm diameter) biomass was estimated to contain 48 kg N ha^{-1} (Majdi and Persson, 1993). If a turnover rate of one per year is assumed for this fraction, the root litter should contain $48 \text{ kg N ha}^{-1} \text{ y}^{-1}$. Provided that this estimate is reasonable (excluding the unknown accumulation of N in coarse roots), the plant uptake should be $80 \text{ kg N ha}^{-1} \text{ y}^{-1}$. This is close to the estimate of the supply of inorganic N. Corresponding data were not available for the other sites. However, the estimates of annual net N mineralization seem reasonable with regard to most of the tree growth figures in Table 1 and estimates on N deposition ($10\text{--}31 \text{ kg N ha}^{-1} \text{ y}^{-1}$) and leaching ($0\text{--}30 \text{ kg N ha}^{-1} \text{ y}^{-1}$).

Conclusions

Nitrification in the litter and humus layers of spruce forests in S Sweden and E Denmark is probably an acid-sensitive, chemoautotrophic process of little importance at pH levels below 4. This implies that low pH will often restrict nitrification in the humus layer, which normally has a higher N mineralization than other soil layers. Forest soils in this geographical region seem to have a considerable nitrification potential in the 10–50 cm soil layer, which mostly corresponds to the B horizon. This would be due to the higher pH in this horizon, but we could not determine whether the nitrification type at greater soil depths was the same as that in the upper soil layers. Our findings do not allow us to draw any conclusions about the actual nitrification

rates in the field, where tree roots are probably better competitors for NH_4^+ than the nitrifiers. The B horizon, rather than the more acid humus layer, might be the source of NO_3^- leaching during periods of reduced root uptake, for example, during wintertime. Deposition of NH_4^+ , followed by a downward transport of NH_4^+ from the organic soil layers to the nitrifying subsoil, can result in an increased risk of nitrate leaching in the future also in Scandinavian forests.

Acknowledgements

Thanks are due to Stefan Andersson, Gerd Färjsjö, Per Gundersen, Riitta Hyvönen, Ulf Johansson, Heléne Lundkvist, Hans-Örjan Nohrstedt, Lars-Göran Olsén, Lennart Rasmussen, Anna Rudebeck and Ulla Wahlund, for practical support, to Göran Ågren and two anonymous referees for valuable comments on the manuscript, to Birgitta Vegerfors for statistical advice, and to David Tilles for the linguistic revision. Grants for the study were received from the National Swedish Environmental Protection Agency and the Swedish Foundation for Plant Nutrition Research.

References

- Anonymous 1976 Determination of the sum of the nitrite and nitrate nitrogen content of water. Svensk Standard SIS 028133, 1–7.
- Beese F 1986 Parameter des Stickstoffumsatzes in Ökosystemen mit Böden unterschiedlicher Acidität. Göttinger Bodenkundl. Ber. 90, 1–344.
- De Boer W, Klein Gunnewiek P J A and Troelstra S R 1990 Nitrification in Dutch heathland soils. II. Characteristics of nitrate production. Plant and Soil 127, 193–200.
- De Boer W, Klein Gunnewiek P J A, Kester R A, Tietema A and Laanbroek H J 1993 The effect of acetylene on N transformations in an acid oak-beech soil. Plant and Soil 149, 292–296.
- Gundersen P and Rasmussen L 1990 Nitrification in forest soils: Effects from nitrogen deposition on soil acidification and aluminium release. Rev. Environ. Contam. Toxicol. 113, 1–43.
- Hallgren Larsson E and Westling O 1993 Luftföroreningar i södra Sverig-nedfall och effekter 1992. Swedish Environmental Research Institute (IVL) Rapport B 1108. 61 p.
- Hyman M R and Wood P M 1985 Suicidal inactivation and labelling of ammonia monooxygenase by acetylene. Biochem. J. 227, 719–725.
- Hynes R K and Knowles R 1982 Effect of acetylene on autotrophic and heterotrophic nitrification. Can. J. Microbiol. 28, 334–340.
- Klemetsson L, Hansson G and Mosier A 1990 The use of acetylene for the quantification of N_2 and N_2O production from biological processes in soil. In Denitrification in Soil Sediments. Eds. N P Revsbech and J Sørensen. pp 167–180. Plenum Press, New York.
- Kriebitzsch W-U 1978 Stickstoffnachlieferung in sauren Waldböden Nordwest-deutschlands. Scripta Geobotanica 14, 1–66.
- Majdi H and Persson H 1993 Spatial distribution of fine roots, rhizosphere and bulk-soil chemistry in an acidified *Picea abies* stand. Scand. J. For. Res. 8, 147–155.
- Nilsson J and Grennfelt P (eds) 1988 Critical loads for sulphur and nitrogen, Report from a workshop held at Skokloster, Sweden, 19–24 March 1988. NORD miljörapport 1988:15, pp 225–268. Nordic Council of Ministers, Copenhagen.
- Nilsson L-O and Wiklund K 1994 Nitrogen uptake in a Norway spruce stand following ammonium sulphate application, fertigation, irrigation, drought and nitrogen-free fertilisation. Plant and Soil 164, 221–229.
- Persson T 1989 Role of soil animals in C and N mineralisation. Plant and Soil 115, 241–245.
- Persson T and Wirén A 1993 Effects of experimental acidification on C and N mineralization in forest soils. Agric. Ecosyst. Environ. 47, 159–174.
- Popovic B 1980 Mineralization of nitrogen in incubated soil samples from an old Scots pine forest. Ecol. Bull. (Stockholm) 32, 411–418.
- Raison R J, Connell M J and Khanna P K 1987 Methodology for studying fluxes of soil mineral-N in situ. Soil Biol. Biochem. 19, 521–530.
- Robertson G P 1982 Factors regulating nitrification in primary and secondary succession. Ecology 63, 1561–1573.
- Schulze E-D, De Vries W, Hauhs M, Rosén K, Rasmussen L, Tamm C O and Nilsson J 1989 Critical loads for nitrogen deposition on forest ecosystems. Water Air Soil Pollut. 48, 451–456.
- Skyllberg U 1993 Acid-base properties of humus layers in northern coniferous forests. Swedish University of Agricultural Sciences, Department of Forest Ecology, Soil Science Section, Dissertation, Umeå 1993. 54 p.
- Svensson G and Anfält T 1982 Rapid determination of ammonia in whole blood and plasma using flow injection analysis. Clin. Chim. Acta 119, 7–14.
- Tamm C O 1990 Nitrogen in terrestrial ecosystems: Questions of productivity, vegetational changes, and ecosystem stability. Ecol. Stud. 81, 1–115.
- Terry R E and Leavitt R W 1992 Enhanced acetylene biodegradation in soil with history of exposure to the gas. Soil Sci. Soc. Am. J. 56, 1477–1481.
- Tietema A, Duijsings J J H M, Verstraten J M and Westerveld J W 1990 Estimation of actual nitrification rates in an acid forest soil. In Nutrient Cycling in Terrestrial Ecosystems. Eds. A F Harrison, P Ineson and O W Heal. pp 190–197. Elsevier Applied Science, London, New York.
- Tietema A, De Boer W, Riemer L and Verstraten J M 1992 Nitrate production in nitrogen-saturated acid forest soils: vertical distribution and characteristics. Soil Biol. Biochem. 24, 235–240.
- Tietema A, Riemer L, Verstraten J M, van der Maas M P, van Wijk A J and van Voorthuyzen I 1993 Nitrogen cycling in acid forest soils subject to increased atmospheric nitrogen input. For. Ecol. Manage. 57, 29–44.
- Tokuchi N, Takeda H and Iwatsubo G 1993 Vertical changes in soil solution chemistry in soil profiles under coniferous forest. Geoderma 59, 57–73.
- White C S and Gosz J R 1987 Factors controlling nitrogen mineralization and nitrification in forest ecosystems in New Mexico. Biol. Fertil. Soils 5, 195–202.
- Vitousek P M, Gosz J R, Grier C C, Melillo J M and Reiners W A 1982 A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecol. Monogr. 52, 155–177.
- Zöttl H 1960 Dynamik der Stickstoffmineralisation im organischen Waldboden-material. I. Beziehung zwischen Bruttomineralisation und Nettomineralisation. Plant and Soil 8, 166–182.