Vertical Patterns of Nitrogen Transformations during Infiltration in Two Wetland Soils

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The ^{15}N isotope dilution and pairing methods were applied to investigate the vertical distribution of nitrogen transformations during infiltration in one peaty soil and one sandy soil. Water containing ^{15}N -nitrate (99.9%; 200 μM) as the only nitrogen fraction was infiltrated through cores containing homogenized soil, with lengths varying from 5.5 to 38 cm. Oxygen and nitrogen dynamics were investigated by measuring inflowing and outflowing water. The experimental design allowed determinations of vertical profiles of aerobic respiration, nitrification, and coupled and uncoupled denitrification and ammonification. In the sandy soil, all oxygen was consumed in the upper 14 cm and nitrate was subsequently consumed and removed, up to a maximum of 70% in the longest core (28 cm). In the peaty soil, oxygen was consumed in the upper 7.5 cm and all nitrate was denitrified in the top 20 cm. In both soils, nitrogen removal by denitrification was counteracted by the release of ammonium and dissolved organic nitrogen. In the sandy soil, net nitrogen removal occurred in cores of 14 cm and longer; in the longest core, 40% was removed. In the peaty soil, release was equal to removal in the top 14 cm but release exceeded removal in the deeper layers, leading to a 100% increase of total nitrogen in the effluent water from the longest core (38 cm).

In southern Sweden, the transport of nitrogen in lowland streams has been of interest during the 1980s and 1990s due to its role in eutrophication of marine coastal ecosystems (15, 16, 18, 25). Wetlands hold promise as effective traps for nitrogen, promoting denitrification, sedimentation, and plant uptake of nitrogen. Consequently, the use of wetlands has been proposed as a measure to reduce nitrogen transport. During the 1990s, several wetlands and ponds have been constructed and restored and existing wetlands have been preserved. In Scandinavia, so-called water meadows have been used from the middle of the 19th century to increase hay production by utilizing nutrient-rich stream water (25). The water was supplied to terrestrial soils, creating a water depth of between 1 and 2 dm which spread horizontally over the meadow surface and subsequently infiltrated the soil or was released into a nearby stream. The management of water meadows ceased with the introduction of commercial fertilizers early in this century. Recent studies have examined the potential of restored water meadows as nitrogen traps (25). It was shown that total nitrogen (Tot-N) removal was poor in the studied soils since denitrification was counteracted by the release of dissolved organic nitrogen (DON) and ammonium.

Nitrogen transformations in flooded soils have been studied by several authors, but data for the effects of water infiltration on nitrogen transformations are few (7, 11). In sediments, diffusion is most important in transferring nitrogen species between overlying water and sites of microbial activity, and excellent illustrations of nitrogen transformation profiles have been presented by Christensen et al. (10) and Jensen et al. (19). Studies by Patrick and Reddy (29) and Reddy et al. (31, 32) illustrate that diffusion is also the predominant transport mechanism in flooded soils. However, wetlands where water percolates through the soil have features that distinguish them from wetlands on more impermeable soils or sediments. Oxy-

gen, nitrate, and other nitrogen species are introduced into the soil mainly by bulk flow, and as water moves down through the soil profile, oxygen is subsequently consumed and other electron acceptors, such as nitrate, manganese, iron, and sulfate, are utilized by microorganisms. Infiltration enhances the contact area among water, bacteria, and substrate, which results in a high potential for nutrient transformations. High transformation rates can lead to either removal or release of nitrogen, as determined by the balance among the processes of denitrification, nitrification, uptake, mineralization, sorption, and desorption. The wetland types where water percolates through the soil include water meadows (25), riparian forests with subsurface flow (36), tidal salt marshes (39), and occasionally flooded agricultural soils.

Here we describe an investigation of nitrogen transformation profiles during infiltration in soil cores of different compositions. We used nitrogen isotope pairing and dilution techniques, which allow estimations of denitrification, nitrification, and ammonification.

MATERIALS AND METHODS

The methods used in this study were essentially the same as those used by Stepanauskas et al. (37). Soil samples from depths of between 5 and 20 cm were collected from two water meadows at Isgrannatorp and Vomb in southern Sweden as previously described (25). At Isgrannatorp, the soil consists of freshwater marsh peat with high organic content and hydraulic conductivity. At Vomb, the soil is composed of a 10-m-thick sand layer with a sandy/organic topsoil. Analyses of soil organic content (measured as the loss on ignition after 4 h at 550°C) and Kjeldahl nitrogen were made with dry soil subsamples. At Isgrannatorp, the organic content was 52.8% and Kjeldahl nitrogen was 18 mg of N g (dry weight)⁻¹. At Vomb, the organic content was 8.6% and Kjeldahl nitrogen was 2.8 mg of N g (dry weight)⁻¹. The collected soil was sieved (2-mm-mesh size), homogenized to remove roots, stones, and large aggregates in order to reduce spatial variability, and stored in sealed plastic bags at 4°C. Nine soil cores of different lengths were prepared for each soil by transferring different portions of soil to Plexiglas tubes (diameter, 70 mm), where they were mixed with artificial lake water (24). The slurries were allowed to settle for 4 h, and the final lengths of soil columns were measured (Table 1). As a result of lower porosities, sandy-soil cores became more compact, which resulted in shorter cores and more overlying water, than were peaty-soil cores. The columns were sealed with gastight caps, and a downward water flow (25 ml h⁻¹) was created with a peristaltic pump and aerated artificial lake water containing 200 μM $^{15}\text{NO}_3^-$. Inflow connections were made of thin silicone tubing, and the outflow consisted

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TABLE 1. Lengths of soil cores after sedimentation and dry weight of the soil mass of each core

Type of soil	Length (cm)	Dry wt (g)
Peaty	7.5	102
	10	147
	14	207
	20	357
	24	485
	28	438
	32	544
	36	619
	38	679
Sandy	5.5	227
	7.5	304
	11.5	412
	14	517
	17	618
	20.5	703
	22.5	795
	25.5	900
	27.5	971

of chloropren rubber tubing, which has low gas conductivity. At the lower ends of Plexiglas tubes, rough-fiber filters and 100- μ m-mesh nets prevented the outflow of particulate matter. The reason for using cores of different lengths instead of sampling at different depths in one core was the fact that the withdrawal of large volumes of water would result in disturbances in the water flow patterns in the soil. Sampling from inflowing and outflowing water was performed with connecting gastight syringes.

The infiltration experiments were conducted at 16°C in darkness. After the ammonium concentration of effluents had reached a steady state (37), which took about 8 days, the full sampling program was performed with sandy-soil cores on the following 3 days. Due to a pump breakdown, sampling of peaty-soil cores was performed only on 2 days. Water samples for chemical analyses were stored frozen in plastic vials except for those that were used for analyses of dissolved gases (see below). Inflow and outflow water samples were analyzed for nitrogen fractions, N_2O sulfate, dissolved oxygen, and isotopic compositions of N_2 , NO_3^- , and NH_4^+ . All compounds were analyzed at every sampling occasion, except for NO_3^- , ^{15}N - NH_4^+ , and Tot-N, which were analyzed on one of the sampling days for each soil. The averages of the 3- and 2-day analyses were used in calculations for sandy and peaty soils, respectively. Nitrate-nitrite, nitrite, ammonium, and Tot-N were analyzed by standard methods (9, 22, 38, 42). DON was estimated as the difference between Tot-N and the inorganic fractions of NO_3^- + NO_2^- and NH_4^+ . Sulfate was determined with an ion chromatograph (Dionex 100). The outflowing water was not turbid (no absorbance at 665 and 750 nm), and no sample filtration was performed before analysis. Tot-N values were therefore considered to represent dissolved nitrogen fractions (37). To analyze N_2O , 6-ml water samples were injected into preevacuated 12-ml glass tubes with rubber membranes (Exetainer; Labco, High Wycombe, England). After vigorously shaking the Exetainers for 1 min, a headspace sample was injected into a gas chromatograph (Varian 3300 with an electron capture detector). To account for N_2O dissolved in the water phase, the Bunsen constant was used (41). Oxygen in the effluent of each soil core was measured by using a macroelectrode flowthrough cell.

^{15}N analyses. Exetainers (12 ml; Labco) were filled with effluent water collected in the sampling syringes, and ZnCl_2 was added to a final concentration of 50 mg liter $^{-1}$, after which Exetainers were stored at 4°C. Before analysis, 2 ml of water sample was replaced with helium and the vials were shaken for 5 min. Fifty microliters of the headspace was injected into a Hewlett-Packard MS engine quadrupole mass spectrometer to measure the production of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ (26, 37). The sensitivity of the mass spectrometer proved to be sufficient, since the use of highly enriched nitrate combined with high denitrification rates resulted in $^{30}\text{N}_2/\text{Tot-N}_2$ ratios that were >54 times as high as the background ratios.

The isotopic composition of NO_3^- + NO_2^- was determined after conversion to N_2 by a denitrifying bacterial culture, NCIMP 1967, by a previously described assay (33, 37). The nitrogen gas produced was analyzed by mass spectrometry as described above, and the ^{15}N fraction in nitrate was calculated by the method of Risgaard-Petersen et al. (33).

The isotopic composition of ammonium was determined by the method of Risgaard-Petersen and Rysgaard (34).

Calculations. The rates of denitrification based on infiltrated nitrate (D_w) and coupled nitrification-denitrification (D_n) were calculated by the method of Nielsen (26) and compensated for nitrogen solubility in water (40) as follows: $D_w = ^{29}\text{N}_2 + (2 \cdot ^{30}\text{N}_2)$ and $D_n = D_w \cdot ^{29}\text{N}_2 / (2 \cdot ^{30}\text{N}_2)$, where $^{29}\text{N}_2$ and $^{30}\text{N}_2$ are the net

FIG. 1. Tot-N concentrations in effluents from peaty- and sandy-soil cores of different lengths. The dashed line shows the concentration in infiltration water.

fluxes of these nitrogen gas isotopes. The total denitrification (D_{tot}) rate was estimated as the sum of D_w and D_n .

The net fluxes of different nitrogen forms (F) were calculated from the equation $F = (C_e - C_i)(V/A)$, where C_e and C_i are effluent and influent concentrations, respectively, V is the water flow rate, and A is the soil surface area in the column.

The rate of the efflux of unlabelled nitrate (R) was estimated from the isotope dilution of labelled NO_3^- by the method of Nishio et al. (27) and Rysgaard et al. (35) as follows: $R = [C_i(e - i)/(0.00366 - e)](V/A)$, where V and A are the same as stated above, C_i is nitrate inflow concentration, i and e are the ^{15}N fractions of inflow and outflow nitrate, respectively, and 0.00366 is the ^{15}N background fraction of nitrified ammonium.

The rate of nitrification was calculated by adding R and coupled nitrification-denitrification. Ammonification was estimated as the sum of ^{14}N -ammonium production and nitrification. ^{14}N -nitrate uptake was estimated as the difference between R and ^{14}N -nitrate in effluent water.

Dissimilatory nitrate reduction to ammonium (DNRA) was calculated by the method of Rysgaard et al. (35) as follows: $\text{DNRA} = \{[(C_e - C_i)(e - 0.00366)]/n\}(V/A)$, where V and A are the same as stated above, C_e and C_i are the concentrations of ammonium in the effluent and influent, respectively, e is the ^{15}N -labelled fraction of NH_4^+ in effluent, 0.00366 is the labelled fraction of soil NH_4^+ , and n is the labelled fraction of nitrate that is reduced to NH_4^+ .

RESULTS

Mass balances. With respect to Tot-N, the peaty soil was a source, whereas the sandy soil was a sink (Fig. 1). In the peaty soil, the Tot-N concentration increased throughout the soil profile; in the effluent of the longest core, the concentration was about 100% higher than that in the inflowing water. In the sandy soil, the Tot-N concentration decreased; in the outflow of the longest cores, about 40% of the inflowing nitrogen was removed.

The oxygen concentration declined rapidly in both soils and was depleted after 7.5 cm in the peaty soil and after 11.5 cm in the sandy soil (Fig. 2). The consumption of oxygen was accompanied by a decline in the nitrate concentration. In the peaty soil, all the nitrate was consumed in the top 20 cm of the soil. In the sandy soil, the nitrate concentration decreased to 36 μM in the outflow of the longest core. The sulfate concentration increased slightly throughout both soils (up to 17% of inflow),

FIG. 2. Oxygen and nitrate concentrations in effluents from peaty- and sandy-soil cores of different lengths.

indicating that sulfate reduction was not an important respiratory pathway (data not shown).

Nitrate removal was counteracted by a release of DON and ammonium (Fig. 3). The concentration of DON increased markedly throughout peaty-soil cores. However, the $\text{NH}_4\text{-N}$ concentration in peaty-soil cores and the DON and $\text{NH}_4\text{-N}$ concentrations in sandy-soil cores tended to level out after 14 to 20 cm, after which no significant changes were recorded. In the longest cores, the peaty soil released seven times as much DON and three times as much $\text{NH}_4\text{-N}$ as did the sandy soil.

Nitrous oxide production occurred at intermediate oxygen concentrations (Fig. 2 and 4). In the peaty soil, N_2O was produced in the top layers (depth, 0 to 7.5 cm) but was totally consumed to levels near air saturation in the deeper parts (between 14 and 20 cm). In the sandy soil, nitrous oxide was produced at depths of between 5.5 and 14 cm but subsequently reached a stable concentration throughout the soil profile. Nitrite concentration profiles showed patterns almost identical to those of nitrous oxide (Fig. 4). In both soils, nitrite accumulated in the top layers. However, similar to nitrous oxide, it was almost completely consumed in the deeper parts of the peaty soil, whereas small amounts remained in the effluent of the longest sandy-soil core. Compared to denitrification rates, the production of intermediate compounds was low, except in the shortest (0- to 14-cm) cores, where the NO_2^-/N_2 production ratio reached 20% in the peaty soil and 10% in the sandy soil. The highest $\text{N}_2\text{O}/\text{N}_2$ production ratios were 6 and 2% in the peaty and sandy soils, respectively.

Process rate. Denitrification was the main nitrate-removing process in all soil cores and accounted for 63 to 90% of nitrate removal, except for the shortest (47%) and longest (57%) cores of the peaty soil. N_2 production was low in the top layer of the peaty soil (phase 1) but increased in the intermediate section (phase 2), below which it levelled out (phase 3; Fig. 5). The pattern for the sandy soil was identical, with the exception that the zone of high denitrification activity (phase 2) was closer to the soil surface in the peaty soil (15 to 20 cm) than it

FIG. 3. Ammonium and DON concentrations in effluents from peaty- and sandy-soil cores of different lengths.

was in the sandy soil (25 to 30 cm). However, the total amounts of N_2 produced in the longest soil cores were similar for the two soil types. Denitrification of nitrate in infiltrated water (D_w) was 1 order of magnitude higher than denitrification coupled to nitrification (D_n). All the N_2 fractions (D_n , D_w , and D_{tot}) exhibited similar patterns and attained equivalent values in both soil types.

Total ammonification in the peaty soil was twice as high as that in the sandy soil (Fig. 6). The proportion of ^{15}N -labelled ammonium in the sandy soil increased with depth up to 10% but was lower in the two longest cores (Fig. 7). In the peaty soil, the proportion of ^{15}N -labelled ammonium was highest in the top soil (5%) and decreased with depth. The ^{15}N labelling of ammonium can be a result of DNRA or rapid turnover of ^{15}N -nitrate. If ^{15}N -nitrate turnover is assumed to be negligible, the maximum DNRA rates were 0.5 and 0.2 $\text{mmol m}^{-2} \text{day}^{-1}$ in the sandy and peaty soils, respectively.

More than 90% of the outflow ammonium was ^{14}N labelled (Fig. 7) and was hence either of soil origin and produced by ammonification or a result of nitrogen fixation. Some of this ammonium was nitrified and either released to the outflow water, denitrified, or taken up by organisms (Fig. 6). The nitrification rates in both soils were about the same but repre-

FIG. 4. Nitrite and nitrous oxide concentrations in effluents from peaty- and sandy-soil cores of different lengths.

sented a larger fraction of the ammonium produced in the sandy soil.

DISCUSSION

In our infiltration systems, the shapes of the profiles of nitrate, oxygen, and denitrification were similar to those found in sediments by Christensen et al. (10) and Jensen et al. (19), with the important exception that they extended deeper (Fig. 2 and 5). Nitrate and oxygen declined with depth, as was the case in previous sediment studies (10, 19). In sediment and soil-water systems where water is stagnant or moves laterally, diffusion is the predominant transport mechanism (10, 19, 29, 31, 32) and nitrogen turnover rates have been shown to be most intense in the uppermost millimeters (10, 19). On the contrary, when water is infiltrated through a soil matrix, electron acceptors are transported down by mass flow and the microbial processes can extend further down the soil profile. In our study, denitrification was highest in a zone somewhat below the surface layer. This was even more evident when the N_2 production rates were fitted to a logistic expression,

$$y = y_0 e^{k_1 x} \left[\frac{1}{1 + \frac{(e^{k_1 x} - 1)y_0}{k_2}} \right]$$

which describes a sigmoidal shape, where y is produced N_2 , x is soil depth, y_0 is the intercept with the y axis, k_1 is the rate of increase, and k_2 is the maximum value of N_2 accumulation. The derivative of the logistic equation, $dy/dx = k_1 y (1 - [y/k_2])$, describes the denitrification rate per unit of soil volume. The rates at different depths can be calculated for different soil types by using the values of the constants from curve fits (Fig. 5). The results reveal that the maximum denitrification rate was higher and occurred closer to the soil surface in the peaty soil than in the sandy soil (Fig. 8). The different phases (phases 1, 2, and 3; Fig. 5) correspond to different factors that regulate the denitrification activities at different soil depths as follows: oxygen inhibition of denitrification during phase 1, rapid nitrate consumption during phase 2, and inhibition of further denitrification by the depleted nitrate pool during phase 3. As for the profiles of nitrate and oxygen, similar patterns for denitrification rates have been found in sediments (10, 19), with the same regulating mechanisms but on a smaller scale. The high input of external nitrate resulted in rates of uncoupled denitrification that made up 91 to 96% of total denitrification. This was much higher than denitrification coupled to nitrification, which has shown to be more important in sediments (10). The coupled and uncoupled processes showed similar patterns with an accumulation of N_2 gas throughout the soil profile.

Nitrite and nitrous oxide concentrations exhibited almost identical patterns (Fig. 4); they were produced where the oxygen level was high to intermediate and consumed where oxygen was depleted. Although no oxygen was found in the effluents of the longest cores of the sandy soil, oxidized nitrogen compounds, i.e., NO_3^- , NO_2^- , and N_2O , were found, indicating less reduced conditions than those in the peaty soil. There is evidence in the literature that denitrification intermediates can accumulate when the soil is exposed to intermediate oxygen availability (2). More reduced soils, however, produce little N_2O and can act as sinks for nitrous oxide (3, 37). Since the experimental setup did not allow measurements of ^{15}N -labelled nitrite and nitrous oxide, we could not conclude whether the NO_2^- and N_2O originated from nitrification or denitrification.

Ammonium and DON accumulated with depth in both soils, although the concentration levelled out in the longest cores of the sandy soil (Fig. 3). These profiles are similar to diffusion profiles of ammonium in flooded soils (32), where ammonium is consumed in the oxidized top layers. However, production and accumulation of ammonium throughout the soil depth, rather than consumption at the surface, were the probable causes of the profile found in our study. We have not found any reported profiles of DON in soil or sediment. The small fraction of ^{15}N -labelled ammonium in the outflow water (Fig. 7) clearly showed that the efflux of reduced nitrogen was produced from unlabelled sources. There are several possible mechanisms by which ammonium and DON can be produced and released, e.g., mineralization of soil organic matter, desorption processes (20), and nitrogen fixation (7). Ammonification, calculated as ^{14}N -ammonium release plus nitrification, in the peaty soil was about twice as high as that in the sandy soil (Fig. 6). On the other hand, the nitrification rates in the two soils were similar and were obviously limited by oxygen. About half of the nitrate produced was further denitrified, and the remaining nitrate was either washed out or retained in the soil.

FIG. 5. Dinitrogen produced in peaty soil cores of different lengths from nitrate in influent water (D_w) and from nitrate formed by nitrification (D_n) and the sum of them (D_{tot}). The rates are expressed on an area basis, i.e., N_2 is accumulated throughout soil profiles. Data are fitted with a logistic expression (see text), and a loss function was designed to make the curve fit where y_0 was close to or equal to zero.

This retention was probably due to nitrate assimilation by microorganisms during growth. The small fraction of ^{15}N -labelled ammonium (<10% [Fig. 7]) may have been caused by either production from the DNRA process or microbial uptake of ^{15}N -nitrate with subsequent release of ^{15}N -ammonium. Hypothetically, if all the ^{15}N -ammonium was produced by DNRA, the rates would be at most $0.5 \text{ mmol m}^{-2} \text{ day}^{-1}$ in the sandy soil and $0.2 \text{ mmol m}^{-2} \text{ day}^{-1}$ in the peaty soil, which are very low activities compared to the corresponding denitrification values of about $17 \text{ mmol m}^{-2} \text{ day}^{-1}$. However, Buresh and Patrick (5) investigated soil amended with different carbon sources and found that highly reduced conditions were required for the DNRA process. Since the soils in our study only gradually became more reduced with increasing depth, nitrate

would probably be consumed by denitrification before DNRA occurred.

Due to the balance among different process rates, the sandy soil acted as a net nitrogen sink and the peaty soil acted as a net nitrogen source (Fig. 1). In the sandy soil, denitrification and uptake of nitrate exceeded DON mineralization and ammonification, whereas the situation was the opposite in the peaty soil (Fig. 3). The difference in rates and profiles between infiltration systems of soils or sediment where water is stagnant or moves laterally is that the major part of the sediment volume does not take part in the removal and release processes. One characteristic of infiltration is that in contrast to the analogous interstitial water, the soil solution is constantly replaced and washed out. This may allow for higher losses since equi-

FIG. 6. Rates of different soil nitrogen processes expressed on an area basis. Ammonium release is the amount of $^{14}\text{NH}_4^+$ found in the outflow of soil cores. Nitrification is the sum of R and D_n (see text). Nitrate release is the $^{14}\text{NO}_3^-$ in effluent. Nitrate uptake is the uptake of $^{14}\text{NO}_3^-$, which is calculated as the difference between R and $^{14}\text{NO}_3^-$ in effluent. (A) Peaty soil; (B) sandy soil. The scales on the x axis are different.

libria between ions adsorbed to soil particles and ions in the soil solution are affected. Moreover, by increasing the amount of electron acceptors in the soil profile (e.g., oxygen, nitrate, and sulfate), the mineralization rate increases and hence an increased efflux of nitrogen compounds is promoted (8, 12, 13). Mineralization that involves oxidation by electron acceptors other than nitrogen often causes net release of inorganic nitrogen, whereas oxidation with nitrate promotes net removal. However, the C/N ratio of the mineralized substrate is of great importance. At a C/N ratio of 10 (recorded for the sandy soil [13a]), denitrification of eight atoms of nitrogen results in mineralization of one atom of nitrogen (21).

Soil core infiltration experiments reported in the literature often employ wastewater infiltration in gravel or other coarse material. Yamaguchi et al. (43) found that nitrate eventually disappeared in soil cores low in organic content. NH_4^+ was low in effluent water, and DON was not analyzed. Lance et al. (23) reported NH_4^+ and DON consumption in 2.75-m-long cores packed with loamy sand and infiltrated with secondary effluent

wastewater high in NH_4^+ . However, the nitrate concentration was low in inflowing water and increased in the core. Although infiltration of water through soil is common, e.g., in farmlands during heavy rains and floods and in natural and constructed wetlands (1, 25), studies of nitrogen transformations during infiltration of natural soils are scarce in the literature. We have previously reported the release of NH_4^+ and DON in effluents from sandy soil cores of the type used here (37) and in groundwater effluents from field experiments at the sampling sites of this study (25). Hedin et al. (17) discussed the export of DON from an old-growth temperate forest and reported that 95% of nitrogen export was in the DON fraction. Valiela et al. (39) measured nitrogen transformations in a tidal salt marsh, and they concluded that equal amounts of DON entered and left the marsh. Inorganic nitrogen was transformed into and exported as particulate N. Such studies illustrate the importance of measuring several nitrogen fractions in mass balance studies. To focus only on nitrate or Tot-N gives incomplete information about the nitrogen removal functions of a wetland.

FIG. 7. Fractions of ^{15}N isotopic labelling of ammonium in effluent water from peaty- and sandy-soil cores. The dashed line represents the natural ^{15}N labelling of ammonium. ND, not detected.

FIG. 8. Simulated denitrification rates (expressed per unit of soil volume) in peaty- and sandy-soil cores. The constants obtained from the curve fit in Fig. 5 were used in the derivative of the logistic expression (see text).

Measuring only nitrate disregards changes in ammonium and DON, and Tot-N estimates give no information as to whether the nitrogen is inorganic or organic, particulate or dissolved, or refractory or labile. Moreover, there are nitrogen turnover processes not studied here that can be important removal and/or release functions of a wetland, e.g., nitrogen fixation (7) and sorption-desorption processes (20). An important issue to investigate is to what extent the released DON fraction is bioavailable. It has been shown that photochemical release of ammonium from DON can be substantial (6). Furthermore, studies by Stepanauskas (36a) and Olofsson (28) show that 0 to 15% of the DON in wetland soil water can be assimilated by bacteria and reenter food chains, thus supporting nitrogen-limited aquatic ecosystems.

A comparison between the results from this laboratory experiment and the field mass balance data of Leonardson et al. (25) reveals several similarities. The distributions of dissolved nitrogen fractions in the outflowing water of the field and laboratory studies were remarkably similar (Fig. 9). Almost complete removal of nitrate was recorded in both laboratory and field studies of the peaty soil. However, in the field experiment, the Tot-N inflow concentrations were about 400 and 200

μM in the peaty and sandy soils, respectively, and were not changed in the outflow. In the laboratory experiment, the inflow had a Tot-N concentration of about 200 μM and the outflow concentration more than doubled in the peaty soil and significantly decreased in the sandy soil. The explanation for this discrepancy is that the effectiveness of nitrogen removal is dependent on the nitrate concentration as discussed above and elsewhere (14). Probably, the peaty soil has a much higher nitrate removal potential than was expressed; a higher concentration would lead to a shift from net release to net removal, whereas a higher nitrate concentration in the sandy soil probably would have a smaller effect on Tot-N removal.

According to the requirements of the isotope pairing technique, the ^{14}N -nitrate produced in oxygenated layers should mix randomly with the ^{15}N -nitrate of the inflow water before being denitrified. In our experiment, this corresponds to a zone of nitrification in the top layer where $^{14}\text{NO}_3^-$ is produced and mixed with $^{15}\text{NO}_3^-$, with the mixed nitrate then transported to an underlying zone where it is denitrified. Since we observed denitrification before the oxygen was totally consumed, probably due to spatial heterogeneity, this assumption was not

FIG. 9. Comparison between the field mass balance data of Leonardson et al. (25) and the laboratory (lab) results in this study. The inflow concentrations of Leonardson et al. (25) were measured in applied stream water, and the outflow concentrations were measured in groundwater effluent, which made up 85 and 90% of the total water volume leaving the peaty and sandy soils, respectively.

totally fulfilled, i.e., $^{14}\text{NO}_3^-$ and $^{15}\text{NO}_3^-$ were not randomly paired. This would have caused an underestimation of the production of $^{28}\text{N}_2$ (26). However, since denitrification of ^{15}N -labelled nitrate accounted for 91 to 96% of total denitrification, this does not significantly affect our D_{tot} values. In addition, it should be remembered that all mathematical operations which involve the subtraction and addition of different parameter estimates reduce reliability since errors in measurements accumulate. All estimates of DON, nitrification, DNRA, ammonification, and uptake are afflicted with this type of error.

In conclusion, subsurface flow in wetlands may provide good conditions for nitrogen removal (4, 30). However, high transformation rates also involve high potential for the production of dissolved organic and inorganic compounds, including nitrogen forms, especially in highly organic soils, which can counteract removal. Vertical infiltration, i.e., mass flow of water and nutrients, shows nitrogen transformation profiles similar to those described for sediments (10), but on a much larger scale. Processes that occur at distances of millimeters in surface sediments can be extended to several decimeters in soils where mass flow occurs.

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