Turnover and fate of $^{15}$N-labelled cattle slurry ammonium-N applied in the autumn to winter wheat

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Abstract

The fate of $^{15}$NH$_4$-N labelled cattle slurry applied before sowing in September of a winter wheat crop was studied on a loamy sand soil. The aim was to quantify immobilization of slurry NH$_4$-N into microbial biomass, the speed at which nitrate derived from the slurry NH$_4$-N was transported down the soil profile, and the utilization of slurry NH$_4$-N by the winter wheat crop. Cattle slurry was applied at a rate corresponding to 75 kg NH$_4$-N ha$^{-1}$, with very little loss by volatilization (<4%) due to rapid incorporation by ploughing. The slurry amendment resulted in a doubling of soil surface CO$_2$ flux, an index of microbial activity, over non-amended soil within the first c. 2 weeks, but ceased again after c. 4 weeks, due to depletion of the easily degradable substances, e.g. volatile fatty acids, in the slurry. Nitrification of the applied NH$_4$-N was fast and complete by 3 weeks from application, and at this time, the maximum immobilization of slurry NH$_4$-N into the microbial biomass (23% of applied $^{15}$NH$_4$-N) was also observed, although no significant increase in total microbial biomass was observed. Rapid turnover of the microbial biomass quickly diluted the assimilated $^{15}$N, with only 6% of applied $^{15}$NH$_4$-N remaining in the microbial biomass by next spring. Downwards transport of nitrate was rapid in spite of lower than normal precipitation, and slurry-derived $^{15}$NO$_3$-N appeared in ceramic suction cups installed at 60 cm depth already 2 months after slurry application. Due to the unusually low winter precipitation in the experimental year, wheat yields were high, and the recovery of N in above-ground plant biomass derived from slurry NH$_4$-N at harvest reached 32%. An additional 45% of the applied slurry NH$_4$-N could be found in the soil to a depth of 100 cm (mostly in organic form in the plough layer), indicating that 23% had been lost by leaching or in gaseous form. It was concluded that although significant immobilization of slurry NH$_4$-N did occur, this was not sufficient to prevent leaching of slurry-derived N over the winter and that the relatively high recovery of slurry-derived N in the wheat crop was due partly to lower than normal winter percolation and partly to a relatively high rooting depth on this particular site. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cattle slurry; $^{15}$N immobilization; N leaching; N-use efficiency; Soil microbial biomass N

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1. Introduction

With the intensification of livestock production in many Western European countries, over the last couple of decades, much attention has been focused on its eventual adverse environmental effects. Intensive livestock production commonly leads to a high excess of nitrogen (N) in animal manures, which is at great risk of being lost, either through gaseous losses, i.e. ammonia volatilization or denitrification, or through leaching. Kirchmann et al. (1998) stated that in Western Europe, an average of 30% of the N excreted by farm animals is released to the atmosphere as ammonia, causing economic losses and problems with acidification and eutrophication in surrounding natural, N-limited ecosystems. Many studies have also shown an increased risk of nitrate leaching from soils receiving high levels of animal manure (e.g. Nielsen and Jensen, 1990; Beckwith et al., 1998). For this reason, legislative regulations have been introduced in many European countries to control these adverse environmental effects, and the EU has implemented an upper limit for the amount of total N in animal manure that may be applied per hectare annually.

In Denmark, current legislation requires farmers to utilize animal manure total N with an efficiency of 45–55% in the first year after application and 10% in the second year. This encourages farmers to use more efficient application techniques with a lower potential for ammonia volatilization losses (Sørensen et al., 1997). Furthermore, spreading of liquid animal manure, e.g. slurry and urine, is generally not allowed in the autumn, to prevent nitrate losses caused by leaching of nitrified slurry ammonium-N. These regulations have forced Danish farmers to apply more than 80% of the liquid manure in the spring season, either before sowing of spring crops or with trailer techniques in between the rows of growing crops (Sommer et al., 1997). This has put a relatively heavy workload on many livestock farms during a few weeks in the spring. Farmers are therefore often tempted to apply slurry slightly too early, when the soils are still wet and prone to soil compaction by the heavy machinery used for slurry application. The issue of adverse effects of spring slurry application on soil fertility is thus emerging, and in this context, the complete ban on autumn slurry application for nearly all arable crops has been questioned.

It is well known, however, that autumn application of fertilizer N results in poor crop recovery of the applied N (Powlson et al., 1986), and it may be expected that autumn application of liquid animal manures, with the major proportion of N in mineral form, would yield similar low recoveries (Jackson and Smith, 1997). However, as animal slurries usually contain a fair proportion of easily degradable substances, such as volatile fatty acids (Sørensen, 1998), immobilization of slurry ammonium-N normally occurs due to the increased biological activity after application (Kirchmann and Lundvall, 1993). Where this may be seen as a major disadvantage by spring application (crop roots and microorganisms compete for the same mineral N), it may pose a potential advantage by autumn application if the immobilization is large enough for retaining a significant proportion of the slurry ammonium-N and provided that the immobilized N is released again the following cropping season.

The appropriate methodology for studying the fate of animal manure N in the soil–plant cropping system, is to label the manure with 15N. Several studies have been published where either the faeces or the urine fraction (e.g. Sørensen and Jensen, 1996, 1998; Thomsen et al., 1997), as well as a synthetic urine (Clough et al., 1998) have been 15N labelled. However, if the aim is to only trace the ammonium-N fraction of an animal manure, the labelling can be carried out by simply spiking it with a small quantity of highly 15N enriched ammonium-N. However, only a few published studies have applied this methodology (Trehan and Wild, 1993; Paul and Beauchamp, 1995; Carey et al., 1997; Morvan et al., 1997).

Our objectives were therefore to study:
1. the turnover and fate of autumn applied slurry ammonium-15N in the soil with a winter wheat crop over 1 year following application;
2. how rapidly nitrified slurry ammonium-15N is transported down the soil profile by percolation to estimate the risk of leaching; and
3. whether significant and persistent microbial
immobilization of the slurry ammonium-\(^{15}\)N does occur to such a degree that it may protect it from leaching.

2. Materials and methods

2.1. Site and soil

The experiment was conducted on a loamy sand soil located at a private farm (Nordkær) at Nørhalne, Denmark (57\(^{∞}\)/10\(^{∞}\)N, 9\(^{∞}\)/50\(^{∞}\)E). Basic topsoil (0–25 cm) properties were 6.5% clay (<2 \(\mu\)m), 12.5% silt (2–20 \(\mu\)m), 67.9% fine sand (20–200 \(\mu\)m), 8.9% coarse sand (<200 \(\mu\)m), bulk density 1.3 Mg m\(^{-3}\), pH (in 0.01 M CaCl\(_2\)) 6.0, 2.80% C, 0.258% N, C/N ratio 10.8. The site had been maintained as arable land for a long period (more than 75 years) and during the last 25 years was cropped with cereals or fodder beets; however, peas were grown in the year preceding the experimental year.

2.2. Experimental design

The study was conducted in a randomized field experiment (four replicates in a block design) with three factors: (1) with or without cattle slurry applied either in autumn or spring at a rate corresponding to 75 kg NH\(_4\)-N ha\(^{-1}\), (2) with or without fertilizer N applied in spring at a rate of 75 kg NH\(_4\)-N ha\(^{-1}\), and (3) conventional or reduced tillage for seed-bed preparation. The experimental plots of the study were placed in the treatments with no fertilizer applied, but with or without cattle slurry applied in the autumn and with conventional or reduced tillage for seed-bed preparation. As the tillage treatments did not show any significant effects in any of the parameters reported here, all the following results have been averaged over tillage treatments (\(n=8\)).

Experimental plots (4 \(\times\) 15 m) were established in mid-September 1995, after the preceding pea crop had been harvested and the straw removed. Cattle slurry (4.5 g total N kg\(^{-1}\), 2.1 g NH\(_4\)-N kg\(^{-1}\), 2.4 g organic-N kg\(^{-1}\) and 85 g dry matter kg\(^{-1}\)) was applied to the soil (at a rate of 3.5 kg slurry m\(^{-2}\)) on 20 September 1995 in the appropriate plots (microplots for \(^{15}\)N were covered) and incorporated immediately by ploughing to a maximum depth of 20 cm, followed by seed-bed preparation. Winter wheat (Triticum aestivum L.) was sown the day after at a target density of 350 plants m\(^{-2}\). The field received a herbicide application in the autumn and two fungicide treatments in the following summer.

A framed microplot (1 m \(\times\) 1 m, 20 cm depth) was established in each of the plots receiving cattle slurry, and \(^{15}\)N-labelled cattle slurry was applied on 22 September 1995 at the same N rate as that applied in the surrounding main plot 2 days earlier. The cattle slurry was spiced with a small quantity of \(^{15}\)N-labelled ammonium-sulphate (69.2 at.%), increasing the NH\(_4\)-N content by only c. 2%, but producing a \(^{15}\)NH\(_4\) enrichment of 2.31 at. \(^{15}\)N. The \(^{15}\)N-labelled cattle slurry was incorporated into the soil in the microplots by hand cultivation, simulating ploughing and to the same depth as in the surrounding main plot.

Two ceramic suction cups were installed c. 60 cm below each microplot and 30 cm apart. The ceramic cups (25 mm diameter) were mounted on 1 m long plastic tubes and inserted from outside the microplot at an angle inclined c. 45\(^{∞}\) to the soil surface (see Nielsen and Jensen, 1990). Ceramic suction cups were also installed in the main plots with and without cattle slurry. A screw auger slightly larger than the diameter of the ceramic cup was used to bore the hole and a kaolinite paste used to ensure good contact between the soil and the ceramic cup. A rubber collar mounted around the tube at the soil surface prevented bypass flow of rainwater from above.

2.3. Sampling

Soil samples were taken from both main plots and \(^{15}\)N-labelled microplots 2, 23, 74 (autumn) and 205 (spring) days after application of the cattle slurry. On each occasion, 16 cores per main plot or microplot were taken to a depth of 20 cm using a steel corer of 30 mm diameter. After harvest of the wheat crop, soil samples were taken in the microplots to a depth of 100 cm in 25 cm depth increments, using a 100 mm diameter auger with two cores sampled in each microplot. At all sam-
plings, the soil cores were thoroughly mixed, stored for a maximum of 24 h at 2–5°C before analyses and were not sieved or pre-incubated to avoid interference of soil handling on biological and biochemical properties before analysis (Ocio and Brookes, 1990). Soil samples for total C, N and 15N were dried at 105°C and finely ground on a ball mill before analyses on an ANCA-MS system as described below. Chemical and biochemical analyses were carried out in duplicate for each soil sample.

Sampling of soil solution started after the field had reached field capacity and was carried out four times during autumn and twice during spring. A vacuum of −0.06 MPa was applied to the ceramic suction cups with a small hand-operated pump 2 days prior to sampling the soil solution. Soil solution samples were frozen immediately until analysis.

Winter wheat plant samples were taken in both main plots and microplots during the growing season (three times in autumn and three times in spring/summer) and at harvest. In the main plots, plants from 2 to 6 m of row were harvested at each sampling, except at harvest where the whole plot was harvested. In the microplots, 15 plants were sampled at random (excluding the outer 10 cm) at each sampling except for the last sampling, where the whole microplot was harvested (excluding the outer 10 cm). Plant materials were dried at c. 70°C and finely ground (<0.5 mm) before analyses for total N and 15N on an ANCA-MS system as described below. Plant dry matter yield and total N uptake were determined from the main plots, and 15N enrichment from the microplots.

2.4. Measurements and analyses

Meteorological data (precipitation and air temperature) were recorded at a meteorological station (Tylstrup) 8 km from the experimental site. The mean annual precipitation and annual mean air temperature (30 year averages) were 668 mm and 7.3°C, with maximum and minimum daily mean temperatures of 15.5°C (July) and −0.7°C (February).

The gravimetric soil water content (drying at 105°C over night) was measured at each sampling date.

Measurements of soil surface CO2 flux as an index of in-situ microbial respiratory activity were made seven times during the first 5 weeks after application of the cattle slurry. A closed chamber (diameter 10 cm) was placed on the soil (inserted 1–2 cm) and connected to an infra-red CO2 gas analyzer (Soil Respiration System SRC-1, PP Systems, Hitchin, UK) in a closed circuit. Normal exposure time was less than 2 min. Thorough mixing of the air in the closed chamber, tubing and IRGA sensor cell was ensured by a small ventilator inside the chamber. Further details have been given by Jensen et al. (1996). In each main plot, five replicate measurements were made at random location. Measurements were made in the daytime (from 8.00 a.m. to noon every time) and treatments randomized in the measuring sequence.

Soil microbial biomass was determined by fumigation-extraction (FE) (Brookes et al., 1985); see Jensen et al. (1997b) for further details of analytical procedures. The total N in the 0.5 M K2SO4 soil extracts was determined by a persulphate-oxidation procedure according to Cabrera and Beare (1993), a method which, for soil extracts, yields total N results similar to total Kjeldahl N. Soil microbial biomass N was calculated from the difference in total N between fumigated and unfumigated samples, using an \( \text{f}_{\text{EN}} = \frac{1}{K_{\text{EN}}} \) factor of 1.85 (Brookes et al., 1985; Joergensen and Mueller, 1996).

Nitrate-N in the soil solution and ammonium-N and nitrate-N in the unfumigated 0.5 M K2SO4 extracts were determined by standard colorimetric methods (Keeney and Nelson, 1982) using flow-injection analysis.

Total 15N of all fumigated and unfumigated 0.5 M K2SO4 extracts and mineral 15N in unfumigated 0.5 M K2SO4 extracts and soil solution sampled from suction cups were determined in aliquots selected to contain c. 100–400 µg N. A micro-diffusion procedure slightly modified from that of Sørensen and Jensen (1991) was used. Recovery of N in the diffusion traps was normally 95–100%. Diffusion traps were analysed for total N and 15N on the ANCA-MS system described below. The measured atom percentage (at.%) of
Total C, N and $^{15}$N in the whole soil, taken from microplots amended with labelled cattle slurry and from main plots with or without cattle slurry, were determined in samples taken initially, in spring and at harvest using an ANCA-MS system (ANCA-SL elemental analyzer and 20–20 mass spectrometer, Europa Scientific Ltd., Crewe, UK).

Nitrogen derived from slurry ammonium-N ($N_{dfs}$) in the different soil nitrogen pools and taken up by the plants, was calculated according to Powlson and Barraclough (1993). Measurements of $^{15}$N in soil and plant samples from the plots without application of cattle slurry were used to determine the background enrichment used in the calculations.

2.5. Statistics

Plant dry matter yield and N uptake, soil microbial biomass N, soil mineral N, soil solution NO$_3$-N and N derived from cattle slurry were analysed statistically by analysis of variance (ANOVA). The significance of differences between slurry application rates and sampling dates was estimated using the Tukey test with $a=0.05$. A statistical analysis was performed using SAS software (SAS Institute, 1985).

3. Results

3.1. Soil surface CO$_2$ flux

After the cattle slurry application and seedbed tillage, the soil surface CO$_2$ flux increased in both the slurry-amended and non-amended treatments (Fig. 1), with significantly ($P<0.01$) higher flux rates in the slurry amended treatment until a maximum was reached c. 2 weeks after application. After that, flux rates decreased and were not significantly different on the last two measuring dates. The soil temperature during this period averaged 11.3°C, and showed no clear correspondence with the CO$_2$-flux pattern.

Fig. 1. Temporal variations in soil surface CO$_2$ flux as a measure of microbial activity in soil non-amended or amended with cattle slurry (at a rate of 3.5 kg m$^{-2}$, corresponding to c. 310 g DM m$^{-2}$) on 20 September 1995 (main plots, indicated by arrow). The first point shown was measured prior to slurry application. Bars represent SE ($n=8$).

3.2. Soil mineral N and N derived from the slurry ($N_{dfs}$)

The soil mineral N content in the non-amended treatment decreased significantly during the autumn [Fig. 2(a)] and remained at a minimum of c. 20 kg N ha$^{-1}$ (0–20 cm) from the December 1995 to the April 1996 sampling. In the amended treatment, the soil mineral N content had increased by 54 kg N ha$^{-1}$ at the first sampling (2 days after the cattle slurry application and sowing of the winter wheat), corresponding to c. 72% recovery of the 75 kg NH$_4$-N applied in the slurry (calculated by difference). Soil mineral N derived from the slurry ammonium-N determined from the $^{15}$N labelling amounted to a similar figure of 59 kg N ha$^{-1}$ [Fig. 2(b)]. Nitrification was fast, at the first sampling c. 2/3 of the increase was as ammonium-N, but at the second sampling 3 weeks later, ammonium-N levels were similar in the amended and non-amended treatments, with nitrate-N levels having increased significantly ($P<0.001$) in the amended treatment [Fig. 2(a)]. However, at the third sampling in December 1995, soil mineral N levels in the amended treatment had decreased to a level similar to the non-amended treatment, probably due to leaching of mineral N below 20 cm depth. Simultaneously,
Fig. 2. Temporal variations in (a) soil mineral N (NO$_3^-$-N + NH$_4^+$-N) and soil ammonium N (0–20 cm), in soil non-amended or amended with cattle-slurry (at a rate of 7.5 g NH$_4^+$-N m$^{-2}$) on 22 September 1995 (to microplots, indicated by arrow) and (b) soil mineral N derived from the slurry (Ndfs) in soil amended with cattle-slurry. Bars represent SE (n=8).

Fig. 3. Temporal variations in (a) soil microbial biomass N (0–20 cm), in soil non-amended or amended with cattle-slurry on 22 September 1995 (to microplots, indicated by arrow) and (b) soil mineral N derived from the slurry (Ndfs) in soil amended with cattle-slurry. Bars represent SE (n=8).

mineral N derived from the slurry NH$_4^+$-N decreased dramatically (P<0.001), and only 3 and 2 kg N ha$^{-1}$ [Fig. 2(b)] were found at the December 1995 and April 1996 samplings.

3.3. Soil microbial biomass N and slurry-derived N (Ndfs)

Soil microbial biomass N was relatively stable at a level between 187 and 143 kg N ha$^{-1}$ in both amended and non-amended treatments [Fig. 3(a)], showing a slightly decreasing trend with time. Slurry application caused a slight, but insignificant, increase in microbial biomass N at the second sampling, c. 3 weeks after application [Fig. 3(a)], but from the $^{15}$N labelled data, a significant immobilization of cattle slurry ammonium-N could be observed, with 17 kg N ha$^{-1}$ derived from the slurry in the microbial biomass, corresponding to 23% of the applied slurry ammonium-N [Fig. 3(b)]. At the third sampling in December 1995, the microbial biomass N derived from slurry NH$_4^+$-N had decreased significantly (P<0.01), followed by a slight decrease until the fourth sampling in April 1996.

3.4. Soil solution nitrate-N and slurry-derived nitrate-N (Ndfs)

Nitrate-N levels in the soil solution at 60 cm depth did not differ significantly between treatments at any time [Fig. 4(a)]. At the last sampling in June 1996, soil solution could only be recovered from suction cups in the slurry amended treatment. There was a marked temporal pattern of soil solution nitrate-N, with concentrations rising sharply from the initial, relatively low level of
season in 1996 and did not differ between treatments [Fig. 5(a)]. Crop growth conditions were very optimal in 1996, however, and dry-matter production increased dramatically during the growing season, reaching a maximum of 19.4 and 17.4 t DM ha\(^{-1}\) (not significantly different) at harvest in August 1996 in the amended and non-amended treatments, respectively (corresponding to a grain yield of 10 100 and 9100 kg ha\(^{-1}\), H.S. Østergaard, pers. commun.). Winter wheat N uptake (Fig. 5b) showed a marked sigmoidal pattern as well, with c. 40 kg N ha\(^{-1}\) being taken up in both treatments in the beginning of May 1996 and a final N uptake of 182 and 150 kg N ha\(^{-1}\) (not significantly different) at harvest in Aug 1996 in the amended and non-amended treatments, respectively. Winter wheat uptake of N derived from the slurry ammonium-N [Fig. 5(c)] showed a similar pattern, although the crop uptake of soil derived N was dominant in the last part of the growing season, with the maximum amount of slurry-derived N, 28.5 kg N ha\(^{-1}\), found in the crop at the early July sampling and a significant decrease (\(P<0.05\)) in slurry-derived N content by harvest. The proportion of winter wheat N content (indicated by arrow) and (b) fraction of soil solution NO\(_3\)-N derived from the slurry in soil amended with cattle-slurry. Bars represent SE (\(n=8\)).

13–14 mg nitrate-N l\(^{-1}\) at the first two samplings, to very high levels of 53–81 mg nitrate-N l\(^{-1}\) at the samplings in November and December 1995 and April 1996, followed by a sharp decrease at the last sampling, probably due to plant uptake. The proportion of slurry-derived nitrate-N in the soil solution [Fig 4(b)] became significant (\(P<0.001\)) from the late November 1995 sampling, where nitrate leaching apparently occurred, and increased significantly at the following early December 1995 and subsequent April 1996 sampling, where it reached a maximum of 6% of the soil solution nitrate-N being derived from the slurry ammonium-N.

3.5 Wheat crop dry matter production and N uptake

Winter wheat dry matter production was less than 1 t DM ha\(^{-1}\) at the beginning of the growing season in 1996 and did not differ between treatments [Fig. 5(a)]. Crop growth conditions were very optimal in 1996, however, and dry-matter production increased dramatically during the growing season, reaching a maximum of 19.4 and 17.4 t DM ha\(^{-1}\) (not significantly different) at harvest in August 1996 in the amended and non-amended treatments, respectively (corresponding to a grain yield of 10 100 and 9100 kg ha\(^{-1}\), H.S. Østergaard, pers. commun.). Winter wheat N uptake (Fig. 5b) showed a marked sigmoidal pattern as well, with c. 40 kg N ha\(^{-1}\) being taken up in both treatments in the beginning of May 1996 and a final N uptake of 182 and 150 kg N ha\(^{-1}\) (not significantly different) at harvest in Aug 1996 in the amended and non-amended treatments, respectively. Winter wheat uptake of N derived from the slurry ammonium-N [Fig. 5(c)] showed a similar pattern, although the crop uptake of soil derived N was dominant in the last part of the growing season, with the maximum amount of slurry-derived N, 28.5 kg N ha\(^{-1}\), found in the crop at the early July sampling and a significant decrease (\(P<0.05\)) in slurry-derived N content by harvest. The proportion of winter wheat N content derived from the slurry ammonium-N decreased continuously over the whole growth period from 43% of plant N at the initial sampling in late Oct 1995 to only 14% of plant N at harvest [Fig. 5(d)].
3.7. Recovery of slurry-derived ammonium-N

Two days after application of the slurry, 79% of the slurry-derived ammonium-N was recovered in the soil mineral N pool to 20 cm depth, while a further 18% was recovered as organic (non-microbial) or clay-fixed N, making the recovery in the plough layer almost complete (Table 1). In the following spring, almost the same amount was recovered as organic (non-microbial) or clay-fixed N, but the soil mineral and microbial biomass N were negligible, and thus only 24% of the applied slurry ammonium-N was recovered to a depth of 20 cm.

However, the recovery of slurry-derived ammonium-N in soil (0–100 cm) and wheat above-ground biomass at harvest was relatively high, 58 kg N ha$^{-1}$, corresponding to 77% of the applied ammonium-N (Table 2). This means that less than a quarter of the slurry ammonium-N was lost by either volatilization, denitrification or leaching, and approximately a third of the applied slurry ammonium-N was removed with the harvested wheat grain and straw. Thus, about 45% was left in the soil, mainly in organic form as mentioned above.

4. Discussion

4.1. Biological activity and microbial N immobilization

The continuous N mineralization-immobilization turnover (MIT) is caused by microbial activity, growth and death, and occurs even without substrate input other than the native soil organic ammonium-N was found below a depth of 50 cm (3 kg N ha$^{-1}$).
matter, albeit at a relatively low rate. However, for a significant microbial immobilization of mineral N to occur, an increase in the microbial activity is required and will be brought about by a substrate input such as animal manure containing a mixture of both easily available and more recalcitrant C sources. In our study, soil surface CO₂ flux also increased after ploughing in the non-amended treatment (Fig. 1), in spite of a generally decreasing temperature trend (data not shown). As shown by Reichsoky (1997), however, soil tillage, and especially mould-board ploughing, may induce very large increases in CO₂ flux. Certainly, the application of cattle slurry in this experiment at a rate equivalent of 35 t FW or c. 3 t DM ha⁻¹ produced a further increase in soil respiration of the amended over the non-amended treatment, but only temporarily. Two week after application of the slurry, the soil surface CO₂ flux reached a maximum in both treatments, with approximately twice the flux rate in the amended treatment compared to the non-amended treatment (Fig. 1). One week later, the flux rates had decreased, and the difference between treatments ceased. In Canada, Gregorich et al. (1998) found similar increases in soil surface CO₂ flux after application of 56 t ha⁻¹ of solid farmyard manure. Their absolute

![Fig. 6. Distribution with depth after wheat harvest of soil mineral, soil microbial biomass and soil organic (non-SMB) N (a) and N derived from the applied cattle slurry (b). In (a), soil mineral N content is insignificant (<20 kg N per layer) and thus omitted. Bars represent SE of the total N (a) or total Ndfs (b) (n=5).](image)

Table 1: Recovery of N derived from slurry NH₄-N in the soil (0-20 cm) 2 days after slurry application and in the following spring [means ± SE (n=8)].

<table>
<thead>
<tr>
<th></th>
<th>Total N</th>
<th>N derived from slurry NH₄-N</th>
<th>Percentage of added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg N ha⁻¹</td>
<td>kg N ha⁻¹</td>
<td></td>
</tr>
<tr>
<td>24 September 1995</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil mineral N</td>
<td>104 ± 9</td>
<td>58.8 ± 9.2</td>
<td>78</td>
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<tr>
<td>Soil microbial biomass N</td>
<td>187 ± 7</td>
<td>0.0 ± 4.3</td>
<td>0</td>
</tr>
<tr>
<td>Organic (non-SMB) or clay-fixed N</td>
<td>605 ± 94</td>
<td>13.2 ± 6.0</td>
<td>18</td>
</tr>
<tr>
<td>Total soil N</td>
<td>6340 ± 88</td>
<td>72.0 ± 6.0</td>
<td>96</td>
</tr>
<tr>
<td>Slurry NH₄-N not recovered 0-20 cm</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>14 April 1996</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil mineral N</td>
<td>21 ± 1</td>
<td>2.0 ± 0.1</td>
<td>3</td>
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<tr>
<td>Soil microbial biomass N</td>
<td>115 ± 3</td>
<td>4.5 ± 0.5</td>
<td>6</td>
</tr>
<tr>
<td>Organic (non-SMB) or clay-fixed N</td>
<td>6057 ± 131</td>
<td>13.6 ± 1.0</td>
<td>15</td>
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<tr>
<td>Total soil N</td>
<td>6234 ± 133</td>
<td>18.0 ± 1.0</td>
<td>24</td>
</tr>
<tr>
<td>Slurry NH₄-N not recovered 0-20 cm</td>
<td>57</td>
<td>76</td>
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Table 2
Recovery of N derived from slurry NH$_4^+$-N in the soil (0–100 cm) and in the wheat crop (above ground) at harvest in August 1996 [means±SE (n=8)]

<table>
<thead>
<tr>
<th></th>
<th>Total N kg N ha$^{-1}$</th>
<th>N derived from slurry NH$_4^+$-N kg N ha$^{-1}$</th>
<th>Percentage of added NH$_4^+$-N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 August 1996</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Soil mineral N</td>
<td>35±1</td>
<td>4.4±0.1</td>
<td>6</td>
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<tr>
<td>Soil microbial biomass N</td>
<td>252±22</td>
<td>3.6±0.4</td>
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</tr>
<tr>
<td>Organic (non-SMB) or clay-fixed N</td>
<td>15319±603</td>
<td>25.8±3.6</td>
<td>34</td>
</tr>
<tr>
<td>Total soil N</td>
<td>15606±605</td>
<td>33.8±3.7</td>
<td>45</td>
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<tr>
<td>Total wheat N (aboveground)</td>
<td>182±8</td>
<td>24.3±1.0</td>
<td>32</td>
</tr>
<tr>
<td>Recovery of slurry NH$_4^+$-N (in soil + crop)</td>
<td>58</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Slurry NH$_4^+$-N not accounted for</td>
<td>17</td>
<td>23</td>
<td></td>
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</tbody>
</table>

Flux rates were also comparable to our study, but the difference between the manure-amended and non-amended treatments persisted for much longer, c. 100 days compared to the c. 25 days in our study. This means that the initial flush of microbial activity within the first 3–4 weeks in our experiment was most likely driven by easily decomposable substances, such as soluble organic C and volatile fatty acids, known to accumulate especially in dry matter rich slurries during anaerobic storage (Sørensen, 1998). At the same time, the level of microbial biomass N derived from the slurry [Fig. 3(b)], i.e. immobilized from the soil mineral N pool, was the highest measured; 23% of the applied slurry ammonium N was immobilized into the microbial biomass during the first 3 weeks. Other studies have shown equally fast immobilization rates; in a pot experiment at 20°C, Trehan and Wild (1993) found maximum immobilization to occur within 3 weeks, and Morvan et al. (1997) found a very rapid immobilization of slurry NH$_4^+$-N within the first week in a field experiment with temperatures ranging between 15 and 25°C. In an incubation experiment, Sørensen and Jensen (1995) also found that maximum immobilization, due to slurry application, occurred within 1–2 weeks at 22°C, and they furthermore observed an increasing degree of N immobilization and decreasing remineralization rate with soil clay content. Both Kirchmann and Lundvall (1993) and Sørensen (1998) showed a close relationship between N immobilization and the content of volatile fatty acids (VFA) in anaerobic slurries, the VFAs decomposing very rapidly within a few days after application to soil. In our study, measurements were not carried out frequently enough, so we cannot exclude the possibility that immobilization has been even higher either during the first 2 weeks or later, but the following measurements in early December and April showed no further immobilization, with values decreasing to roughly a quarter of the maximum value by next spring, indicating a rapid turnover of microbial N derived from the slurry. This is more rapid than the microbial turnover observed by Jensen et al. (1997a), who found 15N-labelled microbial biomass half-lives of 230–570 days. Total microbial biomass N is hardly a temporary increase in MIT (Trehan and Wild, 1993; Paul and Beauchamp, 1995), without any significant net growth of the microbial biomass, but with a significant proportion of the assimilated microbial N being transformed into dead organic
N (e.g. microbial metabolites) within the first few weeks (Mueller et al., 1998).

4.2. Losses of slurry ammonium-N

The rapid incorporation of the slurry into the soil apparently prevented ammonia volatilization, as can be seen from the high recovery of slurry-derived N (96%) at the initial sampling (Table 1). Sørensen and Jensen (1995) found similar low apparent loss rates. Some immobilization of ammonium-N did occur during the first 2 days from application to the initial sampling (18%, Table 1), however, but it seemed to be mainly abiotic, i.e. ammonium fixation, as no measurable assimilation of \(^{15}\)N into the soil microbial biomass was observed at that time. Sørensen and Jensen (1995) also found ammonium fixation to occur at rates between 0.6 and 19% on sandy and sandy loam soils.

Climatic conditions during the experimental period were somewhat unusual and were characterized by a winter precipitation much lower than normal, only half of the long-term average of 326 mm from September to March. Furthermore, winter temperatures were lower than normal, mostly below 0°C from December to February (2.8°C below the long-term average), and this altogether decreased the risk of N losses from leaching. As a consequence, very favourable crop growth conditions prevailed in the spring with an ample supply of mineral N in the profile available for the winter wheat corresponding to c. 150 kg mineral N ha\(^{-1}\) to a depth of 100 cm (H.S. Østergaard, pers. commun.). This also reflected in the unusually high grain yield of more than 9000 kg ha\(^{-1}\) in all treatments, including the unfertilized, unmanured treatment (H.S. Østergaard, pers. commun.). However, this should also have reduced the risk of leaching of slurry ammonium-N once this was nitrified [within 3 weeks, see Fig. 2(a), but the contrary was indicated by the disappearance from the plough layer [Fig. 2(b)] and the appearance in the soil solution at 60 cm depth of slurry-derived \(^{15}\)N already in late November [Fig. 4(b)], c. 2 months after slurry application. Transport of nitrate derived from the slurry ammonium-N down the profile thus occurred even under such low leaching risk conditions, but we do not know to what depth below 60 cm it was leached before winter percolation ceased. The soil solution nitrate concentrations [Fig. 4(a)] were extraordinarily high in November, December and April, regardless of manure application, probably due to large N mineralization from roots and rhizodeposits of the previous pea crop. This further accentuates the fact that the slurry-derived \(^{15}\)N signature could be picked out on such a high background of mineral N so early after slurry application.

Beckwith et al. (1998) estimated from a number of experiments on loamy sandy soils in the UK that application of animal slurries in September resulted in leaching losses of 20–30% of the applied 200 kg total N ha\(^{-1}\) and that delaying this until December or January could minimize losses to less than 5%. However, Thomsen et al. (1997) found that by spring application, slurry \(^{15}\)N did not seem much more prone to leaching in the following winter than applied fertilizer \(^{15}\)N, with 3–5 and 1–3% of the applied \(^{15}\)N being leached, respectively. However, as larger amounts of total N were applied with the slurry, absolute leaching losses were larger with slurry.

4.3. Crop uptake and soil profile distribution of N derived from slurry ammonium-N

Considering the observed rapid movement of slurry-derived \(^{15}\)N down through the soil profile, it may seem somewhat surprising that the winter wheat crop was still capable of taking up a maximum 38% (in early July) of the autumn applied slurry ammonium-N. Sørensen and Jensen (1996) found a cumulated recovery of 52–62% of \(^{15}\)N labelled urine applied before sowing in three cuts of Italian rye grass on different soil types. However, Thomsen et al. (1997) found that cattle slurry selectively \(^{15}\)N-labelled in the urine fraction applied to a coarse sand and a sandy loam soil in spring before sowing of barley resulted in only 32 and 36%, respectively, of the applied \(^{15}\)N to be recovered in the crop, a figure similar to our findings with autumn application. Powlson et al. (1986), in England, also found similar recoveries of autumn applied fertilizer N to winter wheat on
four soils. However, Jackson and Smith (1997) evaluated autumn, winter and spring applications of animal slurries in England on seven sites in 4 years and found that apparent N recovery rates (difference method) were halved by autumn compared to spring application. In the current study, the major proportion of the \(^{15}\text{N}\) was taken up between 7 May and 5 June, corresponding well with the difference in N uptake between the amended and non-amended treatments [Fig. 5(b) and (c)]. One explanation for the relatively high recovery in our study could be that the winter wheat rooting depth was sufficiently large to catch the leached slurry-derived N. At soil sampling after harvest, a relatively high wheat root density, even at a depth of 100 cm, was observed visually, indicating a rooting depth of somewhat more than 100 cm, which is unexpected on this loamy sand soil normally classified in Denmark as having a maximum rooting depth of 75 cm.

After harvest, very little slurry-derived \(^{15}\text{N}\) was found in the soil profile as mineral N, less than 5 kg N ha\(^{-1}\) [Fig. 6(b) and Table 2]. The major proportion of slurry-derived \(^{15}\text{N}\) in the top 25 cm was now present as organic N (82\%), which was an increase from the 64\% organic \(^{15}\text{N}\) in the top 20 cm in the spring (calculated from Table 1). Furthermore, the majority of the slurry-derived N was present in the top 25 cm, with less than 20\% in the 25–100 cm depth. This is similar to the findings from a lysimeter study, where a grass sward received \(^{15}\text{NH}_4\text{-N}\) labelled pig slurry (Carey et al., 1997), and indicates the importance of biological activity for the transformation of applied mineral N into organic forms. Although the level of total microbial biomass N is relatively stable over time [Fig. 3(a)], the rapid turnover of the microbial biomass caused microbial biomass N derived from the slurry to decrease more or less exponentially after the initial maximum immobilization [17 kg Ndfs ha\(^{-1}\) in October, decreasing to only 3 kg Ndfs ha\(^{-1}\) [Fig. 6(b)] the following August. These observations also confirm the general conclusions made by Sørensen et al. (1994) that the residual N value does not differ between various sources, whether they are mineral fertilizer, manure or crop residues.

5. Conclusions

It is concluded that the application of cattle slurry increased microbial activity temporarily and thereby promoted very rapid MIT and significant immobilization of slurry \(\text{NH}_4\text{-N}\) into the microbial biomass. However, rapid microbial turnover depleted the microbial biomass \(^{15}\text{N}\) before next spring and leaching of slurry-derived N was also rapid, even in the experimental year with a low leaching risk. Due to the lower-than-normal winter precipitation and probably also due to a high rooting depth of the winter wheat crop, a relatively high recovery of slurry-derived N in the plant biomass was found. Post-harvest residual N derived from the slurry \(\text{NH}_4\text{-N}\) could mainly be found in organic form in the topsoil, as also commonly found with fertilizer N, indicating the importance of microbial activity in N cycling through MIT.

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