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Mineralization-immobilization and plant uptake of nitrogen as influenced by the spatial distribution of cattle slurry in soils of different texture

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Abstract

The effect of incorporating cattle slurry in soil, either by mixing or by simulated injection into a hollow in soil, on the ryegrass uptake of total N and $^{15}NH_4^+$-N was determined in three soils of different texture. The N accumulation in Italian ryegrass (*Lolium multiflorum* L.) from slurry N and from an equivalent amount of NH_4^+-N in ($^{15}NH_4$) SO_4 (control) was measured during 6 months of growth in pots. After this period the total recovery of labelled N in the top soil plus herbage was similar in the slurry and the control treatments. This indicated that gaseous losses from slurry NH_4^+-N were insignificant. Consequently, the availability of slurry N to plants was mainly influenced by the mineralization-immobilization processes. The apparent utilization of slurry NH_4^+-N mixed into soil was 7%, 14% and 24% lower than the utilization of $(NH_4)_2SO_4$-N in a sand soil, a sandy loam soil and a loam soil, respectively. Thus, the net immobilization of N due to slurry application increased with increasing soil clay content, whereas the recovery in plants of ^{15}N-labelled NH_4^+-N from slurry was similar on the three soils. A parallel incubation experiment showed that the immobilization of slurry N occurred within the first week after slurry application. The incorporation of slurry N by simulated injection increased the plant uptake of both total and labelled N compared to mixing the slurry into the soil. The apparent utilization of injected slurry NH_4^+-N was 7% higher, 8% lower and 4% higher than the utilization of $(NH_4)_2SO_4$-N in the sand, the sandy loam and the loam soil, respectively. It is concluded that the spatial distribution of slurry in soil influenced the net mineralization of N to the same degree as did the soil type.

Introduction

The amount of nitrogen (N) in animal manure that is available to plants is influenced by N mineralization-immobilization processes in the soil after manure application, and by losses of N due to NH_3 volatilization, NO_3^- leaching and denitrification. Several investigations have indicated that the net mineralization of manure N is negatively correlated with the soil clay content (Castellanos and Pratt, 1981; Chescheir et al., 1986; Hébert et al., 1991; Van Faassen and van Dijk, 1987). The influence of soil texture on the turnover of organic matter in soil may be due to 1) differences in the physical protection of organic matter, e.g. by adsorption of organic matter on inorganic clay surfaces and entrapment of materials in aggregates inaccessible to microbes and/or 2) soil texture effects on microbial turnover processes, e.g. by increasing protection of microbial biomass with increasing soil clay content (Van Veen and Kuikman, 1990). Sørensen et al. (1994) found almost no effect of soil texture on mineralization and plant accumulation of ^{15}N-labelled organic manure N, while the immobilization and remineralization of N due to manure was influenced by the soil texture. Thus, the latter mechanism seems to be most important for the turnover of manure N. Similarly Vilsmeyer and Gutzer (1990) observed a positive correlation between immobilization of $^{15}NH_4^+$-N from slurry and the soil clay content. A considerable net immobilization of N has been observed in soils after application of anaerobically stored manures (Amberger et al., 1982; Flowers and

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Arnold, 1983; Kirchmann, 1991). This immobilization may be caused by soil microbes utilizing carbon in volatile fatty acids of the manure (Kirchmann and Lundvall, 1993).

The N efficiency of slurry injected into soil has been reported to be higher than that of surface applied slurry. This difference in N efficiency is mainly due to a reduction in the volatilization of NH_3 from injected slurry (Thompson et al., 1987). On the other hand soil injection of slurry may increase the potential for denitrification (Comfort et al., 1988; Petersen, 1992; Thompson et al.,1987).

The aims of the present work were: (i) to study the immobilization of slurry NH_4^+-N and net mineralization of N after application of cattle slurry to three soils of different texture; and (ii) to study effects of simulated slurry injection compared to mixing slurry into the top soil on plant N uptake from slurry NH_4^+-N and total N in soils of different texture.

Materials and methods

Experimental slurry

The slurry was collected from an anaerobic storage pit on a farm with milking cows after mixing of the slurry. The slurry had been accumulated during an 8 month period and contained chopped straw from bedding and run-off water from a silage store. The slurry sample was stored in a closed container at 4 °C for about one month and then homogenized by blending. The slurry contained 6.20% dry matter, 4.94% total N, 3.42% NH_4^+-N and 42% total C in dry matter, and pH (H_2O) was 6.8. Immediately before soil application a small amount of $(^{15}NH_4)_2SO_4$ (90.2 atom % ^{15}N) was applied to the slurry and the slurry was mixed for one hour resulting in an enrichment of 1.060 atom % ^{15}N excess of the slurry NH_4^+-N and a NH_4^+-N content of 3.46%.

Pot experiment

The experimental soils were sampled from the plough layer of three arable fields. The soils were air-dried to a water content of about 10% and sieved (<9 mm). Soil characteristics are shown in Table 1. Basic nutrients except N were mixed with the soil, and 19 kg dry soil was filled into 20L pots with a diameter of 0.25 m. The 10–30 cm soil layer in pots was compacted to a soil density of 1.3 g dry soil cm^{-3} and water was added to 70% of the water holding capacity (WHC). The top 10 cm of the soil (equivalent to 6.3 kg dry soil) was treated as follows:

1. Control, no N added.
2. 0.76 g $(^{15}NH_4)_2SO_4$-N pot^{-1} with 1.136 atom % ^{15}N excess was mixed into the soil.
3. 354 g cattle slurry pot^{-1} (0.76 g $^{15}NH_4^+$-N) was mixed into the soil.
4. 354 g cattle slurry pot^{-1} (0.76 g $^{15}NH_4^+$-N) was poured into a cylindrical hollow in the soil (9 cm dia, 10 cm height) simulating a slurry injection, and after one hour the remaining soil was filled into the hollow.

The upper soil layer was also compacted to a density of 1.3 g dry soil cm^{-3}. The experiment was designed as a randomized complete block experiment with four replicates. Pots were sown with Italian ryegrass (*Lolium multiflorum* L., cv. Ninak). The soil surface was covered by 1 cm of quartz sand, the pots were placed outdoor at Risøand irrigated to 70% WHC. The pots were irrigated regularly by weight. The grass was cut 1 cm above soil surface after 2, 3 and 6 months, oven-dried and analysed for total N content and ^{15}N enrichment. After the final cut, the top soil (0–8 cm) of treatment 2 and 3 were sampled for determination of ^{15}N recovery. Soil was not sampled for ^{15}N determination in treatment 4 because the uneven distribution of ^{15}N made representative sampling very difficult. Roots (including stubble) were washed free from soil in all treatments, oven-dried and analysed for total N content and ^{15}N enrichment.

Incubation experiment

The three soils were sieved (< 2 mm) and 50 g samples (oven-dry weight basis) were incubated in 250 mL polyethylene bottles. Deionized water was added in order to keep a soil moisture content of 55% WHC. The bottles were covered with aluminium foil with holes for aeration. After 13 days of pre-incubation the soils were given the following treatments:

1. Control (no amendments).
2. Ammonium sulfate, 120 μg N g^{-1} soil added in 1 mL water.
3. Freeze-dried cattle slurry (38 μg NH_4^+-N g^{-1}) and 82 μg $(NH_4)_2SO_4$-N g^{-1} soil added in 1 mL water. The added $(NH_4)_2SO_4$-N corresponded to the loss of NH_3-N during freeze-drying of the slurry.

Soils were incubated at 22 °C in dark chambers. The water content was adjusted weekly by weight to 55% WHC. After 0, 1, 2, 4, 7, 12 and 20 weeks of

Table 1. Characteristics of experimental soils

		Soil		
		Lundgård	Risø	Ølby
Total N	(% of DW)	0.11	0.14	0.16
Total C	"	1.4	1.3	1.7
Organic matter	"	2.2	2.0	2.1
Clay (<0.002 mm)	"	4.5	17	26
Silt (0.02–0.002mm)	"	4.2	16	27
Fine sand (0.02–0.2mm)	"	19	37	24
Coarse sand (0.02–2mm)	"	70	28	21
Clay fixed NH_4^+-N	(μg g^{-1} soil)	4	75	157
CEC	(cmol+ kg^{-1} soil)	9.9	15.0	20.7
WHC	(g H_2O g^{-1} dry soil)	0.36	0.42	0.43
pH (H_2O)		6.2	6.8	7.9
Soil classification		Sand	Sandy loam	Loam

incubation three replicates per treatment were analysed for inorganic N. For determining possible clay fixation of the added $^{15}NH_4^+$-N in the pot experiment, 116 μg $(^{15}NH_4)_2SO_4$-N g^{-1} soil (10 atom % ^{15}N excess) were added to the soils. After one week of incubation, NH_4^+ -N fixed by clay was determined.

Analytical methods

Total N and C were determined in air-dried soil, freeze-dried slurry and oven-dried plant samples by elemental analysis using a Carlo Erba NA1500 N/C analyzer according to Jensen (1991). The ^{15}N enrichment was determined on a mass spectrometer (Delta, Finnigan MAT) coupled on-line to the elemental analyzer (Jensen, 1991). The soil ^{15}N determinations were corrected for the soil backgrounds (0.369 atom % ^{15}N).

Inorganic N was extracted by shaking 1 g of freeze-dried slurry, 10 g of fresh slurry or 10 g of soil with 100 mL 2 *M* KCl for 1 h (Keeney and Nelson, 1982). Ammonium-N and NO_3^--N + NO_2^--N in filtered extracts were measured on a Technicon Auto-Analyzer II using the sodiumsalicylate-sodium nitroprusside-hypochlorite method (Technicon, 1974) for NH_4^+-N and the sulphanilamide-naphthyl-ethylenediamine method for NO_3^- -N plus NO_2^--N after having reduced nitrate to nitrite with hydrazine (Kamphake et al., 1967).

Total N in fresh slurry was determined as total N in freeze-dried slurry plus NH_4^+-N lost by freeze-drying.

Fixed $^{15}NH_4^+$-N in clay was determined by total N and ^{15}N analysis of the dried soil after destruction and extraction of organic matter from the soil as described by Silva and Bremner (1966).

Ammonium-N in slurry extracts was concentrated for ^{15}N analysis using a diffusion procedure. After adding MgO to the extract, ammonium-N in the extracts was diffused as NH_3 to an acidified glass filter enclosed in polytetrafluoroethylene (teflon) tape floating in the extract (Sørensen and Jensen, 1991).

Results are expressed on an oven-dry basis (soil, 105 °C, 24h; manure and plants, 80 °C, 24h).

Calculations and statistical analysis

The uptake efficiency of $(NH_4)_2SO_4$-N was calculated by difference: (N uptake in $(NH_4)_2SO_4$ treatment - N uptake in control)$\cdot$100 / added NH_4^+-N (Jenkinson et al., 1985). The uptake efficiency of $(NH_4)_2SO_4$-N differed in the 3 soils. In order to distinguish the effect of slurry N from the effect of ammonium-N alone, the apparent utilization of slurry NH_4^+-N was compared to the apparent utilization of $(NH_4)_2SO_4$-N by calculating the difference: (N uptake in herbage after slurry treatment - N uptake in herbage after $(NH_4)_2SO_4$ treatment)$\cdot$ 100 / added NH_4^+-N.

Analysis of variance was carried out on data by using the SAS procedure for ANOVA (SAS, 1989). Least significant differences (LSD values) were calculated from the mean square errors, if the main effects or interactions were significant ($p < 0.05$).

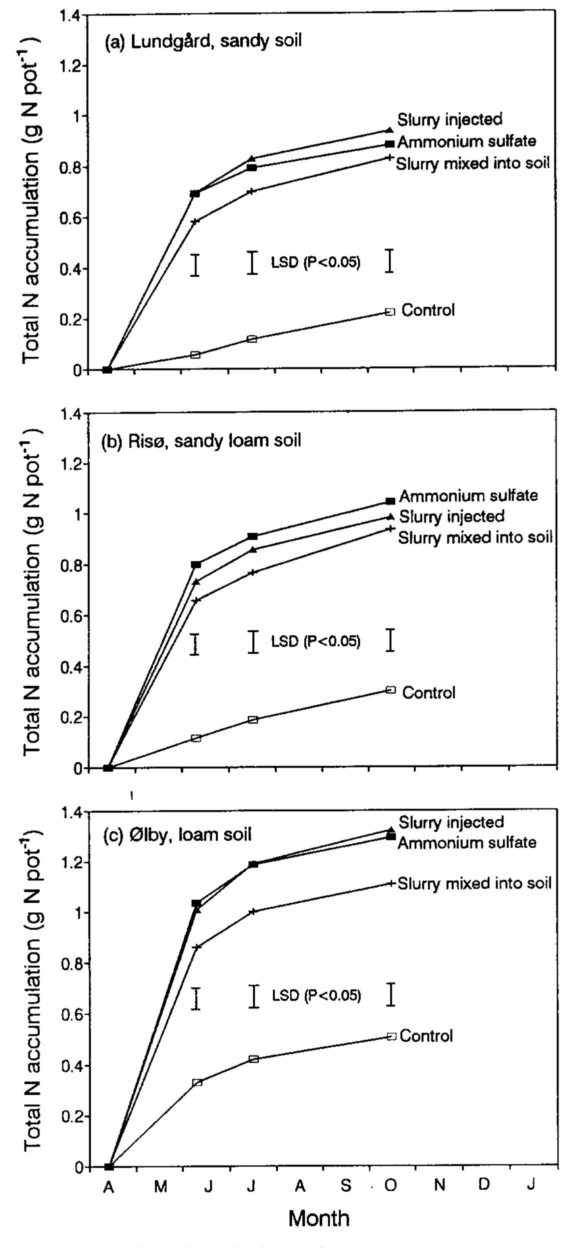

Fig. 1. Total N uptake in herbage of ryegrass grown in three soils receiving either no N, 0.76g $(NH_4)_2SO_4$-N pot^{-1} or 0.76g NH_4^+-N pot^{-1} in cattle slurry mixed into soil or added by simulated injection.

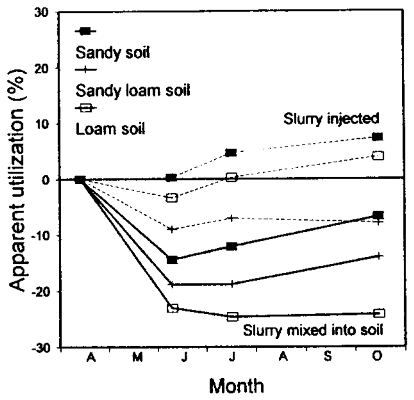

Fig. 2. Apparent utilization by ryegrass of NH_4^+-N in cattle slurry applied to three soils by soil mixing (solid line) or by simulated injection (dashed line) compared to apparent utilization of $(NH_4)_2SO_4$-N, calculated by difference in ryegrass herbage N uptake.

Results

Pot experiment

The fertilization with $(NH_4)_2SO_4$ resulted in an increase in plant accumulation of N almost corresponding to the amount of applied N after two months (Fig. 1). After 6 months, the uptake efficiency of $(NH_4)_2SO_4$-N in herbage calculated by difference was 88% in the sand, 99% in the sandy loam and 105% in the loam soil. Application of the same amount of NH_4^+-N with slurry mixed into soil resulted in a lower N accumulation in herbage on all soils; the reduction being highest in the loam soil (Fig. 1). In contrast, the same amount of slurry applied by simulated injection resulted in a total N uptake similar to the treatment with $(NH_4)_2SO_4$.

The apparent utilization of NH_4^+-N in slurry was compared to the utilization of $(NH_4)_2SO_4$-N by the difference of total N uptake in herbage. After 6 months, the apparent utilization of NH_4^+-N was 7%, 14% and 24% lower for soil-mixed slurry than for $(NH_4)_2SO_4$ in the sand, the sandy loam and the loam soil, respectively (Fig. 2). The apparent utilization of slurry NH_4^+-N added by simulated injection was 7% higher, 8% lower and 4% higher than for $(NH_4)_2SO_4$ in the sand, the sandy loam and the loam soil, respectively (Fig. 2).

After 6 months of growth, 73% to 84% of the labelled $(NH_4)_2SO_4$-N was recovered in the herbage with the highest recovery in the sandy loam soil (Fig. 3). The recovery in herbage of labelled NH_4^+-N applied with soil-mixed slurry was 20 to 30% lower than in the $(NH_4)_2SO_4$ treatment, and it was similar in the three soils (Table 2). The recovery in herbage of labelled NH_4^+-N from injected slurry was intermediate between the two other treatments (Fig. 3).

After 6 months, 8–22% of the labelled $(NH_4)_2SO_4$-N and 23–39% of the soil-mixed slurry ^{15}N was recovered in the top soil. In both treatments ^{15}N in the top soil increased with increasing soil clay content (Table 2).

Table 2. Recovery of ^{15}N-labelled $(NH_4)_2SO_4$-N and cattle slurry NH_4^+-N in ryegrass roots and herbage and in the top soil (0–10 cm, including roots) in pots 6 months after N application

Soil	Treatment	Recovery of ^{15}N (%)			
		In roots	In herbage	In soil	Total[a]
Sand	$(NH_4)_2SO_4$	7.1±0.7[b]	73±2	8±0.9	81±3
	Slurry, mixed	6.2±0.2	54±2	23±2	77±4
	Slurry, simulated injection	7.9±0.9	60±5	ND[c]	ND
Sandy loam	$(NH_4)_2SO_4$	8.3±0.3	84±5	15±0.4	99±5
	Slurry, mixed	7.1±0.6	56±5	37±1	93±6
	Slurry, simulated injection	8.1±1.0	66±1	ND	ND
Loam	$(NH_4)_2SO_4$	6.2±0.7	79±4	22±3	101±5
	Slurry, mixed	6.1±0.5	58±2	39±3	97±3
	Slurry, simulated injection	6.0±0.4	71±4	ND	ND

[a] Total = soil + herbage.
[b] ± standard deviation.
[c] ND: Not determined.

The total recovery of labelled N in top soil and herbage was close to 100% in the two soils with the highest clay content, while it was only about 80% in the sandy soil (Table 2). There was no significant difference in the total recovery of labelled N from $(NH_4)_2SO_4$ and soil-mixed slurry.

The accumulation of unlabelled N in herbage plus roots was significantly higher in the $(NH_4)_2SO_4$ amended soils than in the control soils (Fig. 4). Slurry application resulted in a further increase of the uptake of unlabelled N compared to $(NH_4)_2SO_4$ application, except for slurry mixed with the loam soil (Fig. 4).

Incubation experiment

During the initial weeks after application of $(NH_4)_2SO_4$ there was a slight net immobilization of N in the two soils, but from week 2 to 4 net immobilization changed to net mineralization (Fig. 5). In the sand soil a net mineralization of N due to the slurry was observed immediately after application of the slurry. The inorganic N content continued ta be higher in the slurry treated soil than in the $(NH_4)_2SO_4$ treatment (Fig. 5a). In the two other soils the slurry application caused an immobilization corresponding to 25% of the applied NH_4^+-N within the first week after application. In the sandy loam soil the initial period of immobilization due to slurry addition was followed by a period of net mineralization, but the inorganic N content was still lowest in the slurry treated soil after 20 weeks (Fig. 5b). In the loam soil there was no net mineralization in the slurry-treated soil and the concentration of inorganic N remained much lower than in the $(NH_4)_2SO_4$ treatment (Fig. 5c).

After one week of incubation with $(^{15}NH_4)_2SO_4$ 0.6%, 19% and 8.0% of the applied ^{15}N was recovered as fixed non-exchangeable $^{15}NH_4^+$ -N in the sand, the sandy loam and the loam soil, respectively. After one week of incubation of the loam soil with $(NH_4)_2SO_4$, the concentration of NH_4^+-N was at the original low level while it took 4 weeks in the sandy loam soil and 7 weeks in the sandy soil before the concentration of NH_4^+-N was at the original level, indicating major differences in nitrification rates in the soils (data not shown).

Discussion

Effect of soil type and spatial distribution of slurry on mineralization - immobilization of N

The major part of the ryegrass uptake of both labelled and total N took place during the initial two months of plant growth (Figs. 1 and 3). At the time of the first cut, the soil was probably exhausted of plant available N, since the plant N accumulation due to $(NH_4)_2SO_4$ almost corresponded to the amount of N applied with the N fertilizer. Therefore it is likely that N harvested in the last two cuts was mainly derived from N miner-

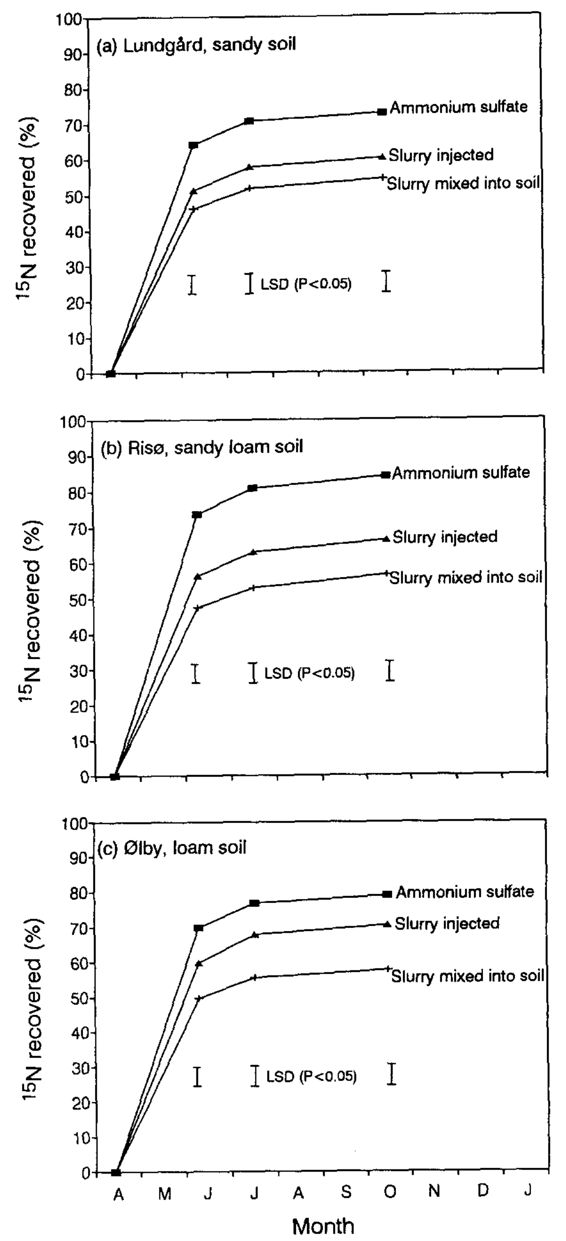

Fig. 3. Cumulative recovery of ^{15}N in herbage of ryegrass grown in three soils applied with ^{15}N-labelled NH_4^+-N (0.76 g NH_4^+-N pot^{-1}) in $(NH_4)_2SO_4$ or cattle slurry mixed into soil or added by simulated injection.

alized after the first cut, and from root N translocated to the top. During the period from the middle of June until the middle of October, N uptake was nearly similar in all treatments (Fig. 1). Hence, the net mineralization of N was only slightly influenced by slurry application during this period.

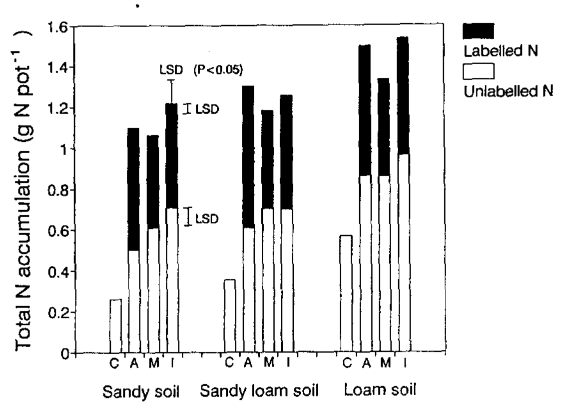

Fig. 4. Accumulation of labelled and unlabelled N in ryegrass (herbage + roots) grown in three soils for 6 months. The treatments were: no N amendment (C), 0.76g $(^{15}NH_4)_2SO_4$-N pot^{-1} (A), 0.76 g $^{15}NH_4^+$-N in cattle slurry mixed into soil (M) and 0.76 g $^{15}NH_4^+$-N in cattle slurry incorporated by simulated injection (I).

The increasing net immobilization of slurry N with increasing soil clay content was probably due to an increasing capacity for protection of the microbial biomass with increasing soil clay content (Amato and Ladd, 1992; Van Veen et al., 1985). When a high amount of N is retained in the microbial biomass, net mineralization of N will be lower. The activity of predators such as bacterivorous nematodes may stimulate the net mineralization of N after slurry application (Opperman et al., 1993).

The plant N uptake was higher after simulated injection than after incorporation of slurry by mixing with soil. We suggest that this is due to the following mechanisms. When slurry is mixed with soil, a high proportion of the microorganisms involved in the slurry decomposition are associated with soil particles. Consequently, a higher proportion of the organisms are protected against predation, while the microorganisms utilizing the injected slurry to a higher degree are unprotected, living directly at the slurry material. Consequently, a lower amount of slurry N is retained in the microbial biomass after injection. When slurry is applied by injection, the water soluble part of the slurry is dispersed in soil by water flow and diffusion, while the fraction of large particles in the slurry remains at the place of application. The largest particles in cattle slurries have a high C/N and may cause immobilization of N in soil (Diaz-Fierros et al., 1988; Whitehead et al., 1989). Jingguo and Bakken (1989) observed that in a heterogeneous soil-plant system with N immobilizing and N mineralizing zones, the overall net mineralization and plant N accumulation can be much higher than

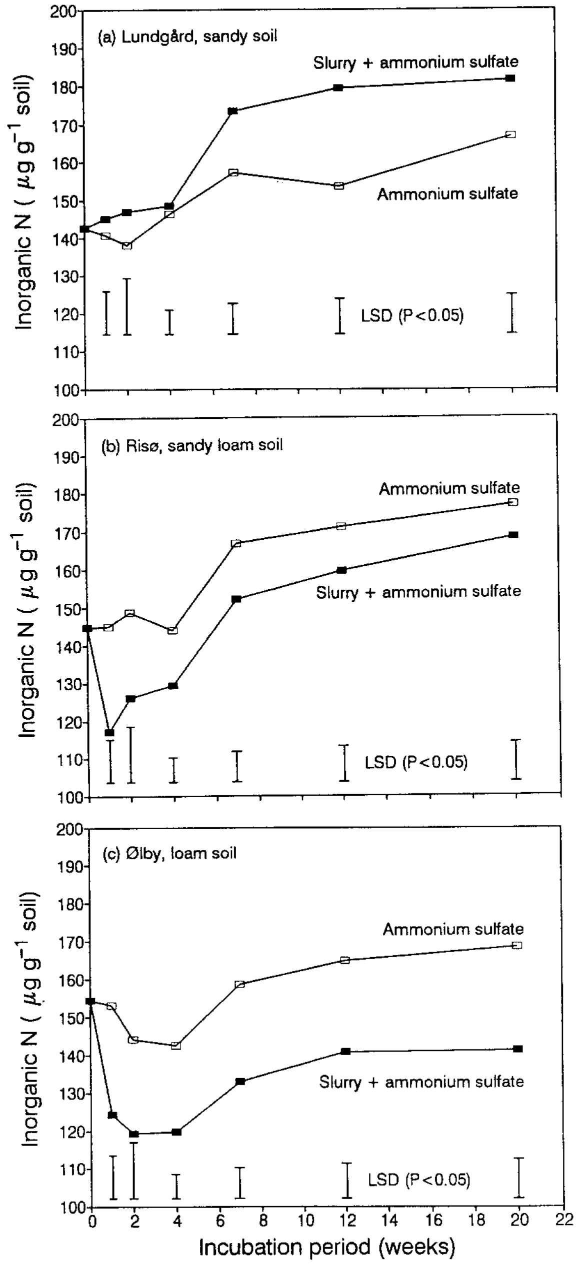

Fig. 5. Inorganic N in three soils during incubation with 120 μg NH_4^+-N g^{-1} soil in freeze-dried cattle slurry (NH_4^+-N lost by freeze-drying replaced with $(NH_4)_2SO_4$-N) or with 120 μg $(NH_4)_2SO_4$-N g^{-1} soil.

in a similar homogenized system, as it was observed in the present experiment.

Comparing the treatment with slurry in the incubation experiment with the incorporation of slurry by soil mixing in the pot experiment, showed a good agreement between the net mineralization of N during incubation and the plant uptake of N in pots. The only exception was that the net mineralization of slurry N in the sandy soil was not reflected in the plant N uptake from slurry mixed into the sandy soil.

The fate of ^{15}N-labelled NH_4^+-N

The immobilization of applied ^{15}N is influenced by the mineralization-immobilization-turnover (MIT) in soil after application (Jansson and Persson, 1982), and later on by the remineralization of immobilized N. After application of slurry, MIT is increased due to the input of carbon, and, as expected, increased immobilization of ^{15}N was observed in the slurry treatments. The rate of nitrification in the soils may also have influenced the amount of immobilized ^{15}N, since N is immobilized mainly from the available NH_4^+-N pool (Jansson, 1958). When the nitrification rate was low the NH_4^+-N pool remained labelled for a longer period, and a higher amount of labelled N was then immobilized. In the loam soil, NH_4^+-N was quickly nitrified or immobilized, so after a short period, N was immobilized from a N pool with a lower enrichment of ^{15}N. Despite of the higher rate of nitrification, the amount of labelled N remaining in the top soil was still highest in the loam soil (Table 2).

Jenkinson et al. (1985) described how plants given ^{15}N-labelled fertilizer take up more unlabelled N from soil than plants not given fertilizer, and they defined this effect as 'added nitrogen interaction' (ANI). In the following the definitions of ANI described by Jenkinson et al. (1985) will be used. In the pot experiment a 'real' positive ANI due to $(NH_4)_2SO_4$ was observed, since the additional total N uptake in plant root and herbage due to $(NH_4)_2SO_4$ was higher than the amount of N applied (Fig. 4). The plant uptake of unlabelled N was also higher in the treatments with labelled NH_4^+-N, compared to the control treatment. This ANI was partly 'apparent' due to MIT (Jansson and Persson, 1982) and partly due to the 'real' ANI observed on the total plant N uptake. The root dry matter was higher in the treatments with N application than in the control (data not shown), and the higher uptake of soil N may have been due to a more extended root system. The higher plant uptake of unlabelled N in the slurry treatments compared to the $(NH_4)_2SO_4$ treatment was due to mineralization of unlabelled organic N from the slurry and to an increased MIT due to the slurry application.

The initial clay fixation was expected to be similar in the $(NH_4)_2SO_4$ treatment and the soil-mixed slurry treatment as the same concentration of NH_4^+

was applied and most clay fixation takes place within a few hours after application of NH_4^+-N (Drury and Beauchamp, 1991). The recovery of $(NH_4)_2SO_4$-^{15}N in plants exceeded 90% in the sandy loam soil, which indicates that a major part of the $^{15}NH_4^+$ which may have been clay fixed became available to plants. In agreement with this, Trehan and Wild (1993) and Scherer and Weimar (1994) observed that a large part of clay fixed NH_4^+-N was available to plants. Thus, clay fixation was considered to be of little importance for the plant N uptake in the present experiment.

The high recovery of labelled N in the loam and the sandy loam soil indicates that no significant gaseous loss of labelled N from $(NH_4)_2SO_4$ and soil-mixed slurry occurred. The labelled N not accounted for in the sand soil was probably leached to the 10 to 30 cm soil layer. There was a high root density even in the deepest soil layer, and the recovery of ^{15}N in roots was similar in the three soils. Therefore, most of the labelled N not accounted for in the sand soil was probably immobilized in the subsoil. The rate of nitrification was low in this soil, so labelled NH_4^+-N remained available for immobilization during a long period.

Conclusion

Mixing of cattle slurry into the top soil resulted in net immobilization of N during a 6 month period. An incubation experiment indicated that the immobilization due to slurry mainly occurred within the first week after soil application. The net immobilization of N due to slurry application increased with increasing soil clay content. If the slurry was applied to a hollow in soil, simulating an injection, the plant N uptake was higher and corresponded to the N uptake in treatments receiving the same amount of NH_4^+-N in $(NH_4)_2SO_4$. Thus, the spatial distribution of slurry in soil influenced the net N mineralization to the same degree as did the soil type.

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