Soil Use and Management, December 2007, **23,** 368–373 doi: 10.1111/j.1475-2743.2007.00126.x

Effects of long-term annual inputs of straw and organic manure on plant N uptake and soil N fluxes

J. Luxhøi[1], L. Elsgaard[2], I. K. Thomsen[2] & L. S. Jensen[1]
[1]*Plant and Soil Science, Department of Agricultural Sciences, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg, Denmark, and* [2]*Department of Agroecology, Faculty of Agricultural Sciences, University of Aarhus, Blichers Allé 20, PO Box 50, DK-8830 Tjele, Denmark*

Abstract

This study investigated the effects of long-term annual inputs of animal manure and straw on the rate of gross nitrogen (N) mineralization–immobilization turnover (MIT), net N mineralization and potential nitrification, and examined how these N transformation rates affect plant N availability. The experiment was conducted during May–June 2001 in long-term field experiments in Askov, Denmark, where organic manure and barley straw had been applied annually for 11 and 20 years prior to the year 2000, respectively. Thus, any differences could be attributed to residual effects from the previous years of application. Inputs of straw and organic manure to soil increased soil organic matter (SOM)-N content in soil in the order: without straw, without manure < without straw, with manure < with straw, without manure < with straw, with manure. The inputs did not change net N mineralization in the soil. There was a distinct but non-significant trend towards higher gross N mineralization with increasing SOM-N. Gross N immobilization was enhanced by straw inputs and to a lesser extent by organic manure inputs, while potential nitrification was enhanced by both amendments. The results show that long-term annual inputs of straw and organic manure can increase MIT rate and potential nitrification rate without influencing net N mineralization rate. MIT and potential nitrification explained 23–31% of the variation in plant N uptake, while net N mineralization rate only explained 1%. Plant N uptake therefore seems to be more influenced by MIT rate and potential nitrification rate than by net mineralization rate, presumably because mineral N in the transition between gross N mineralization and gross N immobilization is available for assimilation by plants.

Keywords: Nitrogen, mineralization, nitrification, straw, manure

Introduction

Environmentally justified regulation of nitrogen (N) inputs has reduced the use of mineral fertilizer N in Danish agriculture by approximately 50% since the 1980s (Statistics Denmark 1; http://www.statistikbanken.dk/statbank5a/default.asp?w = 1024; click: English flag → Agriculture → Intermediate consumption → KVAEL2). Inputs of animal manure N have remained approximately stable since the 1980s (Statistics Denmark 2; http://www.statistikbanken.dk/statbank5a/default.asp?w = 1024), but management strategies to increase their fertilizer value have been implemented, i.e. application in spring instead of in autumn. Furthermore, autumn incorporation of straw with a high C:N ratio has been used to prevent N losses during winter, because it promotes microbial immobilization of N.

The amount of fertilizer N potentially replaceable by manure N in the year of application has been shown to correspond to the amount of ammoniacal N in slurry (Petersen, 1996). However, some manure N will remain in the soil (Jensen *et al*., 2000; Sørensen & Amato, 2002), and therefore long-term annual inputs of organic manures cause an accumulation of soil organic matter (SOM)-N (Sommerfeldt *et al*., 1988; Chang *et al*., 1991; Thomsen & Christensen, 2004). Likewise, long-term annual incorporation of straw results in an accumulation of SOM-N, partly from N in the straw and partly due to straw-induced N immobilization (Thomsen, 1995; Power *et al*., 1998). This residual N is gradually mineralized. Thus long-term annual inputs of straw

Correspondence: J. Luxhøi. E-mail: jelu@life.ku.dk
Received May 2007; accepted after revision July 2007

and organic manure could potentially cause soil mineralization to increase (Chang *et al*., 1991; Whalen *et al*., 2001; Luxhøi *et al*., 2004).

To maximize the utilization of crop residue N and manure N, it is therefore important to take into account the fate of the residual N from these sources in the years after application. To develop fertilizer strategies that achieve a high and predictable crop uptake of residual N in intensively managed soils, we need an improved understanding of the mechanisms and rate-determining processes that actually control mineral N release during the crop growth period.

The objective of the present study was to determine how long-term annual inputs of animal manure and crop residues affect rates of gross N mineralization–immobilization turnover (MIT), net N mineralization and potential nitrification, and to investigate how these N transformation rates affect plant availability of N.

Materials and methods

Site description

The experiment was conducted on a light sandy loam in one of the long-term field experiments in Askov, Denmark (Thomsen, 1995). The soil is classified as an Alfisol (Typic Hapludalf), and the Ap-horizon contains 11% clay, 16% silt and 69% sand. The soil N content is shown in Table 1. Soil was sampled from field plots (2.5 × 10 m) that had received 0 or 12 t barley straw ha^{-1} $year^{-1}$ from 1980 to 1999 combined with organic manure treatments: (i) without organic manure; and (ii) with organic manure (catch crop + 35 t pig slurry ha^{-1} $year^{-1}$) from 1989 to 1999. The experiment was laid out in a split-plot design with three replicates. Straw was the main plot factor and organic manure the sub-plot factor. From 1980 to 1999, spring barley (*Hordeum vulgare* L.) was grown every year, and from 1989 to 1999, perennial ryegrass (*Lolium perenne* L.) was undersown in the plots receiving organic manure. In the years 2000 and 2001, straw and organic manure were no longer applied, and instead of spring barley, winter wheat (*Triticum aestivum* L.) was grown in both years.

Table 1 Soil content of organic N (ton N ha^{-1}) in the four treatments

	Straw	
Manure	Without	With
Without	2.9 (0.06)	3.4 (0.07)
With	3.2 (0.03)	3.7 (0.08)

Numbers in brackets are standard errors (n = 24).

Soil sampling

On 2 May and 6 June 2001, 12 soil cores (diameter 4 cm and depth 20 cm) were collected from each plot for the determination of MIT. Simultaneously, eight soil cores (diameter 4 cm and depth 20 cm) and eight additional smaller soil cores (diameter 2 cm and depth 20 cm) were collected from the same plots for the determination of net N mineralization and potential nitrification.

Gross N mineralization–immobilization turnover

The soil cores were transferred to the University of Copenhagen and stored at ambient soil temperature by inserting them (with sealed bases and open tops), 20 cm into garden soil under shelter (Luxhøi *et al*., 2004). The soil cores were divided into two subsets, used in two subsequent incubation periods of 3 days. Before each incubation period $(^{15}NH_4)_2$ SO_4 solution, equivalent to 10 mg N kg^{-1} soil with five atom % ^{15}N, was added to the soil cores. Each core was injected four times, and for each injection, 4 × 0.4 mL solution was delivered as the needle was slowly pulled from the soil. One day after the injections, half the injected soil cores (i.e. three cores per field plot) were sampled for the determination of initial contents of NH_4^+, $^{15}NH_4^+$, NO_3^-, $^{15}NO_3^-$, SOM-N and SOM-^{15}N. The remaining soil cores were incubated for an additional 72 h before sampling for the determination of the final N contents as above. Soil temperatures at the University of Copenhagen site and at the field site were monitored hourly at 10-cm depth by wireless Gemini temperature loggers (type: Tinytalk, TK-0023, Metric, Denmark).

At sampling, soil from the three soil cores was bulked, sieved (8 mm) and mixed. Twenty grams (dry weight basis) of portions of the mixed soil were extracted in 80 mL 1 M KCl for 45 min in an end-over-end shaker. The extracts were filtered (Advantec no. 5) and frozen. NH_4^+-N and NO_3^--N in the soil extracts were analysed on a flow injection analyser (Lachat Instruments Division). NH_4^+-N was determined by a gas diffusion method, and NO_3^--N by a cadmium reduction. ^{15}N enrichment of NH_4^+-N and NO_3^--N was determined using a diffusion technique (Brooks *et al*., 1989) slightly modified according to Sparling *et al*. (1995). NH_4^+ in the soil extracts was converted to NH_3 by alkaline reaction with MgO, and trapped from the solution on an acid glass fibre filter in the lid of the container. Subsequently, Devarda's Alloy was applied to the solution to reduce NO_3^- to NH_4^+, which was trapped on a new acid glass fibre filter placed in the lid. ^{15}N enrichment of the NH_4^+ or NO_3^- in the filter was measured on a mass spectrometer (type 20–20) coupled to an elemental analyser sample preparation module (ANCA-SL, both instruments from Europe Scientific, Crewe, UK). According to the method of Recous *et al*. (1999), the remaining soil on the filters and in the extraction bottles was shaken in 40 mL of 1 M KCl for 15 min and centrifuged at 2500 g. The supernatant was discarded and the procedure was repeated four times, which in a preliminary test had been found to remove any residual mineral N. Remaining SOM-N and SOM-^{15}N enrichment in the soil were measured on the mass spectrometer as described above.

Net N mineralization

The eight soil cores from each plot for the determination of net N mineralization were transferred to the Faculty of Agricultural Sciences, University of Aarhus. Soil from the smaller soil cores was used immediately for the determination of initial mineral-N content (NO_3^- + NH_4^+). The larger soil cores were incubated for 3 weeks under shelter at ambient soil temperature, with sealed bases and open tops, before sampling for the determination of the final mineral N content. Temperature was not recorded at the Foulum site.

At sampling, the soil from the eight soil cores was sieved (6-mm mesh), and bulked. Soil portions of 100 g (on a dry weight basis) were added to 200 mL of 1 M KCl and extracted for 1 h in an end-over-end shaker. The extracts were filtered (Advantec no. 7) and frozen. NH_4^+-N and NO_3^--N in the soil extracts were determined by a flow injection analyser system, as described earlier.

Potential nitrification

In addition to initial mineral N determination, soil from the smaller soil cores collected in June was used immediately for the determination of potential nitrification. Ten grams (fresh weight) of sieved and mixed soil was put into 250-mL conical flasks with 100 mL of 0.5 mM $(NH_4)_2SO_4$ in 1 mM K_2HPO_4 (pH 7.2). Then, 1 mL of 1 M $NaClO_3$ was added to inhibit NO_2^- oxidation (Belser & Mays, 1980). The flasks were incubated on a rotary shaker at 20 °C in the dark. After 15 min and again after 5 h, 3 mL of the soil slurries were transferred to 10-mL test tubes with 3 mL of 4 M KCl to stop microbial activity. The samples were centrifuged (2000 *g* for 5 min) and 2 mL of filtered (0.45 μm) supernatant were transferred to a cuvette for colorimetric NO_2^- analysis (Baush and Lomb, Spectronic 2000) by the diazotization method of Hanson & Phillips (1981). Results were corrected for blanks and compared with a standard curve prepared from $NaNO_2$ in phosphate buffer.

Calculations and statistics

Gross N mineralization and gross N immobilization rates in the soil cores injected with $(^{15}NH_4)_2SO_4$ solution were estimated from the change in N pool size and the change in ^{15}N abundance in the pools. The calculations were carried out with the numerical model FLUAZ (Mary *et al*., 1998). Net N mineralization rate is the difference between gross N mineralization rate and gross N immobilization rate, and was calculated as the change in mineral N content over time in the non-injected soil cores (Figure 1). Potential nitrification was calculated from the NO_2^- produced in the interval between t = 15 min and 5 h. FLUAZ provides mean rate estimates of gross N mineralization and gross N immobilization and the weighted 90% confidence interval of rate estimates, using the experimental variation in the measured parameters. After recalculating the confidence interval into variance, the N transformation data were subjected to weighted ANOVA in the Proc GLM for least squares of mean in SAS eighth edition, as suggested by Luxhøi & Brockhoff (2004). Net N mineralization, potential nitrification and SOM-N were subjected to ANOVA in Proc GLM in SAS eighth edition. The significance of differences between wheat straw and organic manure application and sampling dates was estimated using the Tukey test with α = 0.05.

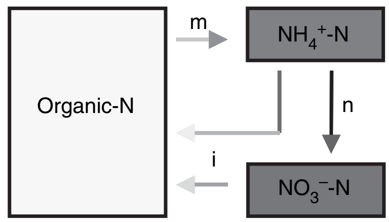

Figure 1 Schematic diagram of nitrogen transformations in the soil, where 'm' denotes gross N mineralization rate, 'i' denotes gross N immobilization rate and 'n' denotes nitrification rate. Net N mineralization is the accumulation of mineral N and is basically the difference between 'm' and 'i'.

Results and discussion

The average daily temperature profile at the University of Copenhagen site (site of incubation) was similar to the temperature profile at the Askov field site (Figure 2a). Soil moisture content was increased by an average of 3.5% by injection of $(^{15}NH_4)_2SO_4$ solution (Figure 2b). The influence of the straw and organic manure amendments on the gross N mineralization rates varied substantially between incubation periods, but was not significantly correlated with abiotic factors such as soil temperature and soil moisture content. Soil temperature and soil moisture content may be interrelated, as higher temperatures can cause decreasing moisture content, and hence it is difficult to separate the influence of these two factors on gross N mineralization rate (Jamieson *et al*., 1999).

As described in the *Materials and methods* section, organic manure was applied annually for 11 years and straw was applied annually for 20 years up to 1999. In 2000 and 2001, no amendments were applied. The present study was conducted in 2001 when most of the easily decomposable N originating from the amendments will already have been

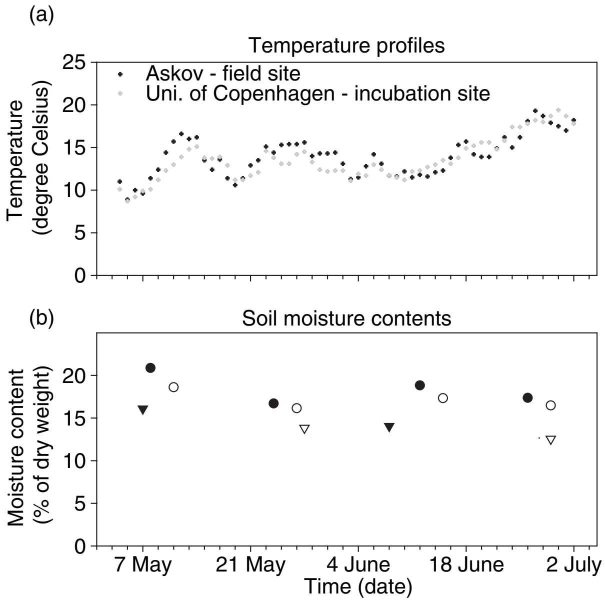

Figure 2 (a) Temperature profile during the experiment at the Askov field site and the University of Copenhagen incubation site. The dots represent daily temperatures, as averaged from hourly measurements. (b) Moisture content measured at the start and end of each incubation period. Circles denote moisture content in the $(^{15}NH_4)_2SO_4$ solution injected into soil cores, while triangles denote moisture content in the non-injected soil cores. Filled and open symbols denote moisture content at the start and end of individual incubation periods, respectively.

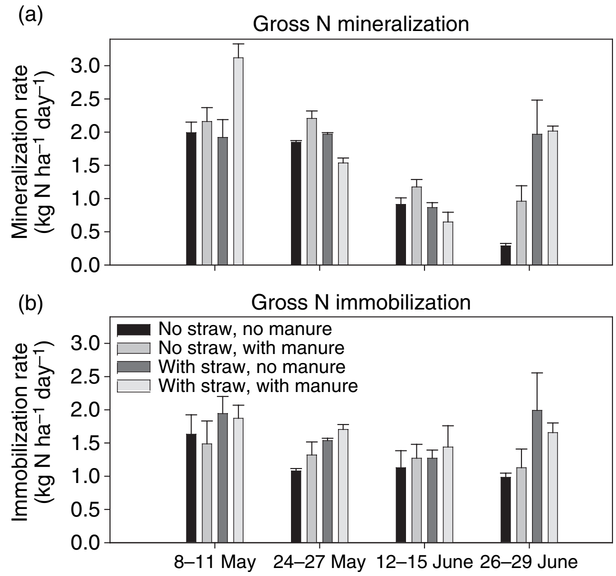

Figure 3 Gross N mineralization and immobilization rates. Error bars indicate standard errors (n = 3).

mineralized before the experiment was started (Vanlauwe *et al.*, 1997). Therefore, the large variation between incubation periods and the variable influence of straw and organic manure amendments (Figure 3a) was not expected. Gross N immobilization rates were significantly increased by straw ($P < 0.01$) and to a lesser extent by organic manure ($P < 0.05$), and these effects were more persistent over time than the effects on gross N mineralization rate (Figure 3b).

Relationships between N fluxes in soil, SOM-N and plant N uptake

Because MIT rates were determined four times during the 2001 season, they were not directly comparable with the other parameters, e.g. net N mineralization rates were determined twice, SOM-N pool sizes were determined eight times and potential nitrification rates and crop N uptake were determined only once during the 2001 season. Hence, to make comparisons, annual averages for each individual field plot were calculated for MIT rates, net N mineralization rates and SOM-N pool sizes. Table 2 shows r^2 values for linear correlations between N fluxes in soil, SOM-N and plant N uptake. There was a weak (r^2 = 0.24) but positive correlation between SOM-N and gross N mineralization rate, while SOM-N and gross N immobilization rate was highly (r^2 = 0.89) positively correlated. This is in agreement with Barrett & Burke (2000) who found that SOM-C explained 32% of the variation in gross N mineralization and 76% of the variation in gross N immobilization. Potential nitrification rates (determined on 12 June, only) were significantly (P < 0.01) increased by the long-term annual application of straw and manure (Figure 4a), and 55% of the variation could be explained by the SOM-N (Table 2). Increased SOM-N may not directly influence nitrification rate, but potential nitrification rate is a measure of the enzymatic potential of the nitrifying population (Belser, 1979). Thus, the results indicate that the nitrifying population was adapted to greater NH_4^+ production in the amended treatments. The results therefore support our earlier assumption, that residual N from previous years affects gross N transformation. There were no significant differences between net N mineralization rates measured in May and June, and the net rates were not significantly affected by the long-term history of straw and organic manure inputs (Figure 4b). There was no correlation between SOM-N and net N mineralization rate, probably because gross N mineralization and gross N immobilization balanced each other to produce very small net N mineralization rates, as also found by Tlustos *et al.* (1998) and Luxhøi *et al.* (2004).

Net N mineralization assays are often used for evaluating plant available N. However, according to Table 2, there was no relationship between plant N uptake and net N mineralization in this study. However, MIT and potential nitrification showed stronger relationships with plant N uptake, although these relationships were insignificant (Table 2). Gross N mineralization and immobilization occur

Table 2 r^2 values for linear correlations

	SOM-N	Gross N mineralization	Gross N immobilization	Potential nitrification	Net N mineralization	Plant N uptake
SOM-N	1	0.24	**0.89**	**0.55**	0.02	0.31
Gross N mineralization	0.24	1	0.21	0.05	0.01	0.23
Gross N immobilization	**0.89**	0.21	1	**0.53**	0.02	0.27
Potential nitrification	**0.55**	0.05	**0.53**	1	0.02	0.31
Net N mineralization	0.02	0.01	0.02	0.02	1	0.01
Plant N uptake	0.31	0.23	0.27	0.31	0.01	1

Values in bold are significant.

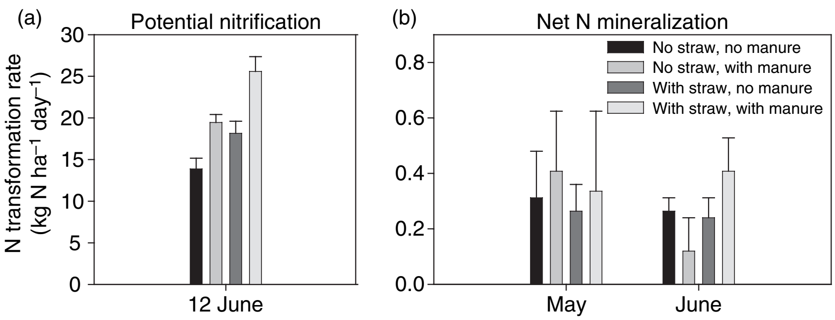

Figure 4 Potential nitrification rates and net N mineralization rates. Error bars indicate standard errors ($n = 3$).

in different 'hot-spots'; consequently, driven by the concentration gradient, NO_3^- can diffuse between them. Plant roots are likely to take up this moving NO_3^- and as a result plants seem to be more dependent on gross production of mineral N than they are on net N mineralization (Wang & Bakken, 1997; Luxhøi *et al.*, 2004; Schimel & Bennett, 2004).

Errors relating to incubation procedure

Because the temperature profiles at the University of Copenhagen site and at the field site in Askov were similar (Figure 2), the incubation of soil cores at the University of Copenhagen site did not create any substantial error. However, as the soil cores were incubated under shelter, they did not receive any precipitation after removal from the field site, and therefore, the soil moisture content was held artificially constant during incubation. Furthermore, injection of $(^{15}NH_4)_2SO_4$ solution for MIT determination increased the average water content from 15 to 18.5%, and increased the NH_4^+ concentration from ~0 to ~10 mg NH_4^+-N kg^{-1} soil, which may have increased microbial activity (Andersen & Jensen, 2001; Luxhøi *et al.*, 2005).

The mean loss of ^{15}N from the soil injected with $(^{15}NH_4)_2SO_4$ solution was 6%. Running the data set through FLUAZ indicated that this loss of ^{15}N was attributable to denitrification, with an average rate of 0.3 mg N day^{-1}. Assuming that the non-injected soil had a similar average rate of denitrification, this would create a relative error in the measured net N mineralization rate of 200%. The injection of $^{15}NH_4^+$ solution could potentially increase denitrification as: (i) the solution could create anaerobic microhabitats; and (ii) nitrification of the applied $^{15}NH_4^+$ could produce the substrate (NO_3^-) for denitrification. Therefore, the denitrification rate in the non-injected soil would probably be less than in the injected soil. This calculation illustrates that gaseous losses could have obscured the net N mineralization rates.

In conclusion, the present results show that long-term annual inputs of straw and organic manure to soil increased the SOM-N content and increased the MIT rates and potential nitrification rate without influencing the net N mineralization rate. Plant N uptake was more influenced by the MIT rate and potential nitrification rate than by the net mineralization rate, presumably because some mineral N, in transition between gross N mineralization and gross N immobilization, was assimilated by the plants.

Acknowledgements

The Danish Ministry of Food, Agriculture and Fisheries funded this study. The authors thank Birthe K. Nielsen and Anja H. Ivø for outstanding laboratory skills.

References

Andersen, M.K. & Jensen, L.S. 2001. Low soil temperature effects on short-term gross N mineralisation–immobilisation turnover after incorporation of a green manure. *Soil Biology and Biochemistry*, **33,** 511–521.

Barrett, J.E. & Burke, I.C. 2000. Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. *Soil Biology and Biochemistry*, **32,** 1707–1716.

Belser, L.W. 1979. Population ecology of nitrifying bacteria. *Annual Reviews of Microbiology*, **33,** 309–333.

Belser, L.W. & Mays, E.L. 1980. Specific inhibition of nitrate oxidation by chlorate and its use in assessing nitrification in soils and sediments. *Applied and Environmental Microbiology*, **39,** 505–510.

Brooks, P.D., Stark, J.M., McInteer, B.B. & Preston, T. 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. *Soil Science Society of America Journal*, **53,** 1707–1711.

Chang, C., Sommerfeldt, T.G. & Entz, T. 1991. Soil chemistry after eleven annual applications of cattle feedlot manure. *Journal of Environmental Quality*, **20,** 474–480.

Hanson, R.S. & Phillips, J.A. 1981. Chemical composition. In: *Manual of methods for general bacteriology* (ed. P. Gerhardt), pp. 328–364. American Society for Microbiology, Washington, DC.

Jamieson, N., Monaghan, R. & Barraclough, D. 1999. Seasonal trends of gross N mineralization in a natural calcareous grassland. *Global Change Biology*, **5,** 423–431.

Jensen, L.S., Pedersen, I.S., Hansen, T.B. & Nielsen, N.E. 2000. Turnover and fate of ^{15}N-labelled cattle slurry ammonium-N applied in the autumn to winter wheat. *European Journal of Agronomy*, **12,** 23–35.

Luxhøi, J. & Brockhoff, P.B. 2004. Analysis of variance on gross nitrogen mineralization data. *Soil Biology and Biochemistry*, **36,** 735–736.

Luxhøi, J., Debosz, K., Elsgaard, L. & Jensen, L.S. 2004. Mineralization of nitrogen in Danish soils, as affected by short-, medium- and long-term annual inputs of animal slurries. *Biology and Fertility of Soils*, **39,** 352–359.

Luxhøi, J., Recous, S., Fillery, I.R.P., Murphy, D.V. & Jensen, L.S. 2005. Comparison of $^{15}NH_4^+$ pool dilution techniques to measure gross N fluxes in a coarse textured soil. *Soil Biology and Biochemistry*, **37,** 569–572.

Mary, B., Recous, S. & Robin, D. 1998. A model for calculating nitrogen fluxes in soil using ^{15}N tracing. *Soil Biology and Biochemistry*, **30,** 1963–1979.

Petersen, J. 1996. Fertilization of spring barley by combination of pig slurry and mineral nitrogen fertilizer. *Journal of Agricultural Science*, **127,** 151–159.

Power, J.F., Koerner, P.T., Doran, J.W. & Wilhelm, W.W. 1998. Residual effects of crop residues on grain production and selected soil properties. *Soil Science Society of America Journal*, **62,** 1393–1397.

Recous, S., Aita, C. & Mary, B. 1999. In situ changes in gross N transformations in bare soil after addition of straw. *Soil Biology and Biochemistry*, **31,** 119–133.

Schimel, J.P. & Bennett, J. 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, **85,** 591–602.

Sommerfeldt, T.G., Chang, C. & Entz, T. 1988. Long-term annual manure applications increase soil organic matter and nitrogen, and decrease carbon to nitrogen ratio. *Soil Science Society of America Journal*, **52,** 1668–1672.

Sørensen, P. & Amato, M. 2002. Remineralisation and residual effects of N after application of pig slurry to soil. *European Journal of Agronomy*, **16,** 81–95.

Sparling, G.P., Murphy, D.V., Thompson, R.B. & Fillery, I.R.P. 1995. Short-term net N mineralization from plant residues and gross and net N mineralisation from soil organic matter after rewetting of a seasonally dry soil. *Australian Journal of Soil Research*, **33,** 961–973.

Thomsen, I.K. 1995. Catch crop and animal slurry in spring barley grown with straw incorporation. *Acta Agriculturae Scandinavica Section B Soil and Plant Science*, **45,** 166–170.

Thomsen, I.K. & Christensen, B.T. 2004. Yields of wheat and soil carbon and nitrogen contents following long-term incorporation of barley straw and ryegrass catch crops. *Soil Use and Management*, **20,** 432–438.

Tlustos, P., Willison, T.W., Baker, J.C., Murphy, D.V., Pavlikova, D., Goulding, K.W.T. & Powlson, D.S. 1998. Short-term effects of nitrogen on methane oxidation in soils. *Biology and Fertility of Soils*, **28,** 64–70.

Vanlauwe, B., Diels, J., Sanginga, N. & Merckx, R. 1997. Residue quality and decomposition: an unsteady relationship? In: *Driven by nature: plant litter quality and decomposition* (eds G. Cadisch & E. K. Giller), pp. 157–166. CAB International, Wallingford, UK.

Wang, J. & Bakken, L.R. 1997. Competition for nitrogen during decomposition of plant residues in soil: effect of spatial placement of N-rich and N-poor plant residues. *Soil Biology and Biochemistry*, **29,** 153–162.

Whalen, J.K., Chang, C. & Olson, B.M. 2001. Nitrogen and phosphorus mineralization potentials of soils receiving repeated annual cattle manure application. *Biology and Fertility of Soils*, **34,** 334–341.