

Research and Development

# Final Project Report

(Not to be used for LINK projects)

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Project title

Indices of nitrate loss from arable soils

MAFF project code

NT1520

Contractor organisation  
and locationSoil Science Department  
IACR-Rothamsted  
Harpenden  
Herts.  
AL5 2JQ

Total MAFF project costs

£

Project start date

01/04/98

Project end date

31/03/00

## Executive summary (maximum 2 sides A4)

- Techniques are now available which use  $^{15}\text{N}$  to measure the rates of the individual processes of the soil nitrogen cycle: i.e. gross rates of mineralisation, nitrification and immobilisation. This allows greater resolution and understanding of the processes that control nitrogen supply for crop uptake and nitrogen losses.
- The main objective of this project was to extend the  $^{15}\text{N}$  isotope dilution techniques, developed under previously funded MAFF research, to determine whether the ratio of gross nitrification to gross immobilisation could be used to indicate the potential of arable soils to 'leak' nitrate.
- Soil was sampled from:
  - four plots of the Broadbalk continuous wheat experiment with a range of long-term stable N management treatments (farmyard manure, 0, 144, and 288 kg N ha<sup>-1</sup>);
  - fifteen sites, where N response trials were also undertaken, which cover the breadth of arable cropping, rotations and soil types in the UK;
  - three plots of the LIFE demonstration experiment at the Royal Agricultural College, Cirencester, where measurements of N losses were also carried out.
- Incubations of soil, with either  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$  added, were carried out under controlled conditions to measure gross rates of mineralisation, nitrification and immobilisation on soil sampled in Spring 1998, Harvest 1998, Spring 1999 and Harvest 1999.
- The size and  $^{15}\text{N}$  enrichment of the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools were measured at regular intervals during incubation periods of up to 14 days.
- Measurements of  $\text{CO}_2$  production and gaseous losses were also made during the incubations.

- As expected, arable soils maintain only a very small  $\text{NH}_4^+$  pool under normal conditions.
- Normal farm practice in the UK does not leave large residues of mineral N in the soil at harvest.
- Long-term stable N management practices lead to changes in the soil N cycle that can be detected by simple measurements of N pools and processes (Broadbalk). However, small and short-term changes in management are more difficult to distinguish using such measurements (LIFE and LINK sites).
- On average, arable soils have a net mineralisation rate of c.  $0.3 \text{ mg N kg}^{-1} \text{ day}^{-1}$  ( $\sim 1.2 \text{ kg N ha}^{-1} \text{ day}^{-1}$ ) during the growing season.
- Very rapid reactions occur when small amounts of N are added to soil as either  $\text{NH}_4^+$  or  $\text{NO}_3^-$ .
- Rapid nitrification of added  $^{15}\text{NH}_4^+$  indicates that nitrification is limited by  $\text{NH}_4^+$  supply in many arable soils and that the rate of nitrification will be overestimated if a  $\text{NH}_4^+$  substrate is added to soil
- During the first 24 hours after addition of  $^{15}\text{N}$  label to soils, equilibrium between added and native pools is not achieved and preferential consumption of the added label may occur.
- In arable soils, where nitrification is rapid, a nitrification inhibitor should be used to create a labelled ammonium pool for the measurement of gross mineralisation.
- The addition of approximately  $1.5 \text{ mg } ^{15}\text{N kg}^{-1}$  soil as  $\text{NH}_4^+$  at approximately 60 atom% in the presence of a nitrification inhibitor is sufficient to create a labelled ammonium pool for the measurement of gross mineralisation.
- The addition of approximately  $1.5 \text{ mg } ^{15}\text{N kg}^{-1}$  soil as  $\text{NO}_3^-$  at approximately 60 atom% is sufficient to create a labelled nitrate pool for the measurement of gross nitrification.
- Isotope dilution does occur in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools when they are labelled with  $^{15}\text{N}$ .
- The decline of the atom% enrichment of either  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$  can be described by exponential decay curves.
- The shape of the decay curve is affected by both site and sampling time.
- Interactions between soil and management factors control the measured rates of gross mineralisation
- On Broadbalk the measured rates of gross mineralisation are highly correlated with the soil organic matter content and other measurements of N pools and processes (Section 4.2).
- On the LINK sites there are significant differences in the rates of gross mineralisation between sites and samplings, but there was no simple pattern with soil texture or management.
- Elevated rates of gross nitrification were measured, where soils had recently received additions of  $\text{NH}_4^+$ , but the background rates of nitrification were not significantly different between sites.
- The  $\text{NH}_4^+$  consumption rates calculated from the dilution of  $^{15}\text{NH}_4^+$  atom% in the presence of  $\text{C}_2\text{H}_2$  can be used to estimate immobilisation, but such estimates are associated with large errors.
- Gross immobilisation and gross mineralisation rates are very highly correlated, indicating that the internal N cycle of arable soils is relatively conservative.
- The Nitrification:Immobilisation (N:I) ratio is greater at harvest than in spring. The soil internal N cycle is less conservative at the harvest sampling, possibly due to a priming effect of  $\text{NH}_4^+$  addition on the nitrifier population in soil.
- The N:I ratio at harvest is significantly correlated with subsequent overwinter N losses, but this relationship is not strong enough to be used for prediction. The N:I ratio and soil mineral N in spring are significantly correlated with subsequent crop uptake in the absence of fertiliser. These relationships are also not strong enough to use for prediction.
- There is a strong relationship between soil mineral N and N:I in spring indicating that the spring soil mineral N is not just a snapshot measurement of one N pool but also an indicator of potential N supply.
- Because of the difficulties of measurement and the high variability of the resulting data, isotope dilution techniques provide a semi-quantitative understanding of the soil N cycle.
- The N:I ratio provides a useful semi-quantitative indicator of the capacity of a soil to produce nitrate that is appropriate in arable, as well as forest, soils.
- However, the measurement of the N:I ratio is complex and expensive and we can foresee no circumstances where it might be used routinely.
- Isotope dilution techniques should be used as a research tool to improve the understanding of the fundamental processes in soil and their interaction with site and soil management.
- Nitrification is dominantly limited by  $\text{NH}_4^+$  supply in arable soils. However, the role of nitrifiers in controlling N losses in soils where  $\text{NH}_4^+$  is not regularly added, e.g. some organic systems, or where  $\text{NO}_3^-$  is used as the only fertiliser form, should be studied further.

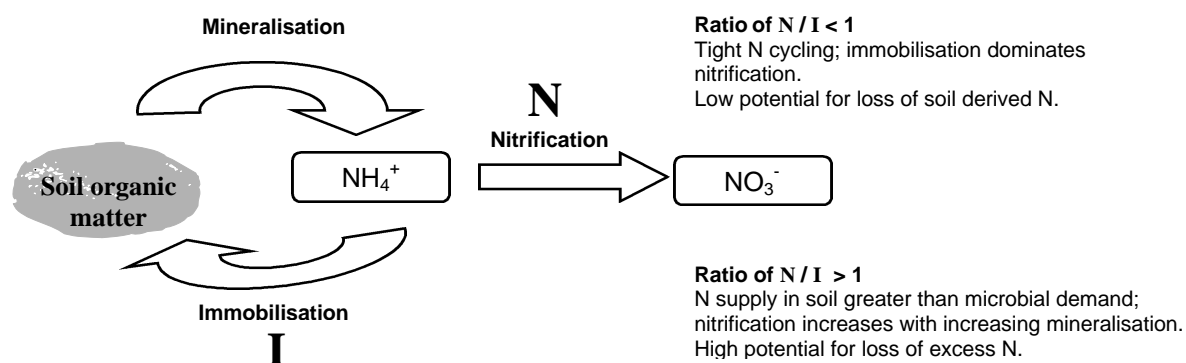
## Scientific report (maximum 20 sides A4)

### 1.0 Background

The potential for soil to 'leak' nitrogen to the environment remains of pressing concern. Leaching of nitrate is environmentally and economically undesirable, and the gaseous loss of nitrogen as nitrous oxide ( $\text{N}_2\text{O}$ ) has important consequences for global warming.

Nitrogen (N) fluxes in soils are dependent on the supply of, and demand for, mineral N (nitrate,  $\text{NO}_3^-$ , and ammonium,  $\text{NH}_4^+$ ). Both are controlled by interacting biological, chemical and physical factors. Mineral N can be supplied to the soil as fertilisers, or returned in animal excreta, as well as through atmospheric deposition and from ammonification (commonly termed N mineralisation) of soil organic matter and crop residues (SOM). Research that addresses the effect of anthropogenic activity on nitrogen cycling within the soil-plant system has been compromised by the lack of a methodology that distinguishes between interacting, competing and sometimes antagonistic processes that cycle N within the soil. The development of  $^{15}\text{N}$  isotopic techniques now enables such processes to be distinguished based on gross transformations. Aber (1992) and Tietema and Wessel (1992) studied the response of forest soils to atmospheric N deposition. Aber (1992) listed the characteristics that might define an 'N saturated' soil, which included the dominance of nitrification over immobilisation. Tietema and Wessel (1992) compared the different N cycling characteristics of N saturated and N limited soils and suggested that a ratio of gross nitrification to gross N immobilisation might offer an index of when a forest soil was saturated and hence likely to lose nitrogen (Figure1).

**Figure 1** Key processes in the soil internal N cycle, showing how the ratio of gross nitrification to gross immobilisation might offer an index of N saturation.



In arable soils, it is generally assumed that nitrification occurs rapidly, when  $\text{NH}_4^+$  supply permits. A large pool of  $\text{NH}_4^+$  is rarely maintained in arable soils. Nitrifiers respond rapidly to additions of  $\text{NH}_4^+$  in fertiliser or manure, when  $\text{NH}_4^+$  supply is in excess of requirements for maintenance and growth of the heterotrophic microbial population (immobilisation), or when this is restricted by carbon availability. In contrast to forests, N uptake by crops can also be rapid in agricultural systems representing a further sink for 'surplus N'. It is therefore only during periods when crop uptake is small that the ratio of gross nitrification to  $\text{NH}_4^+$  immobilisation might form an index of potential N loss. In humid temperate climates, such periods regularly occur, so that indices of potential loss can be applied in agricultural systems. Goulding *et al.* (1998) preliminarily examined the potential of this approach using existing data sets and showed an increase in the ratio of gross nitrification to gross immobilisation in a cultivated ley compared to an adjacent continuous arable soil. At these sites there was no discernible difference in nitrate pool sizes, or the gross rate of N mineralisation.

## 2.0 Scientific objectives

### 2.1 Original objectives

The main objective of this project was to extend  $^{15}\text{N}$  isotope dilution techniques developed under previously funded MAFF research (NT1510, 1514, 1906) to determine whether the index of gross nitrification to gross immobilisation could be used to indicate the potential of arable soils to 'leak' nitrate. This would be achieved by:

- measuring gross mineralisation, gross nitrification and  $\text{CO}_2$  production at a range of sites under arable cropping (approximately 20), 4 times over 2 cropping seasons;
- calculating indices from measurements made;
- comparing indices with crop yields, measured and predicted nitrate losses; and,
- assessing the suitability of indices for the practical indication of N saturated soils.

### 2.2 Achievement of objectives

The objectives of this research project were met on time and in full. The methods used, results obtained and a discussion of their implications follow in the remaining sections of this report.

## 3.0 Materials and methods

### 3.1 Site selection

The nitrogen cycle of the long-term continuous winter wheat experiment on Broadbalk field, IACR-Rothamsted, has been studied since 1843. Drainage is currently being collected and assessed for losses of mineral and dissolved organic N (NT1319). The site is therefore ideal for testing our hypothesis. Four plots in Section 9 (continuous wheat) of the experiment, representing a range of treatments (Table 1), were selected for study in this project. The soil is a silty clay loam of the Batcombe series.

**Table 1** Plot management and soil characteristics of the soils sampled in the Broadbalk experiment

| Plot number | Management   | pH  | %N    | Available P<br>$\text{mg kg}^{-1}$ | Available K<br>$\text{mg kg}^{-1}$ |
|-------------|--|-----|-------|------------------------------------|------------------------------------|
| 2.2         | 35 t $\text{ha}^{-1}$ FYM applied in autumn from bullocks        | 7.8 | 0.307 | 102                                | 684                                |
| 3           | None   | 8.1 | 0.095 | 7                                  | 81                                 |
| 8           | 144 kg N $\text{ha}^{-1}$ applied in April<br>P, K, Mg in autumn | 7.3 | 0.120 | 78                                 | 274                                |
| 16          | 288 kg N $\text{ha}^{-1}$ applied in April<br>P, K, Mg in autumn | 7.8 | 0.132 | 75                                 | 235                                |

As part of the LINK Technologies for Sustainable Farming Systems Project (P164, Decision support systems to design whole farm rotations that optimise the use of available N in mixed arable and horticultural systems: on farm testing, CSA 3629), N response trials were established on approximately 40 sites in 1998 and continued in 1999. Data were therefore available for site management; N uptake of unfertilised plots and local meteorological data had also been compiled. To extend the focussed studies on Broadbalk, 15 of the LINK sites were selected for soil sampling in this project, which represent the breadth of arable cropping, rotations and soil types in the UK (Table 2).

Measurements of nitrogen losses have been made on the demonstration plots of the LIFE experiment at the Royal Agricultural College, Cirencester since 1995 (NT1822). The soil at the site is a calcareous (pH 8.1) clay loam with organic matter varying from 2.7-5.7% across the site. Three plots of this experiment were selected for sampling for this project (A, C, and F). All had a crop of winter wheat harvested in 1999, but in 1998 had crops of winter beans, grass/clover ley or spring peas, respectively. These sites allowed us to test for subtle, short-term impacts of management on N loss.

**Table 2** Location, cropping and soil characteristics for LINK sites

| Site | Grid reference | Crop        |           |            | Topsoil (0-30 cm) |     |       |
|------|----------------|-------------|-----------|------------|-------------------|-----|-------|
|      |                | 1997        | 1998      | 1999       | Texture           | pH  | %N    |
| 4    | TQ883625       | W. wheat    | W. wheat  | W. OSR     | Silty loam        | 5.2 | 0.140 |
| 5    | SP955248       | Peas        | W. wheat  | W. wheat   | Sandy loam        | 6.9 | 0.241 |
| 6a   | TF074009       | W. wheat    | Potatoes  |            | Clay loam         | 7.7 | 0.164 |
| 6b   | TF074009       |             | Peas      | W. wheat   | Clay loam         | 7.6 | 0.161 |
| 8    | SD306053       | Peas        | W. wheat  | W. barley  | Silty clay loam   | 6.9 | 0.101 |
| 9    | SJ429461       | W. OSR      | W. wheat  | S. OSR     | Sandy clay loam   | 5.9 | 0.173 |
| 10   | TF074009       | Peas        | W. wheat  | Potatoes   | Clay loam         | 7.7 | 0.167 |
| 11   | TL401350       | W. OSR      | W. wheat  | W. wheat   | Clay              | 7.8 | 0.168 |
| 12   | TL465342       | W. wheat    | W. wheat  | W. OSR     | Clay              | 7.4 | 0.164 |
| 13   | TL399607       | W. wheat    | W. barley | W. OSR     | Clay              | 7.8 | 0.164 |
| 14   | TM247672       | W. wheat    | W. OSR    | W. wheat   | Sandy clay loam   | 7.9 | 0.138 |
| 15   | TM251167       | Vining peas | W. wheat  | Sugar beet | Sandy clay loam   | 6.6 | 0.219 |
| 16   | TF577254       | Set-aside   | W. wheat  |            | Silty loam        | 6.9 | 0.070 |
| 17   | SP429957       | W. beans    | W. wheat  | W. wheat   | Clay              | 7.0 | 0.332 |
| 18   | TQ872025       | Celery      | W. wheat  |            | Silty clay loam   | 6.9 | 0.121 |
| 19   | SP311709       | W. OSR      | W. wheat  | Potatoes   | Sandy loam        | 6.3 | 0.064 |

### 3.2 Sampling and preparation

On the Broadbalk experiment, three replicate topsoil (0-23 cm) samples were taken in each plot; ten cores taken using a 2.5 cm diameter auger were bulked for each replicate. Samples were taken on 7/12/98, 26/2/99 and 2/11/99.

For the LINK sites, three replicate topsoil (0-30 cm) samples were taken at each site; twelve cores taken using a 2.5 cm diameter auger were bulked for each replicate. Samples were taken 1) in spring before the first application of fertiliser, and 2) following harvest in a) control plots, which had received no fertiliser, and b) in the main bulk of the field, which had received standard fertiliser applications. For some sites it was not possible to sample in both 1998 and 1999. Dates of sampling and fertiliser applications are given in Table 3.

On the LIFE experiment, soils were sampled from plots A, C and F in early September in 1998 and 1999 and in early March before fertiliser application in 1999. Four replicate topsoil (0-20 cm) samples were taken in each plot; 4 cores taken using a 5 cm diameter auger were bulked for each replicate.

All soils were stored at < 5°C for less than a week before sieving. After sieving, sub-samples were taken to determine soil moisture content by drying in an oven for 24 hours at 105°C. Soil mineral N was determined by extracting c. 20 g of soil with 80 ml of 2 M KCl and shaking for 1 hour. NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations in the extract were determined colorimetrically by flow injection analysis (Skalar SAN<sup>PLUS</sup>) and soil concentrations of mineral N calculated. Potentially mineralisable N (PAN) was also estimated using the anaerobic incubation method: c. 20 g of soil was incubated in 80 ml of distilled/deionised water for 7 days at 40°C and then extracted with 2 M KCl. NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations in the extract were determined colorimetrically by flow injection analysis (Skalar SAN<sup>PLUS</sup>). PAN was calculated from the increase in the NH<sub>4</sub><sup>+</sup>-N pool during incubation. Values of mineral N and PAN are presented on a dry weight basis.

### 3.3 Measurement of gross N transformations

To minimise the effect of disturbance due to sampling and soil sieving on the gross rates of N transformations, sieved soil (300 g of moist soil) was weighed into incubation vessels (Kilner Jars with lids adapted for gas sampling) immediately after sieving and pre-incubated at field temperatures for two weeks.

The <sup>15</sup>N isotopic dilution technique requires labelled <sup>15</sup>N to be distributed throughout the soil so that the unlabelled soil NH<sub>4</sub><sup>+</sup> pool (or in the case of nitrification the NO<sub>3</sub><sup>-</sup> pool) becomes uniformly enriched with <sup>15</sup>N. This was achieved by applying 20 ml of (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> at 60.9 atom % or K<sup>15</sup>NO<sub>3</sub> at 61.2 atom % at a rate equivalent to 1.5 mg N kg<sup>-1</sup> dry soil to pre-incubated soil. Solutions were added dropwise while rotating the incubation vessels, ensuring that soil depth was never greater than 2 cm during addition (see Willison *et al.*, 1998). One incubation with <sup>15</sup>NH<sub>4</sub><sup>+</sup> and one with <sup>15</sup>NO<sub>3</sub><sup>-</sup> were set up for each replicate of soil sampled from the

field (3 replicate incubations for Broadbalk and LINK sites; 4 replicate incubations for LIFE). In total c. 725 incubations were carried out during the project.

A period is allowed after addition of  $^{15}\text{N}$  to allow for any abiotic fixation of  $^{15}\text{N}$  and allow the added and indigenous  $\text{NH}_4^+$  or  $\text{NO}_3^-$  pools to equilibrate (Davidson *et al*, 1991; Murphy *et al*, 1997). To ensure equilibration was complete, and for convenience, we used an equilibration period of 24 hours (in common with other workers, e.g. Ledgard *et al*, 1998). Soil was therefore removed from the incubation vessels after 1, 2 or 3 (not Spring 1998), and 7 days for  $^{15}\text{NH}_4^+$  labelled soil and after 1, 7 and 14 days for  $^{15}\text{NO}_3^-$  labelled soil. At each sampling c. 80 g of soil was removed for mineral N extraction in 160 ml of 2 M KCl.  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations were determined colorimetrically by flow injection analysis (Skalar SAN<sup>PLUS</sup>). The  $^{15}\text{N}:^{14}\text{N}$  isotopic ratio of the  $\text{NH}_4^+$ -N or  $\text{NO}_3^-$ -N within soil extracts was determined by mass spectrometry (Europa Tracermass) after preparation by diffusion.  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N were diffused on to separate acidified glass fibre disks, using a modification of the procedure of Brooks *et al*. (1989). Where necessary an unlabelled N 'spike' was used to increase N concentration or reduce  $^{15}\text{N}$  enrichments to within suitable detection limits for the mass spectrometer and reference materials. In total c. 4500 extracts were analysed in this way during the project. Soil moisture content was also determined at each sampling time. Values are presented on a dry weight basis.

$\text{CO}_2$  production was measured for two 24 hour periods during the incubations. Gas samples were taken from each incubation immediately after sealing the incubation vessels after addition of label and 24 hours later before the jars were opened; similarly gas samples were taken after opening and resealing the incubation vessels after 7 days incubations and 24 hours later. In addition, at Spring and Harvest 98, samples were taken after 0, 1 and 3 days to determine the headspace concentrations and  $^{15}\text{N}:^{14}\text{N}$  isotopic ratio of  $\text{N}_2\text{O}$  and  $\text{N}_2$  directly using the ANCA-G gas preparation module and 20-20 isotope ratio mass spectrometer in order to measure gaseous losses of  $^{15}\text{N}$ .

**Table 3** Sampling dates and fertiliser applications for LINK sites in 1998 and 1999.

| Site | 1998                    |                          |                                    | 1999                    |                          |                                    |
|------|-------------------------|--------------------------|------------------------------------|-------------------------|--------------------------|------------------------------------|
|      | Spring<br>Sampling date | Harvest<br>Sampling date | N applied<br>kg N ha <sup>-1</sup> | Spring<br>Sampling date | Harvest<br>Sampling date | N applied<br>kg N ha <sup>-1</sup> |
| 4    | 13/02/98                | 16/07/98                 | 246                                | 16/02/99                | 02/07/99                 | 250                                |
| 5    | 18/02/98                | 10/08/98                 | 222                                | 24/02/99                | 04/08/99                 | 125                                |
| 6a   | 27/02/98                | 24/09/98                 | 240                                | Not sampled             | Not sampled              |                                    |
| 6b   | Not sampled             | Not sampled              |                                    | 22/02/99                | 18/08/99                 | 190                                |
| 8    | 16/02/98                | 04/08/98                 | 174                                | 18/02/99                | 08/07/99                 | 170                                |
| 9    | 17/02/98                | 18/08/98                 | 122                                | 10/04/99                | 17/08/99                 | 125 <sup>2</sup>                   |
| 10   | 03/03/98                | 21/08/98                 | 190                                | 31/03/99                | 01/10/99                 | 213                                |
| 11   | 23/02/98                | 15/08/98                 | 200                                | 02/03/99                | 05/08/99                 | 235                                |
| 12   | 23/02/98                | 19/08/98                 | 223                                | 15/02/99                | 01/07/99                 | 285                                |
| 13   | 24/02/98                | 14/07/98                 | 188                                | 15/02/99                | 15/07/99                 | 250                                |
| 14   | 25/02/98                | 15/07/98                 | 166                                | 01/03/99                | 03/08/99                 | 123 <sup>3</sup>                   |
| 15   | 25/02/98                | 25/08/98                 | 84 <sup>1</sup>                    | 09/04/99                | 19/11/99                 | 192 <sup>4</sup>                   |
| 16   | 27/02/98                | 14/08/98                 | 170                                | Not sampled             | Not sampled              |                                    |
| 17   | 18/02/98                | 11/08/98                 | 180                                | 17/02/99                | 09/08/99                 | 220                                |
| 18   | 02/03/98                | 31/07/98                 | 160                                | 26/02/98                | Not sampled              |                                    |
| 19   | 03/03/98                | 05/08/98                 | 128                                | 19/04/99 <sup>5</sup>   | 01/10/99                 | 136                                |

<sup>1</sup> Additional N was applied in Autumn 1997 in 45 t ha<sup>-1</sup> Pig FYM

<sup>2</sup> Additional N was applied in January 1999, when slurry was spread (unknown rate)

<sup>3</sup> Additional N was applied in Autumn 1998 in 40 t ha<sup>-1</sup> Pig FYM

<sup>4</sup> Additional N was applied in Autumn 1998 in 30 t ha<sup>-1</sup> Duck FYM

<sup>5</sup> In Spring 99, Site 19 was sampled after the application of ammonium nitrate fertiliser during bed preparation for potato planting, zero plots therefore received this fertiliser but no subsequent N.

Gross N mineralisation and gross nitrification rates were calculated from the change in pool size, and the decline in atom %  $^{15}\text{N}$  excess, of the  $^{15}\text{N}$ -labelled  $\text{NH}_4^+$  or  $\text{NO}_3^-$  pool, as soil-derived organic- $^{14}\text{N}$  was ammonified or indigenous  $^{14}\text{NH}_4^+$  was nitrified (Kirkham and Bartholomew, 1954; Barraclough, 1991). The rate of consumption of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  was also calculated.

Treatments with and without a nitrification inhibitor ( $\pm$  acetylene treatments) added to the headspace above soil labelled with  $(^{15}\text{NH}_4)_2\text{SO}_4$ , were compared at Spring 98, Harvest 98 and Spring 99 to combat the problems associated with the rapid decline of the  $^{15}\text{NH}_4^+$  during the first 24 hours of the incubation. 8 ml of  $\text{C}_2\text{H}_2$  was injected into the headspace above the soil through the gas sampling port (to give a concentration of approx. 10% v/v) within half an hour of  $^{15}\text{NH}_4^+$  addition and then every time a jar was opened and sealed. Gas measurements, as described earlier were only made in the absence of  $\text{C}_2\text{H}_2$ .

### 3.4 Modelling and statistical analysis

For the LINK sites, set-up files were compiled for each site and SUNDIAL Version-93-7 (Smith *et al*, 1996) was used to simulate the soil-crop N cycle during 1998 and 1999 and to estimate losses of N overwinter.

Statistical analysis, including t-test, curve fitting and analysis of variance, was carried out on GENSTAT 5, release 4.1.

## 4.0 Results and discussion

Each sub-section below describes the results in detail and then lists, simply, the main implications of the research. Those not interested in the detail may wish to read only the sections that begin "These data show that:".

### 4.1 Recovery of added $^{15}\text{N}$ label on Day 1

Where labelled nitrate ( $^{15}\text{NO}_3^-$ ) was added to soil, 76% on average was recovered in the extracted  $\text{NO}_3^-$  pool after 1 day's incubation. There was no significant amount of  $^{15}\text{NH}_4^+$  recovered.

Sampling from the LINK sites showed very highly significant differences between sites and samplings with no interaction between site and sampling. There were no differences between the recovery of  $^{15}\text{NO}_3^-$  in field soil and the zero N plots at harvest. Most  $^{15}\text{NO}_3^-$  was recovered in soils sampled at Harvest 98 (98%); smaller recoveries were measured at Spring 98 (78%), Harvest 99 (67%) and Spring 99 (54%). Measurements of gaseous losses and  $^{15}\text{N}$  atom% from the incubations at Spring and Harvest 98 showed greater losses from the added label in spring than at harvest. However, the estimates of  $^{15}\text{N}$  in gaseous losses were up to 5 times greater than those estimated from the recovery of  $^{15}\text{N}$  in the soil. Smaller recoveries of  $^{15}\text{NO}_3^-$  in Spring may be due to larger or 'primed' populations of denitrifying organisms at spring compared to harvest. While differences between sites were consistent there was no discernible pattern with soil texture or management (Table 4).

When labelled ammonium ( $^{15}\text{NH}_4^+$ ) was added to soil, on average only 0.5% of this was recovered in the extracted  $\text{NH}_4^+$  pool after 1 day's incubation. However, 63% on average of the added  $^{15}\text{NH}_4^+$  was recovered as  $^{15}\text{NO}_3^-$ . There were very highly significant differences between sites and sampling dates, with significantly more of the added  $^{15}\text{N}$  recovered as  $^{15}\text{NO}_3^-$  in Spring 99 (68%).  $^{15}\text{N}$  was measured in gaseous losses ( $\text{N}_2\text{O}$  and  $\text{N}_2$ ) where  $^{15}\text{NH}_4^+$  was added. However, losses were slightly smaller than where  $^{15}\text{NO}_3^-$  was added. In Spring 99 more  $^{15}\text{NO}_3^-$  was recovered where  $^{15}\text{NH}_4^+$  was added to the soil than where  $^{15}\text{NO}_3^-$  was added (Table 4). This may reflect a protection of the  $^{15}\text{N}$  from gaseous losses (unfortunately there were no measurements of  $^{15}\text{N}$  in gaseous losses made at this sampling).

To allow dilution of  $^{15}\text{NH}_4^+$  to be measured during the incubation, a significant pool of  $^{15}\text{NH}_4^+$  needs to remain in the soil. Acetylene ( $\text{C}_2\text{H}_2$ ) is known to be a strong inhibitor of nitrification (McCarty, 1999) and can be easily added to the headspace above soil incubations. Recently  $\text{C}_2\text{H}_2$ -infused cores have been used to simultaneously estimate net and gross rates of mineralisation in grassland systems (Hatch *et al*, 2000). Where acetylene ( $\text{C}_2\text{H}_2$ ) was added to the headspace above the soil incubations, recovery of  $^{15}\text{NH}_4^+$  was significantly increased (Table 4): 26.5% on average of the added  $^{15}\text{NH}_4^+$  was recovered as  $^{15}\text{NH}_4^+$  after 1 day's incubation. A small amount of  $^{15}\text{NO}_3^-$  was recovered in the presence of  $\text{C}_2\text{H}_2$  and it is likely that some nitrification occurred between the addition of  $^{15}\text{N}$  and  $\text{C}_2\text{H}_2$  and during equilibration of the  $\text{C}_2\text{H}_2$  throughout the soil. There were highly significant differences in the recovery of  $^{15}\text{NH}_4^+$  between sites and sampling times. Most  $^{15}\text{NH}_4^+$  was recovered in soils sampled at Harvest 98 (38%); less at Spring 98 (27%), Spring 99 (20%) and Harvest 99 (18%). However, there was no difference between the recovery of  $^{15}\text{NH}_4^+$  in the field and zero plots at harvest. The rapid decline in the recovery of  $^{15}\text{NH}_4^+$ , even 15 minutes after addition has often been attributed to abiotic ammonium fixation (Davidson *et al*, 1991). Site differences in the recovery of  $^{15}\text{NH}_4^+$  may result from differences in the  $\text{NH}_4^+$  fixation capacity of the soil. However, the interaction between site and sampling time

also indicate that biological processes (in addition to nitrification) are involved in the consumption of  $^{15}\text{NH}_4^+$  during the first 24 hours. The rapid decline of the added  $^{15}\text{NH}_4^+$  also suggests that preferential consumption of added  $^{15}\text{N}$  might occur before equilibration of added and native pools.

**Table 4** Recovery of added  $^{15}\text{N}$  after 1 day of incubation in soil, as a percentage of the amount added (c. 1.5 mg  $^{15}\text{N}$  kg $^{-1}$  soil). Data from the LINK sites sampled in Spring 1999.

| Site | $^{15}\text{NO}_3^-$ added |                           | $^{15}\text{NH}_4^+$ added             |                           | $+C_2H_2$                 |                           |
|------|----------------------------|---------------------------|--|---------------------------|---------------------------|---------------------------|
|      | % as $^{15}\text{NO}_3^-$  | % as $^{15}\text{NH}_4^+$ | $-C_2H_2$<br>% as $^{15}\text{NO}_3^-$ | % as $^{15}\text{NH}_4^+$ | % as $^{15}\text{NO}_3^-$ | % as $^{15}\text{NH}_4^+$ |
| 4    | 34.9                       | 0.1                       | 61.4                                   | 0.4                       | 4.6                       | 22.7                      |
| 5    | 72.4                       | 0.1                       | 63.5                                   | 0.3                       | 3.7                       | 17.1                      |
| 6b   | 41.9                       | 0.1                       | 64.9                                   | 0.4                       | 5.9                       | 34.5                      |
| 8    | 37.5                       | 0.1                       | 62.4                                   | 0.3                       | 13.8                      | 15.7                      |
| 9    | 72.3                       | 0.1                       | 92.4                                   | 0.0                       | 22.1                      | 30.1                      |
| 10   | 38.8                       | 0.1                       | 74.2                                   | 0.2                       | 4.2                       | 24.9                      |
| 11   | 63.7                       | 0.1                       | 69.1                                   | 0.2                       | 4.2                       | 11.5                      |
| 12   | 39.4                       | 0.1                       | 57.4                                   | 0.7                       | 4.6                       | 15.8                      |
| 13   | 38.6                       | 0.1                       | 61.9                                   | 0.6                       | 6.3                       | 14.8                      |
| 14   | 73.0                       | 0.1                       | 57.4                                   | 0.2                       | 6.9                       | 23.3                      |
| 15   | 73.8                       | 0.1                       | 61.9                                   | 0.1                       | 20.2                      | 17.2                      |
| 17   | 65.9                       | 0.1                       | 60.7                                   | 0.2                       | 8.5                       | 17.2                      |
| 18   | 65.8                       | 0.1                       | 72.1                                   | 0.2                       | 4.5                       | 23.7                      |
| 19   | n/a                        | 0.1                       | 61.9                                   | 0.3                       | 3.5                       | 58.2                      |

These data show that :

- Very rapid reactions occur when small amounts of N are added to soil as either  $\text{NH}_4^+$  or  $\text{NO}_3^-$
- $^{15}\text{NH}_4^+$  disappears rapidly from the extractable pool even when nitrification is prevented
- During the first 24 hours after addition of  $^{15}\text{N}$  label to soils, equilibrium between added and native pools is not achieved and preferential consumption of the added label may occur. This is particularly seen in the very rapid nitrification of added  $^{15}\text{NH}_4^+$  in the absence of a nitrification inhibitor.
- In arable soils, where nitrification is rapid, a nitrification inhibitor should be used to create a labelled ammonium pool for the measurement of gross mineralisation.
- The addition of approximately 1.5 mg  $^{15}\text{N}$  kg $^{-1}$  soil as  $\text{NH}_4^+$  at approximately 60 atom% in the presence of a nitrification inhibitor is sufficient to create a labelled ammonium pool for the measurement of gross mineralisation.
- Rapid nitrification of added  $^{15}\text{NH}_4^+$  indicates that nitrification is limited by  $\text{NH}_4^+$  supply in many arable soils and that the rate of nitrification will be overestimated when a  $\text{NH}_4^+$  substrate is added to soil
- The addition of approximately 1.5 mg  $^{15}\text{N}$  kg $^{-1}$  soil as  $\text{NO}_3^-$  at approximately 60 atom% is sufficient to create a labelled nitrate pool for the measurement of gross nitrification. Lower enrichments of  $^{15}\text{NO}_3^-$  to 10 atom% are also likely to be sufficient.

#### 4.2 N pools in soil and net mineralisation rates

At all sites the  $\text{NH}_4^+$  pool was always small (0.1 – 2.0 mg kg $^{-1}$ ), except where slurry (LINK site 9, Spring 99, 18.0 mg  $\text{NH}_4^+\text{-N}$  kg $^{-1}$  soil) or ammonium nitrate fertiliser had recently been applied (LINK site 20, Spring 99, 26.7 mg  $\text{NH}_4^+\text{-N}$  kg $^{-1}$  soil). At some sites the  $\text{NH}_4^+$  pool was slightly increased at Harvest, possibly due to some inhibition of nitrification in dry soils. The  $\text{NO}_3^-$  pool was significantly different between sites and samplings and there was significantly more  $\text{NO}_3^-$  in the field soil than in the zero plots at harvest, but this only amounted to an increase of 3 kg N ha $^{-1}$  on average. The mineral N pool and PAN on Broadbalk showed the very clear differences between plots, which might be expected from their long-term history (e.g. Table 5). PAN values from the LINK sites range from 7 to 161 mg N kg $^{-1}$ , and are significantly lower on average at harvest than in spring.



**Table 5** Soil N pools and mineralisation rates for Broadbalk. Data collected from samples taken in Autumn 1999, as an example. Means of three replicates with standard errors in brackets.

| Plot (N applied)  | 2.2 (FYM)    | 3 (0)        | 8 (144)      | 16 (288)     |
|---|--------------|--------------|--------------|--------------|
| NH <sub>4</sub> <sup>+</sup> (mg N kg <sup>-1</sup> )                   | 1.8 (0.85)   | 3.2 (1.22)   | 1.9 (0.55)   | 0.7 (0.04)   |
| NO <sub>3</sub> <sup>-</sup> (mg N kg <sup>-1</sup> )                   | 12.2 (2.23)  | 2.8 (0.15)   | 5.2 (1.41)   | 9.6 (1.28)   |
| PAN (mg N kg <sup>-1</sup> )  | 51.2 (2.42)  | 3.5 (1.11)   | 17.3 (0.36)  | 28.3 (5.08)  |
| Net mineralisation rate<br>(mg N kg <sup>-1</sup> day <sup>-1</sup> )   | 1.11 (0.561) | 0.06 (0.391) | 0.38 (0.056) | 0.43 (0.135) |
| Gross mineralisation rate<br>(mg N kg <sup>-1</sup> day <sup>-1</sup> ) | 0.89 (0.021) | 0.08 (0.019) | 0.23 (0.025) | 0.39 (0.024) |

During the incubations, the change in the soil mineral N pool can be used to estimate net mineralisation rates. Data from the incubation with <sup>15</sup>NO<sub>3</sub><sup>-</sup> added were used, because the addition of <sup>15</sup>N resulted in the smallest proportional change to the pool and this incubation ran for the longest period. Data for the LINK sites showed both net mineralisation and net immobilisation during the incubations ranging from -3.2 to 3.9 mg N kg<sup>-1</sup> day<sup>-1</sup>, with a mean of 0.28 mg N kg<sup>-1</sup> day<sup>-1</sup>. There was no significant difference in the net mineralisation rate between sites or samplings; the measurement had high variability (e.g. Table 5) and this masked differences. In the presence of C<sub>2</sub>H<sub>2</sub>, the increase in the size of the NH<sub>4</sub><sup>+</sup> pool can also be used to give an independent estimate of net mineralisation, which is not affected by nitrate consumption processes. The range of net mineralisation rates calculated in this way for the LINK sites was similar (-2.3 to 0.8 mg N kg<sup>-1</sup> day<sup>-1</sup>, mean 0.15 mg N kg<sup>-1</sup> day<sup>-1</sup>). However, the net mineralisation rates calculated in the presence of C<sub>2</sub>H<sub>2</sub> were significantly lower than those calculated where <sup>15</sup>NO<sub>3</sub><sup>-</sup> was added. The addition of C<sub>2</sub>H<sub>2</sub> might provide a carbon source and increase microbial activity. These results confirm the findings of Terry and Duxbury (1985) that C<sub>2</sub>H<sub>2</sub> does not provide an additional source of carbon in incubations of less than 7 days.

These data show that:

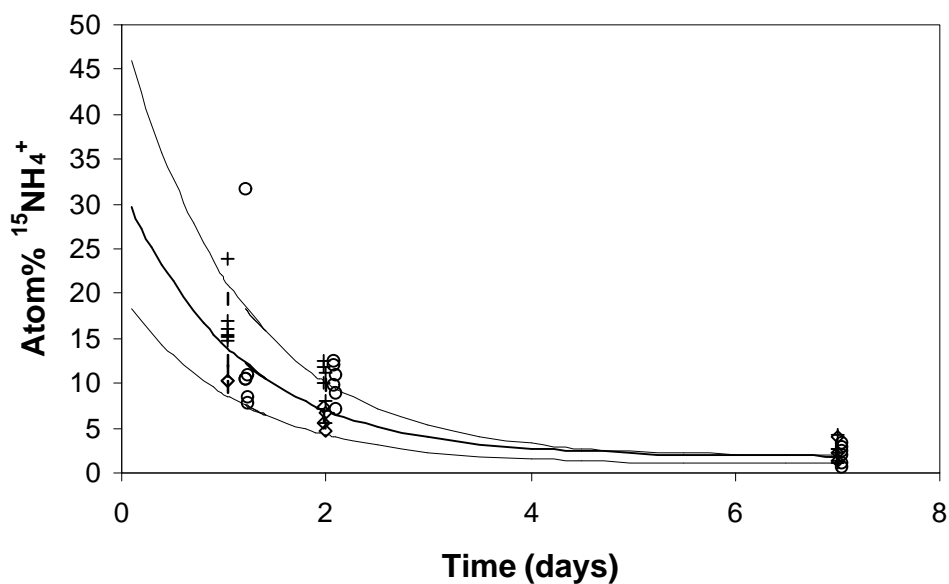
- As expected, arable soils maintain only a very small NH<sub>4</sub><sup>+</sup> pool under normal conditions.
- Normal farm practice in the UK does not leave large residues of mineral N in the soil at harvest.
- Long-term stable N management practices lead to changes in the soil N cycle that can be detected by simple measurements of N pools and processes. However, small and short-term changes in management are more difficult to distinguish using such measurements.
- On average, arable soils have a net mineralisation rate of c. 0.3 mg N kg<sup>-1</sup> day<sup>-1</sup> (~1.2 kg N ha<sup>-1</sup> day<sup>-1</sup>) during the growing season.

#### 4.3 Dilution of labelled pools during the incubation

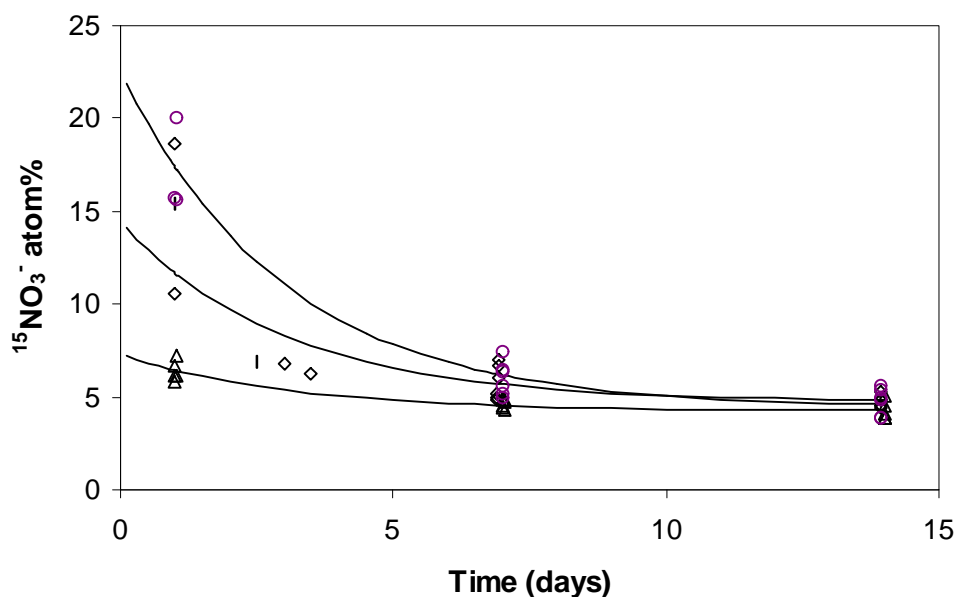
In presence of C<sub>2</sub>H<sub>2</sub>, the decline in <sup>15</sup>NH<sub>4</sub><sup>+</sup> atom% during the incubation after the equilibration period (1 to 7 days) can be fitted by an exponential decay curve ( $y = a + (b * r^x)$ ) e.g. Figure 2). Similarly the decline in the <sup>15</sup>NO<sub>3</sub><sup>-</sup> atom%, where either <sup>15</sup>NO<sub>3</sub><sup>-</sup> was added (1 to 14 days) or where <sup>15</sup>NO<sub>3</sub><sup>-</sup> results from the addition of <sup>15</sup>NH<sub>4</sub><sup>+</sup> in the absence of C<sub>2</sub>H<sub>2</sub> can also be fitted by exponential decay curves (e.g. Figure 3).

For each sampling a family of exponential decay curves was fitted with a single exponential parameter ( $r$ ). Values of  $r$  were significantly larger for the decline in <sup>15</sup>NO<sub>3</sub><sup>-</sup> atom% (0.7161 – 0.9632) than for the decline in <sup>15</sup>NH<sub>4</sub><sup>+</sup> atom% (0.4111 - 0.6121), indicating that the decline in <sup>15</sup>NO<sub>3</sub><sup>-</sup> atom% was less rapid, as expected. Where <sup>15</sup>NO<sub>3</sub><sup>-</sup> was added, the atom% <sup>15</sup>NO<sub>3</sub><sup>-</sup> declined most rapidly at Harvest 98 and where <sup>15</sup>NO<sub>3</sub><sup>-</sup> resulted from the addition of <sup>15</sup>NH<sub>4</sub><sup>+</sup> in the absence of C<sub>2</sub>H<sub>2</sub>, the atom% <sup>15</sup>NO<sub>3</sub><sup>-</sup> declined more rapidly than when <sup>15</sup>NO<sub>3</sub><sup>-</sup> was added. Even in the presence of C<sub>2</sub>H<sub>2</sub>, the <sup>15</sup>NH<sub>4</sub><sup>+</sup> atom% declined rapidly and an intermediate sampling time 2 or 3 days after <sup>15</sup>N addition was introduced after Spring 98. The atom% enrichment of the NH<sub>4</sub><sup>+</sup> pool was not significantly greater than natural abundance after 7 days incubation in the presence of C<sub>2</sub>H<sub>2</sub> and there was no indication of an increase in the amount of labelled NH<sub>4</sub><sup>+</sup> or the atom% enrichment of the NH<sub>4</sub><sup>+</sup> pool due to remineralisation of immobilised <sup>15</sup>N during the incubations. The atom% <sup>15</sup>NH<sub>4</sub><sup>+</sup> declined more rapidly in Spring than at Harvest samplings. There was no significant difference between the shape of the exponential decay curves fitted to the field and zero plots at harvest. However, where <sup>15</sup>NO<sub>3</sub><sup>-</sup> was added to soils the intercept of the curves was lower for the field than the zero plots, as the higher initial nitrate pool diluted the <sup>15</sup>NO<sub>3</sub><sup>-</sup> addition, reducing the initial <sup>15</sup>NO<sub>3</sub><sup>-</sup> atom%.

There were significant correlations between the linear parameters (a and b) fitted to each site at different sampling times, but the interaction between site and sampling time was also significant, indicating that the decline of atom% enrichment at any site was dependent on both soil type and management/season.



**Figure 2** Data points for 3 sites (6b, 9, 11) and fitted curves ( $r^2 = 0.885$  for all site curves fitted) for exponential decline in  $^{15}\text{NH}_4^+$  atom% for soils taken from the LINK sites at Harvest 1999. Exponential parameter ( $r$ ) is 0.6121 for all fitted curves.



**Figure 3** Data points for 3 sites (9,11,14) and fitted curves ( $r^2 = 0.714$  for all site curves fitted) for exponential decline in  $^{15}\text{NO}_3^-$  atom% for soils taken from the LINK sites at Harvest 1998. Exponential parameter ( $r$ ) is 0.7161 for all fitted curves.

These data show that :

- Isotope dilution does occur in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools when they are labelled with  $^{15}\text{N}$ .
- The decline of atom% enrichment of either  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$  can be described by exponential decay curves.
- The shape of the decay curve is affected by both site and sampling time.
- Since exponential decay curves fit the data well, simple analytical solutions can be used to estimate gross rates of mineralisation and nitrification.

#### 4.4 Gross rates of N turnover processes

Gross mineralisation rates were calculated from the decline in atom% enrichment of the  $^{15}\text{NH}_4^+$  pool between Day 1 and Day 2 or 3 of the incubation. Rapid depletion of the  $^{15}\text{NH}_4^+$  pool during the first 24 hours where  $^{15}\text{NH}_4^+$  was added without  $\text{C}_2\text{H}_2$  meant that there was insufficient  $^{15}\text{NH}_4^+$  remaining to accurately measure any dilution in these incubations. Measured rates of gross mineralisation in the presence of  $\text{C}_2\text{H}_2$  ranged from 0 to  $5.88 \text{ mg N kg}^{-1} \text{ day}^{-1}$  (Table 6). This is a wider range than has previously been measured in arable soils (Recous and Mary, 1988; Murphy *et al*, 1998; Watkins and Barraclough, 1996), but the quartiles of the distribution for all sites and samplings ( $0.56$  to  $1.66 \text{ mg N kg}^{-1} \text{ day}^{-1}$ ) did reflect the range of values for gross mineralisation typically measured. Gross mineralisation was significantly higher in Spring 99 than at Harvest 98 or 99. While there were significant differences between sites there was no clear pattern with soil texture or management. A significant interaction between site and sampling indicates that soil and management factors interact to control the rate of gross mineralisation. For example, soil compaction during the drilling of winter wheat at site 17 in the wet autumn of 1998, seems to have markedly increased gross rates of mineralisation, but not net rates of mineralisation. De Neve and Hofman (2000) also found that net mineralisation rate were not affected by compaction. Three sites (11,12,13) on the same soil series with similar rotations, but at contrasting points in the rotation during the sampling period, did not have significantly different rates of gross mineralisation, despite the use of minimal cultivations at one site (12) in contrast to ploughing at the other sites

Gross mineralisation rates on Broadbalk (Table 5) showed significant differences between plots, in line with their total organic matter content and measurements of mineral N, PAN and net mineralisation. On the LIFE experiment (Harvest 99) Plot C had a significantly lower rate of gross mineralisation than either Plot A or F, which were not significantly different (Table 7). This paralleled small, but not significant, differences in soil mineral N, PAN and net mineralisation between the plots.

$\text{CO}_2$  production during the incubations was greatest in Spring 98, but variations between sampling dates may reflect different storage times of samples before  $\text{CO}_2$  analysis, as much as any seasonal or management effects. There were significant differences between sites and  $\text{CO}_2$  production was significantly correlated with gross mineralisation rates (e.g.  $r = 0.617$  for Harvest 98).  $\text{CO}_2$  production was significantly different between the Broadbalk plots in line with their total organic matter content and measurements of mineral N, PAN and net mineralisation. However,  $\text{CO}_2$  production was not significantly different between the LIFE plots.

Gross nitrification rates were calculated from the decline in  $^{15}\text{NO}_3^-$  atom% following the addition of  $^{15}\text{NO}_3^-$  between Day 1 and Day 7 of the incubations. Where gross nitrification rates were calculated from the decline in  $^{15}\text{NO}_3^-$  atom% following the addition of  $^{15}\text{NH}_4^+$  without  $\text{C}_2\text{H}_2$ , these were not significantly different. Gross nitrification rates ranged from 0 to  $9.47 \text{ mg N kg}^{-1} \text{ day}^{-1}$  (Table 6). Two very high rates of nitrification were measured in Spring 99 for Sites 9 and 19; these were associated with the addition of  $\text{NH}_4^+$  in slurry and as ammonium nitrate, respectively, before the soils were sampled. Following the addition of fertiliser on the Broadbalk experiment, very high rates of nitrification were also measured,  $8 \text{ mg N kg}^{-1} \text{ day}^{-1}$  (Mendum, 2000), compared to  $0.21 \text{ mg N kg}^{-1} \text{ day}^{-1}$  in the same plot before fertiliser addition. The nitrifying population is able to respond very rapidly to any addition of  $\text{NH}_4^+$ . Again the quartiles of the distribution for all sites and samplings ( $0.05 - 1.5 \text{ mg N kg}^{-1} \text{ day}^{-1}$ ) did reflect the range of values for nitrification commonly measured in arable soils with no recent  $\text{NH}_4^+$  addition (Willison *et al*, 1998). When the high rates (Spring 99, Sites 9 and 19) are excluded, there were no significant differences in gross nitrification rates between sites. Gross nitrification rates measured at Harvest 98 are significantly, but only slightly greater than those measured at the other sampling times.

**Table 6** Gross mineralisation rates ( $\text{mg N kg}^{-1} \text{ day}^{-1}$ , M) and gross nitrification rates ( $\text{mg N kg}^{-1} \text{ day}^{-1}$ , N) for LINK sites in Spring and at Harvest in 1998 and 1999. Gross mineralisation rates could not be calculated for Spring 98, other missing values indicated by n/a. Means of three replicates (SE in brackets). Although negative values are indicated, these are never significantly different from zero. Negative values for gross rates are not possible.

| Site | Spring 98       |                 | Harvest 98       |                 |                 | Spring 99       |                 | Harvest 99       |                  |                  | Zero plot        |  |
|------|-----------------|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|------------------|------------------|------------------|------------------|--|
|      | N               | M               | Field<br>N       | Zero plot<br>M  | Zero plot<br>N  | M               | N               | Field<br>M       | Field<br>N       | Zero plot<br>M   | Zero plot<br>N   |  |
| 4    | 0.04<br>(0.019) | 0.91<br>(0.112) | 0.97<br>(0.148)  | 1.09<br>(0.071) | 1.20<br>(0.011) | 1.28<br>(0.097) | 0.37<br>(0.253) | 0.88<br>(0.024)  | 0.45<br>(0.033)  | 0.83<br>(0.202)  | 0.49<br>(0.268)  |  |
| 5    | 1.10<br>(0.235) | 0.40<br>(0.052) | 0.93<br>(0.041)  | 0.71<br>(0.031) | 0.90<br>(0.051) | 0.76<br>(0.101) | 0.36<br>(0.107) | 0.35<br>(0.097)  | 0.42<br>(0.065)  | -0.12<br>(0.180) | 0.32<br>(0.329)  |  |
| 6a   | 0.52<br>(0.053) | 0.40<br>(0.051) | 0.80<br>(0.079)  | 0.42<br>(0.007) | 0.76<br>(0.210) |                 |                 |                  |                  |                  |                  |  |
| 6b   |                 |                 |                  |                 |                 | 1.05<br>(0.082) | 0.77<br>(0.136) | 0.49<br>(0.089)  | 1.78<br>(1.064)  | 1.46<br>(0.346)  | 0.13<br>(0.422)  |  |
| 8    | 0.50<br>(0.003) | 0.90<br>(0.088) | 0.53<br>(0.492)  | 1.02<br>(0.347) | 1.08<br>(0.142) | 0.80<br>(0.051) | 0.18<br>(0.040) | -0.29<br>(0.092) | 0.10<br>(0.087)  | -0.48<br>(0.125) | 0.18<br>(0.106)  |  |
| 9    | 0.97<br>(0.151) | 1.18<br>(0.209) | 0.80<br>(0.104)  | 1.07<br>(0.098) | 0.82<br>(0.029) | 1.50<br>(0.208) | 2.95<br>(0.518) | 1.24<br>(0.095)  | 0.97<br>(0.394)  | 1.35<br>(0.848)  | 0.68<br>(0.256)  |  |
| 10   | 0.79<br>(0.063) | 0.93<br>(0.089) | 3.06<br>(2.473)  | 0.93<br>(0.048) | 1.00<br>(0.114) | 0.76<br>(0.102) | 0.02<br>(0.153) | 1.18<br>(0.302)  | 0.51<br>(0.023)  | 1.73<br>(0.323)  | 0.59<br>(0.069)  |  |
| 11   | 0.45<br>(0.182) | 0.89<br>(0.178) | n/a              | 1.12<br>(0.087) | 1.43<br>(0.255) | 1.05<br>(0.290) | 0.02<br>(0.088) | 0.01<br>(0.053)  | 0.69<br>(2.245)  | 0.18<br>(0.235)  | 0.18<br>(0.073)  |  |
| 12   | 1.24<br>(0.164) | 1.09<br>(0.412) | 1.15<br>(0.224)  | 0.71<br>(0.025) | 1.06<br>(0.393) | 0.88<br>(0.084) | 0.56<br>(0.187) | 0.21<br>(0.199)  | -0.03<br>(0.083) | 0.28<br>(0.092)  | -0.35<br>(0.120) |  |
| 13   | 0.57<br>(0.057) | 0.63<br>(0.049) | 1.09<br>(0.171)  | 0.96<br>(0.524) | 0.88<br>(0.103) | 0.96<br>(0.030) | 0.56<br>(0.056) | 0.64<br>(0.123)  | -0.21<br>(0.975) | 0.41<br>(0.281)  | -0.04<br>(0.395) |  |
| 14   | 0.47<br>(0.064) | 0.54<br>(0.047) | 0.84<br>(0.025)  | 0.76<br>(0.188) | 1.03<br>(0.031) | 0.56<br>(0.131) | 0.17<br>(0.073) | 1.78<br>(0.933)  | 0.48<br>(0.015)  | -0.14<br>(0.145) | 0.39<br>(0.152)  |  |
| 15   | 0.87<br>(0.081) | n/a             | n/a              | n/a             | n/a             | 0.95<br>(0.196) | 0.64<br>(0.597) | n/a              | n/a              | n/a              | n/a              |  |
| 16   | 0.49<br>(0.056) | 0.14<br>(0.050) | -0.02<br>(0.105) | 0.60<br>(0.202) | 0.66<br>(0.057) |                 |                 |                  |                  |                  |                  |  |
| 17   | 1.52<br>(0.087) | 1.05<br>(0.119) | n/a              | 0.51<br>(0.087) | 1.76<br>(0.170) | 3.47<br>(1.818) | 0.29<br>(0.021) | 2.75<br>(0.473)  | 0.21<br>(0.047)  | 5.88<br>(1.409)  | 0.13<br>(0.105)  |  |
| 18   | 0.14<br>(0.215) | 0.47<br>(0.017) | 0.91<br>(0.072)  | 0.54<br>(0.081) | 0.73<br>(0.010) | 0.57<br>(0.170) | 0.19<br>(0.107) |                  |                  |                  |                  |  |
| 19   | 0.39<br>(0.337) | 1.27<br>(0.062) | 0.72<br>(0.043)  | 1.09<br>(0.243) | 1.02<br>(0.055) | 1.35<br>(0.267) | 9.47<br>(2.113) | 0.91<br>(0.238)  | 0.82<br>(0.242)  | 1.28<br>(0.023)  | 0.91<br>(0.276)  |  |

**Table 7** Soil N pools and gross N transformation rates for the LIFE experiment sampled in Autumn 1999. Means of four replicates with standard errors in brackets.

| Plot  | A            | C            | F            |
|---|--------------|--------------|--------------|
| $\text{NH}_4^+$ ( $\text{mg N kg}^{-1}$ )                               | 0.81 (0.10)  | 0.61 (0.29)  | 0.08 (0.07)  |
| $\text{NO}_3^-$ ( $\text{mg N kg}^{-1}$ )                               | 7.22 (1.47)  | 3.28 (0.25)  | 4.85 (0.26)  |
| PAN ( $\text{mg N kg}^{-1}$ )   | 69.58 (5.70) | 66.48 (5.32) | 68.83 (2.26) |
| Net mineralisation rate<br>( $\text{mg N kg}^{-1} \text{ day}^{-1}$ )   | 0.14 (0.12)  | 0.08 (0.09)  | 0.19 (0.05)  |
| Gross mineralisation rate<br>( $\text{mg N kg}^{-1} \text{ day}^{-1}$ ) | 1.27 (0.32)  | 0.34 (0.20)  | 1.23 (0.82)  |
| Gross nitrification rate<br>( $\text{mg N kg}^{-1} \text{ day}^{-1}$ )  | 0.43 (0.18)  | 0.25 (0.08)  | 0.43 (0.10)  |
| N:I index   | 0.38         | 0.89         | 0.40         |

The consumption rates of  $\text{NH}_4^+$  calculated from the dilution in atom% enrichment of the  $^{15}\text{NH}_4^+$  pool in the presence of  $\text{C}_2\text{H}_2$  should approximate to the gross immobilisation of  $\text{NH}_4^+$ . However, the inhibition of nitrification results in an elevated pool size of  $\text{NH}_4^+$  that might stimulate immobilisation. It has been argued that, since immobilisation is driven by C availability, the presence of additional  $\text{NH}_4^+$  is not likely to regulate microbial immobilisation (Chalk *et al*, 1990). A separate estimate of gross immobilisation was calculated by difference from the rates of gross mineralisation and the net rate of mineralisation where  $^{15}\text{NO}_3^-$  was added. The two estimates are not significantly different, which indicates that the consumption rate of  $\text{NH}_4^+$  in the presence of  $\text{C}_2\text{H}_2$  can be used to estimate gross immobilisation rates. However, estimates of immobilisation by either method will result in high degrees of error due to the combination of measurement errors with relatively large multiplication factors (Tietema and Wessel, 1992). Gross immobilisation ranged from 0.09 to 5.2 mg N  $\text{kg}^{-1} \text{day}^{-1}$  and was highly significantly correlated with gross mineralisation ( $r = 0.85$ ), so that high rates of gross mineralisation tended to be matched by high rates of gross immobilisation, e.g. at Site 17 in 1999. The pseudo-residence time (Tietema and Wessel, 1992) for  $\text{NH}_4^+$  in all the soils is less than 1 day, except where  $\text{NH}_4^+$  has been added in slurry or fertiliser.

These data show that :

- Interactions between soil and management factors control the measured rates of gross mineralisation
- On Broadbalk the measured rates of gross mineralisation are highly correlated with the soil organic matter content and other measurements of N pools and processes (Section 4.2).
- On the LINK sites there are significant differences in the rates of gross mineralisation between sites and samplings, but there was no simple pattern with soil texture or management.
- Elevated rates of gross nitrification were measured, where soils had recently received additions of  $\text{NH}_4^+$ .
- The background rates of nitrification were not significantly different between sites. Nitrification is controlled by  $\text{NH}_4^+$  supply in arable soils.
- The  $\text{NH}_4^+$  consumption rates calculated from the dilution of  $^{15}\text{NH}_4^+$  atom% in the presence of  $\text{C}_2\text{H}_2$  can be used to estimate immobilisation, but such estimates are associated with large errors.
- Gross immobilisation and gross mineralisation rates are very highly correlated, indicating that the internal N cycle of arable soils is relatively conservative.

#### 4.5 Indices of soil N turnover and relationship to crop uptake and N losses

Nitrification to Immobilisation ratios (N:I) were calculated for each site and sampling. There were no significant differences between sites, with an average index of 1.42 across all the LINK sites and samplings, reflecting a relatively conservative cycling of N in these arable soils. However, there were significant differences between samplings and for the LINK sites there was a significant interaction between site and sampling. The N:I ratio was increased at harvest compared to spring, and there was some indication that the ratio was lower in the zero plots than in the field. In spring it seems that the soil internal N cycle is more conservative than at harvest, i.e. N is tightly held in the system and less prone to loss. This may reflect a priming of the nitrifying population due to fertiliser addition during the growing season, which does not occur where no additional N is supplied (most zero plots).

There was a significant linear correlation between the N:I ratio and the simulated leaching loss overwinter ( $r^2 = 0.1493$ , Table 8); none of the other measurements correlated significantly with the simulated leaching loss. However, Site 8 was a clear outlier from the relationship. From these data it is not clear whether this represents a failure of the model simulation or a true breakdown in the relationship between the N:I ratio and potential N loss. Comparison of the N:I ratio with leaching losses on Broadbalk shows a strong correlation. However, this cannot be used to prove that the N:I ratio can be used to indicate leaching loss, as all measurements of N pools and processes correlate strongly.

Where N:I was calculated for the spring sampling, both N:I and soil mineral N correlated significantly with the N uptake on the zero plots of the N response experiments ( $r^2 = 0.2875$  and  $0.3349$  respectively), despite the range of crops covered by the trials (Table 2). N:I and soil mineral N were very highly significantly correlated for spring sampling ( $r^2 = 0.895$ ) but this relationship was dominated by a group of points at low N:I and low mineral N (c. 10 mg N  $\text{kg}^{-1}$ ) and three points with much higher mineral N and N:I (Sites 15, 19 and 9; Table 9). When data for the sites which have received additional mineral N (9 and 19) are removed there is a strong correlation between PAN and mineral N for the spring sampling ( $r^2 = 0.8143$ ). Mineral N measured in spring represents the accumulation of mineral N in the soil after the cessation of drainage and as a result is not just the measurement of a particular pool at a particular time (snapshot measurement, Stockdale *et al* 1997) but also relates to the N supply potential of the site.

**Table 8** Soil mineral N, PAN and N:I ratio for LINK sites for Harvest 98 and simulated N losses overwinter 1998-99.

| Site |       | Mineral N<br>(mg kg <sup>-1</sup> ) | PAN<br>(mg kg <sup>-1</sup> ) | Net mineralisation<br>rate (mg N kg <sup>-1</sup> day <sup>-1</sup> ) | N:I ratio | Simulated overwinter<br>losses (kg N ha <sup>-1</sup> ) |
|------|-------|-------------------------------------|-------------------------------|---|-----------|---|
| 4    | Field | 8.3 (0.59)                          | 14.9 (0.93)                   | 0.3 (0.03)  | 1.6       | 16  |
|      | Zero  | 8.0 (0.34)                          | 14.4 (1.30)                   | 0.4 (0.11)  | 1.7       | 18  |
| 5    | Field | 10.9 (1.24)                         | 37.3 (0.34)                   | 0.3 (0.03)  | 6.2       | 75  |
|      | Zero  | 6.0 (0.09)                          | 22.5 (3.48)                   | 0.3 (0.24)  | 1.5       | 15  |
| 6a   | Field | 3.4 (0.44)                          | 15.4 (3.25)                   | 0.5 (0.34)  | 6.2       | 61  |
|      | Zero  | 4.3 (0.85)                          | 12.9 (2.12)                   | 0.3 (0.29)  | 3.5       | 35  |
| 8    | Field | 9.4 (1.74)                          | 23.0 (6.35)                   | 0.7 (0.39)  | 1.4       | 113   |
|      | Zero  | 8.8 (1.92)                          | 13.5 (1.49)                   | 0.3 (0.03)  | 1.8       | 79  |
| 9    | Field | 5.0 (0.25)                          | 33.6 (3.61)                   | 0.6 (0.03)  | 1.0       | 17  |
|      | Zero  | 5.5 (0.48)                          | 47.9 (18.8)                   | 0.4 (0.12)  | 0.9       | 17  |
| 10   | Field | 8.2 (1.06)                          | 19.8 (3.67)                   | 0.2 (0.07)  | 3.6       | 66  |
|      | Zero  | 2.3 (0.07)                          | 22.9 (6.65)                   | 0.5 (0.44)  | 1.6       | 71  |
| 11   | Field | 6.1 (0.14)                          | 11.1 (2.39)                   | 0.2 (0.02)  | n/a       | 124   |
|      | Zero  | 4.7 (0.22)                          | 12.1 (0.75)                   | 0.1 (0.07)  | 1.4       | 15  |
| 12   | Field | 16.8 (1.71)                         | 21.9 (2.91)                   | 0.1 (0.10)  | 1.3       | 77  |
|      | Zero  | 20.6 (1.14)                         | 14.1 (0.93)                   | 0.03 (0.17)   | 1.5       | 15  |
| 13   | Field | 5.2 (0.69)                          | 15.1 (3.52)                   | 0.5 (0.04)  | 2.0       | 22  |
|      | Zero  | 4.1 (0.31)                          | 7.8 (2.50)                    | 0.03 (0.02)   | 0.9       | 21  |
| 14   | Field | 9.0 (0.58)                          | 4.4 (0.24)                    | 0.1 (0.02)  | 1.8       | 43  |
|      | Zero  | 6.1 (0.37)                          | 7.5 (1.04)                    | 0.1 (0.02)  | 1.3       | 39  |
| 16   | Field | 3.2 (0.17)                          | 14.8 (6.79)                   | 0.2 (0.03)  | 0.2       | 22  |
|      | Zero  | 3.2 (0.24)                          | 2.3 (0.34)                    | 0.3 (0.02)  | 1.2       | 22  |
| 17   | Field | 7.3 (0.58)                          | 17.4 (1.26)                   | 0.2 (0.01)  | n/a       | 57  |
|      | Zero  | 9.4 (0.39)                          | 19.2 (2.77)                   | 0.1 (0.17)  | 3.8       | 42  |
| 18   | Field | 1.6 (0.25)                          | 9.3 (0.12)                    | 0.1 (0.07)  | 2.2       | 24  |
|      | Zero  | 1.2 (0.16)                          | 10.0 (0.78)                   | 0.5 (0.14)  | 2.2       | 24  |
| 19   | Field | 4.4 (0.34)                          | 16.0 (0.48)                   | 0.2 (0.05)  | 0.7       | 24  |
|      | Zero  | 4.9 (0.63)                          | 20.6 (5.63)                   | 0.3 (0.01)  | 1.0       | 24  |

**Table 9** Soil mineral N, PAN, net mineralisation rate and N:I ratio for LINK Sites sampled in Spring 99 and the N uptake by unfertilised crops.

| Site | Mineral N<br>(mg kg <sup>-1</sup> ) | PAN<br>(mg kg <sup>-1</sup> ) | Net mineralisation rate<br>(mg N kg <sup>-1</sup> day <sup>-1</sup> ) | N:I ratio | N uptake on zero<br>plots (kg N ha <sup>-1</sup> ) |
|------|-------------------------------------|-------------------------------|---|-----------|--|
| 4    | 8.0 (0.69)                          | 23.0 (0.92)                   | 0.1 (0.32)  | 0.4       | 105.4  |
| 5    | 8.1 (1.91)                          | 25.6 (3.13)                   | 0.5 (0.12)  | 0.5       | 51.4   |
| 6b   | 5.4 (0.63)                          | 6.3 (0.99)                    | 0.4 (0.08)  | 1.0       | 63.7   |
| 8    | 7.8 (0.35)                          | n/a                           | 0.02 (0.03)   | 0.2       | 85.9   |
| 9    | 62.5 (9.81)                         | 17.2 (6.59)                   | 0.5 (0.12)  | 3.2       | 156.4  |
| 10   | 7.3 (0.32)                          | 16.8 (3.56)                   | 0.7 (0.07)  | 0.1       | 157.4  |
| 11   | 8.6 (0.49)                          | 17.5 (3.11)                   | -0.2 (0.01)   | 0.02      | 68.1   |
| 12   | 6.9 (0.43)                          | 8.5 (2.08)                    | 0.4 (0.12)  | 0.6       | 53.8   |
| 13   | 8.7 (0.70)                          | n/a                           | -0.1 (0.07)   | 0.6       | 43.1   |
| 14   | 8.4 (0.67)                          | 16.6 (1.00)                   | 0.2 (0.04)  | 0.4       | 153.7  |
| 15   | 22.5 (1.75)                         | 22.0 (12.3)                   | 0.4 (0.07)  | 1.2       | 208.7  |
| 17   | 11.0 (2.23)                         | 37.0 (2.32)                   | 0.4 (0.05)  | 0.1       | 48.4   |
| 19   | 50.0 (13.29)                        | 19.9 (11.9)                   | -0.5 (0.89)   | 5.1       | 183.5  |

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These data show that :

- The N:I ratio is greater at harvest than in spring. The soil internal N cycle is less conservative at harvest, possibly due to a priming effect of  $\text{NH}_4^+$  addition on the nitrifier population in soil in spring.
- The N:I ratio at harvest is significantly correlated with subsequent overwinter N losses, but this relationship is not strong enough to be used for prediction. However, this relationship does show that it is appropriate to use this measurement as an indicator in arable, as well as forest, soils.
- The N:I ratio and soil mineral N in spring are significantly correlated with subsequent crop uptake in the absence of fertiliser. These relationships are not strong enough to use for prediction.
- There is a strong relationship between soil mineral N and N:I in spring indicating that the spring soil mineral N is not just a snapshot measurement of one N pool but also an indicator of potential N supply.

## 5.0 Implications

### 5.1 Reliability and limitations of results

Because of the difficulties of measurement and the high variability in the resulting data, isotope dilution methods, such as those used in this project, can yield only a semi-quantitative insight into the internal soil N cycle (Tietema and Wessel, 1992). As a result of the incubation methodology used in this study, the gross mineralisation and nitrification rates measured represent only the potential range of values likely to be encountered under field conditions. *In situ* measurements are much more difficult and subject to even greater variability. However, it is reassuring that the range of data presented here does include the range of values previously measured both in incubations and using *in situ* techniques. While there were no significant differences between sites or samplings in the rate of net mineralisation, isotope dilution measurements showed a significant difference in N dynamics between spring and harvest samplings. There was an increase in the dominance of nitrification over immobilisation at harvest, which has significant implications both for the fate of N following harvest (increased risk of loss, in addition to that caused by climatic factors) and for the cycling of N in systems which do not receive large additions of ammonium to stimulate the nitrifying population (some organic farming systems, and, where  $\text{NO}_3^-$  is used as the only fertiliser form). The data also indicates that soil damage through compaction in a wet autumn can significantly change soil N dynamics increasing the rate of mineralisation turnover in soil, but without affecting net mineralisation rates (Site 17).

The wide range of sites selected for study (LINK sites) clearly created a wide range of interacting soil, management and climatic factors, which, perhaps not surprisingly, could not be resolved into simple relationships between soil texture or the effect of a previous crop on soil dynamics. However, where the techniques were applied to plots managed in contrasting ways at the same site (Broadbalk, LIFE) isotope dilution techniques provided valuable information, supplementing that provided by simpler measurements of soil N pools and processes.

### 5.2 Implications

The N:I ratio does seem to provide a semi-quantitative measure of the capacity of a soil to produce nitrate relative to its capacity to immobilise N and hence a measure of the risk of N loss to the environment in arable soils. The N:I ratio is not a fixed characteristic of a soil but varies with season and management and it is sensitive to the disruption of the soil internal cycle by external inputs of  $\text{NH}_4^+$ . However, this measurement is complex and expensive and we can foresee no circumstances in which it might be used routinely to assess the potential for N loss or N supply for crops.

However, as a research tool to improve the understanding of the N cycle in soils, isotope dilution techniques have great value. Measurements of the gross processes of N cycling in soils are vital to improve our understanding of the vulnerability of the soil N cycle to disruption (N deposition, compaction, use of organic manures) and to improve the process-based description of N cycling in SUNDIAL and other models of the soil N cycle to refine the management requirements of a particular soil. In semi-natural systems, such indicators may be crucial to determine points of critical load from N deposition. However, in arable soils where nitrifier populations react rapidly to any addition of  $\text{NH}_4^+$ , the residence time of  $\text{NH}_4^+$  in soil is usually less than 1 day, and the N:I ratio seems limited only by  $\text{NH}_4^+$  supply.

The limited work carried out in this project also supports the value of measurements of spring mineral N to guide fertiliser recommendation. A more detailed consideration of this and other data will be presented in the report of the LINK project (CSA 3629).

## 6.0 Future opportunities

The isotope dilution techniques used and developed in this project should be applied to study the cycling of N in systems where nitrification is restricted (perhaps due to low pH) and to systems which do not receive large  $\text{NH}_4^+$  additions (some organic farming systems, and where  $\text{NO}_3^-$  is used as the only fertiliser). Ideally gross N transformations should be characterised as part of additional work at well-established experimental sites, so that relationships with soil texture and management can be drawn out clearly (particularly the influence of low impact tillage techniques and residue return and of soil compaction). Such work should be linked to an evaluation, and possible improvement, of the performance of process-based models of the soil N cycle e.g. SUNDIAL.

## 7.0 Actions resulting from the project

### Scientific papers

Willison, T. W., Baker, J. C., Murphy, D. V and Goulding K. W. T (1998) Comparison of a wet and dry  $^{15}\text{N}$  isotope dilution technique as a short-term nitrification assay. *Soil Biology and Biochemistry*, 30, 661-663 (attached as appendix)

Murphy, D., Dise, N., Goulding, K., MacDonald, J., Peake, C., Redfern, P. and Stockdale E. (2000) Can concepts of N saturation developed for forest systems be applied in arable soils? In: *Sustainable Management of Soil Organic Matter* CABI, in press. (attached as appendix)

Willison, T. W., Murphy D. V., Stockdale E. A. and Goulding K. W. T. (2000) Gross N transformations in soils under different land uses. *Journal of Environmental Quality*, submitted

### Conference presentations

Murphy, D. V., Willison, T. W., Baker, J. C. and Goulding, K. W. T. (1998) Assessing the potential for nitrogen loss in agricultural soils. 5<sup>th</sup> Congress of the European Society of Agronomy, Nitra, Slovak Republic.

Murphy, D. V., Willison, T. W., Baker, J. C. and Goulding, K. W. T. (1998) Gross nitrification to gross immobilisation ratios offer a means of assessing potential N loss from soils. 16<sup>th</sup> World Congress of Soil Science. Montpellier, France.

Murphy, D. V., Willison, T. W., Stockdale, E. A. and Goulding, K. W. T. (1998) Can we develop indices of N loss for agricultural soils? New Zealand Society of Soil Science, Gisborne.

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