Nitrogen mineralization in agricultural soils

Proceedings of a symposium held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993

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AB-DLO Thema's 1

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Proceedings of a symposium held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993

J.J. Neeteson and J. Hassink (eds.)

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DLO-Instituut voor Agrobiologisch en Bodemvruchtbaarheidsonderzoek
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Abstract

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The publication contains 28 papers presented at the symposium 'Nitrogen mineralization in agricultural soils', organized by the former DLO Institute for Soil Fertility Research, now DLO Research Institute for Agrobiology and Soil Fertility, in Haren, the Netherlands in spring 1993. The following topics in nitrogen mineralization in agro-ecosystems are discussed:

i) methods to predict nitrogen mineralization,
ii) measurement of nitrogen mineralization and immobilization, and microbial biomass, and
iii) modeling nitrogen mineralization and immobilization.

Key words: nitrogen mineralization, agricultural soils, nitrogen immobilization, microbial biomass, modeling

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Contents

Preface

Nitrogen, which is essential for crop growth, is taken up by plant roots from the soil solution mainly in the form of nitrate and ammonium ions. Soil nitrate and ammonium originate from mineralized soil organic matter, from inorganic and organic fertilizers, and to a much lesser extent from wet and dry atmospheric deposition. To be able to assess the amount of fertilizer to be applied it is necessary to account for the amount of nitrogen which will be mineralized during the growth period. This is even more important when food has to be produced cost-effectively without damage to the environment. Since nitrogen net mineralization, i.e. nitrogen mineralization minus nitrogen immobilization, may vary widely among fields, there is a strong need for methods to predict the quantitative contribution of the process to the availability of nitrogen for crops.

A symposium on "Nitrogen mineralization in agricultural soils" was organized by the former DLO Institute for Soil Fertility Research, IB-DLO (now DLO Research Institute for Agrobiology and Soil Fertility, AB-DLO) in Haren, the Netherlands in spring 1993. There were 110 participants from 16 different European countries.

The papers presented at the symposium are included in these proceedings.
The following topics of nitrogen mineralization in agro-ecosystems are discussed:
(i) methods to predict nitrogen mineralization,
(ii) measurement of nitrogen mineralization and immobilization, and microbial biomass, and
(iii) modeling nitrogen mineralization and immobilization.

The papers give an excellent review of the current state of nitrogen mineralization research. We hope that the information presented will contribute to a better understanding of the process of nitrogen mineralization/immobilization and that it ultimately will lead to reliable predictions of net nitrogen mineralization in agricultural fields.

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Active organic matter fractions and microbial biomass as predictors of N mineralization

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Abstract

It was tested whether light, intermediate and heavy macro-organic N (> 150 µm) fractions and active and total microbial biomass are good predictors of N mineralization when soils differing in organic matter input (grassland vs arable) and soil texture were compared. We found that the relative difference between arable and grassland soils decreases in the order light, intermediate and heavy macro-organic N, active microbial biomass, total microbial biomass and total soil organic N. The relative changes in heavy macro-organic N and active microbial biomass were similar to the relative change in N mineralization.

The percentage of soil N that mineralized in grassland soils was lower in fine-textured soils than in coarse-textured soils. The same difference was found when the amount of N that was mineralized per unit of microbial biomass was considered. It is assumed that this difference in mineralization is caused by the higher physical protection of organic matter and microorganisms in the fine-textured soils. However, both the size of the light macro-organic matter fraction and the active part of the microbial biomass correlated very well with the rate of N mineralization when grassland soils of different textures were compared. We assume that the relationship between the light fraction and the active microbial biomass and N mineralization was the same for fine- and coarse-textured soils, because the light fraction and the active microbial biomass are not physically protected in the soil.

The incorporation of the active fractions obtained by physical fractionation and the degree of physical protection of organic matter pools into organic matter models might contribute to the analysis of N mineralization in different agricultural soils.

INTRODUCTION

During the last two decades, many mathematical and simulation models have been constructed to describe the dynamics of soil organic matter (Van Veen and Kuikman, 1990). Generally, small pools with a high turnover rate and pools of greater size and slower turnover rate are distinguished (Cambardella and Elliott, 1992). However, physical or chemical isolation and determination in terms of decomposability of these pools has

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been difficult (Tiessen *et al.*, 1984). The fractions with high turnover (i.e. active organic matter fractions) and mineralization rates have been found to be more sensitive to differences in management (i.e. input of residues) than total organic C and N (Doran, 1980; Dalal and Mayer, 1987; Hassink *et al.*, 1991). The active fractions of the soil organic matter are assumed to play a prominent role in soil nutrient dynamics and various methods have been proposed to characterize the active fraction (Janzen *et al.*, 1992).

One approach is to quantify the microbial biomass. Several techniques, such as the fumigation-incubation (FI) and fumigation extraction (FE) methods (Jenkinson and Powlson, 1976; Brookes *et al.*, 1985) and the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978) have been developed to quantify microbial biomass. The microbial biomass represents only a small fraction of the total amount of soil organic matter, but it has a relatively rapid turnover. In some studies the size of the microbial biomass was found to be a good indication of the rate of N mineralization (Paul and Voroney, 1984; Azam *et al.*, 1986).

Another approach to measure the active fraction is the use of densiometric techniques to isolate density fractions of the macro-organic matter (Janzen *et al.*, 1992). It has been observed that organic C in the macro-organic matter fraction is much more labile than organic C in the clay and silt size fractions (Dalal and Mayer, 1986; Tiessen *et al.*, 1984). The so-called light fraction of the macro-organic matter is considered to be decomposing plant and animal residues with a relatively high C:N ratio and a rapid turnover (Christensen, 1992). The heavy fraction includes the organomineral complexed soil organic matter which consists of comparatively more decomposed products ("true humus") with a low C:N ratio, a slower turnover rate and a higher density due to its intimate association with soil minerals (Greenland and Ford, 1964). It has been found that the amount of light fraction is affected by the amount of litter production (Christensen, 1992). Obviously, the light fraction is relatively important in forests and permanent grasslands (Garwood *et al.*, 1972; Whitehead *et al.*, 1975). Density fractionation might become an important tool for predicting nutrient availability in low-input farming systems, which depend entirely on the recycling of residues.

The first objective of this paper is to test whether microbial biomass and macro-organic matter fractions are better indicators of N mineralization than total organic N, when soils which receive different amounts of organic materials (i.e. grassland and arable soils) are compared.

The last decade has shown an increasing awareness of the importance of soil texture and structure for organic matter and nitrogen turnover. Net mineralization of soil organic matter and decomposition of plant material was often observed to be more rapid in sandy soils than in clay soils (Catroux *et al.*, 1987; Hassink *et al.*, 1990; Ladd *et al.*, 1990). The lower net mineralization in clay soils may be partly caused by a greater physical protection of soil organic matter and microbial biomass (Van Veen *et al.*, 1985; Verberne *et al.*, 1990). Physical protection may be caused by adsortion of organics to surfaces of clays or coating of organics by clay particles (Tisdall and Oades, 1982) and entrapment of organics in small pores in aggregates inaccessible to microbes (Elliott and Coleman, 1988). The observation that disruption of soil structure increases N mineralization to a greater extent in clay soils than in loams, while in sandy soils no increase was observed at all (Hassink, 1992) supports the hypothesis that physical protection strongly affects the rate of N mineralization.

During the decomposition of plant residues in the soil, the extent of physical protection increases. The light fraction of the soil organic matter is not bound to the mineral particles and is therefore not yet protected by clay minerals (Janzen *et al.*, 1992; Young and Spycher, 1979). As the light fraction is an active pool of the soil organic matter

which is physically not more protected in fine-textured soils than in coarse-textured soils, it might give a good indication of N mineralization in different soils.

It has also been suggested that the active part of the microbial biomass (determined by the SIR method) is not physically protected in the soil. So, the active microbial biomass might correlate better with N mineralization than total microbial biomass. In agreement with this the amount of active microbial biomass was found to be a much better indicator of microbial activity than the amount of total microbial biomass (determined by the FI method; Hassink, 1993).

The second objective of this paper is to test whether the light fraction and the active microbial biomass correlate better with N mineralization than any other organic matter fraction when soils with different textures are compared.

MATERIALS AND METHODS

Soil sampling

In March, June, September and December 1992 samples were collected from a sandy and loamy grassland and a sandy and loamy arable field. Sets of grassland and arable fields were located in the same region and on soils with the same texture. The grassland soils were grazed by dairy cattle; the fields received 400-500 kg fertilizer-N per hectare per year and had been under grass for at least eight years. The arable soils had a crop rotation of winter wheat - sugar beet - spring barley - potatoes. In 1992 the crop was winter wheat for both soils.

In March 1991 samples were collected from five coarse-textured and six fine-textured grassland soils which had been under grass for at least eight years. The land was grazed by dairy cattle and received 400-500 kg fertilizer-N per year.

In 1992 the top 10 cm of the grassland and arable soils were sampled. In 1991 samples were taken from the 0-10 and 10-25 cm layer of each grassland location. In both years three mixed samples, each consisting of 20 bulked cores, were taken from each layer. The samples were sieved (< 8 mm); roots and stubble were removed and the samples were analyzed separately. Some characteristics of the grassland soils sampled in 1991 and the arable and grassland soils sampled in 1992 are given in Tables 1 and 2, respectively. The differences in pH and granular composition between both layers of the grassland soils (1991) were small so that only the values for the top 10 cm are given.

Determination of N mineralization rates

N mineralization was determined by measuring the increase in mineral N after incubation of soil samples in glass jars at 20 °C for 12 weeks. Drying of the samples was prevented by covering the jars with a polyethylene seal permeable to air, but impermeable to water. Mineral N was measured colorimetrically after extraction with 1N KCl solution for 1 h using a soil:water ratio of 1:2.5.

Table 1. Some characteristics of the 0-10 and 10-25 cm layer of the grassland sites sampled in 1991.

	C (%)		C/N		pH (K-Cl)	Granular comp., 0-10 cm layer, % particles <		
	0-10	10-25	0-10	10-25		2 µm	16 µm	50µm
Sand								
Cranend.	2.96	2.25	17.1	19.3	5.4	3.2	4.8	21.2
Dalfsen	3.88	5.49	20.8	40.5	5.1	1.9	3.1	9.8
Maarheeze	2.61	2.29	18.3	18.6	5.0	2.6	4.5	11.8
Drentse Aa	5.75	4.21	17.4	20.0	4.8	5.3	8.4	36.9
Tynaarlo	3.8	2.97	19.7	21.1	4.3	3.1	5.1	22.3
Loam								
Lelystad	3.15	1.44	11.1	12.1	7.2	21.7	34.9	65.8
Mijnsh.l.	2.20	1.40	9.6	9.1	7.2	20.1	33.5	79.9
Burum	5.31	2.25	10.5	9.1	4.8	27.6	41.3	82.0
Clay								
Haskerdijk	5.60	4.04	11.1	11.1	4.9	54.0	77.2	88.5
Finst.wolde	3.23	1.80	10.1	9.3	7.1	45.8	65.8	85.1
Zaltbommel	3.98	3.28	9.5	8.9	5.4	51.0	74.3	90.6

Table 2. Some characteristics of the 0-10 cm layer of the arable and grassland sites sampled in 1992.

	C (%)	C/N	pH (K-Cl)	Granular composition, % particles <		
				2 µm	16 µm	50µm
Sand						
arable	4.4	21.8	6.2	3.9 6.4	35.9	
grassland	5.9	19.7	4.2	2.9 4.9	39.4	
Loam						
arable	1.7	11.5	6.6	17.3	30.1	94.0
grassland	2.7	10.9	6.7	22.1	36.7	81.5

Determination of the microbial biomass

The amount of N in the microbial biomass was determined by the chloroform fumigation incubation technique (Jenkinson and Powlson, 1976). A *k* value of 0.4 was used to calculate the biomass N from the flush. The active microbial biomass was determined by the substrate induced respiration method (Anderson and Domsch, 1978). The exact procedures for both determinations have been described earlier (Hassink *et al.*, 1991; Hassink, 1993).

Determination of the light, intermediate and heavy macro-organic matter fractions

Washing of the soil samples. Dried sieved soil samples of 300 g were rewetted with tap water overnight and subsequently wet-sieved over a 250 μm and a 150 μm mesh sieve. The sample was placed on the top sieve (250 μm) and washed with tap water. The macroaggregates were destroyed by pushing the soil through the top sieve during the washing procedure until the water passing the sieve became clear. The material present on both sieves was washed into a bucket. The material in the bucket was swirled and the organic material was separated from the mineral material by decantation. The organic material was poured into a small tray with a 150 μm mesh sieve at the bottom and sides of 10 cm height. The mineral material was retained on the bottom of the bucket. Swirling and decantation was repeated several times until there were no more visible organic particles in the mineral fraction. The mineral fraction was discarded.

Density fractionation. The organic material was fractionated in Ludox TM. Ludox is an aqueous colloidal dispersion of silica particles produced by Du Pont. The tray containing the organic material was placed in Ludox with a density of 1.37 g cm^{-3}, and was stirred several times. The floating fraction was collected and placed in a similar tray that was placed in Ludox with a density of 1.13 g cm^{-3}. Mixing was repeated until the quantity of floating material became negligible. The organic material placed into the 1.13 g cm^{-3} Ludox was also separated into a floatable and a sinking fraction. Again, mixing was repeated several times until the quantity of floatable material became negligable. Finally, three fractions were obtained: a light fraction with a density < 1.13 g cm^{-3}; an intermediate fraction with a density between 1.13 and 1.37 g cm^{-3}, and a heavy fraction with a density > 1.37 g cm^{-3}. The three fractions were washed with tap water and dried. A more extensive description of the procedure and the characteristics of the Ludox is given by Meijboom *et al.* (1994).

Characterization of the light, intermediate and heavy fraction. The ash content of the fractions was defined as the fraction retained after heating oven-dry samples at 700 °C for 24 hours. The C content in the fraction was calculated as (100-ash content)/2. Total N was determined according to Deys (1961) by destruction with sulfuric acid and salycylic acid.

Statistical analysis

The relationships between N mineralization and soil organic N, N in microbial biomass, biomass-SIR and N in the light, intermediate and heavy fractions of the macro-organic matter were analyzed with correlation and linear regression analysis.

RESULTS

Characteristics of the light, intermediate and heavy fractions

The organic matter present in the light fraction consisted of recognizable plant residues. The intermediate fraction consisted partly of recognizable plant residues mixed with soil particles and partly undefined particles, while the heavy fraction consisted completely of

undefined organic material (Figure 1[A,B,C]). The ash content was low in the light fraction (18-35 %), higher in the intermediate fraction (35-45 %), and highest in the heavy fraction (60-87 %).

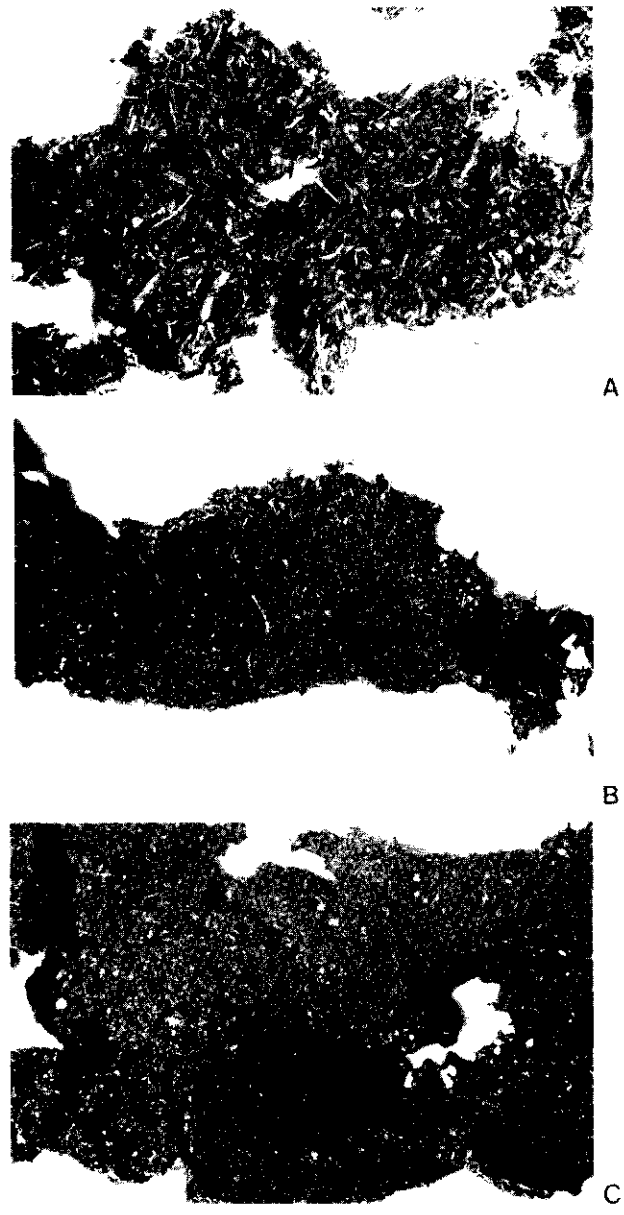

Figure 1. Light (A), intermediate (B) and heavy (C) fraction of the macro-organic matter in the top 10 cm of a sandy grassland soil.

In all soils, the C:N ratio of the fractions decreased in the order light, intermediate and heavy. In the light fraction the C:N ratio ranged between 18 and 24; in the intermediate fraction between 15 and 21 and in the heavy fraction between 13 and 16. There were no differences between the grassland and arable soils.

Comparison of N mineralization, microbial biomass, density fractions and total organic N in grassland and arable soils

To compare N mineralization rates in arable and grassland soils, the rate of N mineralization in the arable soil was divided by the rate of N mineralization in the corresponding grassland soil. For the other parameters (microbial biomass, active microbial biomass, light, intermediate and heavy macro-organic N and total organic N) the same ratios were calculated (Figure 2).

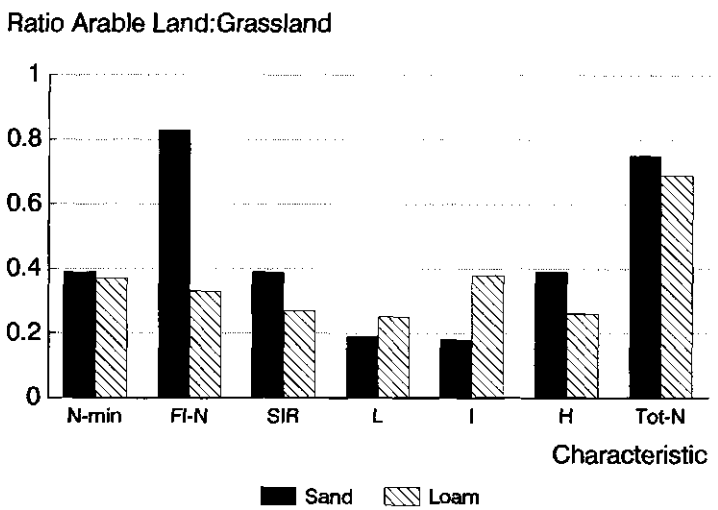

Figure 2. Amounts of active and total microbial biomass (SIR and Fl-N, respectively), light (L), intermediate (I) and heavy (H) macro-organic N and total organic N (Tot-N) and rates of N mineralization (N-min) in the top 10 cm of a sandy and loamy arable soil divided by the amounts and rates of the same parameters in the corresponding grassland soils.

The average values of the four sampling data were taken. For N mineralization the ratio was almost 0.4 for both soil types. The ratio was considerably higher for total organic N (about 0.7 for both soils). The ratio for the active microbial biomass (SIR) was almost identical to the ratio for N mineralization. The ratio for total microbial biomass (Fl) differed; 0.8 for the sandy soil and 0.3 for the loam. The ratio generally increased in the order light, intermediate and heavy macro-organic matter and was in the same range as for N mineralization (heavy fraction) or slightly lower (light fraction).

Relationship between soil organic N, N in the microbial biomass, N in light, intermediate and heavy macro-organic matter fractions, and N mineralization in grassland soils of different textures

There was a significant ($P < 0.05$) positive correlation between N mineralization and soil organic N content of the grassland soils. The correlation coefficient between N mineralization and soil organic N, however, was only 0.38 (Table 3).

Table 3. Correlation coefficients between N mineralization and total organic N, microbial biomass N (FI), active microbial biomass (SIR), and the light, intermediate and heavy fractions of macro-organic matter (mom) in the top 10 cm and 10-25 cm layer of grassland soils.

Soil organic N	0.38
Microbial biomass N (FI)	0.41
Active microbial biomass (SIR)	0.59
Light fraction of mom	0.62
Intermediate fraction of mom	0.45
Heavy fraction of mom	0.39

All correlations are significant ($P < 0.05$).

The amount of N mineralized per amount of soil organic N was significantly ($p < 0.05$) higher in sandy soils than in loams and clays (Figure 3).

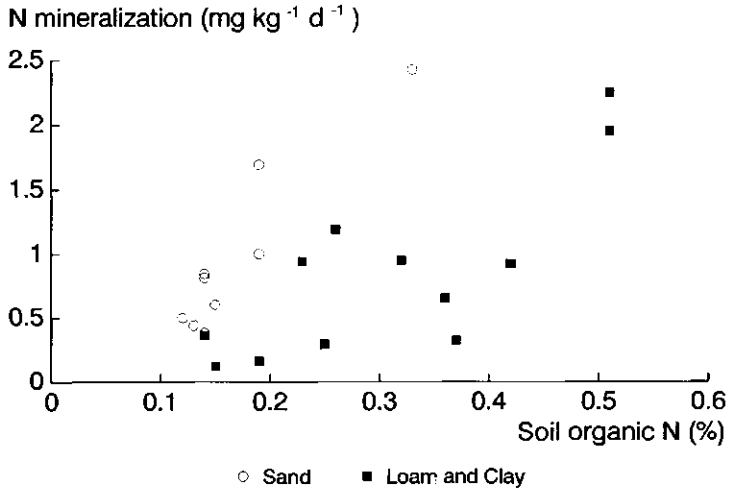

Figure 3. Relationship between the soil organic N content of the top 10 cm and the 10-25 cm layer of grassland soils and their rate of N mineralization.

The microbial biomass also showed a significant positive correlation with N mineralization (Table 3). Again, the amount of N mineralized per unit of microbial biomass was significantly ($P < 0.05$) higher in sandy soils than in loams and clays (Figure 4).

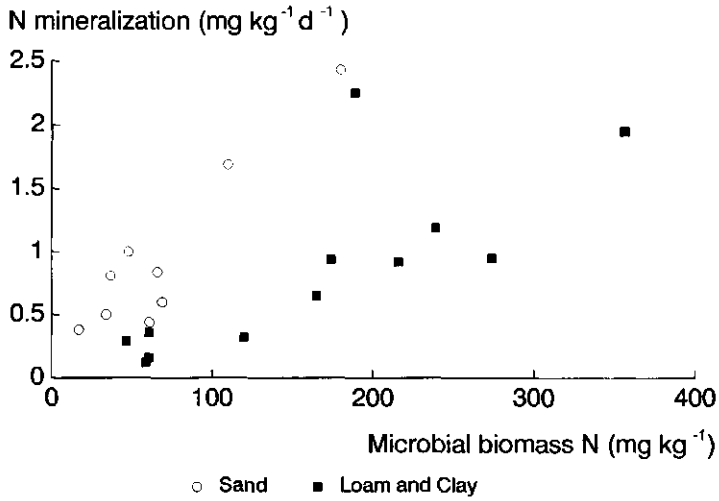

Figure 4. Relationship between the amount of N in the microbial biomass in the top 10 cm and the 10-25 cm layer of grassland soils and the rate of N mineralization.

The active microbial biomass as determined by the SIR method had a higher correlation coefficient with N mineralization than the amount of microbial biomass determined by the fumigation incubation method (Table 3). Besides, the relationship between SIR and N mineralization was the same for sandy soils, loams and clays (Figure 5).

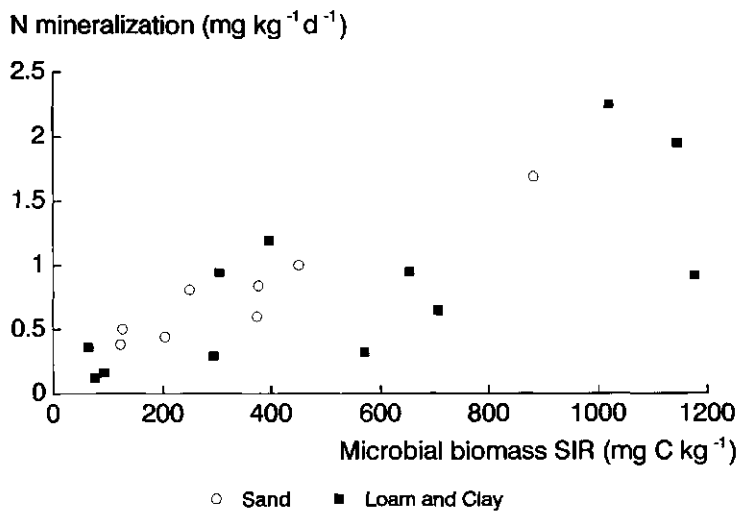

Figure 5. Relationship between the amount of active microbial biomass (SIR) in the top 10 cm and the 10-25 cm layer of grassland soils and their rate of N mineralization.

The correlation coefficient between N mineralization and the amount of N in the macro-organic matter fractions decreased in the order light, intermediate and heavy (coefficients of 0.62, 0.45 and 0.39, respectively; Table 3). Contrary to the situation for total organic N and N in the microbial biomass, but in agreement with the active microbial biomass, the relationship between the amount of N in the light fraction and N mineralization was the same for the sandy soils and the loams and clays (Figure 6).

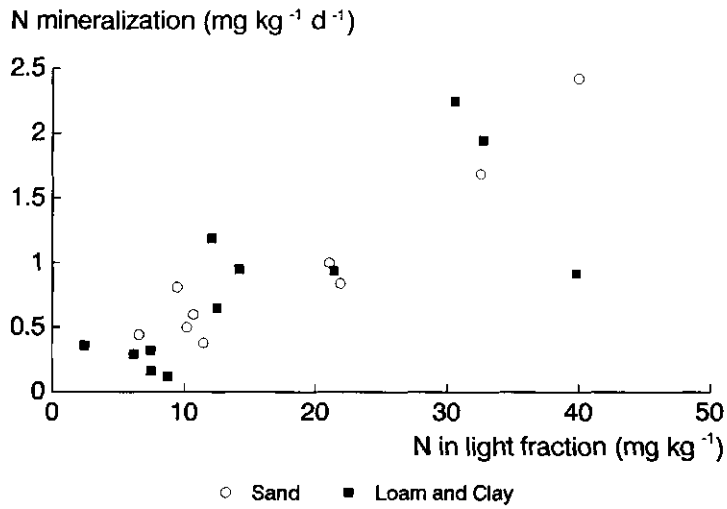

Figure 6. Relationship between the amount of N in the light fraction of the macro-organic matter in the top 10 cm and the 10-25 cm layer of grassland soils and their rate of N mineralization.

Contribution of soil organic matter fractions to the total amount of soil organic N; grassland soils

The percentages soil organic N present in the macro-organic matter fractions (> 150 μm) generally increased in the order light, intermediate and heavy (Table 4). The percentages soil organic N in the three fractions were higher in the top 10 cm of the grassland soils than in the 10-25 cm layer and higher in the grassland soils than in the corresponding arable soils (Table 4). In the top 10 cm of the grassland soils on average 17 % of the total amount of soil organic N was present in the three macro-organic matter fractions; for the 10-25 cm layer of the grassland soils this was 13 % and for the top 10 cm of the arable soils 4 %. So, most of the soil organic N was not present in the macro-organic matter fractions.

The percentages soil organic N present in the light and intermediate fractions in the top 10 cm of the grassland soils were higher in the sandy soils than in the fine-textured soils (Figures 7, 8). In the sandy soils 1-2 % of the amount of soil organic N was present in the light fraction. In the loams and clays this ranged from 0-5 to 1 %. The intermediate fraction comprised 6-17 % of the soil organic N in the sandy soils and 2-4 % in the loams and clays. The percentage soil organic N present in the heavy fraction was not affected by soil texture. For the 10-25 cm layer differences between the sandy soils and the fine-textured soils were less clear.

Table 4. Average percentage (%) soil organic N present in the light, intermediate and heavy fractions of the top 10 cm and the 10-25 cm layer of grassland soils sampled in 1991 and in the top 10 cm of the arable and grassland soils sampled in 1992.

	Light	Intermediate	Heavy
Grassland soils 1991			
0-10	0.9	5.2	10.6
10-25	0.5	3.8	8.2
Arable and grassland soils 1992			
Sand			
arable	1.2	0.9	3.1
grassland	4.2	7.3	11.0
Loam			
arable	0.7	0.8	1.8
grassland	2.7	1.9	5.0

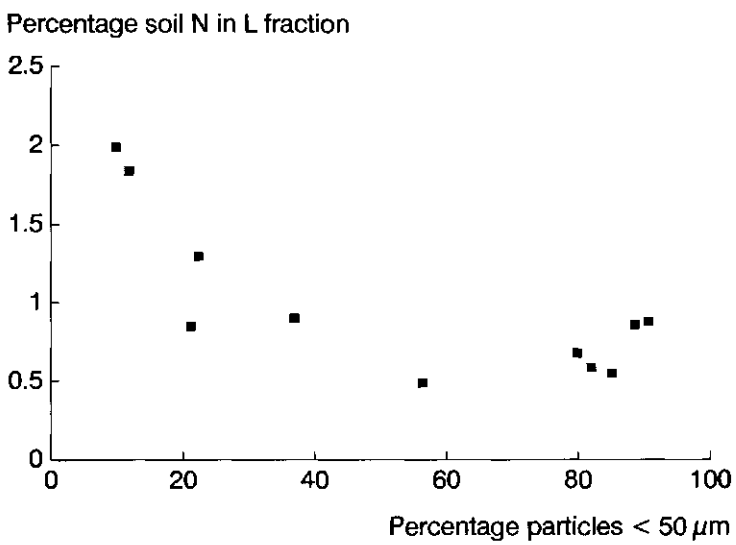

Figure 7. Relationship between soil texture and the percentage soil organic N present in the light fraction of the macro-organic matter in the top 10 cm of grassland soils.

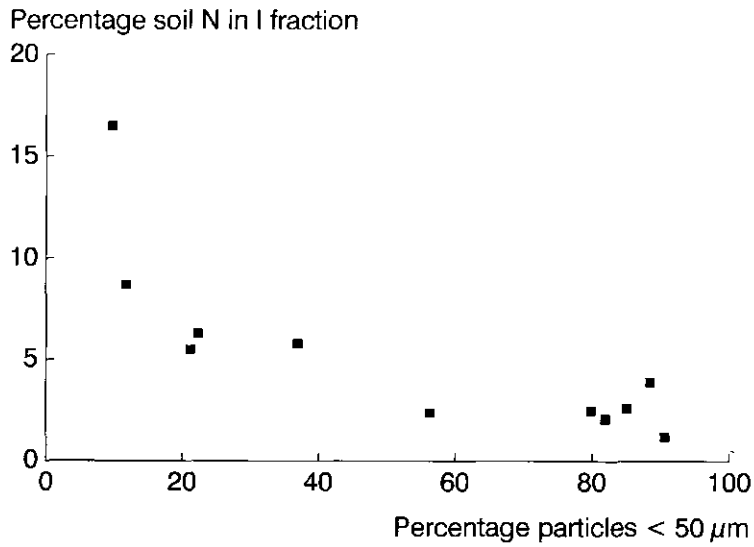

Figure 8. Relationship between soil texture and the percentage soil organic N is present in the intermediate fraction of the macro-organic matter in the top 10 cm of grassland soils.

DISCUSSION

The density fractionation method enables us to measure the active fractions of the soil organic matter, whereas the fumigation and substrate induced respiration methods enable us to measure the living component of the organic matter. This is of great importance for the further development and verification of models that describe dynamics in soil organic matter and N mineralization.

The first objective was to test whether microbial biomass and macro-organic matter fractions are better indicators of N mineralization than total organic N when comparing soils which receive different amounts of organic materials (i.e. grassland and arable soils). It was found that the relative changes in microbial biomass and light, intermediate and heavy macro-organic N correlated better with the relative changes in N mineralization than changes in total organic N when comparing grassland and arable soils. This indicates that the active fractions are potentially better predictors of N mineralization than total soil organic N when systems differing in organic matter input are compared. The relative change decreased in the order light, intermediate and heavy macro-organic matter, indicating that the activity of the fractions decreases in this order.

The second objective was to test whether the light fraction and the active microbial biomass correlate better with N mineralization than any other organic matter fraction when soils with different textures are compared. We found the highest correlations with N mineralization for the amount of light fraction of the macro-organic matter and the amount of active microbial biomass, when comparing grassland soils of different textures. The light fraction has a low ash content, indicating that it is not associated with soil minerals (Meijboom *et al.*, 1994), indicating a possibly low degree of physical protection. During decompositon the degree of physical protection will increase, since the heavy fraction includes the organomineral complexed soil organic matter (Sollins *et al.*, 1984). Moreover, organics bound to clay and silt particles might be even more physically

protected (Dalal and Mayer, 1986). The importance of the light fraction for N mineralization has been recognized before. Ford and Greenland (1968) found that the light fraction accounted for 25-60 % of N mineralization. The percentage soil organic N in the light and intermediate macro-organic matter fractions was higher in coarse-textured soils than in fine-textured soils. This might explain the higher mineralization rates in coarse-textured soils than in fine-textured soils.

We observed that although the microbial biomass is generally considered as an active organic matter pool, it is not a good indicator of N mineralization when comparing soils of different textures. N mineralization per unit of microbial biomass N was higher in sandy soils than in fine-textured soils. It was hypothesized that a higher proportion of the microbes is physically protected in small pores in loams and clays than in sandy soils (Hassink *et al.*, 1993[a]). Due to this protection the grazing intensity on the microbes by the soil fauna might be smaller in clays than in sandy soils, and this might result in a population that is less active in clays (Hunt *et al.*, 1977). The correlation coefficient between N mineralization and the amount of active microbial biomass was higher than between N mineralization and total microbial biomass. Moreover, we observed that contrary to the total amount of microbial biomass, N mineralization per unit of active microbial biomass was not affected by soil texture. We suggest that this is caused by the fact that the active part of the microbial biomass is not physically protected in the soil.

Our conclusion is that the light fraction and the active microbial biomass are the best indicators of N mineralization when soils differing in residue input and soil texture are compared.

Suggestions for improving the models that describe the dynamics of soil organic matter and nitrogen mineralization

The incorporation of the active organic matter fractions obtained by physical fractionation, the active microbial biomass, and the degree of physical protection of organic matter pools and microbial biomass in organic matter models might improve the simulation of N mineralization by these models. This might increase our understanding of differences in N mineralization in different agricultural soils. Studies with labelled material in different soils would be useful to collect information on the turnover rates of the density fractions that are obtained. An important aspect is the C:N ratio of the microbial biomass and of the different organic matter pools. The C:N ratio is generally higher in fresh residues than in more decomposed material. It has often been found that the C:N ratio decreases in the order light, intermediate and heavy fraction of the macro-organic matter, while the lowest C:N ratios are observed for the organic matter bound to clay an silt size particles (Swift and Posner, 1972; Christensen, 1985; Hassink *et al.*, 1993[b]). According to Tezuka (1990) the C:N ratio of the microbes may depend on the C:N ratio of their food. Microbes decomposing fresh residues might then have a higher C:N ratio than microbes decomposing organic matter bound to clay and silt size particles. The observation in a previous study that the C:N ratio of the bacteria is generally higher in sandy soils than in loams and clays (Hassink, 1994; Hassink *et al.*, 1994), corresponds with the results of the present study that the percentage of N found in the light and intermediate fraction is higher in the sandy soils than in the loams and clays.

Future research should indicate if it is necessary to distinguish different pools of microbial biomass with different C:N ratios. Techniques are available to extract bacteria and fungi from the soil (Bakken, 1985). This would enable us to directly determine the C:N ratio of the microbes in different soils.

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Fractionation of vegetable crop residues in relation to *in situ* N mineralization

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Abstract

The N mineralization from soil organic matter and from incorporated crop residues is the most uncertain factor in modern N recommendation systems. In the vegetable region of West Flanders large amounts of crop residues are ploughed in yearly. In a field trial crop residues of six vegetable crops were incorporated into a soil and the evolution of mineral N was followed to a depth of 120 cm. The Stevenson fractionation was applied to all crop residues. An incubation experiment was set up using five of the crop residues of the field trial. Results of the incubation experiment and the field trial were in good agreement. Linear regressions were calculated between amount of N mineralized in the field and chemical properties. Highly significant correlations ($P < 0.001$) were obtained between net amount of N mineralized after two weeks and lignin content ($R^2 = 0.949$). The net amount of N mineralized after four weeks was best correlated with the C:N ratio times the lignin content over the square root of the water soluble carbohydrates ($R^2 = 0.862$, $P = 0.001$).

INTRODUCTION

During the past few decades Central West Flanders (Belgium) has known an explosive growth of vegetable crop production destined for the surrounding industry. Yearly large amounts of crop residues are ploughed in. These crop residues contain large amounts of nitrogen (N). Figures cited in the literature range from 5 to 25 kg N per ha for crops like spinach, to more than 100 kg per ha for, e.g., blanching celery and different types of cabbages (Breimer, 1988; Demyttenaere, 1991). After incorporation into the soil part of this nitrogen will be mineralized. This mineralization process is as yet far from being fully understood. In areas with an important vegetable crop production the nitrogen contained in crop residues can play an important part in the nutrition of subsequent crops. If no subsequent crop is planted (late autumn or winter) the nitrogen mineralized will be leached to the groundwater.

In this paper an attempt is made to relate the N mineralization of some important vegetable crop residues in the field to their chemical composition as determined by the

In: J.J. Neeteson and J. Hassink (eds.), 1994. Nitrogen mineralization in agricultural soils; proc. symp. held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993. AB-DLO Thema's, AB-DLO, Haren, pp. 17-25.

Stevenson fractionation (Stevenson, 1965). Some of the results are verified by an incubation experiment in the laboratory.

MATERIALS AND METHODS

Field trial

At the beginning of October a field trial of the N mineralization of vegetable crop residues was carried out during a period of 5 months. The field is located in the vegetable growing region of West-Flanders (Pittem). The upper 30 cm has a loamy sand texture with a pH_{KCl} of 5.75, a carbon content of 1.14 %, and the C/N ratio of the soil organic matter was 11.8, making it a representative soil for that region. The field was planted with chicory as a pre-crop. The mineral N content of the soil at the beginning of the trial (after harvest of the chicory) was 22 kg N per ha in the layer 0-120 cm. The vegetable crop residues incorporated into the soil included residues of cauliflower (leaves and stems separately), broccoli (leaves), white cabbage (leaves and stems combined), spinach (lower leaves and stems), chicory (leaves) and leek (leaves). The incorporated amounts (Table 1) are based on determinations of the amounts of crop residues in that region.

Table 1. Data on amounts of crop residues used in the field trial (CaL (CaS) = leaves (stems) of cauliflower, BrL = leaves of broccoli, WcL (WcS) = leaves (stems) of white cabbage, Sp = spinach, Le = leek, Ch = chicory), FM = fresh matter, DM = dry matter. Plot size is 10 m².

	CaL	CaS	BrL	WcL	WcS	Sp	Le	Ch
FM per plot (kg)	22.5	13.4	37.4	36.6	19.8	21.8	25	35
DM (%)	15.5	15.2	12.1	14.9	22.6	11.2	15	9.9
DM per plot (kg)	3.48	2.04	4.54	5.44	4.47	2.45	3.75	3.48
N (% of DM)	3.28	2.82	2.91	1.64	1.91	3.26	2.15	1.91
N (g per plot)	114	57	132	89	85	80	80	66

Because of the low amounts of crop residues for spinach, twice the amount of crop residues determined after harvest was incorporated into the soil. The trial was carried out in three replicates, including three blanks, i.e. three plots without residues. The individual plot areas were 10 m². The crop residues were spread out on the field and then incorporated into the soil to a depth of approximately 15 cm. Soil samples were taken regularly to a depth of 120 cm in layers of 30 cm. The samples from the upper 30 cm layer were extracted with KCl and analyzed colorimetrically for both NO_3^--N and NH_4^+-N with a continuous flow auto-analyzer (Beernaert *et al.*, 1987). The samples from the lower layers were extracted with $KAl(SO_4)_2$ and analyzed for NO_3^--N with a specific NO_3^--electrode.

Fractionations

The Stevenson fractionation (Stevenson, 1965) was applied to the crop residues used in the field trial. Six fractions are obtained (hereafter referred to as FR1 to FR6): fats, waxes and oils (ether extraction, 24 hours), resins (alcohol extraction, reflux for 2 hours), water-soluble polysaccharides (water extraction, reflux for 2 hours), hemicellulose and structural

protein (hydrolysis with 2 % HCl, reflux for 5 hours), cellulose and structural protein (hydrolysis with 80 % H_2SO_4 at 15 °C for 2.5 hours, followed by dilution with distilled water to 1.42 M and reflux for 5 hours) and lignin (residue). The first three fractions were determined gravimetrically, fractions 4 and 5 by an analysis of reducing sugars and an analysis of total N (protein was calculated as amount of N × 6.25) and fraction 6 is considered as the final residue. Total N content of each fraction was determined with the Kjeldahl method. The fractionations were done in three replicates. Ash content is the residue after ignition of the dried plant material for 4.5 hours at 450 °C.

Incubations

An incubation experiment using soil from the field trial was set up for leaves and stems of broccoli, cauliflower and white cabbage. The soil used in the incubation experiment was not air-dried and not sieved in order not to disturb microbial activity. Stones and visible plant material were removed and large soil aggregates were crumbled. Plastic tubes with a diameter of 4.6 cm were filled with a soil-crop residue mixture (6 g of chopped fresh crop residues per tube mixed thoroughly with 342 g of wet soil). The soil-crop residue mixture in the tubes was pressed to obtain a bulk density of 1.45 g cm^{-3}. Soil moisture content was kept constant at 12 % (weight basis, 75 % of field capacity). Sampling took place by removing intact tubes. Samples in 3 replicates (3 tubes per crop residue) were removed after 7, 21, 34, 50, 69, 83 and 99 days and analyzed for NO_3^--N and NH_4^+-N in the same way as described for the field trial.

RESULTS

Field trial

N mineralization during the field trial is given in Figures 1 and 2.

Calculations using the leaching model of Burns (1974) showed that leaching of N out of the upper 120 cm started in the first days of November, i.e. from the second month after incorporation. From the N-mineralization pattern of the residues it was suspected that some denitrification had taken place from the second month of the trial (due to heavy rainfall in November, December and January). Because N losses by leaching or denitrification were not measured during the field trial, only the data of the first and second sampling (2 and 4 weeks after incorporation, respectively, hereafter referred to as WE2 and WE4) were used to establish quantitative relationships with chemical composition. These data were not affected by losses by leaching or denitrification. Spinach, leaves of cauliflower and leaves of broccoli show a similar N mineralization (Table 2). For spinach, the net amount of mineralized N after 2 weeks is more than 45 % of the total added N), indicating that it is a very easily degradable material. Two months after incorporation into the soil no leafy material could be found for any of the above three crop residues.

The other crop residues clearly mineralize their N much more slowly (Table 2). The slight decrease in net mineralized N between the second and the fourth week is within the measuring error. The residues of chicory and stems of white cabbage seem to be particularly resistant to N mineralization, with less than 10 % of the total added N being mineralized within the first 4 weeks.

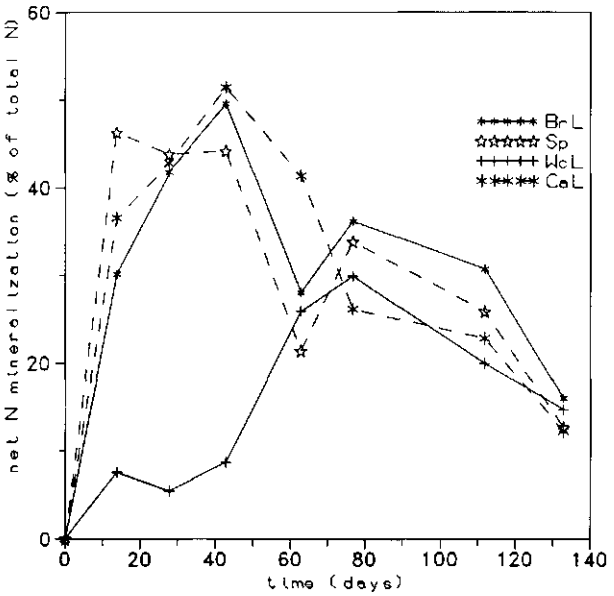

Figure 1. Net N mineralization (layer 0-120 cm) during the field trial for leaves of cabbages and spinach (see Table 1 for abbreviations).

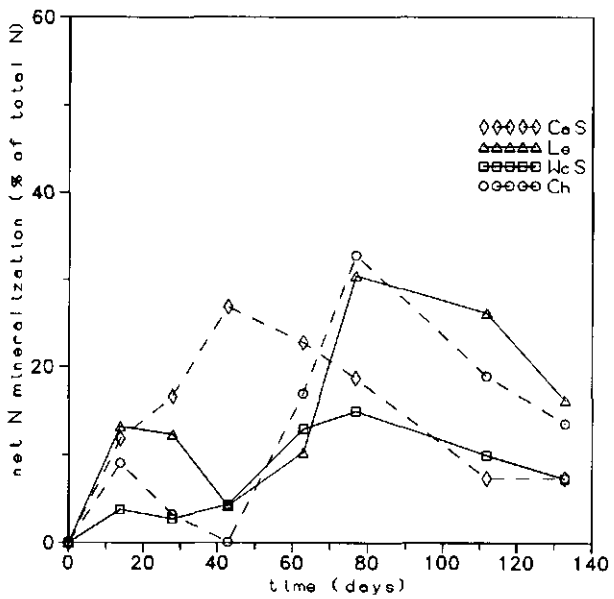

Figure 2. Net N mineralization (layer 0-120 cm) during the field trial for stems of cabbages and leaves of leek and chicory (see Table 1 for abbreviations).

Table 2. Net amount of N mineralized (layer 0-120 cm) in the field trial, 2 (WE2) and 4 (WE4) weeks after incorporation of the crop residues (bracketed values represent standard errors) (see Table 1 for abbreviations).

	CaL	CaS	BrL	WcL	WcS	Sp	Le	Ch
WE2	36.6	11.9	30.1	7.6	3.8	46.3	13.3	9.1
	(9.9)	(22.6)	(2.7)	(4.8)	(2.4)	(9.5)	(2.7)	(3)
WE4	42.8	16.6	41.9	5.5	2.7	43.8	12.4	3.3
	(15.3)	(5.2)	(10.3)	(1.2)	(0.6)	(11.1)	(2.3)	(3.7)

Fractionations

The results of the fractionations of the crop residues used in the field trial are given in Table 3.

Table 3. Chemical composition and results of the fractionation (in % of dry matter) of the crop residues used in the field trial (FR1 to FR6 = fraction 1 to fraction 6; see text for further explanation).

	CaL	CaS	BrL	WcL	WcS	Sp	Le	Ch
Ash (%)	23.1	17.9	16.1	25.2	9.3	44.0	19.3	28.9
Total C (%)	42.8	45.6	46.6	41.5	50.4	31.1	44.9	39.5
Total N (%)	3.28	2.82	2.91	1.64	1.91	3.26	2.15	1.91
C:N	13.2	16.2	16.0	25.3	26.4	9.6	20.9	20.7
FR1 (fats, waxes, oils)	2.9	0.7	3.9	3.4	0.9	2.1	4.3	3.5
FR2 (resins)	4.6	3.3	8.9	7.3	6.3	4.0	10.0	5.2
FR3 (watersol. fraction)	32.7	15.3	24.8	23.7	18.8	18.5	21.6	22.6
FR4 (hemicellulose)	17.8	19.5	15.9	12.5	21.8	22.2	14.2	12.5
FR5 (cellulose)	11.3	21.7	18.8	6.9	20.5	5.9	15.4	6.7
FR6 (lignin)	7.6	21.6	11.7	20.9	22.4	3.4	15.1	20.4
N (%) in FR3	21	44	20	29	49	20	26	25
N (%) in FR4	62	43	56	50	36	73	56	53
N (%) in FR5	12	6	16	1	9	5	6	5
N (%) in FR6	5	7	8	20	6	2	12	17

Spinach and leaves of cauliflower and broccoli have the lowest lignin content and the lowest C:N ratio. Chicory and leaves and stems of white cabbage have a higher lignin content and high C:N ratios. No N is extracted in FR1 and FR2. Most of the N is recovered in FR3 and FR4. As reported by Frankenberger and Abdelmagid (1985) there is essentially no relationship between lipid composition of the residues and their N mineralization.

Incubations

The evolution of the net amount of N mineralized is shown in Figures 3 and 4.

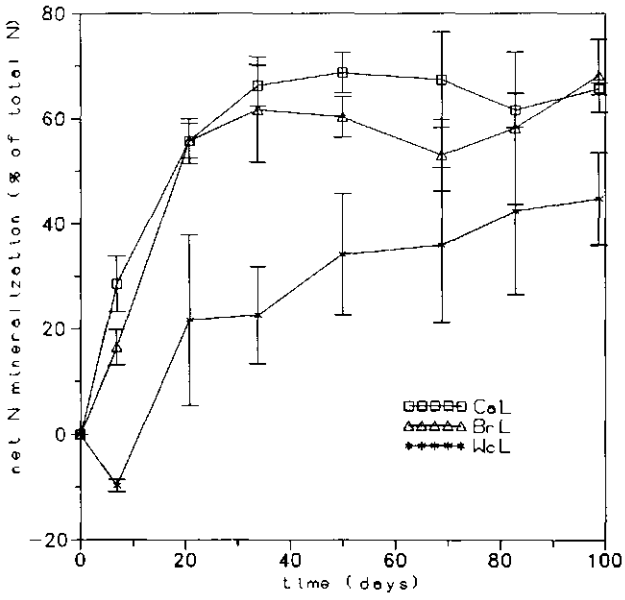

Figure 3. Net N mineralization (± 1 standard deviation) for leaves of cabbages during the incubation trial (see Table 1 for abbreviations).

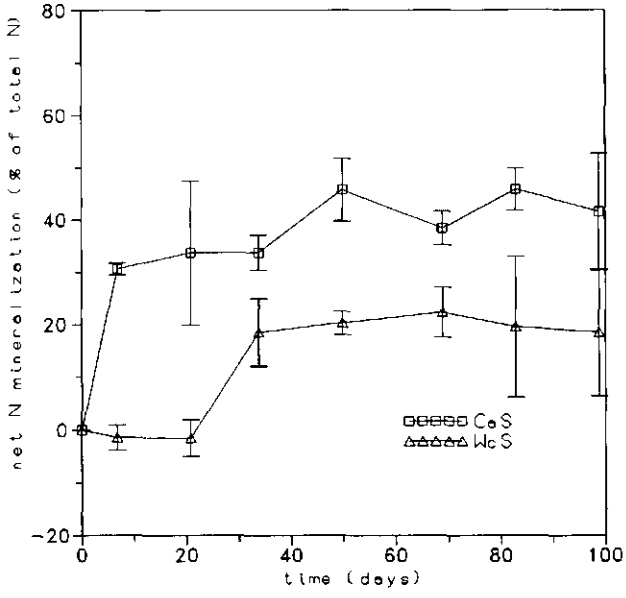

Figure 4. Net N mineralization (± 1 standard deviation) for stems of cabbages during the incubation trial (see Table 1 for abbreviations).

For all crop residues the N mineralization pattern is in agreement with the findings of the field trial and the fractionations. The percentages of N mineralized are higher than in the field trial because of higher temperatures, optimal moisture content, and because there are no N losses. An initial net immobilization of N is observed for both stems and leaves of white cabbage. From the N mineralization pattern of leaves and stems of white cabbage it was concluded that the contribution of the leaves to N mineralization in the field was about twice that of the stems.

Relations between plant chemical composition and N mineralization in the field

Several authors tried to relate N mineralization of plant material to its initial chemical composition. Frankenberger and Abdelmagid (1985) reported highly significant relationships between cumulative N mineralization and total N content for different leguminous crops in incubation experiments. Kirchmann and Bergqvist (1989) found a highly significant linear relationship between lignin content and cumulative amount of N mineralized during incubation of white clover.

Chemical composition of the crop residues was expressed in six different forms, namely the total N content (factor 1), the total C:N ratio (factor 2), the lignin content (factor 3), the sum of resins, hemicellulose, cellulose and lignin content (factor 4), the lignin content times the total C:N ratio over the square root of the water-soluble carbohydrates (factor 5) and the C:N ratio of the cellulose + lignin fractions (factor 6).

These relationships are based on the assumptions that fractions 2, 4, 5 and 6 are resistant to mineralization, whereas the water-soluble carbohydrates (FR3) are an easily available carbon source. Factor 5 was derived from a relationship used by Herman *et al.* (1977). They found that the product of lignin and C:N ratio over (total carbohydrates)^{½} was very effective in predicting the decomposition of grass roots in an incubation experiment. Here, we used water-soluble carbohydrates rather than total carbohydrates in the denominator of factor 5, because cellulose and hemicellulose were determined differently than by Herman *et al.* (1977).

The regressions between WE2 and all factors, except factor 4, are significant at the P = 0.01 level (Table 4). Net N mineralization after 4 weeks (WE4) is less well related to chemical composition, although factors 1, 2, 3 and 5 are still significant at P = 0.01.

Table 4. Slopes, intercepts and coefficients of determination for the linear regressions between factors expressing chemical composition and net amount of N mineralized 2 weeks (WE2) and 4 weeks (WE4) after incorporation. Bracketed values are not significant at the P = 0.01 level.

	WE2			WE4		
	Slope	Intercept	R^2	Slope	Intercept	R^2
Factor 1	21.1	-32.5	0.780	26.3	-44.1	0.860
Factor 2	-2.44	65.1	0.827	-2.81	73.3	0.782
Factor 3	-2.12	52.5	0.949	-2.35	57.2	0.824
Factor 4	-0.85	64.3	(0.441)	-0.75	60.2	(0.242)
Factor 5	-0.33	42.8	0.916	-0.38	47.7	0.862
Factor 6	-0.54	44.3	0.700	-0.61	48.7	(0.633)

DISCUSSION

The results of the field trial clearly show that even in autumn, N mineralization from fresh organic residues is still important. Particularly for crop residues of cauliflower and broccoli, with a high total N content and a fast N mineralization, losses by leaching during winter can amount to 100 kg or more. In summer, N mineralization will undoubtedly be more complete. An important amount of mineral N will be released within the first month after incorporation into the soil.

There is a striking difference between N mineralization patterns of cauliflower and broccoli on the one hand and white cabbage on the other. This means that botanical similarity is no guide to predicting N mineralization of crop residues.

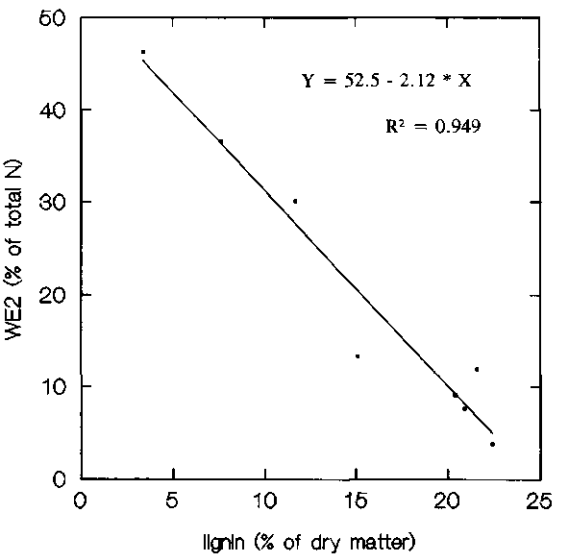

Figure 5. Linear regression between net amount of N mineralized after 2 weeks (WE2) and lignin content of the crop residues.

Short-term N mineralization (WE2) can be predicted well by lignin content (Figure 5), which is easy to determine. Combination of lignin content and total N content would be very useful as a first estimate of initial N mineralization for use in N recommendation systems. N mineralization after 4 weeks (WE4) is best predicted by a combination of C:N ratio, lignin and content of water-soluble carbohydrates (factor 5), but total N content (factor 1) is almost as good a predictor (Figure 6).

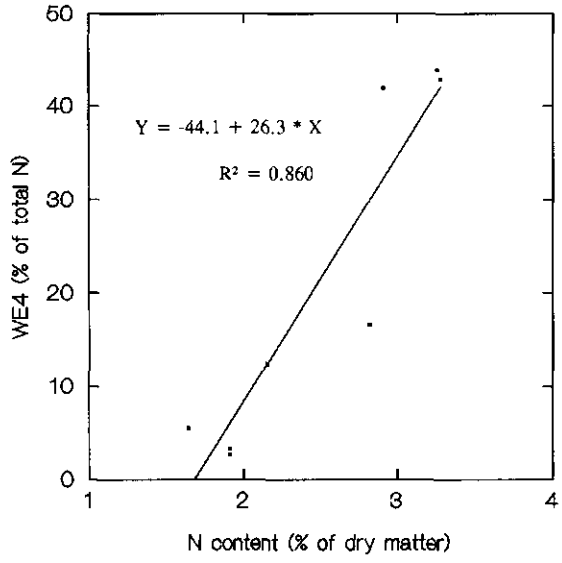

Figure 6. Linear regression between net amount of N mineralized after 4 weeks (WE4) and total N content of the crop residues.

ACKNOWLEDGEMENTS

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Methods to fractionate organic matter in various pools

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Abstract

A short overview is given of methods to fractionate organic matter in different pools. The relationships between the research objectives and the concepts behind the experimental fractionation procedures are emphasized.

Three categories of methods are distinguished: chemical extraction methods, procedures for physical fractionation of soil organic matter, and methods to determine the chemical composition of organic residues.

Prospects for future research are indicated. Methods used to study the effects of inorganic soil constituents on organic matter dynamics, and combinations of different procedures seem to be most promising. The need for methods to separate biologically meaningful fractions is stressed.

INTRODUCTION

As in other fields of research, a standard way of gaining understanding in soil organic matter cycling is dividing the material considered into smaller units that behave homogeneously, and study the properties of these fractions and their mutual relationships. This kind of approach is at the base of a wide gamut of methods to fractionate soil organics and plant residues. The details of the different procedures vary widely, depending on the purposes for which the methods are used, i.e. the specific aspects of the decomposition/mineralization processes that are investigated. Topics of research range from the study of the relation between the 'quality' or chemical composition of plant residues and nutrient release; over the examination of the effects of the inorganic soil constituents on the transformation of organic components, to the determination of the structure and chemical characteristics of the 'end product', stabilized soil organic matter.

Outlining the various methods of organic matter fractionation, adopted for different research needs, is the first objective of the present contribution. The second aim is to indicate how results obtained by different methodologies can be compared and integrated, hinting at prospects for future research.

In: J.J. Neeteson and J. Hassink (eds.), 1994. Nitrogen mineralization in agricultural soils; proc. symp. held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993. AB-DLO Thema's, AB-DLO, Haren, pp. 27-32.

CHEMICAL EXTRACTION METHODS TO FRACTIONATE SOIL ORGANIC MATTER

Complete extraction of soil organic matter

Traditionally, soil organic matter studies have been focusing on the chemical composition and properties of the soil organic colloids. To do this, the organic fraction had to be separated from the inorganic soil particles, which is usually done by extraction. Several extractants have been used (Mortensen, 1965). Most popular is alkali (NaOH), which is commonly used in the first step of the 'classic' method of fractionating soil humus into humin, humic acids (HA) and fulvic acids (FA) (Stevenson, 1965; Schnitzer, 1982). The partitioning is based on a pH-dependent solubility of the different organic molecules. Complete extraction of organic matter from soils is not possible by this procedure. Nevertheless, a lot of valuable information on the chemical characteristics of soil organic matter has been obtained.

Two other methods, also with almost 'classic' pretensions, and aiming as well at an exhaustive extraction of organics from soils, are (1) the proximate analysis for estimating compounds characteristic of plant material (Stevenson, 1965), and (2) the acid hydrolysis used to liberate N-components from soils for later fractionation and characterization (Bremner, 1965; Stevenson, 1982). For the first method, a soil sample is subsequently extracted with the same reagents that are used to determine the content of a class of organic compounds in plant tissues, i.e. waxes, resins, water-soluble polysaccharides, hemicellulose, cellulose, and a large, rather vague pool of proteins, lignin and humus. Acid hydrolysis, usually with 3 or 6 N HCl, separates soil organic N into acid-insoluble and hydrolyzable N. The latter pool can be subdivided into amino acid-, amino sugar- and ammonia-N.

Extraction of a specific pool of soil organic matter

Chemical extraction is also used to isolate components from the soil organic matter pool, e.g. volatile fatty acids, amino acids, or soluble carbohydrates (Paul and Beauchamp, 1989; Christensen and Christensen, 1991). The rationale behind such procedures is mostly the determination of the availability of the considered substances for plants or microorganisms. The extracting reagents are normally mild in comparison to those used for complete extraction of soil organic matter, e.g. salt solutions (such as K_2SO_4 or $CaCl_2$) (Stanford and Smith, 1976; Christensen and Christensen, 1991), or even water (Paul and Beauchamp, 1989).

An organic carbon pool with a special meaning can be extracted from soils after fumigation with chloroform. The correlation that exists between the size of this pool and the amount of C bound in microorganisms is the basis of commonly used methods to determine microbial biomass C (Brookes et *al.*, 1985; Vance et *al.*, 1987; Amato and Ladd, 1988). Microorganisms have a key role in the cycling of nutrients in soils. They are decomposers of organic material, and can be considered as a temporarily reservoir of plant nutrients. Thus, biomass C clearly is a soil organic matter fraction relevant to the functioning of C and N dynamics in soils.

PHYSICAL FRACTIONATION OF SOIL ORGANIC MATTER

A second category of methods to fractionate soil organic matter comprises procedures by which whole soils are physically separated into fractions. Studies using this type of experi-

mentation emphasize the role of inorganic soil constituents in organic matter cycling. The objectives are to elucidate the relations between the location of the organics in the soil structure and their stabilization and turnover.

Recently two reviews on physical soil fractionation have been published (Stevenson and Elliott, 1989; Christensen, 1992). Methods to separate soils by physical means vary according to the disruption techniques applied before fractionation, and the principle of separation, being by density or by particle size. Ultrasonic vibration has been extensively used to disrupt soil aggregates (Turchenek and Oades, 1979 and references herein). An alternative, more gentle procedure for soil dispersion is shaking in water (Brückert, 1979).

Density fractionation is based on the assumption that free, macro-organic material - being decomposing plant and animal debris - has a lower specific weight than the more processed, humus-like products, which are intimately associated with soil minerals, and therefore have a higher density. Heavy liquids are most often used to obtain density fractions, but they cause considerable problems, for instance, their high potential toxicity can substantially alter the biological characteristics of the fractions (Christensen, 1992). Another way to isolate the 'light' fraction is flotation in water (Anderson and Ingram, 1993).

The underlying concept of particle size fractionation is the notion that organic matter associated with particles (or aggregates) of different size differ in structure and function, and therefore have different roles in soil organic matter dynamics (Christensen, 1992). Organomineral complexes can be separated in size classes by wet or dry sieving (fractions > 50 *um*), gravity sedimentation and centrifugation (fractions < 50 *um*). Regularly, combinations of particle size and density fractionation methods are used.

Characterization of organic matter in physical soil fractions

To determine the chemical properties of organic material associated with physical soil fractions, a large variety of assays have been applied. These methods are usually similar to those used for total soil organic matter characterization, and are extensively discussed elsewhere (see references in previous section).

If one aims at determining microbiological properties of fractions, soils should be only mildly dispersed before fractionation. Disruption techniques as ultrasonic vibration can cause death of cells. The decomposability or bioavailability of organic components in soil separates has been estimated by incubation and determination of mineralized C and N (Lowe and Hinds, 1983; Cameron and Posner, 1979). By measuring specific products of microbial origin indications about the distribution of soil microorganisms in physical fractions were obtained (Turchenek and Oades, 1979; Christensen and Bech-Andersen, 1989). But recently, using improved methods of biomass C determination (Amato and Ladd, 1988), it has also been possible to directly measure microbial biomass contents of soil fractions (Jocteur Monrozier *et al.*, 1991).

To study the dynamics of soil organic matter in size and density fractions the application of isotope-labelled material has been proven to be very useful. For example, Christensen and Sørensen (1986) demonstrated that the distribution of labelled C and N over soil fractions was distinguishable from this of native C and N, even after 5 years of incubation. Ladd *et al.* (1977) compared the distribution of immobilized ^{15}N over soil fractions during incubation of different soils with glucose and wheat straw, and concluded that the nature of the organic C amendment, but not the soil type, influenced the distribution of ^{15}N and the pattern of change of organic ^{15}N of soil fractions with time.

CHEMICAL COMPOSITION OF ORGANIC RESIDUES

Methods to determine the chemical composition of plant material are a third group of procedures to fractionate organic matter in different pools. In some studies plant residues are analyzed before amendment to soils (Melillo *et al.*, 1982; Palm and Sanchez, 1991; Kachaka *et al.*, 1993). The aim is to predict their decomposition rate, the release of nutrients, and their part in the formation of 'stable' soil organic matter from the content of certain chemical components. Exemplary groups of components are water-soluble polysaccharides, (hemi)cellulose, lignin, polyphenols. Several procedures to determine the chemical composition of plant residues have been described (Waksman and Tenney, 1927; King and Heath, 1967; Van Soest and Wine, 1968).

CONCLUSIONS AND PROSPECTS FOR FUTURE RESEARCH

In this brief overview of the methodology of organic matter fractionation, we stressed the relation between the research objectives and the concepts behind the experimental procedures.

It should be noted that the actual fractions obtained are often less homogeneous than is assumed by the principles on which the method of fractionation is based. For example, by the alkaline extraction of soil organic matter, the 'fulvic acid' pool can contain a considerable amount of smaller molecules that do not have the typical 'fulvic acid' properties. Or by density fractionation, organic components complexed with mineral particles may be also present in the 'light' fraction, which is assumed to consist only of free vegetal and animal parts (Christensen, 1992). The occurrence of such 'artefacts' should be considered by the interpretation of data of fractionation studies.

It may be very informative to look for relations that may exist between the fractions from the same soil obtained by different procedures. For instance, in case of physical soil fractionation, the 'light' fraction which is isolated based on density, is to a large extent recovered in the coarse fractions of particle size fractionation. Similar functioning of both fractions in soil organic matter turnover can be anticipated.

Purposes for which organic matter fractionation procedures have been used are either to formulate predictive and immediately applicable managing recommendations (e.g. based on the prediction of the amount of N that will be mineralized during the next season), or to obtain better understanding of the processes involved. Regarding the first category, usually simple methods are preferred, depending on the degree of precision required. Experimental procedures applied for the latter group of purposes vary according to the direction in which research progresses or on the specific aspects that are stressed.

In this respect, the key role of soil microorganisms in decomposition/mineralization processes has been recognized for some time, and is usually expressed in models simulating these processes. But although the biological relevance of partitioning of soil organic matter (e.g. distinguishing 'active', 'resistant' and 'recalcitrant' pools (Van Veen *et al.*, 1985; Dendooven, 1990)) has been taken into consideration conceptually, adequate experimental procedures to separate and measure these fractions are still to be developed.

Since long, microbiological properties such as the decomposability of organic components, have been approached by and translated into chemical characteristics. More recently, emphasis is also given to physical protection of organic matter. Interactions of organics with mineral soil constituents of varying size and properties largely influence transformations of organic matter by microbial communities. Such spatial relationships can

be studied using physical methods to fractionate soil organic matter, which may be also combined with other methods.

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Field test of biological and chemical methods to estimate the soil nitrogen supply under a temperate climate

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Abstract

Usually, long-term incubations are used to estimate the organic nitrogen which is potentially mineralizable in the soil. Our purpose was to find a chemical method correlated to the biological method to predict the potential mineralizable nitrogen. Tests were carried out on a loamy soil in Grignon (Ile-de-France), by three different crop managements: bare fallow, maize crop with and without nitrogen fertilization. Soil mineral nitrogen, microbial biomass and chemical extractions were determined every month. Long-term soil incubations were used to compare with the chemical measurements. Two incubation kinetics were commenced in spring and in summer. The mineralized N represented 4 % of the total nitrogen and the mineralized C 6 % of the total organic carbon, giving a C/N ratio of 13. The amount of nitrogen mineralized was lower in summer than in spring for the cultivated treatments. Nitrogen and carbon biomass decreased in summer. Carbon and nitrogen extracted by the different methods ranged from 0.34 % ($NaHCO_3$) to 19 % (autoclaving) of total nitrogen and carbon contents, respectively. Regarding their fluctuations with time, two groups of extracts could be distinguished: one decreasing until harvesting as the microbial biomass (autoclaving), the others ($NaHCO_3$, boiling water and borate phosphate buffer) remained stable between May (sowing) and October.

INTRODUCTION

Up to 40 % of the crop nitrogen originates from the soil organic nitrogen mineralization by the microbial biomass (Paul and Voroney, 1980; Marumoto *et al.*, 1982). Soil organic N is not homogeneous but several pools have to be considered with more or less rapid turnovers (Jenkinson and Rayner, 1977; Molina *et al.*, 1983). Mineral nitrogen produced by the microbial biomass during the growing season originates from the mineralization of a labile pool of organic nitrogen (Paul and Juma, 1981; Carter, 1991). Long-term soil incubations are often used as a biological way to estimate potentially mineralizable soil nitrogen (N_o) (Carter and Macleod, 1987). But it is not a very convenient method for prediction and many authors have developed and tested chemical methods to provide

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indexes of soil nitrogen availability. Usually, these methods are evaluated by relating laboratory test values to crop yield and uptake of nitrogen (Smith, 1965; Keeney and Bremner, 1966; Jenkinson, 1968; Giroux and Sen Tran, 1987) to the field nitrogen availability (Fox and Piekielek, 1978; Hong *et al.*, 1990) or to the nitrogen mineralized during long-term incubations (Juma and Paul, 1984; Gianello and Bremner, 1988). They are also considered as valid methods when they well discriminate the soils studied (Gianello and Bremner, 1988). If N_0 or a chemical index represents an active fraction of organic matter, seasonal fluctuations should occur (Bonde and Rosswall, 1987) following biological activity. Only a few studies compare the evolution of the microbial biomass and the organic pools extracted in field experiments (Vong *et al.*, 1990).

In the field experiment presented, we have tested five different methods to measure the potentially mineralizable nitrogen and carbon: chemical extractions (boiling water, sodium bicarbonate, borate phosphate buffer and autoclaving) and aerobic incubations as reference method. These chemical extraction methods were selected from the literature for their strong correlation with yields, plant nitrogen uptake, soil nitrogen supply or the nitrogen mineralized during an incubation kinetic. They have been screened by considering their sensitivity to fluctuate during the crop growth as compared to the microbial biomass. They also have been compared with the soil nitrogen supply and the incubation results.

MATERIALS AND METHODS

Field experiment

The experiment was located at Grignon (Yvelines, FRANCE). In April 1992, three treatments were commenced: F, bare fallow, MN, maize crop with nitrogen fertilization (128 kg N ha^{-1}), and MON, maize crop without nitrogen fertilization. The loamy soil was typic Eutroquept. The pH(H_2O) was 7.3. The percentages of organic carbon and nitrogen were 10.8 and 1.3, respectively, and the sand : silt : clay ratio was 0.5 : 73.0 : 22.0 in the upper horizon (30 cm). The bulk density was 1.33. The soil temperature was daily measured at 10 cm; the soil water content was determined every two weeks. The maize was sown in May and harvested in October. We have considered only one plot per treatment. Their surfaces were 500 m^2 for the bare fallow and the unfertilized plots, and 5500 m^2 for the fertilized maize plot.

Soil sampling

Ten soil cores were sampled, from each treatment, in the first layer (0-30 cm) every month from May to December 1992. The soil cores were mixed and sieved (< 5 mm). All analyses were performed on this representative sample in order to obtain comparable results (Stanford, 1982). Extractions were done during the week after sampling. During this time the soil was stored at 4 ^{0}C. For the borate-phosphate buffer extraction, the soil was air-dried before the analysis. For each laboratory analysis, three replicates were considered. At harvest, the spatial variability of some parameters (nitrates, humidity, microbial biomass and autoclaving carbon contents) was evaluated doing the analysis in triplicate on each of the ten cores (data not shown).

Soil incubations

Two long-term incubations were started in May and August. In May, the soils were sampled a few days after sowing. Only two treatments could be considered: with (MN) and without nitrogen (F, MON) as all the plots were bare. Mineral nitrogen and CO_2 produced by 45 g of fresh sieved soil were measured during six months. The soils were moistened to field capacity considered as optimum for biological activity (Stanford and Epstein, 1974) and incubated under aerobic conditions at 28 °C. A one-week pre-incubation was performed to prevent overestimation of the mineralization after microbial activity stimulation by mechanical (sieving) and physical (water addition) disturbances. Mineralized nitrogen and carbon were measured colorimetrically on a Skalar autoanalyzer.

Microbial biomass

The microbial biomass C and N content were determined using the chloroform fumigation-extraction method (Vance *et al.*, 1987). Fresh soil (25 g) was fumigated with ethanol-free chloroform for 16 hours. Then control unfumigated and fumigated samples were extracted with 100 ml of 0.1M K_2SO_4 solution for 1 hour. After centrifugation, organic carbon was analyzed in the supernatant by persulfate oxidation under UV (Dorhman DC 80). Total N in the extracts was analyzed using the Kjeldahl method. The microbial biomass expressed in C was calculated as $B_C = (Cf\text{-}Cnf)/K_C$ with Cf and Cnf, the organic carbon extracted in the fumigated and unfumigated samples, respectively, and $K_C = 0.48$ (Chaussod *et al.*, 1986). The microbial biomass expressed in N was calculated as $B_N = (Nf\text{-}Nnf)/K_N$ with Nf and Nnf, the organic nitrogen extracted in the fumigated and unfumigated samples, respectively, and $K_N = 0.45$ (Ocio and Brookes, 1990).

Chemical extractions

Four chemical extraction methods were tested in this study.

The boiling water extraction method was essentially the method described by Livens (1959). In the procedure used, 50 g of fresh soil was placed with 200 ml of water in a 500-ml Erlenmeyer flask fitted with a glass funnel. The Erlenmeyer flasks were heated on a rheostat-controlled electric hot plate until the soil-water mixture had boiled for 60 minutes. The mixture was then cooled and pooled before being centrifugated in 500 ml centrifuge tubes.

The autoclaving extraction (Stanford, 1968; Juma and Paul, 1984) has been modified as follows. It was performed in the fumigated samples after microbial biomass extraction, 100 ml of 0.1M K_2SO_4 solution was added to the soil pellets, shaken for 15 min, autoclaved for 16 hours ($T^0 = 120$ °C, P = 1 bar), cooled and centrifuged.

The extraction in 0.01M bicarbonate sodium solution was proposed by MacLean (1964). Moist soil (150 g) was placed directly into 500-ml centrifuge tubes with 200 ml 0.01M $NaHCO_3$ solution. Centrifuge tubes were shaken on a horizontal agitator for 15 min at room temperature and centrifuged.

All extracts were centrifuged at 7000 rpm. The N content of the aliquot was estimated by a Kjeldahl procedure in which 20 ml of the filtrate was heated with 2 ml of concentrated sulfuric acid and 0.7 g of mineralization catalyst in a Kjeldahl flask (without reduction

of NO_3^--N) until the solution became clear, and digestion was continued for 1 hour. The content of the flask were then steam-distilled with 10 ml of 10 N NaOH (Keeney and Bremner, 1966). The ammonium liberated by distillation was collected in 0.005N sulfuric acid solution and titrated with 0.005N NaOH until pH 4.8 (Lathwell *et al.*, 1972). The organic carbon extracted was determined as previously.

The procedure followed for the borate-phosphate buffer method was described in detail by Gianello and Bremner (1988). Soil (4 g) was steam-distilled with 40 ml of pH 11.2 phosphate-borate buffer for 8 min, and the ammonia-N released was determined. The ammonia-N initially present in the soil sample was determined by distilling 4.0 g soil with 0.2 g MgO and 20 ml 2M KCl for 3.3 min. The ammonium-N produced by buffer extraction was calculated as the difference between these two analyses.

RESULTS

Field results

In the bare fallow plot, the amount of mineral nitrogen in the top 30 cm of the soil remained constant from May to July because of the balance between mineralization, immobilization and leaching (Figure 1).

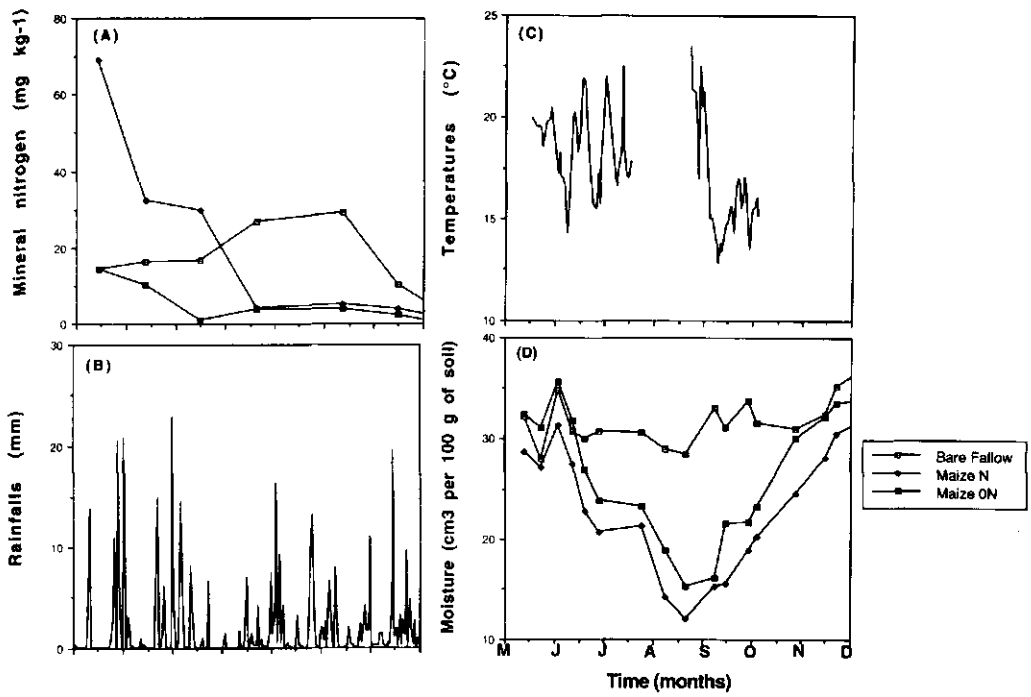

Figure 1. Variations in soil mineral nitrogen during the growing season in mg kg^{-1} dry soil (A), rainfall (B), soil temperature (C), and humidity (D).

Then the mineral nitrogen content markedly increased until the middle of August. Mineralization was important since the soil humidity was at field capacity and the temperature was approximately 20 °C, both favorable conditions for microbial activity. No

further variations appeared until October. In the cultivated plots, the maize nitrogen uptake occurred from sowing to August. After harvest, mineral nitrogen strongly decreased in all plots.

The maize yield was higher in the MON plot (18 300 kg dry matter ha^{-1}, 277 kg N ha^{-1}) than in the MN plot (16 600 kg dry matter ha^{-1}, 210 kg N ha^{-1}). The mineral nitrogen supplied by the soil can be estimated as the "N uptake by plants - mineral N at sowing + mineral N at harvesting" (Lindén *et al.*, 1992): 79 kg N ha^{-1} was found in the MON plot, -187 kg N ha^{-1} in the MN plot 91 kg N ha^{-1} in the bare fallow plot.

Laboratory incubations

After six months of incubation, 3 to 4 % of total organic nitrogen and 6 % of total organic carbon were mineralized. Zero-order kinetics were the best mathematical model for nitrogen mineralization (Table 1).

Table 1. Carbon and nitrogen mineralization in the long-term incubations started in spring and summer: kinetics parameters.

Plot	CO_2-C = $C_1(1-\exp(-k_1 * t))+k_2 * t$				N = k * t		
	C_1 (mg kg^{-1})	k_1 (d^{-1})	k_2 (mg kg^{-1} d^{-1})	r^2	N_{168d*} (mg kg^{-1})	k_n (mg kg^{-1} d^{-1})	r^2
Spring							
F (MON)	213	0.029	2.96	0.999	54	0.33	0.992
MN	122	0.036	3.15	1.000	50	0.30	0.998
Summer							
F	71	0.051	3.26	1.000	51	0.29	0.969
MN	98	0.048	2.47	0.999	39	0.22	0.977
MON	165	0.046	3.13	0.998	44	0.26	0.981

* mineral nitrogen after 168 days of incubation.

In spring and summer, the mineralization constant rate (k_n) was higher, although not significantly, in the F and MON plots compared to the MN plot. Fertilization seemed to depress the biological activity in soil. In the cultivated plots, k_n decreased between spring and summer. Two pools could be distinguished in the carbon mineralization kinetics. The mineralization of a labile and a more resistant pool followed first-order and zero-order kinetics, respectively. Between spring and summer, the carbon mineralization constant rate of the labile pool k_1 increased to a greater extent in the F plot than in the MN and MON plots. The highest value of C_1, the labile pool, was found in the MON plot. It decreased in the F and MON plots. The mineralization constant rate k_2 of the stable pool increased in the F and MON plots and decreased in the MN plot.

Microbial biomass

The microbial biomass represented about 2.5 % of organic carbon and 2.6 % of total nitrogen content. No differences were found between the plots in N and C values (Table 2). The C/N ratio was approximately 8 in the three plots. During the growing season, from

May to October, both carbon and nitrogen microbial biomass decreased by 21, 17 and 22 % in the F, MN and MON plots, respectively (Figure 2). However, some fluctuations occurred during the summer. After both harvesting and crop residue incorporation into the soil, microbial biomass increased.

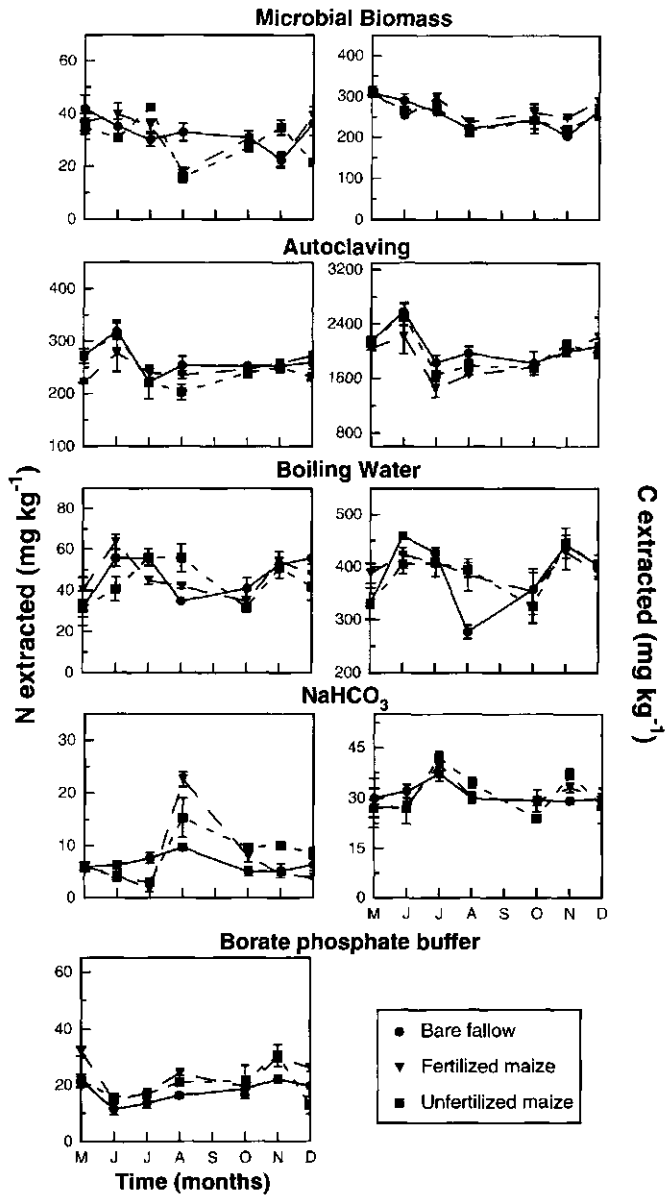

Figure 2. Fluctuations in the amounts of nitrogen and carbon (mg kg^{-1}) contained in the biomass and extracted by autoclaving, boiling water, borate phosphate buffer and $NaHCO_3$. Error bars are deduced from the three replicates done in the laboratory on the homogenized sample.

Chemical extractions

The organic carbon extracted varied from 0.3 % ($NaHCO_3$) to 19.4 % (autoclaving) of total organic carbon (Table 2). The organic nitrogen extracted varied within the same range. The C/N ratios were similar for all the tested methods (between 7 and 9), except the bicarbonate sodium extract which had a lower C/N ratio. No significant differences between the plots were found. With all the methods, the C and N extracted fluctuated in the same manner (Figure 2). In October, the carbon extracted by autoclaving decreased by 15, 13, and 18 % from their initial values in the F, MN and MON plots, respectively. The nitrogen extracted only decreased by 7 and 12 % of their initial values in the F and MON plots, respectively. No decrease was observed in the MN plot. The nitrogen content of the boiling water, $NaHCO_3$ and borate phosphate buffer extracts were similar at sowing and harvesting time. However, a decrease in the nitrogen extracted with the borate phosphate buffer was observed at the beginning of the growing season.

Table 2. Nitrogen and carbon (mg kg^{-1} dry soil) in microbial biomass and extracted by the different methods tested (means and standard deviations calculated with the values obtained from May to December).

Plot	Biomass	Autoclaving	Boiling water	Borate Phosphate	$NaHCO_3$
Nitrogen (mg N kg^{-1})					
F	34 ± 6	262 ± 27	40 ± 12	17.6 ± 4	8 ± 7
MN	32 ± 13	250 ± 19	51 ± 10	23.4 ± 6	9 ± 9
MON	28 ± 8	247 ± 33	40 ± 9	20.0 ± 5	9 ± 7
Carbon (mg C kg^{-1})					
F	251 ± 34	2084 ± 219	390 ± 70		32 ± 4
MN	274 ± 28	1924 ± 242	372 ± 42		32 ± 4
MON	252 ± 29	1999 ± 246	375 ± 52		33 ± 6
C/N					
F	7.4	7.9	7.7		4.0
MN	8.6	7.7	7.6		3.5
MON	8.9	8.1	9.8		3.7

The nitrogen extracted with $NaHCO_3$ strongly increased in August. After harvest, the nitrogen extracted with the borate phosphate buffer increased with the microbial biomass while the boiling water and autoclaving extracts decreased. In winter, after crop residue incorporation in cultivated plots and ploughing, the nitrogen in borate phosphate buffer decreased while it increased in the boiling water and autoclaving extracts. The $NaHCO_3$ extract remained constant during these three months.

DISCUSSION

Nitrogen accumulation in maize plants occurs from July to August (Lubet and Juste, 1985). The active mineralization observed in the bare fallow plot coincided with the nitrogen uptake in the cultivated plots (Figure 1). The percentages of total N mineralized during

the incubations (4 %) were similar to reported literature values (Bonde and Rosswall, 1987). The best model for nitrogen mineralization followed zero-order kinetics. Linear kinetics, already reported by Houot *et al.* (1989), did not allow the determination of the potentially mineralizable nitrogen (N_o), as the classical first-order kinetics did (Dalal and Carter, 1987). The smaller constant rate k_n in the MN plot compared to the MON plot confirmed the depressing effect of fertilization on microbial activity (Wardle, 1992). By this method a labile and a more resistant pool of organic carbon were identified in the carbon mineralization. The labile organic matter decreased between spring and summer as shown by the lower labile pools of carbon (C_1) and smaller rate constants of nitrogen mineralization in the cultivated plots (k_n). This was probably due to the plant nitrogen uptake. In the bare fallow plot, the decrease of microbial biomass could explain the strong decrease of the labile pool C_1 of carbon and allowed the nitrogen mineralization to remain constant. Microbial C and N contents represented 2 % of total organic carbon and nitrogen, which was in the same range as obtained by Dalal and Mayer (1987).The microbial biomass could be considered as a "stabilized one" (Dalal and Mayer, 1987), as its C/N ratio was similar to the soil C/N ratio. Nevertheless, the C/N ratio tends to vary widely as the microbial biomass N extracted after fumigation k_N varies from 0.2 to 0.68. In our study, the k_N of 0.45 may be underestimated. In fact, the microbial biomass could be considered as an active pool since it decreased during the growing season (of 43 kg N ha^{-1} in the F plot, 37 kg N ha^{-1} in the MN plot and 31 kg N ha^{-1} in the MON plot) and increased after harvesting.

Many factors could be involved. In cultivated plots, the root development from June to August (Tardieu and Manichon, 1987) could reduce the microbial biomass carbon and nitrogen contents, perhaps through root competition (Wardle, 1992). Soil drying could also explain the biomass decrease (Wardle and Parkinson, 1990). In the bare fallow plot, the high temperatures and humidities emphasized the mineralization and the decrease of labile organic pools such as the microbial biomass. The microbial biomass could be identified as a labile pool with a rapid turnover which could immobilize and liberate large amounts of mineral nitrogen (Paul and Juma, 1981; Myrold, 1987). If the spatial variability was considered, the decrease of the microbial biomass between May and October could be estimated as 13, 8 and 6 % of the initial value in the F, MN and MON plots respectively, corresponding to a decrease of 12 % of active biomass found by Paul and Voroney (1980). After harvest, the residue incorporation constituted a readily hydrolyzable carbon source which stimulated the microbial growth and activity (Wardle, 1992).

The nitrogen and carbon amounts extracted by all the tested methods (Table 2) were within the range of results published by Fox and Piekielek (1978). The autoclaving method extracted the highest percentage of carbon and nitrogen (20 %), other extracted fractions represented 0.3 to 3.9 % of the total organic carbon or nitrogen. The C/N ratios of the extracts were very similar to those of the biomass (7.4 to 9.8), except in the incubations (C mineralized / N mineralized = 13) and the extraction with sodium bicarbonate (C/N = 4). The nitrogen turnover in biomass could explain the high C/N ratio in these incubations. In the sodium bicarbonate extraction, C/N ratios of 15 have been previously found (Michrina *et al.*, 1982; Fox and Piekielek, 1978). The autoclaving extract varied similar to that of the microbial biomass. However, some differences appeared in summer. Borate phosphate buffer, sodium bicarbonate and boiling water extractions remained constant from May to October. Carbon and nitrogen extracted by autoclaving and boiling water methods increased in June. The soil humidity and temperature would have furthered the adventive root residue immobilization in these two fractions. Boiling water and $NaHCO_3$ extracts fluctuated similarly because of their similar turnover rate which is more rapid than those

of the autoclaving extract and biomass. The two methods extract "glucose" from the same source (polysaccharides) with different effectiveness (Jenkinson, 1968).

The soil nitrogen supply estimated from the field results could be compared with the incubation results and with the chemical extractions (or to their decrease) during the growing season. As the most active period of soil and plant biological activities is between May and August (Figure 1), we considered the decrease of the biomass and of extractable carbon and nitrogen during this period (Table 3).

Table 3. Comparison between soil nitrogen supply, nitrogen mineralized during spring incubation and nitrogen extracted by the chemical methods (amounts at sowing time in May and decreases between May and August). Amounts of nitrogen expressed in kg ha^{-1}.

Plot	Soil supply	N* 168d	Biomass		Autoclaving		Boiling water		$NaHCO_3$		Bor./ Phos.**	
			May	May-Aug	May	May-Aug	May	May-Aug	May	May-Aug	May	May-Aug
F	91	96	133	35	1021	70	179	-	31	-	69	20
MN	-187	89	125	73	975	-	182	-	35	-	91	30
MON	79	96	110	74	963	269	169	-	35	-	78	-

* N after 168 days of incubation, extrapolated to the field considering a Q_{10} of 2
** Borate Phosphate
\- no decrease

The mineralized nitrogen during the spring incubation was extrapolated to the field considering a Q_{10} of 2 (Clark and Gilmour, 1983). The result of this extrapolation was approximately equal to the soil nitrogen supply in the bare fallow plot. In the F and MON plots, the soil nitrogen supply was similar to the nitrogen extracted with the borate phosphate buffer and to the nitrogen mineralized during the spring incubations. The nitrogen decrease in the microbial biomass and in the autoclaving extract between May and August could explain the soil nitrogen supply in the MON plot and the bare fallow plot respectively.

After harvest, the labile organic pools were reconstituted from the degradation of the crop residues and the organic matter liberation by ploughing. The spring incubations results did not explain the field differences. Boiling water and $NaHCO_3$ methods showed clear discrimination between different soils and agricultural situations (Gianello and Bremner, 1988) but did not allow us to predict soil nitrogen mineralization. The borate phosphate buffer method has correctly predicted the soil nitrogen supply in the bare fallow soil and the unfertilized maize. The autoclaving extract and the microbial biomass could be considered as the biological active pools involved in plant nutrition. The same behavior of the extracts in the three plots could be due to the recent differentiation of the treatments.

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Biological relevance of easily extractable organic nitrogen (EUF-N_{org}) for the estimation of nitrogen mineralization

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Abstract

Amounts of extractable organic, microbial and potentially mineralizable nitrogen were determined by electro-ultrafiltration (EUF-N_{org}), fumigation extraction (N_{mic}) and aerobic incubation (N_0) in arable soils. The variability and relation of these fractions were studied on a uniformly cultivated humic gley-pseudogley and on various luvisols and gleysols after changing management. Potentially mineralizable nitrogen comprised between 6 and 17 % of total N (N_t), whereas EUF extracted between 0.9 and 2.6 % of N_t. The spatial variability of all the fractions was related to total nitrogen distribution in the field under continuous rape-wheat rotation. The variability of the potential N mineralization rate was better predicted by total N (r^2 = 0.765) as compared to that by EUF-N_{org} (r^2 = 0.625) and N_{mic} (r^2 = 0.455). Management change such as 'grassland ploughing' and 'ploughing depth increase' were reflected in total and potentially mineralizable N rather than in the fraction EUF-N_{org}. For two independent data sets, the zero-order mineralization rate for the recalcitrant nitrogen fraction could be predicted from total N by similar regression equations. EUF-N_{org} appeared less suitable to predict N supply in the field, as it showed less differentiation, was inconsistently related to total and mineralizable N and failed to describe crop rotation effects on nitrogen turnover.

INTRODUCTION

Several biological and chemical methods have been employed to assess the availability of nitrogen to plants in arable soils under field conditions (Stanford, 1982). Mild extractants like dilute calcium chloride solution (Houba *et al.*, 1987) and electro-ultrafiltration (EUF) (Nemeth, 1985) have been suggested to determine the fraction of organic nitrogen (N_{org}) susceptible to mineralization in the following season. The respective N_{org} in the extracts, however, have proved to be poorly correlated with the computed nitrogen supply during the season (Olfs, 1992). Also questions arise concerning the origin and the dynamics of the extracted organic nitrogen.

To evaluate a chemical extractant it is critical to know whether the extracted fractions

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represent potentially mineralizable nitrogen and biological activity in the soil, i.e. microbial biomass. Juma and Paul (1984) found that mineralizable nitrogen was more strongly related to biomass-derived nitrogen than any chemically extracted fraction. According to Kohl and Werner (1986), extracted N (EUF and $CaCl_2$) and biological activity were correlated. This was confirmed by the findings of Recke *et al.* (1990) and Danneberg *et al.* (1989) which showed that nitrogen compounds derived from microbial cells were present in the extracts. However, these compounds comprise only a minor fraction in the extracts (Nemeth *et al.*, 1986).

Furthermore, it is the question whether these compounds which may be released during sample preparation (drying) also will be important under field conditions. Richter *et al.* (1989a) showed by incubation experiments with dried and field-moist soils that the N flush after drying could be related to the autolyzed biomass, which did not appear in field-moist samples. It is not known how natural, *in-situ*, nitrogen mineralization rates relate to EUF-extractable N and the biomass, and whether biomass and extractable N are correlated. Further, the variability of the N_{org}-fraction in the field and its ability to reflect different management practices is yet to be determined.

The present study was, therefore, conducted with the following objectives: (i) to assess the relationship between different N availability indices, namely extractable organic nitrogen, nitrogen in the microbial biomass and the potentially mineralizable N accumulated during incubation, (ii) to compare the spatial variability of the above fractions on the field scale and to relate it to soil properties, and (iii) to assess whether the long-term and the short-term changes in management practices are reflected by the different fractions.

MATERIALS AND METHODS

Soils

The data were collected in 1989 from two sets of soil samples which will hereafter be called (i) 'Spatial Variability' and (ii) 'Management Change' sites. The soil was collected with a 3-cm diameter core sampler, sieved (< 6.5 mm), gently homogenized and partitioned for incubation, fumigation and chemical extraction (drying at 40 °C) studies. Organic carbon was determined by dry combustion, and total nitrogen by the micro-Kjeldahl method.

Spatial Variability Site

The site was located on an arable field of 1.1 hectare size located on a pseudogley-gley of varying organic matter (2-8 %) and clay content (12-18 %). It had a moderate slope (< 1 %) with westward inclination coinciding with a difference in water table depth ranging from 1.5 to 2.7 m below surface. The site has been cultivated uniformly with a crop rotation of oil-seed rape-wheat-barley since 1960. Before 1960, part of the field had been used as grassland.

The topsoil (0-25 cm) was sampled in January, 1989 at 41 locations on a 15 by 15 m square grid containing 13 columns and 4 rows, randomly omitting 11 samples. Each sample was a composite of three cores. The samples were taken to the laboratory in a cooler and processed immediately.

Management Change Sites

Two sets of soils were included in the comparison of extractable and potentially mineralizable organic N: (a) 12 gleysols which had previously been used as grassland and turned into arable land between 1956 and 1988, completed by three permanent arable soils and (b) luvisols with an increased depth of ploughing introduced between 1967/70 and 1980/82.

The luvisols showing the decline of net nitrogen mineralization after deepening the plough-layer were earlier described in detail (Richter *et al.*, 1989a). The samples were typical for North-west German loess soils (Hannover) used for sugar beet - winter wheat cultivation containing 11 to 18 % clay and approximately 2 % organic matter. The gleysols originated from the same region and a South-West German catchment (Karlsruhe). The gleysols could be grouped into sandy loam (10-20 % clay) and clay loam (25-37 % clay) containing 2-13 % organic matter.

Electro-Ultrafiltration (EUF)

The EUF technique, as described by Nemeth (1982, 1985), involves continuous extraction of an aqueous soil suspension using variable temperature (20 and 80 °C) and voltage (200 and 400 V, equivalent to ≤ 15 and ≤ 150 mA, respectively). Five grammes dry soil were analyzed in duplicate. Nitrate, ammonium and total nitrogen were determined in the combined anode and cathode filtrates by a continuous flow system (Technicon Auto-Analyzer). Total N was determined as nitrate after oxidation by UV radiation in the presence of potassium persulfate under alkaline conditions. N_{org} was calculated as the difference between total and mineral N in the solution.

Fumigation-Extraction-Method (FEM)

Field-moist soil equivalent to 50 g dry soil was extracted with 200 ml 0.5 M K_2SO_4 (Brookes *et al.*, 1985). A parallel sample was fumigated with 30 ml of ethanol-free chloroform for 24 hours at 25 °C in the dark. After evaporating the chloroform the samples were extracted with 0.5 M K_2SO_4 as described above. Total nitrogen in the extracts was determined in an aliquot of 30 ml by steam distillation (Kjeldahl). The microbial nitrogen, N_{mic}, was calculated from the difference between total N in the fumigated and unfumigated sample. The value so obtained was multiplied by a k_N-factor of 2.22 to correct for the incomplete extractability of autolyzed microbial N (Brookes *et al.*, 1985).

Incubation experiments

The incubation procedure introduced by Stanford and Smith (1972) was slightly modified in that field-moist instead of dried soil was used (Richter *et al.*, 1982). Triplicate samples were incubated per soil, in the spatial variability study a single sample at each location was used. Soil (20 g) was mixed with quartz sand, filled into leaching tubes and incubated at 35 °C. During ten to twelve weeks of incubation the samples were leached 5 to 9 times with 100 ml 0.01 M $CaCl_2$-solution followed by the addition of 20 ml nitrogen-free nutrient solution. Nitrate and ammonium in the leachate were assayed by an automated continuous-flow system (Chemlab Instruments).

Parameter estimation

The cumulative amount of nitrogen mineralized, N(t), at any time, t, during the incubation may be described by a double exponential model (Eq. 1).

$$N(t) = N_d (1 - e^{-k_d * t}) + N_r (1 - e^{-k_r * t}) \quad (1)$$

Where, N_d and N_r (mg kg^{-1}) represent an easily decomposable and a more resistant organic nitrogen fraction and k_d and k_r (day^{-1}) are the respective rate coefficients (Richter *et al.*, 1982). The sum of N_d and N_r represents the potentially mineralizable N, N_0.

Due to large errors associated with the estimated parameters of the resistant organic nitrogen fraction (N_r, k_r) a degenerate form of the double exponential model (Eq. 2) assuming zero-order kinetics, may be derived (Richter *et al.*, 1989a).

$$N(t) = N_d (1 - e^{-k_d * t}) + C_r * t, \quad (2)$$

where C_r (mg kg^{-1} day^{-1}) is the approximate product of N_r and k_r.

The parameters for the two equations were estimated from the cumulative amount of nitrogen mineralized, N(t), (mg kg^{-1}), at different times of incubation, t (days), by an iterative non-linear regression procedure using the Marquardt algorithm (Statgraphics). The estimates and mean soil properties were tested for homogeneity of variance by Bartlett's test. The differences between groups were evaluated using the Scheffe test (Sachs, 1980). Linear regression analysis was employed to relate parameter estimates and soil properties.

RESULTS

Spatial variability of N availability indices

The means of the major nitrogen fractions based on the individual estimates were significantly different for the pseudogley-gleysol and the humic gleysol (Table 1). On an average EUF extracted 1.1 and 1.55 % of the total nitrogen, whereas 2 and 3.5 % of the total N in the respective soils was microbially bound. The average cumulative nitrogen mineralized during the incubation, N_{cum}, comprised 4.6 and 3.8 % of N_t for both soil subunits. For the parameters of the slow-release potentially mineralizable nitrogen (N_r, k_r), no statistically sound estimate could be found in 90 % of the individual cumulative mineralization curves. The mean potential mineralization rate, C_r, describing the mineralization between day 28 and 79, was significantly higher ($p \leq 0.001$) in the humic gleysol used as grassland until 1960. However, there were no differences in the parameters for the easily decomposable N fraction (N_d, k_d), which is supposed to reflect the crop residue effects. The parameters estimated from the mean mineralization curves (Figure 1) were only slightly different from the means of the individual estimates (Table 1).

Table 1. Nitrogen availability indices on the soil subunits of the "Spatial Variability Site". Average ± standard deviation, significance level of the difference. Total soil N (N_t); EUF-extractable organic N (EUF-N_{org}); microbial N (N_{mic}); potential and rate constant of decomposable N (N_d, k_d); potential mineralization rate of recalcitrant N (C_r).

Soil Index	[Pseudo]-Gley long-term arable (n = 25)		Humic Gley ploughed-in grassland (n = 16)		Sig. level
N_t (g kg^{-1})	1.42	± 0.17	2.78	± 0.73	≤ 0.001
EUF-N_{org} (mg kg^{-1})	22.0	± 2.0	31.0	± 3.5	≤ 0.01
N_{mic} (mg kg^{-1})	49.1	± 5.8[1)]	55.8	± 9.9	≤ 0.05
N_d (mg kg^{-1})	24.3	± 10.0	26.7	± 9.6[2)]	n.s.
k_d (day^{-1})	0.153	± 0.24	0.083	± 0.063[2)]	n.s.
C_r (mg kg^{-1} day^{-1})	0.530	± 0.11	1.03	± 0.30[2)]	≤ 0.001

[1)] n = 24; [2)] n = 13

All nitrogen availability indices showed a distinct spatial distribution following the trend of low values in the upslope located pseudogley-gleysol increasing towards the humic gleysol. The spatial trend of these data followed the total nitrogen distribution in the field (Figure 2).

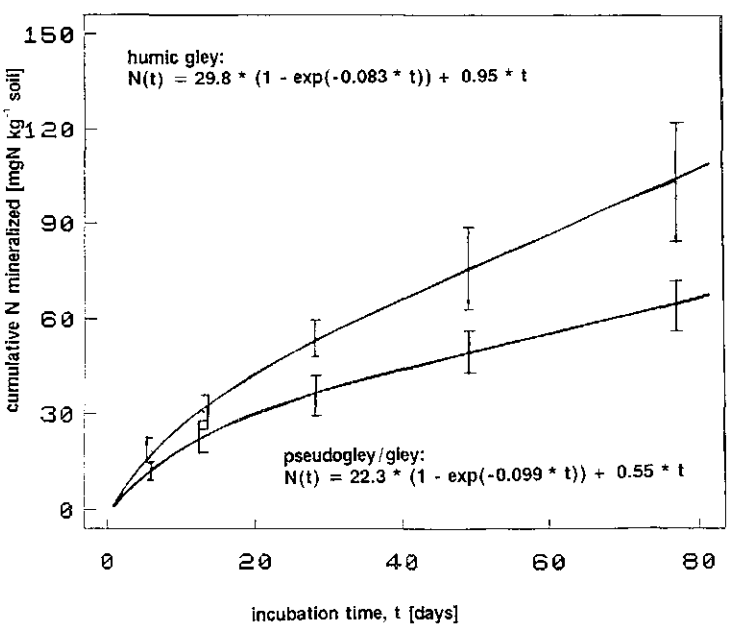

Figure 1. Mean cumulative nitrogen mineralization of the two soil subunits in the pseudogley - gleysol arable field ("Spatial Variability Site").

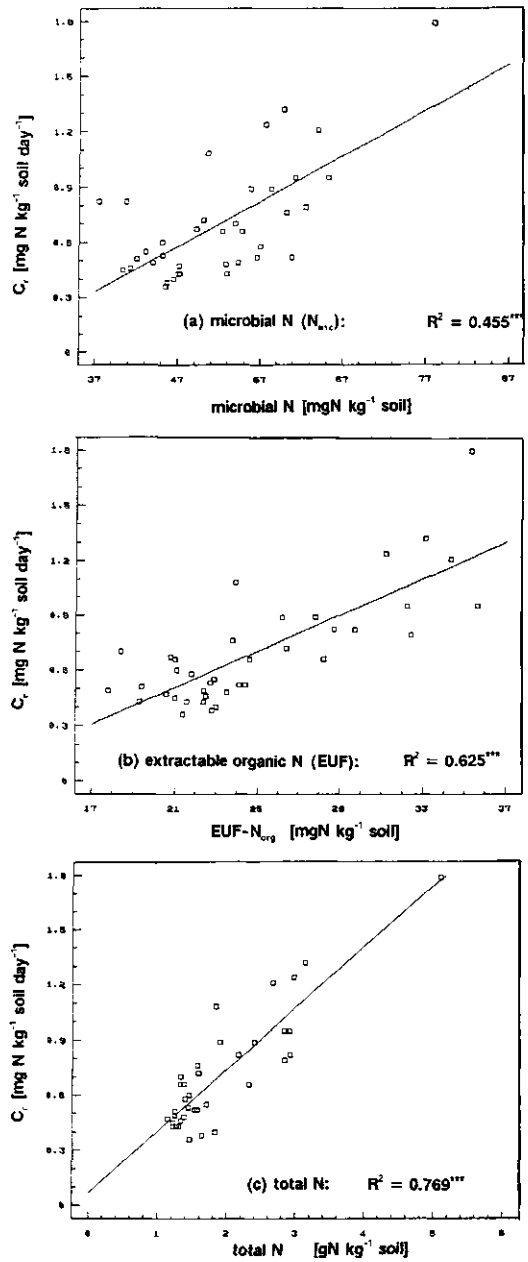

Figure 2. Regression of the potential mineralization rate, C_r, on (a) microbial N, (b) EUF-extractable N_{org} and (c) total nitrogen for the "Spatial Variability Site".

For practical purposes, these results suggest, however, that such arable sites have to be divided and treated separately with respect to the nitrogen dynamics and organic matter turnover. The separate units again, may be represented by mean values.

Management Change Sites

Grassland ploughing. There were distinct differences in nitrogen mineralized and extracted between soils of different texture, regional origin and management history (Table 2).

Table 2. Properties and nitrogen fractions for soils under permanent arable (A) and previous grassland use (GU) until different years (Yr) of turnover in the catchments of Karlsruhe (K) and Hannover (H).

Region & site	Use	Yr	Texture 1)	%clay	N_t (g kg^{-1})	N_{org} 2) (mg kg^{-1})	N_0 3)	C_r (mg $kg^{-1}d^{-1}$)
K 39	GU	88	cU	22	2.90	42.1	234 ± 16	0.83
K 37	GU	86	suL	21	2.69	34.2	270 ± 18	1.19
K 41	GU	62	cU	20	3.32	41.6	301 ± 6	1.18
K 38b	GU	56	suL	15	1.39	19.6	165 ± 19	0.96
K 45	A	-	lS	10	1.20	20.9	138 ± 7	0.68
K 36b	GU	88	uL	21	2.91	36.3	188 ± 20	1.05
K 36a	GU	86	ucL	37	4.52	40.0	350 ± 21	1.69
K 38a	GU	71	scL	37	2.74	39.7	239 ± 50	1.12
K 42	GU	65	ucL	31	2.55	44.2	275 ± 17	1.09
K 46	A	-	cL	-	2.90	42.0	260 ± 13	1.42
H 8	GU	84	slU	12	1.31	34.6	390 ± 46	0.94
H 5	GU	75	uL	24	2.05	36.5	240 ± 12	0.78
H 2	GU	60	uL	19	3.00	33.6	172 ± 10	0.46
H 7	GU	60	slU	17	1.82	31.7	198 ± 13	0.65
H 2	A	-	sU	7	0.85	14.4	118 ± 6	0.32

1) S(s) sand(y); U(u) silt(y); C(c) clay(ey); L(l) loam(y); class adapted from AG Bodenkunde (1982), pp. 80-84.
2) EUF-extractable N_{org}
3) Mean of the estimate and standard error

For several arable sites where grassland had been ploughed in the past (1956-1988) the mineralization potential (N_0) could be estimated by equation (1). With one exception (K 38a), the standard error of the estimate (± SEE) ranged between 6 and 21 mg kg^{-1}, corresponding to approximately 5 to 10 %. It was largely aligned with the decomposable nitrogen fraction, N_d, which could not be estimated for two soils (K 41, 46). Especially in the sandy and silty loam soils (s, uL), more nitrogen was mineralized from the ploughed grassland samples during incubation in comparison to some permanent arable soils of the same region (K 45, 46 and H 2).

Between 6 and 12 % of total N was estimated to be potentially mineralizable. The effect of the ploughing date was apparent although not significant. On average, in recently ploughed grassland (1984-1988) 270 ± 86 mg N kg^{-1} of soil were potentially mineralizable, compared to 209 ± 62 mg N kg^{-1} in early ploughed grassland (1956-1960) and 128 ± 10 mg N kg^{-1} in permanent arable soils. For the clay loam sites (cL) the time-dependent decrease of total N and mineralization potential according to ploughing date, were not as

distinct.

By EUF, between 14 and 44 mg kg^{-1} organic N may be extracted, corresponding to 0.9 and 2.6 % of the total nitrogen. Little differences were found between former grassland soils according to their ploughing date, especially among soils with greater clay content. In sandy loam soils (K 45, H 2), the EUF-extractable nitrogen is much lower in permanent arable soils, representing a larger percentage of N_t (2.6 and 1.7 %, respectively).

The regional means of the estimated potential mineralization, N_0, and rate, C_r, were significantly higher ($p \leq 0.05$ and $p \leq 0.01$, respectively) for the 'Karlsruhe' than for the 'Hannover' samples. EUF-extractable organic nitrogen was not significantly different. It is obvious that there is greater differentiation for these sites by total and mineralizable nitrogen than by extractable organic nitrogen (EUF-N_{org}). Especially, for the clay loam samples there was almost no difference in EUF-N_{org} (CV = 8 %), whereas the mineralization potential varied considerably (CV = 27 %).

Ploughing depth increase. Generally, all nitrogen availability indices studied here showed the dilution effect in soils with more recently increased ploughing depth (Table 3).

Table 3. Total, cumulative (N_{cum}) and potentially mineralizable nitrogen (N_0) during incubation (35 °C) and EUF-extractable nitrogen of arable soils with variable date for increasing the ploughing depth from 25 to 35 cm. Mean ± standard deviation.

Period	Total N (g kg^{-1})	N_{cum}	N_0 (mg kg^{-1} soil)	EUF-N_{org}
1967/70	1.11 ± 0.07	72 ± 6	188 ± 47	47.2 ± 1.9
1970/72	1.07 ± 0.27	65 ± 7	146 ± 23	44.5 ± 2.3
1980/82	0.93 ± 0.06	58 ± 3	143 ± 24	44.6 ± 2.7

Again, the EUF-extractable nitrogen, both organic and total N, did not vary as much as the total soil N and the fractions determined by incubation. While 16-19 % of the total soil nitrogen was estimated to be potentially mineralizable, only 4.1-4.8 % could be extracted by EUF. The small differences between the 'treatments' were due to variability of preceding crops and plant residues (wheat, sugar beet) and stubble treatments (liming and N fertilizer application). The crops influenced extractable (EUF-N_{org}) as well as potentially mineralizable nitrogen (N_{cum}, N_0) preventing significant differences for either between the treatments. It may also be noted that EUF-N_{org} in this batch was about twice that of the other permanent arable sandy loam soils.

Relationship between nitrogen availability indices

In the "spatial variability site", a strong relationship between the slow-release mineralization rate (C_r) and the various nitrogen fractions was found (Table 4). Obviously, the den-

sity of the microbial population affects the turnover rates (C_r, k_d), which also depends on nitrogen supply (N_t) as the correlation between total N and N_{mic} indicates. The EUF-extractable fraction (EUF-N_{org}) was significantly ($p \leq 0.001$) related to N_t ($R^2 = 0.76$). In comparison, organic N extracted by 0.5 M K_2SO_4 was not a good indicator of N turnover, thus indicating that EUF-N_{org} may have some meaning for microbial activity. The correlation between microbial nitrogen, N_{mic}, and EUF-N_{org}, however, was weak ($R^2 = 0.26$). Despite the fact that these fractions were of the same order of magnitude as the easily decomposable nitrogen (N_d), they were not significantly related to this fraction.

Table 4. Correlation matrix for N availability indices in the "Spatial Variability Field". Organic N in the 0.5 M extract, N_{org} (K_2SO_4), for other variables see Table 1.

	N_d	k_d	N_{mic}	N_t	N_{org} (EUF)	N_{org} (K_2SO_4)
C_r	-0.248	0.120	0.675#	0.877#	0.791#	0.306
N_d		-0.419	-0.002	0.061	0.083	-0.159
k_d			0.405*	-0.037	0.057	0.037
N_{mic}				0.605#	0.509#	0.282*
N_{total}					0.872#	0.409+
N_{org} EUF						0.383*

Significance levels: * $p \leq 0.05$, + $p \leq 0.01$, # $p \leq 0.001$; n = 36-41.

For the 'spatial variability' field, the potential mineralization rate, C_r, may be predicted with increasing certainty in the order N_{mic} < EUF-N_{org} < total N (Figure 2). The relationship between EUF-N_{org} and total N was highly significant ($R^2 = 0.76$), but its slope was small. While values for total N varied by a factor of 5, the organic nitrogen in the EUF extracts varied by a factor of approximately only 2 which was true for microbially bound nitrogen, N_{mic}. The extracted fractions, therefore, represent only part of the available nitrogen released through the mineralization process.

In the data set of "ploughed grassland" soils according to the correlation matrix, the mineralization potential, N_0, and mineralization rate of the recalcitrant fraction, C_r, can be predicted with similar certainty from total and EUF-extractable N (Table 5).
In this respect, it is important to note the strong correlation between clay and EUF-N_{org}.

Table 5. Correlation matrix for N availability indices of arable soils with former grassland use.

	EUF-N_{org}	N_0	C_r	Clay[1)]
N_t	0.746+	0.857#	0.657+	0.662+
EUF-N_{org}		0.799#	0.618*	0.741+
N_0			0.836#	0.748+
C_r				0.706+

Significance levels: * $p \leq 0.05$, + $p \leq 0.01$, # $p \leq 0.001$.
1) n = 14.

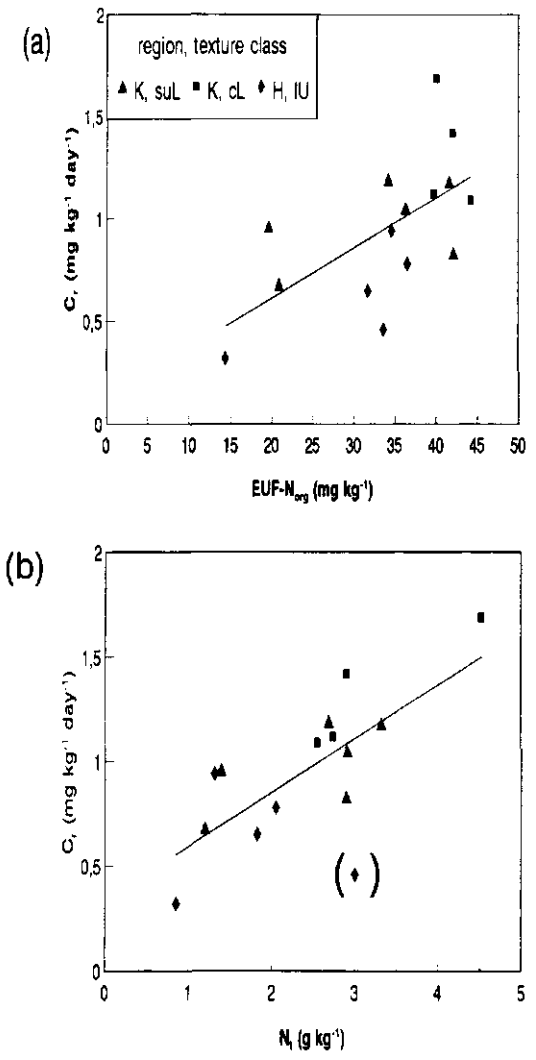

Figure 3. Regression of the potential mineralization rate (C_r) on (a) extractable (EUF-N_{org}) and (b) total soil nitrogen (N_t) in different sandy loam and clay loam arable fields after grassland ploughing.

The regressions shown in Figure 3 confirm the above finding that the mineralization rate, C_r, can be predicted from total nitrogen with greater certainty than from EUF-N_{org} (R^2 = 0.43; R^2 = 0.38, respectively). The mineralization rate, C_r, for soil "2 GU" in the "Hannover" region was lower than the mean value of the same humic gley (Table 1). Changing the "()"-value, the correlation of C_r with N_t improved more than with EUF-N_{org} (R^2 = 0.63; R^2 = 0.43, respectively). The slopes of the regression between C_r and N_t were similar for the data sets of the spatial variability site ($3.4* 10^{-4}$) and grassland ploughing sites ($2.6*10^{-4}$). In both data sets, the sensitivity of the methods can be seen from the relative scatter of the nitrogen fractions which decreased from microbial N and EUF-N_{org} towards total nitrogen, N_t.

DISCUSSION

Statistically, EUF-N_{org} has some favorable properties, which became apparent on the "spatial variability site". Due to small spatial variation, one needs fewer samples to represent a field average, similar to that calculated for total N (Widmer and Richter, 1989). The minimum number of samples to be taken for a tolerable deviation of 10 or 5 % were calculated based on the respective standard deviations and 95 % certainty of the mean. For long-term arable fields the number of samples for a correct estimation of EUF-N_{org} were small ($n_{p\leq0.10}$ = 5; $n_{p\leq0.05}$ = 20) compared to that for the potential mineralization rate C_r ($n_{p\leq0.10}$ = 13; $n_{p\leq0.05}$ = 44). The spatial analysis of the parameters describing nitrogen dynamics also showed that the variability in EUF-N_{org} could simply be explained by a trend along the rows. For extractable nitrogen, there was no short-range, isotropic spatial variability as detected for total and mineralizable N on basis of the detrended data (Richter *et al.*, 1989b, 1994).

In a mechanistic sense, however, the EUF-extractable fraction of organic nitrogen should be characterized by its sensitivity to reflect the changes in soil nitrogen dynamics. Sensitivity, defined as the size of deterministic variation relative to the background variation ('noise'), is lower for EUF-extractable nitrogen compared to total nitrogen. EUF-N_{org} varied less over a broad range of samples. Predicting the mineralization rate, its sensitivity was about half of that determined for total nitrogen. Appel and Mengel (1992) also found a greater prediction certainty for N-uptake by cereals using total nitrogen instead of extractable organic N (EUF-N_{org}, $CaCl_2$-N_{org}).

Furthermore, the relationship between EUF-N_{org} and other soil properties was inconsistent. The "spatial variability" data highlight the interdependence of various N availability indices without the interference of variable management, crops and residue inputs. Thus, the sole mineralization of soil organic N became apparent, which may be termed as "baseline mineralization". The correlation between EUF-N_{org} and total N was not as strong for widely varying soils as on the "spatial variability" plot (R^2 = 0.56 and 0.76, respectively). The variations in EUF-N_{org} were related to texture and were not consistent with management dependent changes in mineralizable nitrogen.

The correlation between potential N mineralization rate, C_r and EUF-N_{org} in the "spatial variability" site suggested the biological relevance of EUF-extractable organic N. However, the weaker correlation with microbial N (N_{mic}) indicates that the two fractions extracted by FEM and EUF may have a completely different character and origin. Fumigation releases mineral nitrogen (25 %), α-amino-N (-20 %) and proteins (-3%) and small peptides as major compounds from cells (Joergensen and Brookes, 1990). These N fractions comprise only a minor part in the EUF extract (Recke *et al.*, 1990). Therefore, we conclude that EUF-N_{org} may primarily represent the unspecific soluble organic nitrogen. This is consistent with the strong correlation observed between EUF-N_{org} and total N on the "spatial variability" site pointing to fulvic and humic acids in the extract. Microbial nitrogen also showed the effect of grassland ploughing. For sandy loam soils, as determined on a subsample (Hannover), microbial nitrogen decreased after ploughing. From 180 mg N kg^{-1} in grassland soil microbial N decreased to 92, 86 to 71 mg N kg^{-1}, depending on the date of ploughing (G 8, 5 and 7, respectively). It was thus significantly ($p \leq 0.001$) higher than in permanent arable soils (42.4 ± 9.6 mg N kg^{-1}) as shown by Widmer (1993). EUF-N_{org} did not show these differences as can be seen in former grassland soils of the Hannover region (Table 2).

None of the measured parameters showed significant differences in the long-term effect of organic matter dilution due to "deeper ploughing". The cumulative nitrogen

mineralization was the most sensitive parameter. It showed the original net mineralization not to be reached before a decade or more had passed (Richter *et al.*, 1989a). Qualitatively, the effect was indicated by EUF-N_{org} similar to total N, with little additional information. More critically, the absolute amount of organic nitrogen, extracted by EUF was about twice that of comparable soils. This examplifies the influence of sample preparation on this nitrogen fraction. The drying process prior to extraction, may have influenced the absolute amount of EUF-N_{org} (Süß and Maier, 1990) thus explaining the difference between batches of similar arable soils.

Considering the short-term effects by crop residues, the fraction of EUF-N_{org} contradicts field observations. The statistical analysis of more than 80 samples used for the comparison of fertilizer recommendations by EUF and N_{min} (Richter *et al.*, 1989c) revealed that the differentiation of EUF-N_{org} according to crop residues was very small (Figure 4).

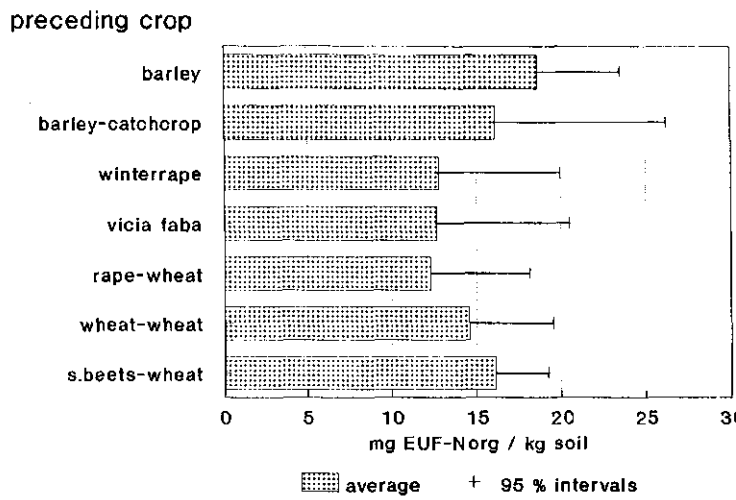

Figure 4. EUF-extractable organic nitrogen for various crop rotations determined after harvest. Mean and 95 % confidence intervals (Scheffe-test).

Moreover, the EUF-extractable nitrogen was higher in those soils where net mineralization of nitrogen was expected to be low due to large carbon inputs (grain stubble + straw). The reverse was true for sites previously used for cover crops with easily mineralizable residues.

In conclusion, the EUF-extractable organic nitrogen (EUF-N_{org}) may predict mineralization of soil nitrogen. However, the sensitivity of this fraction is lower than total nitrogen which has to be determined only once in a "steady state" system. Changes in the N dynamics due to management alteration are not as distinctively expressed by EUF-N as by total N, especially in clay loam soils. Therefore, due to lack of a mechanistic explanation of EUF-N_{org}, this method cannot reliably estimate soil nitrogen supply to the crop. For practical purposes, it seems promising to relate mineralization of soil nitrogen to total N and clay content. Short-term nitrogen turnover may be related to the amount and quality of nitrogen and carbon fluxes following crop growth, residue inputs and management.

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Amino sugar N in soil extracts and its relationship to the net N mineralization of 20 soils

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Abstract

To elucidate the chemical nature of the 'N_{org}' of EUF (electroultrafiltration) and $CaCl_2$ extracts, a procedure for the determination of amino sugars in soil extracts was developed. Net N mineralization of 20 soils was determined in a pot experiment and the extracts of the soil samples were analyzed for their concentration of amino sugar N. Although the amino sugar N concentrations of EUF and $CaCl_2$ extracts were low with a mean of 1 mg N kg^{-1} for both procedures, there was a significant correlation between net N mineralization and extracted amino sugar N in the treatments without N fertilizer application.

INTRODUCTION

The extraction methods electroultrafiltration (EUF) (Nemeth, 1979) and $CaCl_2$ (Houba *et al.*, 1986) are well known for the determination of soil nitrogen. With both techniques NO_3^-, NH_4^+ and an organic N fraction (N_{org}) are obtained. NO_3^-, NH_4^+ and N_{total} are analyzed and N_{org} is calculated as amount of N_{total} minus amount of inorganic N. Both methods extract identical amounts of inorganic N, whereas with EUF two times higher concentrations of N_{org} were obtained as compared with $CaCl_2$ (Appel and Steffens, 1988). It is assumed that the extracted organic N compounds are amino acids, amino sugars and heterocyclic N compounds (Mengel, 1991). EUF N_{org} was found to contain only low amounts of free amino acids but about 23-55 % of N_{org} was protein N (Hütsch and Mengel, 1993; Nemeth *et al.*, 1988).

The objective of this investigation was to determine the concentration of amino sugars in EUF and $CaCl_2$ extracts and their relationship to the net N mineralization of 20 soils.

In: J.J. Neeteson and J. Hassink (eds.), 1994. Nitrogen mineralization in agricultural soils; proc. symp. held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993. AB-DLO Thema's, AB-DLO, Haren, pp. 59-63.

MATERIALS AND METHODS

N mineralization assay

A pot experiment was carried out to determine the net N mineralization of 20 soils. Field-fresh samples in three treatments and four replications were taken between 30th March and 29th October 1990. The main soil characterisitics at the beginning of the experiment are shown in Table 1.

The treatments were: 1. non cropped/without N fertilization, 2. planted with *Lolium multiflorum*/without N fertilization, and 3. planted with *Lolium multiflorum*/with N fertilization to give a final mineral N concentration of 100 mg N kg^{-1} soil.

In the soil samples the amounts of EUF N according to Nemeth (1979) and $CaCl_2$ N (Houba *et al.*, 1986) were determined, and the N concentration in the plant material was analyzed. Net N mineralization was calculated as amount of N taken up by the plants minus inorganic N at the beginning plus inorganic N at the end of the experiment.

Determination of amino sugars in EUF and $CaCl_2$ extracts

Amino sugar determination was carried out by means of a DIONEX 4000i ion exchange chromatograph equipped with a pulsed amperometric detector. Separation was performed on DIONEX CarboPac PA1 columns at room temperature. A 10 mM sodium hydroxide solution was used as eluent with a flow rate of 1 ml min^{-1}. Calibration was performed with standard mixtures of galactosamine, mannosamine and glucosamine. Sample preparation is demonstrated in Figure 1.

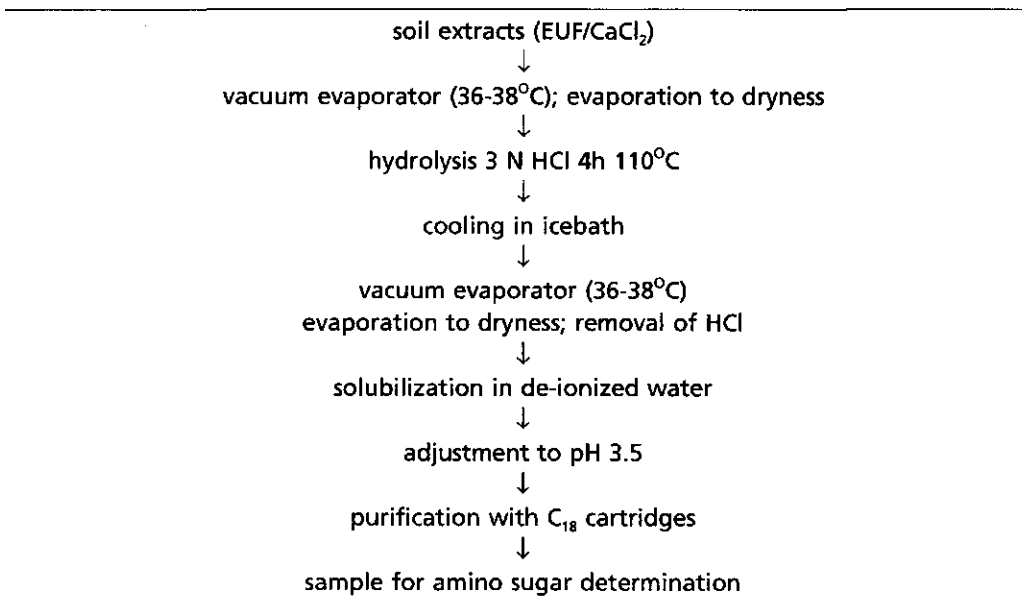

Figure 1. Sample preparation for the determination of amino sugars in soil extracts.

Table 1. Characteristics of the 20 experimental soils.

Soil no.	Land* use	Texture			pH ($CaCl_2$)	EUF N_{org}	$CaCl_2$ N_{org}
		sand (%)	silt (%)	clay (%)		(mg N kg^{-1})	
1	A	13	69	18	6.4	22.8	6.6
2	A	11	70	19	6.1	18.0	7.1
3	A	20	66	14	6.5	17.9	7.8
4	G	9	74	17	5.8	22.6	15.1
5	A	9	80	11	5.9	18.4	8.4
6	A	6	76	18	5.5	19.7	8.7
7	A	8	80	12	7.2	18.5	6.4
8	A	9	80	11	7.2	21.6	7.4
9	A	17	65	18	5.6	21.9	9.0
10	A	56	36	8	5.4	15.2	7.9
11	A	28	55	17	5.5	19.0	10.3
12	A	29	47	24	5.1	11.9	7.5
13	A	11	74	15	6.0	17.2	7.5
14	A	31	50	19	6.3	19.8	8.4
15	G	31	48	21	6.4	28.0	15.8
16	A	5	87	8	4.1	15.9	9.7
17	F	19	70	11	4.9	18.9	16.5
18	A	73	23	4	5.5	11.1	6.2
19	A	69	25	6	6.8	13.7	5.6
20	A	67	26	7	5.2	12.0	10.4
mean		26	60	14	5.9	18.2	9.1

* A: arable soil; G: grassland; F: forest soil

RESULTS AND DISCUSSION

Net N mineralization was significantly higher in treatment 1 (48-140 mg N kg^{-1} soil) than in the two other treatments. In treatment 2 net N mineralization was much lower (9-36 mg N kg^{-1} soil), whereas in treatment 3, with the exception of the forest soil, net N mineralization was negative (0 to -28 mg N kg^{-1} soil), which means that inorganic N was immobilized.

There were no free amino sugars, neither in EUF nor in $CaCl_2$ extracts. The described sample preparation allowed the determination of galactosamine, mannosamine and glucosamine in hydrolyzed soil extracts. The mean amino sugar N concentration of EUF and $CaCl_2$ extracts was 1 mg N kg^{-1} soil for the soil samples from March 1990 (Figure 2). These results are in agreement with other authors, who found amino sugars in soil hydrolysates and assume that these amino sugars mainly originate from bacteria and fungi (Kögel and Bochter, 1985).

Amounts of amino sugars extracted by EUF were almost the same as extracted by $CaCl_2$ and both fractions were closely correlated ($r = 0.83^{***}$). EUF N_{org} contained 3-9 % amino sugar N and $CaCl_2$ N_{org} 6-16 % amino sugar N. The same results were found for the samples from October 1990 (end of the experiment). This may be explained by the transitional character of the organic N fraction, i.e. the amount of amino sugars mineralized and produced during the incubation procedure were similar. The amino sugar N content of both extraction procedures at the end of the experiment correlated with $r = 0.94^{***}$.

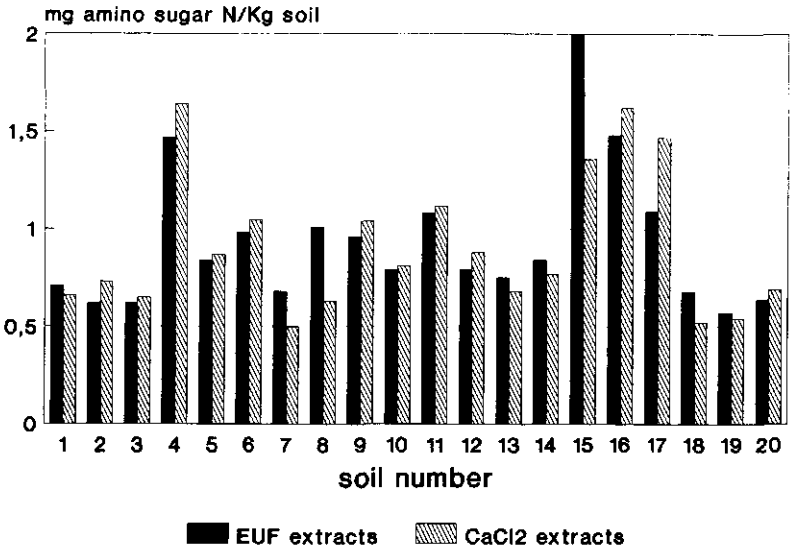

Figure 2. Amino sugar N in hydrolyzed EUF and $CaCl_2$ extracts of 20 soils from a pot experiment (March 1990).

Although the amounts of amino sugar N in the soil extracts were low, there was a positive relationship to the net N mineralization of the treatments without N fertilization (Table 2).

The result that there was no correlation between the net N mineralization of the N fertilized treatment with the amino sugar N may be explained by the low amounts of amino sugars compared to the total mineral N concentration of the soil.

Table 2. Correlation coefficients (r) of N fractions March 1990 versus net N mineralization in three different treatments.

	Net N mineralization		
	Treatments		
	1 non-cropped/- N	2 planted/- N	3 planted/+ N
EUF			
Amino sugar N	0.54*	0.55*	-0.11
N_{min}			-0.49*
N_{org} 1.fraction	0.29	0.45*	0.25
N_{org} 2.fraction	0.53*	0.65**	0.36
n_{org} 1.+ 2.fraction	0.36	0.52*	0.28
$CaCl_2$			
Amino sugar N	0.27	0.49*	0.06
N_{min}			-0.46*
N_{org} 1.fraction	0.64**	0.78***	0.39*
N_{org} 2.fraction[1)]	0.74***	0.80***	0.36
N_{org} 1.+ 2.fraction[1)]	0.75***	0.86***	0.42*

[1)] $CaCl_2$ 2.fraction according to Appel and Mengel (1990).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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Measurement of nitrogen mineralization and immobilization fluxes in soil as a means of predicting net mineralization

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Abstract

The usual methods proposed to predict net mineral N available to crops, i.e. chemical and biological methods and long-term incubations, are analyzed. The failure in the extrapolation of laboratory data and models to true N fluxes *in situ* mainly results from the role of gross mineralization and immobilization. The processes determining rates of gross fluxes are discussed. Many methodological and theoretical problems are posed by the measurements of true N fluxes under field conditions. First estimations of these fluxes in different crop systems show that the net mineralization rate results from high rates of gross *mineralization and immobilization.*

INTRODUCTION

There has been considerable research over the last thirty years into the factors and conditions determining net mineralization of nitrogen in soil. The main practical application of this research was the development of methods for predicting the soil nitrogen supply to a crop, on a year or a half-year basis, in order to improve fertilizer recommendations. Such methods are still needed, but the new environmental constraints placed on agricultural production, e.g. minimizing nitrate leaching, also make it necessary to predict nitrogen availability in soil over a shorter timescale. According to Johnston and Jenkinson (1989) 'we need to know more about the amount and time at which nitrate is supplied from soil reserves, organic inputs and the atmosphere'. This paper analyzes the methods used to assess net mineralization in soil, discusses the processes determining net mineralization, and presents some results on the gross mineralization and immobilization fluxes under field conditions.

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EVALUATION OF METHODS FOR ASSESSING NET MINERALIZATION

Two types of methods are used to assess net mineralization in soils: i) methods providing 'N availability indexes', and ii) methods defining 'potentially mineralizable N'.

A great number and variety of methods for defining N availability indexes have been suggested. These indexes are used as reference values of soil mineralization capacities. The methods, which include chemical and biological tests, have been reviewed by Stanford (1982). Gianello and Bremner (1986b) have compared different tests: the chemical methods vary in the nature of the extractant used (neutral, acidic or alkaline), and the temperature and time of extraction; the biological methods are incubation tests, under aerobic or anaerobic conditions, with or without leaching.

The validation of chemical tests is checked by reference to one or more incubation tests. Table 1 shows the correlation between the chemical and biological methods.

Table 1. Correlation coefficients for the relationships between the results of the chemical and biological methods for defining N availability indices (Gianello and Bremner 1986b).

		Biological methods				
		13	14	15	16	17
Chemical method						
		Correlation coefficient (r)*				
1	Organic C wet oxidation	0.75	0.62	0.77	0.71	0.82
2	Total N Kjeldahl	0.79	0.65	0.80	0.73	0.86
3	Soil-KCl mixture, 100 °C, 4 h	0.95	0.88	0.94	0.94	0.95
4	Soil-KCl mixture, 95 °C, 16 h	0.94	0.84	0.93	0.90	0.96
5	Soil-KCl mixture, 80 °C, 20 h	0.91	0.84	0.88	0.87	0.93
6	Soil-KCl mixture, boil, 1 h	0.83	0.76	0.83	0.79	0.81
7	Soil-KCl mixture, 100 °C, 1 h	0.83	0.79	0.85	0.81	0.83
8	Phosphate-borate buffer	0.93	0.84	0.95	0.91	0.95
9	$CaCl_2$-autoclave	0.87	0.70	0.82	0.77	0.92
10	Acid $KMnO_4$	0.84	0.77	0.89	0.82	0.85
11	Alkaline $KMnO_4$	0.48	0.31	0.52	0.36	0.48
12	$NaHCO_3$ UV	0.69	0.76	0.76	0.74	0.69
Biological method						
		Correlation coefficient (r) **				
13	Anaerobic incub., 40 °C, 7 days	-	0.85	0.95	0.91	0.96
14	Aerobic incub., 30 °C, 14 days	-	-	0.89	0.95	0.81
15	Aerobic incub., 35 °C, 12 weeks	-	-	-	0.96	0.96
16	Method 15 only during 2 weeks	-	-	-	-	0.90
17	N mineralization potential	-	-	-	-	-
	Calculated from method 15	-	-	-	-	-

* R-values between 0.47 and 0.61 are significant at the 1 % level; r-values above 0.61 are significant at the 0.1 % level.

** All r-values reported are significant at the 0.1 % level.

All chemical methods showed a good correlation with all biological methods, except for method 11 (alkaline permanganate extractant). Method 3 (NH_4 extraction in hot KCl) proposed by Gianello and Bremner (1986a) correlates best with incubation tests and is therefore claimed to be the best index of N availability. However, the incubation tests do not necessarily reflect the actual N mineralization capacity of the soil, as pointed out by

Harmsen and Van Schreven as early as 1955. Fox and Piekielek (1984) found a very poor correlation between N mineralized in incubation tests and 'field-measured N availability'.

Another chemical test has recently been developed in Austria by Nemeth (1979); this is the electro-ultrafiltration (EUF) technique. The method yields the nitrate N and organic N extracted, usually at two temperatures and electrical potentials. These values have been combined in different ways to obtain a N index that is believed to represent the whole soil N supply to a crop (Mengel, 1991; Appel and Mengel, 1992). The date of soil sampling may also vary, from harvest time of the previous crop until time of fertilizer application. In an experiment reported by Nemeth *et al.* (1987), the grain yield of winter wheat in fertilized and unfertilized plots was compared to the total EUF (NO_3^--N + organic N) measured in the soil in March, a few weeks after fertilizer application (Figure 1).

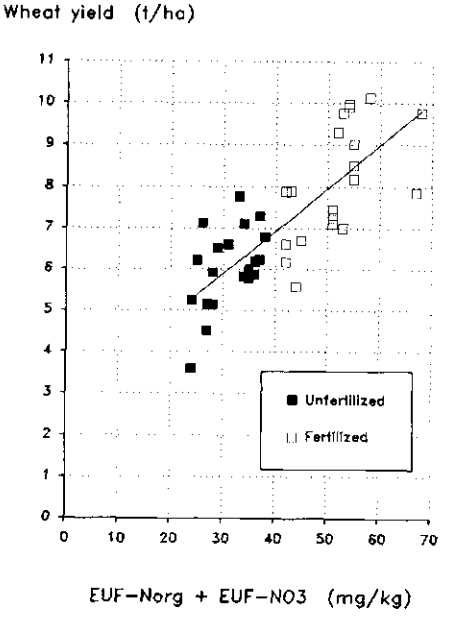


Figure 1. Relationships between grain yield of winter wheat (fertilized and unfertilized plots) and the index value of soil N supply EUF (measured in March). Data from Nemeth et al. (1987).

The results showed that the grain yield was dependent on the EUF-N present in the soil in March. However, variations in the EUF-N were mainly due to variations in NO_3^--N, so that the correlation resulted from the grouping of two populations (fertilized and unfertilized; Figure 1). This does not demonstrate that EUF-extractable N is a valuable indicator of mineralizable N. More generally, the major criticism of most studies on indexes of N availability is that they have not been properly checked. If the index value is a measure of the nitrogen mineralization capacity, it must be correlated with net N mineralization obtained under field conditions. Very few studies have tried to quantify net mineralization *in situ*. The N index has often been compared to crop yield or to plant N uptake in unfertilized plots. The problem is that this uptake depends not only on N mineralization, but also on the amount of mineral N present in the rooting zone in soil when significant crop N uptake begins, and these two factors are largely independent. Machet (1991)

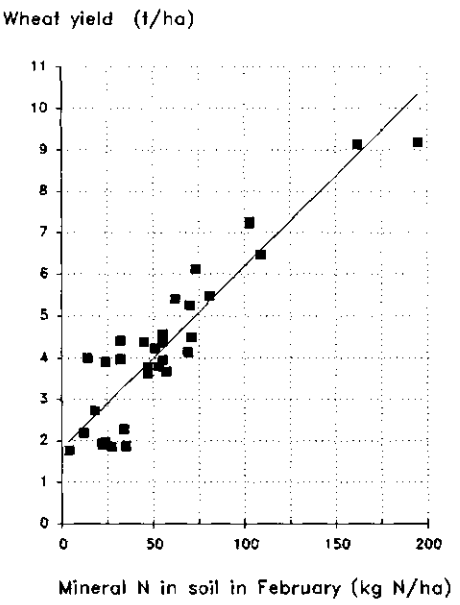


Figure 2. Relationships between winter wheat yield and residual mineral-N in soil at the end of winter in various fields, without fertilizer-N. Data from Machet (1991).

showed that the main source of variation in the yield of unfertilized winter wheat was not soil mineralization, but the residual inorganic N in soil at the end of the winter (Figure 2).

Stanford and Smith (1974) used another approach for determining N availability in soil, which received considerable attention in the 80's. These authors used a long-incubation technique at rather high temperature (35 ^{0}C), with periodic leaching of the soil to remove N mineralized. The N mineralization kinetics obtained by this technique show that the amounts of N mineralized are important and that the mineralization rates decrease smoothly and continuously with time. The kinetics fit relatively well with a single exponential model, given by the equation :

$$N = No\ (1 - e^{-k\ t}).$$

The method has been used to define the 'potentially mineralizable nitrogen' (No) and calculate this value from the incubation data. The effects of soil temperature (T) and moisture (Θ) on the rate constant k have been investigated (Stanford and Epstein, 1974; Stanford *et al.*, 1973, 1975; Oyanedel and Rodriguez, 1977). Stanford *et al.* (1977) suggested that the actual mineralization under field conditions could be estimated versus time from the parameters No and k(T,Θ). This approach is attractive, and it is not surprising that many have tried to use it. Cabrera and Kissel (1988) first determined No and k in their soils. They monitored soil temperature and moisture for 2-4 months in the field and were able to predict N mineralization from the above equation. However, they calculated mineralized N, under field conditions from the following mineral N balance:

$$N\ mineralized = N_2 - N_1 + N_u - N_g + N_e$$

where N_1 and N_2 are the amounts of inorganic N in soil (1.2 or 1.5 m depth) at the

beginning and the end of the period; N_u is plant N uptake; N_g is the N gain (atmospheric and seed inputs) and N_e the loss (volatilization, denitrification and leaching). In their calculation, N_g and N_e were minor corrective terms.

Their results are shown in Figure 3.

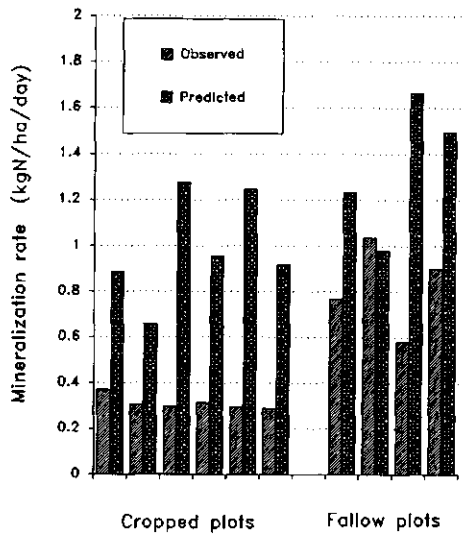


Figure 3. Observed and predicted values of N mineralization rates in different soils, cropped or fallow. Data from Cabrera and Kissel (1988).

The measured mineralization rates on the cropped plots varied little between plots, around 0.3 kg N ha^{-1} day^{-1}. The predicted values were much higher: 0.7 - 1.2 kg N ha^{-1} day^{-1}. The model also overestimated the net mineralization in the fallow plots, but the difference was smaller. Indeed, the mineralization rate was markedly higher in fallow soils than in cropped soils. The model therefore appears to overestimate net mineralization.

The concept of No has been criticized with good reason. Even if there is a very good fit of the model to the data, the No value cannot be determined accurately. The optimized parameters No and k are strongly dependent on the incubation time; this is clearly shown by results of Dendooven (1990) and Dendooven *et al.* (1990). Figure 4 is taken from this study. It indicates that the two parameters were strongly correlated: each couple (k,No) of the curve defined approximately the same mineralization kinetics. The correlation can be decreased only if N mineralized approaches the asymptote No, which requires very long incubation times. Then the method of Stanford and Smith (1974) may be valuable for practical applications, but it is impossible to assign any definite value to the 'mineralizable pool'.

We believe that the leaching test may be useful for estimating gross mineralization. Figure 5 shows the results obtained by two incubation methods. Graph a shows the N mineralization kinetics obtained with the leaching incubation test by Nordmeyer and Richter (1985); graph b is from Muller and Mary (1981) using an incubation method without leaching. The mineralization kinetics in the control soils were comparable, but were very different when sugar-beet tops were added.

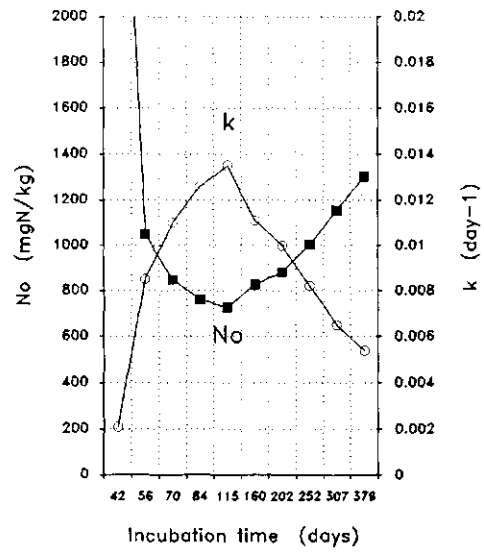


Figure 4. Influence of the incubation time on the calculated parameters N_0 and k of the single exponential mineralization model. Data from Dendooven (1990).

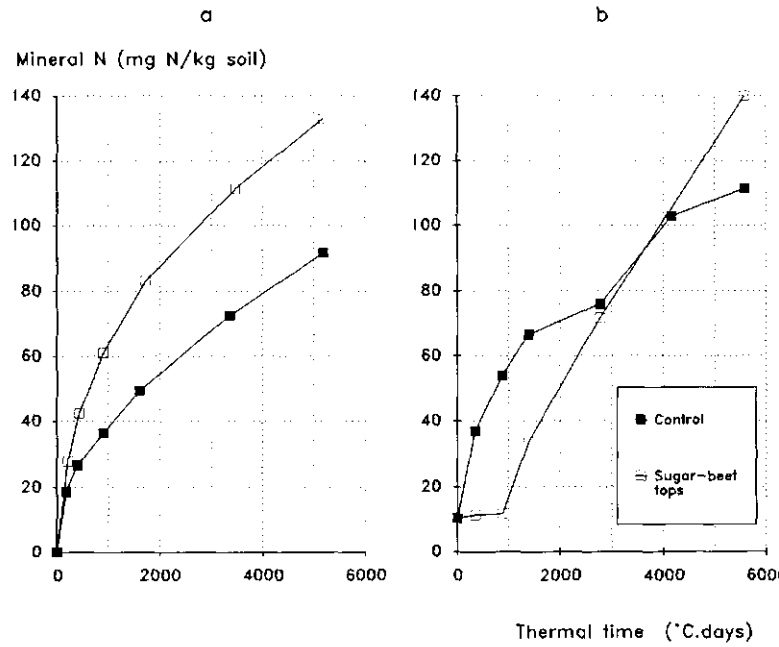


Figure 5. N mineralization kinetics in soils incubated with (open symbols) or without sugar-beet tops (closed symbols). N accumulation is expressed versus a thermal time.
Graph a: incubations made with periodic leaching. Data from Nordmeyer and Richter (1985);
Graph b: incubations made without leaching. Data from Muller and Mary (1981).

The decomposition of sugar-beet tops resulted in net immobilization with the second method, whereas it produced continuous net mineralization with the leaching incubation test. This is probably due to leaching of soluble C compounds, reducing or even blocking N immobilization. This could be the main reason for the overestimation of net mineralization by Cabrera and Kissel (1988) using the leaching test.

PROCESSES DETERMINING NET MINERALIZATION

Net mineralization can be described as the result of four different processes: flush effects, basal mineralization, remineralization (these three fluxes constituting gross mineralization) and biological immobilization.

The *flush effects* likely to occur under field conditions are caused by sequences of soil drying-rewetting or freezing-thawing. Figure 6 shows the change in mineral N in soil incubated fresh or dried and rewetted every two weeks (Van Schreven, 1968).

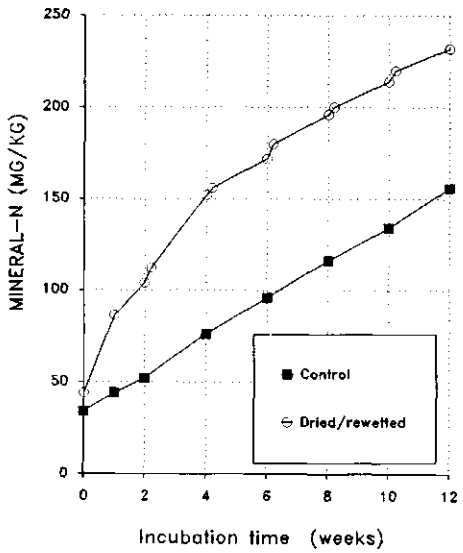


Figure 6. Effect of soil drying and rewetting on N mineralization after subsequent incubation at 29 °C. The soil was dried at 35 °C at weeks 0, 2, 4, 6, 8 and 10. Data from Van Schreven (1968).

A marked flush effect occurred during the first two cycles. The size of the flush decreased in the third cycle and became negligible thereafter. The flush is caused partly by microbial death and subsequent decomposition of microbial cells. It probably also results from physical disturbance of the soil, decreasing physical protection of soil organic matter. The flush effect might be the main source of N mineralization in dry areas, but it probably occurs only in the first 2-3 cm of the soil in wet Northern Europe where the water potential reaches high values in summer.

The three other processes determining net mineralization are illustrated in Figure 7.

Basal mineralization can be defined as the gross mineralization of the soil organic matter in a soil that has not received crop residues recently, and where N immobilization, measured by ^{15}N, is negligible.

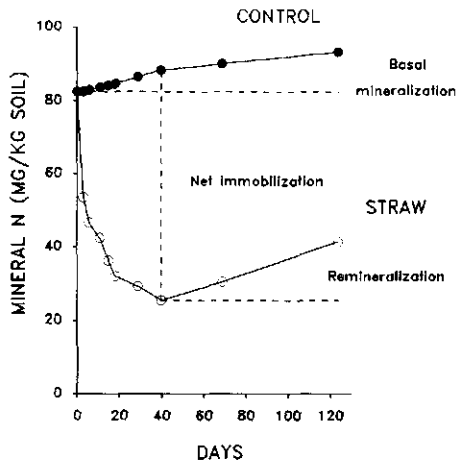


Figure 7. Evolution of mineral N in soil incubated at 15 °C with addition of straw at rate 1.75 g C kg^{-1} soil (open symbols) or without straw (closed symbols). Data from Robin et al. (1992).

This was the case in an incubation reported by Mary *et al.* (1993): basal mineralization rate decreased from 0.30 to 0.19 mg N kg^{-1} day^{-1} during incubation for 6 months at 25 °C, and was very highly correlated with carbon mineralization.

Biological N immobilization corresponds to the assimilation of mineral nitrogen by soil heterotrophic microflora. It is closely related to carbon decomposition in soil and occurs particularly during decomposition of crop residues. Gross immobilization can be only estimated with ^{15}N techniques whereas net immobilization can be calculated from the difference in mineral N contents of amended and unamended soils.

Remineralization takes place after the main decomposition phase, due to recycling of biomass N during microbial death and predation. Remineralization kinetics usually consists of a rapid phase, followed by a much slower phase: only a small part of the N is recycled over a short term.

These processes do not have the same kinetics. Flush effects due to drying and freezing are threshold events, basal mineralization is a rather continuous process, immobilization and remineralization vary with time because the return of crop residues to soil is discontinuous. These processes may also respond differently to environmental conditions. This especially applies to the soil inorganic N concentration, which strongly affects immobilization and remineralization (Fog, 1988), but not gross mineralization (Hart *et al.*, 1986). When the microbial needs for mineral N (NO_3-N + NH_4-N) in soil are large, this pool is rapidly depleted and the decomposition rate of organic compounds is reduced. In return, N immobilization is affected and remineralization is delayed.

The intensity and the kinetics of net immobilization and remineralization are also very dependent on the biochemical composition and nitrogen content of organic residues decomposing in soil. We found that the decomposition of root mucilage resulted in much more N immobilization and remineralization than did glucose decomposition. Roots that were rich in N decomposed slower than these two substrates and induced a lower net immobilization; the remineralization rate was comparable to that of glucose (Mary *et al.*, 1993).

ESTIMATION OF GROSS MINERALIZATION AND IMMOBILIZATION FLUXES *IN SITU*

Mineralization and immobilization at the year scale

We have attempted to estimate the order of magnitude of gross mineralization and immobilization fluxes in a continuous wheat rotation on a loamy soil with the usual organic restitutions in Northern Europe (Table 2).

Table 2. Estimation of annual organic restitutions and potential N immobilization in a simulated continuous wheat rotation.

Residue addition	Dry Matter (kg ha^{-1})	N (kg ha^{-1})	C (kg ha^{-1})	Potential N immobilization (kg ha^{-1})
Straw	8000	35	3500	120
Roots	1500	10	600	20
Rhizodeposits	3500	20	1500	110
Total	-	65	5600	250

Rhizodeposition was estimated on the basis of 10-15 % of the carbon fixed by photosynthesis being returned to the soil via the root system (Keith *et al.*, 1986), and was assumed to be mainly root mucilage. We used the immobilization ratio obtained under laboratory conditions, i.e. 34 g N kg^{-1} C for straw and roots (Nommik, 1962; Robin *et al.*, 1992) and 72 g N kg^{-1} C for root mucilage (Mary *et al.*, 1993). Total immobilization potential calculated over the year was 250 kg N ha^{-1}. The annual net mineralization flux in this soil was estimated at around 120 kg N ha^{-1} (Hofman, 1988; Machet *et al.*, 1990). The remineralization was estimated to be between 20 and 50 % of immobilized N during a year, depending on the residue. It included all of the nitrogen contained in crop residues (considering that fresh organic matter is at steady state). The nitrogen flush was estimated at 20 kg N ha^{-1}, half of it due to drying/rewetting in summer and half due to freezing/thawing during winter. The basal mineralization was the result of the balance between the four other fluxes, and amounted to 205 kg N ha^{-1} $year^{-1}$. Net mineralization, gross immobilization and gross mineralization would then be: 0.33, 0.68 and 1.02 kg N ha^{-1} day^{-1} respectively, corresponding to a 1:2:3 ratio (Table 3).

The actual quantification of N fluxes *in situ* is difficult and poses both theoretical and methodological problems. Immobilization has often been measured at harvest in field experiments done with ^{15}N-labelled fertilizers. This kind of experiment gives estimations of the proportion or the quantity of fertilizer-N immobilized in the soil. Data have been obtained with different experimental procedures and N pools (Table 4) so that comparisons are difficult. The measurements made at short intervals after labelled-N application can be used to calculate N immobilization rates until mineral ^{15}N has disappeared in soil. The mean rates observed under winter wheat during the two weeks following fertilizer application were 0.25 at the tillering stage and 1.5 kg N ha^{-1} day^{-1} at the stem-elongation stage (Recous *et al.*, 1988).

Table 3. Estimation of potential gross mineralization, gross immobilization and net mineralization in a continuous wheat rotation on a loamy soil (Northern France) receiving usual organic restitutions.

N flux	N (kg ha^{-1} $year^{-1}$)		N (kg ha^{-1} day^{-1})	
Immobilization	- 250		- 0.68	
Remineralization	+ 145		+ 0.22	
Flush mineralization	+ 20	+ 370	+ 0.18	+ 1.02
Basal mineralization	+ 205		+ 0.62	
Balance net mineralization	+ 120		+ 0.33	

Quantification of gross fluxes by the isotope dilution technique

There have been several recent attempts to calculate *in situ* gross immobilization and mineralization fluxes using the isotope dilution technique. The N fluxes which enter a N pool (production) or deplete it (consumption) can be estimated from differential equations describing the rate of change of the N and ^{15}N pools and solving these analytically or numerically (Kirkham and Bartholomew, 1954; Myrold and Tiedje, 1986; Bjarnason, 1988, Barraclough, 1991; Davidson *et al.*, 1991; Tietema and Wessel, 1992). The methods may differ, but they all assume that throughout the period of measurements:

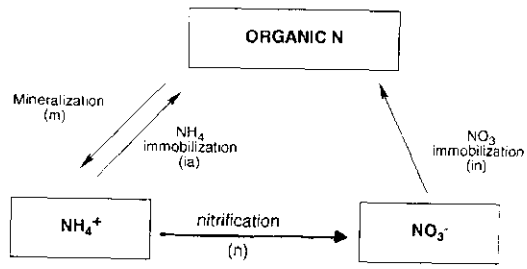
(H1) the N pools involved are isotopically homogeneous,

(H2) the N transformation rates are constant,

(H3) the recycling of biomass-N is negligible.

Experiments in which $^{15}NH_4^+$ tracer is added to the soil allow calculation of mineralization and ammonium consumption (nitrification + ammonium immobilization + crop uptake + possible losses). Parallel $^{15}NO_3^-$ tracer experiments enable the calculation of nitrification and nitrate consumption (nitrate immobilizatien + crop uptake + possible losses) (Figure 8). Most authors use the difference between NH_4^+ consumption fluxes, calculated with $^{15}NH_4^+$, and nitrification flux, calculated with $^{15}NO_3^-$, to calculate gross immobilization. It is assumed that no ammonium is lost and that immobilization of nitrate is negligible. These two assumptions are often nonvalid, at least *in situ* (Recous *et al.*, 1988, 1992a; Schimel *et al.*, 1989). The calculation of gross immobilization using biomass ^{15}N measurements (Davidson *et al.*, 1991) or organic ^{15}N measurements (Recous *et al.*, 1992b) eliminates such assumptions.

The search for suitable conditions in which to apply the isotopic dilution method has led to make many injections of highly enriched ^{15}N solutions into the soil (to satisfy assumption H1) and to measure isotopic dilution over a short time scale of 1, 2 or 7 days (Schimel *et al.*, 1989; Davidson *et al.*, 1991; Davies *et al.*, 1992; Watkins, 1992; Recous *et al.*, 1992b), to satisfy assumptions H2 and H3. In fact, the conditions of short-term experiments, homogenous distribution and uniform mixing of soil N and added 15N are somehow contradictory.



- Estimation of gross mineralization $^{15}NH_4$ enrichment experiment

$$m = -\frac{\Delta A}{\Delta t}\,\frac{\ln\left(\frac{e_{a\,2}}{e_{a\,1}}\right)}{\ln\left(\frac{A_2}{A_1}\right)}$$

- Estimation of gross immobilization

$$\begin{cases} i_a = \dfrac{\Delta\,{}^{15}N_{org}}{\bar{e}_a \cdot \Delta t} \\ i_a + i_n = \dfrac{\Delta\,{}^{15}N_{org}}{\bar{e}_i \cdot \Delta t} \end{cases}$$

(1) $^{15}NH_4$ enrichment experiment

$$i_n = \frac{\Delta\,{}^{15}N_{org}}{\bar{e}_n \cdot \Delta t}$$

(2) $^{15}NO_3$ enrichment experiment

Figure 8. Quantification of N transformation rates by isotope dilution techniques during a time interval ∂ t = t2-t1. m: gross mineralization; n: nitrification; ia: immobilization from ammonium; in: immobilization from nitrate. A = NH_4 pool, ea = ^{15}N isotopic excess of NH_4 pool, ea mean isotopic excess of NH_4 pool between t2-t1 time interval; en = mean isotopic excess of NO_3 pool between t1-t2 time interval; in = mean isotopic excess of (NH_4+NO_3) pool between t2-t1 time interval.

Some authors have used very small amounts of labelled nitrogen in soil so as not to disturb true fluxes of mineralization and immobilization. Tietema and Wessel (1992) determined N transformation rates in the upper soil layers of coniferous and deciduous forests at several sampling times. They observed very high rates of mineralization and immobilization, with fluctuations throughout the year, although the net resulting mineralization flux was rather low (Table 5). Jackson *et al.* (1989) measured smaller, but still important immobilization fluxes from both ammonium and nitrate pools in annual grassland.

The two major difficulties encountered with this approach are (i) obtaining homogenous distribution in soil, and (ii) that concurrent processes like NH_4^+ fixation or NH_3 volatilization can become dominant in $^{15}NH_4^+$ depletion.

Another approach is to apply much larger amounts of labelled N to obtain better distribution of ^{15}N and minimize the concurrent processes (Recous and Mary, 1990; Recous *et al.*, 1992b).

Table 4. Estimation of fertilizer-N net immobilization at harvest time using ^{15}N.

Authors	Crop	N pool measured	N added (kg ha^{-1})	Net immobilization % N applied	kg N ha^{-1}
Balabane and Balesdent (1992)	Maize	Organic N associated with particle size fraction	160	25.6	41
Bristow *et al.* (1987)	Perennial ryegrass	Organic N < 200 µm (microbial biomass)	60	37.0	22
Powlson *et al.* (1992)	W. Wheat	Organic N (microbial biomass + roots)	100-200	16-21	16-40
Recous *et al.* (1988)	W. Wheat	Organic N (roots eliminated)	80	20-25	16-20
Stevens and Laughlin (1989)	Ryegrass	Organic N (microbial biomass + roots)	100	14.5	14

Table 5. Estimation of gross mineralization (m) and immobilization fluxes (i) in various ecosystems. ia = immobilization from NH_4 pool; in = immobilization from NO_3 pool). [a] ia + in.

	N fluxes (kg ha^{-1} day^{-1}) except for Tietema and Wessel (mg kg^{-1} day^{-1}) m	ia	in	Experimental conditions
Tietema and Wessel (1992)				
Feb. 90	78	60	0	Soils from deciduous forest
Nov. 90	48	36	0	$^{15}NH_4$ and $^{15}NO_3$ exp. 2 days
Schimel *et al.* (1989)				
Day	14.9	16.4	4.1	Annual grass top 9 cm
Night	14.0	8.0	3.3	$^{15}NH_4$ and $^{15}NO_3$ exp. 1 day (night/day)
Recous and Mary (1990) 8 dates tillering-flowering (7/03 -14/06)	0.15 - 0.77	0.58 - 0.81[a]		W. wheat; top 5 cm $CO(^{15}NH_2)_2$ exp. 7 days

We used Barraclough's procedure (Barraclough, 1988) and measurements of mineral and organic ^{15}N to calculate N fluxes after eight ^{15}N pulses applied to winter wheat between March and June (Table 4). The immobilization fluxes calculated during one week after N

applications were 0.6-0.8 kg N ha^{-1} day^{-1} for the top 5 cm soil. The mineralization rate was estimated with less precision at 0.15-0.77 kg N ha^{-1} day^{-1}, with a mean rate of 0.34 kg N ha^{-1} day^{-1} during the same period. Extrapolation to the arable layer gives a mean rate of 1.70 kg N ha^{-1} day^{-1}. A direct comparison of immobilization and mineralization rates would lead to the conclusion that net immobilization has occurred. However, we believe that the immobilization rates measured were in fact potential rates. They were not limited by N deficiency since plenty of mineral N was present. The actual immobilization rates without addition of N, would probably have been much lower. In contrast, the mineralization rates were correctly estimated, at least if we accept the hypothesis of no real added nitrogen interaction due to N application (Jenkinson *et al.*, 1985).

CONCLUSIONS

Techniques such as ^{15}N analysis (Preston, 1992) and isotope tracing (Myrold and Tiedje, 1986; Wessel and Tietema, 1992) have greatly improved recently, allowing the accurate determination of N fluxes in soil under laboratory conditions. But estimating the actual gross mineralization and immobilization fluxes under field conditions is more difficult, and will require very detailed studies. Nevertheless, the first estimations obtained *in situ,* together with extrapolations from laboratory experiments, indicate that gross N mineralization and immobilization fluxes *in situ* are probably much greater than net mineralization.

The two processes are not determined by the same factors. Gross mineralization is the sum of very transient processes such as the flush effect, the fairly rapid recycling of microbial biomass-N (remineralization) and the slow process by which humified organic material is mineralized. Gross immobilization is essentially due to the biological assimilation of N by microorganisms and is driven by the C dynamics of recent inputs of organic matter to soil. Its importance and kinetics strongly depend, in the short term, on the nature and the input rate of crop residues (including rhizodeposits); it is also affected by soil inorganic N concentration in decomposition zones.

This is why the recent history of soil plays a major role in the 'N availability index' or the 'potentially mineralizable N' values obtained, whatever the method employed, and is probably the cause of most of the failures in the extrapolation of laboratory data. The prediction models of net mineralization should take into account at least two functions: the first defines the basal mineralization as a function of soil texture and long-term C and N inputs; the second one describes immobilization-remineralization as a function of the amount and nature of recently added organic residues and mineral N.

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Methodology for the study of N mineralization in the field

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Abstract

Field methods used to measure nitrogen mineralization are reviewed. The relative importance of net and gross measures of mineralization are considered in relation to field studies. Some of the problems with *in situ* core incubations are discussed, particularly that of the disruption of plant roots, and immobilization of mineralized N. A technique applying pool dilution to the study of mineralization and nitrification in field plots planted with spring barley is described. Mineralization rates increased during the growing period from less than 0.5 to 3.2 kg N ha^{-1} day^{-1}. Gross mineralization was found to be correlated with soil organic matter content at six sites. Negative values of gross immobilization were calculated, which were ascribed to problems with the application of this method in the field, in particular remineralization of immobilized N.

INTRODUCTION

The current interest in nutrient cycling in both managed and natural ecosystems has led to renewed attempts to develop reliable methods for measuring N mineralization in the field. This plays a fundamental role in determining the amount of nitrogen which is available to be taken up by plants. In arable soils, even where large fertilizer applications are made, a substantial part of the crop's nitrogen comes from non-fertilizer sources, much of which is derived from mineralization (Powlson, 1988). Mineralization also contributes to N losses from the soil/plant system, particularly when it does not coincide with plant uptake. Improved methods of mineralization measurement could potentially improve fertilizer N recommendations by providing more detailed information about the quantity and timing of N release from the soil's organic matter, and help to minimize N loss from the soil by denitrification, leaching or volatilization.

Methods for quantifying mineralization in soils can be divided roughly into those carried out in the laboratory, and field methods. A variety of laboratory methods has been proposed, including aerobic incubations where soils are either leached (Stanford and Smith, 1972), or incubated in closed containers (Keeney, 1982) and anaerobic incubations (Waring and Bremner, 1964). These methods are useful for comparative purposes, in that

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they can rank the potential for mineralization from different soils. They have proved useful in the study of mineralization kinetics and the understanding of how N is released from individual organic matter fractions (Paustian and Bonde, 1987). Laboratory incubations are of little value in quantifying rates of mineralization in the field, as the conditions under which they are carried out (often under high temperatures and constant water contents) are far removed from the conditions in the environment from which they were taken. The remainder of this paper will consider the various methods that are available to quantify mineralization in the field, and some of the problems associated with their development.

MATERIALS AND METHODS

The distinction between gross mineralization, the total quantity of NH_4^+-N released from the soil organic matter, and net mineralization, the gross mineralization rate less the total return of N (by microbial processes) to the soil organic matter by gross immobilization, is important. Where studies are made of nutrient flow through an ecosystem the net transformation is often of greatest interest, and historically, most attempts to measure mineralization have measured the net rate, although this has also been a result of the absence until recently of suitable methods to measure gross rates in the field. The size of the available-N pool in soil changes constantly as a result of inputs and outputs (Figure 1).

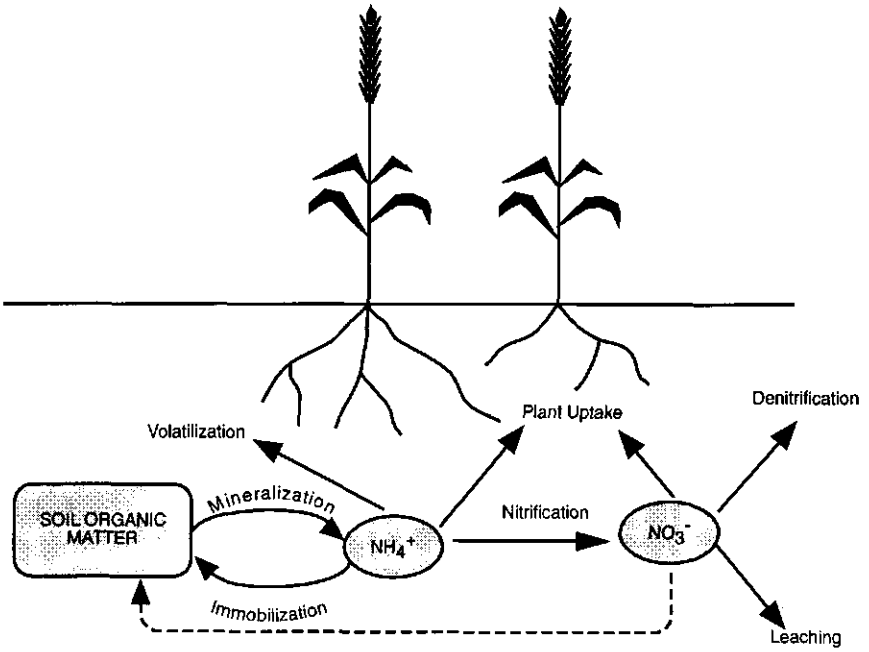

Figure 1. A schematic diagram of the soil nitrogen cycle.

The size of the ammonium pool for example is controlled by up to five processes of consumption and production. Measurements of individual pool sizes are therefore usually inadequate to quantify individual rate processes.

Net mineralization measurements using *in situ* soil cores

In situ incubations of soil cores have been used for many years to make measurements of net mineralization (e.g., Eno, 1960; Popovic, 1980; Raison *et al.*, 1987). The principle of this method is that the mineral nitrogen content (NH_4^+- and NO_3^--N) is determined on a sample of fresh soil at the start of the measurement period. A core of soil (usually between 20-60 mm diameter, and 100-300 mm deep) is isolated from the surrounding soil either by encasing in a sleeve (Raison *et al.*, 1987), in which case the upper surface of the core is covered to prevent leaching, or by placing it within a plastic bag, and returning it to the point from which it was sampled (Nadelhoffer *et al.*, 1985). The cores are incubated for periods ranging from 1-6 weeks, and mineral N determinations are made at the end of that period. The net mineralization rate is defined as:

$$N_{min} = \frac{\Delta NH_4^+\text{-}N + \Delta NO_3^-\text{-}N}{t} \quad (1)$$

where N_{min} = net N mineralization, t = time, and ΔNH_4^+-N, ΔNO_3^--N = net change in NH_4^+-N or NO_3^--N.

The method has the advantage of minimizing disruption to the soil structure, at the same time as allowing mineralization to proceed under conditions similar to those existing in undisturbed soils. By comparing covered with uncovered cores Raison *et al.* (1987) suggested that estimates of plant N uptake can be made as follows:

$$N_{uptake} = N_{min} - (\Delta \text{ soil mineral N}) - (\text{losses}) \quad (2)$$

Using this approach, Smethurst and Nambier (1989), in a study of N uptake by young *Pinus radiata* trees, found a significant correlation ($r^2 = 0.57$, $P < 0.01$) with N uptake as determined by accumulation of N in the plant biomass. Comparisons between plant uptake and net N mineralization in an arable soil were made by Redman *et al.* (1989) in a study of the growth of winter barley receiving different fertilizer and manure treatments. Net mineralization was determined by using core incubations and applying equation (1). An independent measure was obtained by constructing an N budget and estimating mineralization by difference:

$$N_{min} = \frac{N_l + N_c + (\Delta \text{ soil mineral N})}{t} \quad (3)$$

where N_l = leaching losses, and N_c = crop uptake.

No differences between methods were observed in mineralization during the winter period; however, between April and July significantly higher estimates of mineralization were obtained using the core incubation. This was ascribed to the higher moisture contents within the cores.

One of the problems in interpreting net mineralization data from soil cores is that of accounting for artefacts introduced by the process of sampling and incubation. Mineralization rates are known to be sensitive to the availability of water (Myers *et al.*, 1982; Nadelhoffer *et al.*, 1991), yet where soil cores are protected from rainfall, or isolated

in any other way from the soil environment, the moisture content of incubated samples is likely to be different from that of the bulk of the soil. A solution proposed by Adams *et al.* (1989) was to use perforated cores. They found that the variation of soil moisture in such containers maintained the soils within 5 % of that of the undisturbed soil.

A more serious problem with the use of core incubations where they are used to relate net mineralization measurements to crop N uptake, is the severing of root connections in the incubated core. Plant roots provide microbial populations with significant quantities of carbon, by the exudation of soluble compounds and the deposition of cellular material (Newman, 1985), and by doing so stimulate the process of mineralization (Hart *et al.*, 1979; Helal and Sauerbeck 1984; Wheatley *et al.*, 1990). Roots also compete with microbial populations for the mineral N produced. Wang and Bakken (1989) found that when plant residues were alternated with layers of soil in a column with plants present, the apparent net mineralization rate increased. This was because the plants were able to take up N that would otherwise have been immobilized. Where plant roots were not present, mineral N was assumed to diffuse to N-deficient regions of the soil and was immobilized. In this respect, plants effectively remove N from the soil and prevent it from cycling within the microbial population. By doing so they increase net mineralization rates but may not affect or actually reduce gross mineralization rates.

Incubating soil cores in isolation from plant roots often results in a rapid increase in mineral N, and the resulting N fluxes may be unrepresentative of the undisturbed soil. Particular problems are likely to be associated with immobilization of mineralized N, and denitrification losses. In a study of N mineralization and crop uptake by spring barley, Rees (1989) found that (as measured by core incubations) in the period between fertilizer application in April and harvest in August the net release of N was -17.6 and 16.1 kg ha^{-1} in soils receiving 160 kg N ha^{-1} as $(NH_4)_2SO_4$ and KNO_3, respectively. The N fertilizers were enriched with ^{15}N, allowing the contributions of soil-derived N to the crop to be determined. These were 81.6 and 106.4 kg N ha^{-1} in the two treatments, respectively. In this experiment the core incubations therefore underestimated net mineralization by at least 90-99 kg ha^{-1} (but more if leaching and denitrification losses were taken into account). Although part of the reason for the low estimates of net mineralization was an apparent consumption of fertilizer-N shortly after application, consistently low estimates may have been related to microbial consumption of the N produced.

In the same study the accumulation of N in the microbial biomass was measured in the incubated cores. It was found that although the quantity of biomass-N in the field remained relatively constant during the period of incubation, the biomass-N content of the incubated cores increased by 80 mg kg^{-1}. If the amounts of carbon released by the severed roots in the soil core continued at the same rate as those from intact roots (this is likely to be a minimum estimate, as the dead roots themselves would add to the C pool), then it might be expected from published data (Newman, 1985; Lambers, 1987) that carbon release from roots contained in the core would be about 5 g kg^{-1} dry soil. Various studies have been made of N immobilization following additions of carbon to soils (Recous and Mary, 1990; Voroney and Paul, 1984; Amhad *et al.*, 1972; Breland and Bakken, 1991) with estimates ranging between 45 and 90 mg N g^{-1} of added C. Thus in the field incubated cores, it is plausible that the amounts of root-derived C would have been sufficient to immobilize much of the N released.

Denitrification losses from incubated soil cores also have the potential to increase as the concentrations of NO_3^--N rise. To the knowledge of the authors, no specific measurements of denitrification losses from cores incubated in mineralization studies have been made. However, Arah *et al.* (1991) used short-term laboratory incubations of intact soil

cores to measure denitrification losses using the acetylene inhibition technique. In the absence of oxygen, they found losses of 5-120 g N ha^{-1} day^{-1}, depending on the soil's availability of mineral N and water. This is equivalent to losses of 0.017-0.40 mg N kg^{-1} day^{-1} from the cores used in the mineralization assay. As the net changes in mineral N in these cores were often less than 20 mg kg^{-1} $month^{-1}$, denitrification losses of this magnitude could result in significant underestimates in net mineralization.

Pool dilution

The method of determining rates of mineralization by measuring the rate of dilution of a pool of ^{15}N was originally developed by Kirkham and Bartholomew (1954, 1955). Relatively little use was made of pool dilution during the 20 years that followed their initial publications. More recently there has been considerable interest in pool dilution as a tool for understanding the complexities of the N cycle, with studies of mineralization, nitrification and N fixation.

Kirkham and Bartholomew's method was originally designed for laboratory use, the principle being that a ^{15}N enriched ammonium salt is mixed with a soil sample. Nitrogen released by mineralization from the soil organic matter then reduces the enrichment of the NH_4^+ pool, such that the rate of decline in enrichment is proportional to the rate of mineralization. Thus:

$$N_{min} = \frac{(A_{t1} - A_{t2})\ \ln(A_{L1}/A_{L2})}{(t_2 - t_1)\ \ln(A_{t1}/A_{t2})} \quad (4)$$

where: A_{t1}, A_{t2} = NH_4^+-N (labelled and unlabelled) at times 1 and 2, respectively, and A_{L1}, A_{L2} = NH_4^+-N (labelled) at times 1 and 2, respectively.

Three assumptions were made in applying these equations, i.e. that: (1) ^{14}N and ^{15}N behave similarly in soils; (2) there is no significant remineralization of N; (3) rates of mineralization remain constant between measurements.

The validity of these assumptions has been critically reviewed in a number of recent publications (Bjarnason, 1988; Barraclough, 1991; Davidson *et al.*, 1991; Wessel and Tietema, 1992). The original equations of Kirkham and Bartholomew (1954) did not take account of naturally occurring ^{15}N released from soil organic matter, and therefore their method was dependent on using salts with relatively high enrichment of ^{15}N. Subsequent modifications to their equations account for background enrichment (Blackburn, 1979) and are now widely used.

Field measurements of mineralization using pool dilution have been developed in two ways. In the first, ^{15}N-enriched salts are applied to field plots, and subsequent crop uptake and the isotopic enrichment of the soil mineral nitrogen pool monitored (Barraclough *et al.*, 1985; Barraclough and Smith, 1987); in the second, ^{15}N is injected into intact soil cores, and following a suitable time period the core is destructively sampled and the ^{15}N content of the mineral N pool determined (Davidson *et al.*, 1991). The use of pool dilution in soil cores is described by Stockdale *et al.* (1994). The following account describes the application of pool dilution to the measurement of gross mineralization in field plots in a number of arable soils in SE Scotland, which was carried out as part of a PhD project at the University of Edinburgh (McTaggart, 1992).

Experiments

Trials were carried out at two sites in 1989 and at six sites in 1990 (site details, the timing of fertilizer applications and sampling dates are given in Tables 1 and 2), in order to compare rates of mineralization at different times and in different soils, all of which were planted with spring barley.

Table 1. Site details and sampling dates.

Site	Soil texture	OM (%)	pH	Soil series
Bush, Lothian (Lower Fulford field)	Sandy clay	3.7	6.5	Unclassified alluvium
Upper Cairnie, Tayside	Sandy clay loam	1.8	6.7	Balrownie
Manorhill, Borders	Sandy loam	2.4	6.1	Smailholm
Quixwood, Borders	Clay loam	5.1	6.2	Ettrick
Bush, Lothian (Crofts field)	Sandy loam	4.7	5.7	Unclassified alluvium
Bush, Lothian (Farmers Holding field)	Sandy clay loam	3.3	6.0	E Bush/ Macmerry
Treaton, Tayside	Sandy loam	5.7	6.4	Darvel
Kettle, Tayside	Loamy sand	2.8	6.7	Eckford

In both years, single-labelled NH_4NO_3 solutions were applied to paired microplots using the procedure described by Barraclough (1988). In 1989 N was applied with a NH_4:NO_3 ratio of 7:3. The first fertilizer application was made immediately after sowing (31 March at Bush, and 4 April at U. Cairnie) at a rate of 120 kg N ha^{-1} and ^{15}N enrichment of 3 atom %. In each of the paired plots, one plot received ^{15}N-labelled NH_4^+, the other ^{15}N-labelled NO_3^-. The paired plots measured 1 x 1 m and were replicated 3 times. The labelled fertilizer solutions were washed into the soil with 5 liters of water, and the surrounding areas received unlabelled fertilizer solutions at the same rate, and in the same form. On two subsequent occasions during the same season, microplots were used in areas that had received unlabelled fertilizer. These received fertilizer N solutions containing only 10 kg N ha^{-1} (to minimize the fertilization effect), with a ^{15}N enrichment of 10 atom %.

In 1990 labelled fertilizers were applied on one occasion only in order to compare mineralization rates in different soils. Unlabelled NH_4NO_3 was applied at sowing to all sites at a rate of 120 kg ha^{-1}. About 1 month later (Table 2), $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ (both at a rate of 10 kg ha^{-1}, and a ^{15}N enrichment of 10 atom %) were applied to paired microplots at each site.

Table 2. Timing of fertilizer applications and mineralization measurements.

1989

Bush (Lower Fulford field)
Main fertilizer application (120 kg N ha^{-1} as NH_4NO_3 at 3 atom %): 31 March
Subsequent fertilizer application (10 kg N ha^{-1} as NH_4NO_3 at 10 atom %): 13 April, 31 May, 13 July
Measurement periods: 13 April-4 May, 31 May-22 June, 13 July-3 August

Upper Cairnie
Main fertilizer application (120 kg N ha^{-1} as NH_4NO_3 at 3 atom %): 4 April
Subsequent fertilizer application (10 kg N ha^{-1} as NH_4NO_3 at 10 atom %): 14 April, 30 May, 12 July
Measurement periods: 14 April-5 May, 30 May-20 June, 12 July-2 August

1990

Manorhill
Main fertilizer application (120 kg N ha^{-1} as NH_4NO_3 unlabelled): 25 March
Subsequent fertilizer application (10 kg N ha^{-1} as NH_4NO_3 at 10 atom %): 15 May
Measurement period: 15 May-6 June

Quixwood

Main fertilizer application (120 kg N ha^{-1} as NH_4NO_3 unlabelled): 31 March
Subsequent fertilizer application (10 kg N ha^{-1} as NH_4NO_3 at 10 atom %): 16 May
Measurement period: 16 May-6 June

Bush (Crofts field)
Main fertilizer application (120 kg N ha^{-1} as NH_4NO_3 unlabelled): 30 March
Subsequent fertilizer application (10 kg N ha^{-1} as NH_4NO_3 at 10 atom %): 25 May
Measurement period: 25 May-15 June

Bush (Farmers Holding field)
Main fertilizer application (120 kg N ha^{-1} as NH_4NO_3 unlabelled): 2 April
Subsequent fertilizer application (10 kg N ha^{-1} as NH_4NO_3 at 10 atom %): 25 May
Measurement period: 25 May-15 June

Treaton
Main fertilizer application (120 kg N ha^{-1} as NH_4NO_3 unlabelled): 28 March
Subsequent fertilizer application (10 kg N ha^{-1} as NH_4NO_3 at 10 atom %): 17 May
Measurement period: 17 May-7 June

Kettle
Main fertilizer application (120 kg N ha^{-1} as NH_4NO_3 unlabelled): 25 April
Subsequent fertilizer application (10 kg N ha^{-1} as NH_4NO_3 at 10 atom %): 4 May
Measurement period: 4 May-25 June

In both years crop and soil samples (0-40 cm) were collected for analysis at the start and end of the measurement period (approximately 3 weeks, see Table 2). A 20 g sample of fresh sieved soil was mixed with 100 ml 1M KCl and shaken for 1 hour. The solution was then filtered through Whatman No 42 filter paper, and NH_4^+ and NO_3^- concentrations determined by continuous flow analysis. Crop samples comprising 2 rows x 0.5 m, and soil samples (0-30 cm) were taken from the microplots for ^{15}N analyses. In 1989 additional crop samples were taken to determine the pattern of uptake of unlabelled N. The ^{15}N contents

of soil extracts and plant samples were determined by mass spectrometry. Steam distillation was used to remove NH_4^+- and NO_3^--N from the soil extracts as described by Hauck (1982). Plant samples were dried overnight at 100 °C, and then ground, first in a hammer mill and then in an agate ball mill, to produce a very fine flour-like consistency. Plant samples and soil extracts were analyzed for their ^{15}N contents using a VG Isogas MM622 mass spectrometer linked to a Carlo-Erba 1400 automatic N analyzer, which converts nitrogen compounds to N_2 by the Dumas oxidation-reduction procedure.

RESULTS AND DISCUSSION

Gross mineralization rates increased more than six-fold between April and July 1989, increasing from 0.42 and 0.19 to 3.19 and 1.59 kg N ha^{-1} day^{-1} at Upper Cairnie and Bush, respectively, in that period (Figure 2).

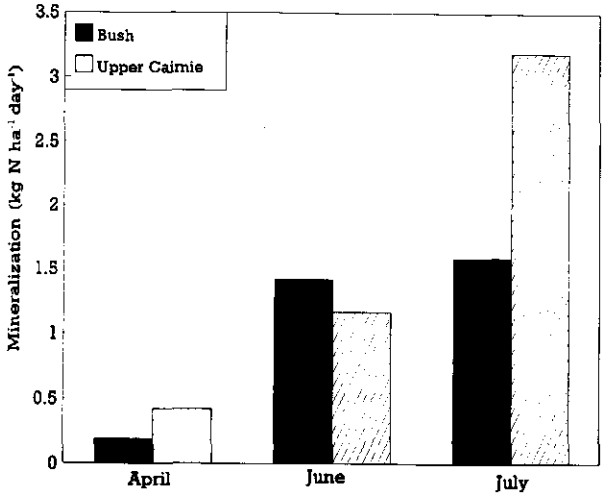

Figure 2. Gross nitrogen mineralization at Upper Cairnie and Bush between April and July, 1989.

The rates of mineralization were usually greater than the uptake of unlabelled N by the crops at both sites (Figure 3). Negative values of ^{14}N uptake are attributed to losses of N from the above-ground parts of the plant, which are commonly observed immediately prior to harvest.

The increased rates of mineralization in the later part of the growing season provided support for the interpretation of previous work on N uptake in this part of Scotland; Smith *et al.* (1984) found that fertilizer N uptake preceded that of soil-derived N, and ascribed this to low spring temperatures (which are characteristic of the Scottish climate), resulting in low rates of mineralization early in the year. Nishio and Fujimoto (1989) found that the major factor controlling gross mineralization in fallow and cropped soils was temperature, with maximum rates occurring in July.

The comparisons of sites that were made in 1990 show that gross mineralization rates ranged from 1.1 kg ha^{-1} day^{-1} at Manorhill, to 2.2 kg ha^{-1} day^{-1} at Kettle (Figure 4). In general, rates of mineralization reflected the organic matter contents of the soils. There was a linear correlation ($r^2 = 0.57$; $P < 0.05$) between mineralization rate and unlabelled N uptake by the crop (Figure 4).

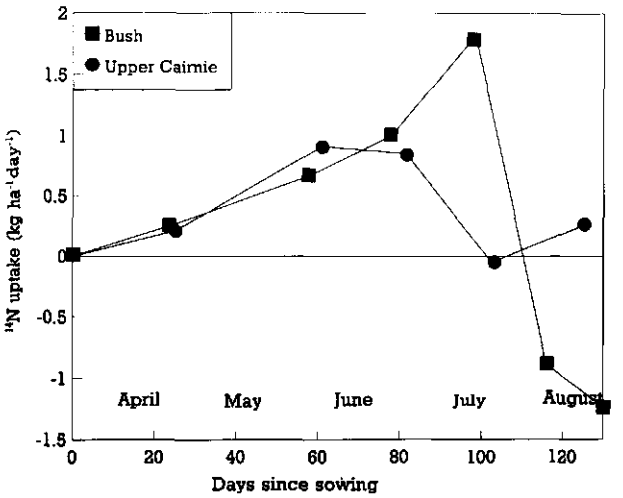

Figure 3. Uptake of ^{14}N by spring barley at Upper Cairnie and Bush, 1989.

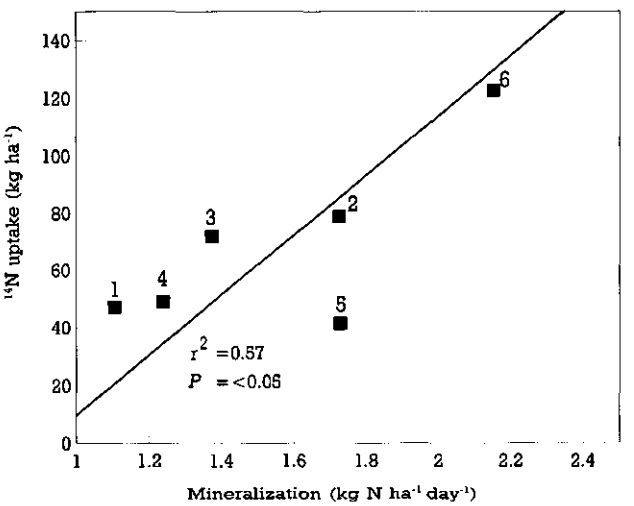

Figure 4. Relationship between gross mineralization and ^{14}N uptake by spring barley at six sites in 1990. 1 Manorhill, 2 Quixwood, 3 Bush (Crofts field), 4 Bush (Farmers Holding field), 5 Treaton, 6 Kettle (see Table 1 for details).

In this season the measured rates of mineralization were more than adequate to account for the amount of unlabelled N that had accumulated in the crop during the growing season.

The rates of gross mineralization calculated in this study (1-4 kg ha^{-1} day^{-1}) are comparable with those found in other studies using the same method (Barraclough and Smith, 1987; Barraclough, 1988). Gross rates calculated from short-term laboratory studies however, generally tend to be much higher (Bjarnason, 1988; Chalk *et al.*, 1990). Low values may result from remineralization of immobilized N (see later).

Dilution of the ^{15}N-labelled NO_3^- in each of the paired plots allowed nitrification rates to be calculated, again using the approach of Barraclough (1988). Gross immobilization rates were then calculated by difference:

$$N_{immob} = \frac{N_{min} - N_{nit} - N_u - \Delta N}{t} \quad (5)$$

where: N_{immob} = gross immobilization rate, N_{min} = amount of N mineralized in time t, N_{nit} = amount of N nitrified in time t, N_u = amount of plant N uptake in time t, and ΔN = change in the size of the mineral N pool.

The nitrification rate varied from 2 kg N ha^{-1} day^{-1} at Bush in 1990 to 4 kg N ha^{-1} day^{-1} at Kettle in 1990 (Table 3). Such rates of nitrification would consume all the NH_4^+ contained in a 120 kg application of NH_4NO_3 in 15-30 days, which is consistent with observations from other fertilizer trials in this area (McTaggart, 1992), given that some NH_4^+-N would also leave the soil as a result of plant uptake.

Calculated gross rates of immobilization ranged from 1.86 to -2.82 kg N ha^{-1} day^{-1} (Table 3).

Table 3. Nitrification and immobilization measured at various sites by pool dilution. Units: kg N ha^{-1} day^{-1}.

Site	Gross nitrification	Gross immobilization
1989		
Bush (Lower Fulford field)		
April	2.0	1.9
June	1.9	-1.3
July	3.3	-2.6*
Upper Cairnie		
April	3.6	-0.8
June	3.6	-2.5
July	2.2	0.8
1990		
Manorhill	3.5	-2.5
Quixwood	3.0	-1.7
Bush (Crofts field)	3.6	-1.8
Bush (Farmers Holding field)	3.1	-2.1
Treaton	4.0	-1.6
Kettle	4.0	-2.8

* This treatment received NH_4:NO_3-N in a ratio of 1:1. All other treatments in this season received NH_4:NO_3 in a ratio of 7:3.

Since by definition gross immobilization rates cannot be less than 0, then fundamental problems with the application of pool dilution in the way described in this experiment need to be considered. Low values of immobilization are likely to be obtained if gross mineralization rates are underestimated, or if nitrification, plant N uptake and the decline in the soil mineral N pool are overestimated.

An assumption that is central to the application of pool dilution both in field and laboratory measurements is that the applied ^{15}N is mixed uniformly with ^{14}N already contained in the soil. It seems likely that even mixing throughout the 30 cm profile was not achieved in this experiment. This would underestimate mineralization and immobilization, because it could occur from a pool containing predominantly ^{14}N rather than a mixture of ^{14}N and ^{15}N. Furthermore, difficulties exist in defining the volume of soil in which the available N occurs. The assumption that ^{14}N and ^{15}N are equally available to the plant throughout the growing season may be difficult to justify, particularly as root growth in spring barley is known to extend to depths beyond 1 m (Russell, 1990).

Remineralization of immobilized ^{15}N would result simultaneously in an underestimate of the gross rates of both mineralization and immobilization. This effect increases with the duration of the experiment. In the present study measurements were carried out over a 3-week period; however, recent laboratory studies suggest that significant remineralization can occur after only a few days in the laboratory (Wessel and Tietema, 1992). When calculating gross mineralization rates, Bjarnason (1988) found his results were "very erroneous" if not corrected for remineralization after two weeks. Although the soil temperatures in Scotland are relatively low even during summer (the mean soil temperature at 30 cm in July at Edinburgh was 15.1 ^{0}C), it seems unwise to discount remineralization for periods of longer than 7 days. Kirkham and Bartholomew (1954) suggest that the errors associated with remineralization are minimized where the organic N pool is large by comparison to the enriched inorganic pool. As it is only the active organic matter pool or biomass that is involved in short-term transformations of N, it is the ratio of this pool to the labelled inorganic pool which is probably most important.

In studies of this type the equations used to describe N transformations are sensitive to estimates of initial pool sizes. In this experiment the initial inorganic-N pool size was assumed to be equal to the sum of the applied fertilizer N plus that already present in the soil, but recent studies (Davidson *et al.*, 1991; Stockdale *et al.*, 1993) have shown that $^{15}NH_4^+$ is consumed by soils in as little as 15 minutes after application. Davidson *et al.* (1991) found that 17-52 % of added NH_4^+ was consumed by abiological processes.

The quantity of mineral N in the soil may also be affected by loss of NO_3^- via denitrification. By washing in fertilizer solutions with water, the potential for denitrification is increased. To account for this possibility, Barraclough (1991) suggested that samples be taken immediately after ^{15}N addition, and then again two or three days later to assess whether any significant losses had occurred.

CONCLUSIONS

Straightforward and reliable methods of measuring rates of mineralization in the field evade us. The interest in net mineralization as a flux relies upon the concept that it provides a measure of N (as NH_4^+) that is available for plant uptake and nitrification. The problem with many measurements of net mineralization as described earlier is that very often the plant is removed from the system under investigation, thereby altering the very process which is being measured. The ability of plants to remove N from the soil varies

between species (Barraclough, 1989) and it therefore seems likely that net mineralization rates will vary between soils planted with different species, even if all other factors in the environment remain constant. As discussed previously, many studies have shown that plant roots are able to increase rates of net mineralization.

Given these constraints, in circumstances where we wish to measure mineralization under a growing crop it seems most appropriate to determine plant N uptake, at the same time as measuring losses from the plant/soil system and changes in the pool of soil N (this approach has been commonly adopted, e.g., Redman *et al.*, 1989). By doing this net mineralization can be determined by difference. The advantage of this approach is that the study can be carried out without alteration to the system; however, there are a number of drawbacks.

First, mineralization is not measured directly, and so errors in any of the other parameters lead to errors in the mineralization estimate. Second, quantitative estimates of N loss by leaching and denitrification are difficult to determine experimentally. Third, even relatively large increases in soil organic N relative to mineralization rates during the period of study are difficult to detect against the background variability in soil organic N.

Measurements of net mineralization using *in situ* core incubations have provided data that are consistent with the knowledge of other N transformations in the systems being studied. In particular, core incubations appear to have been particularly useful in forest ecosystems (Nadelhoffer *et al.*, 1985; Raison *et al.*, 1987). They have also proved useful in arable systems during winter periods when root growth is not active (Redman *et al.*, 1989), and in their ability to rank different sites or treatments according to their capacity to release mineral N (Van Vuuren and Van der Eerden, 1992). They are less useful in soils with actively growing crops, and where recent fertilizer-N applications have been made. It is helpful to carry out other N transformation measurements in particular plant uptake, as in theory net mineralization (minus the change in the mineral N pool of the planted soil) must always exceed plant N uptake.

It might be hoped that the inadequacy of net mineralization measurements might be overcome by the new techniques that have been developed to study gross mineralization. Unfortunately many technical difficulties seem to surround their implementation in the field. These difficulties have been discussed in relation to whole plot applications of the pool dilution technique in this paper. Problems in quantifying gross mineralization rates in intact cores are discussed by Stockdale *et al.* (1993). Until further progress is made it seems inappropriate to apply existing pool dilution techniques to studies of mineralization in the field. However, such techniques offer a useful tool for quantifying gross transformations in the laboratory, provided that the assumptions underlying the methods are observed.

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Assessment of the problems associated with measuring gross mineralization rates in soil cores

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Abstract

Intact soil cores from three sites were injected with a small amount of 99 atom% enriched ammonium sulphate and incubated at a range of temperatures or moisture potentials for between 1 and 40 days in the laboratory. The experiments were aimed at assessing the problems associated with measuring field rates of gross mineralization in intact cores, following work done by Davidson *et al.* (1991).

Very large drops in the $^{15}NH_4^+$ were seen in the first 24 hours after injection, which were not characteristic of the whole incubation period. This rapid fall may be caused by slow equilibriation of NH_4^+ between the fixed and exchangeable pools. The added $^{15}NH_4^+$ and pre-existing NH_4^+ pools may also be subject to different consumption rates during this period due to the incomplete equilibrium and spatial separation of the two pools. Recycling of ^{15}N was indicated in our experiments by an increase in the amount of $^{15}NH_4^+$. This was seen after seven days at 14 °C, and took longer to occur at the lower temperatures.

Use of pool dilution methods must be accompanied by a check that the underlying assumptions are valid and experimental procedures should be chosen to minimize error. Spatial variability of mineral nitrogen in the field can lead to inaccuracies in calculated rates. Where the method is used with care, however, it will lead to a greater understanding of the processes of the soil nitrogen cycle.

INTRODUCTION

The soil nitrogen cycle involves a large number of identifiable pools of nitrogen linked by complex, often simultaneous and opposing, processes (Jansson, 1958; Paul and Juma, 1981). Simple observations mapping the sizes of the nitrogen pools involved over time are therefore not adequate to describe the full dynamics of the system (Jansson and Persson, 1982). Use of ^{15}N has provided a powerful tool, where direct tracer methods determine the location of ^{15}N after a period of exposure and pool dilution methods estimate flow rates

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through a given nitrogen pool (Nason and Myrold, 1991). Where labelled NH_4^+ is added to the NH_4^+ pool in the soil and comes rapidly into equilibrium with it, the decline in the ^{15}N enrichment of the pool, as NH_4^+ at natural abundance is introduced by mineralization of soil organic nitrogen, can be used to calculate the gross mineralization rate. In the same way addition of labelled NO_3^- can allow measurement of gross nitrification rates.

As in all isotopic work, it is necessary to assume that ^{14}N and ^{15}N are not discriminated by processes occurring in soils and that the added ^{15}N equilibriates rapidly with the pool to which it has been added, creating a single homogeneous pool. Whilst some microbial transformation processes do discriminate between ^{14}N and ^{15}N (Cheng *et al.*, 1964; Heaton, 1986), the assumption probably holds for incubations of enriched samples over a short time period. The heterogeneity of ^{15}N in the organic nitrogen pool has not been studied and it is assumed that the ^{15}N abundance is naturally at background levels (Wessel and Tietema, 1992). There are some indications from plant uptake studies (McTaggart, 1992) that the ^{15}N natural abundance of the active pool of soil organic nitrogen is higher (0.370-0.377 atom%) than the value of 0.3663 atom%, and where possible the ^{15}N natural abundance should be measured at the study site.

When experiments are carried out in the laboratory with sieved and well mixed soils or litters (e.g. Bjarnason, 1988; Wessel and Tietema, 1992) it is relatively easy to achieve uniform addition of ^{15}N. However, in the field it is almost impossible to achieve such uniform applications (Barraclough, 1991; Davidson *et al.*, 1991). The heterogeneity of soils in the field, particularly the spatial variability of mineral nitrogen, is a serious problem for the development of *in situ* applications of isotope dilution, since rate estimates are calculated using differences, which amplify errors (Myrold and Tiedje, 1986).

To simplify calculations, mineralization and immobilization rates are usually assumed to be constant (Kirkham and Bartholomew, 1954; Blackburn, 1979), or to vary according to some known relationship (Nason and Myrold, 1991), between measurements of pool size and enrichment. The change in the ^{15}N enrichment of the NH_4^+ pool as mineralization and consumption processes proceed is complex and simple averages of the ^{15}N enrichment between measurements (Shen *et al.*, 1984; Guiraud *et al.*, 1989) can only give approximations of gross rates. The enrichment of ^{15}N only declines linearly for very short periods of time even where mineralization and immobilization are proceeding at constant rate (Bjarnason, 1988).

Table 1. List of symbols used in the equations.

AT_1	Total size of NH_4^+ pool, μg N g^{-1}, at time 1.
AT_2	Total size of NH_4^+ pool, μg N g^{-1}, at time 2.
AL_1	Size of labelled NH_4^+ pool, μg N g^{-1}, at time 1.
AL_2	Size of labelled NH_4^+ pool, μg N g^{-1}, at time 2.
t	Time between measurements, days.
@	Natural ^{15}N enrichment of mineralising NH_4^+.
m	Rate of mineralization / production of NH_4^+, μg N g^{-1} day^{-1}.
c	Rate of NH_4^+ consumption, μg N g^{-1} day^{-1}.

A formal mathematical treatment to allow the calculation of gross mineralization and consumption rates has existed since 1954 (Kirkham and Bartholomew, 1954), where the changes in pool sizes are described by differential equations, and solved analytically. Remineralization of immobilized mineral nitrogen is disregarded and the change in

amount of ^{15}N in the NH_4^+ pool is derived only from the consumption process. Symbols are defined in Table 1.

$$\frac{d\,AL}{d\,t} = -c\,\frac{d\,AL}{d\,AT}$$

Then

$$m = \frac{(AT_2 - AT_1)}{t}\,\frac{\log (AL_1AT_2/AL_2AT_1)}{\log (AT_2/AT_1)}$$

This framework is only valid where ^{15}N addition to the soil is high and where the ^{15}N enrichment of the NH_4^+ pool does not approach background by the end of the incubation period. However, it is still widely used for the calculation of gross mineralization rates (e.g. Davidson *et al.*, 1991; Ambus *et al.*, 1992). The model was extended to allow for nitrogen mineralizing at natural or any fixed ^{15}N abundance from the organic nitrogen pool for anoxic sediments (Blackburn, 1979) and for aerobic soils (Nishio *et al.*, 1985), deriving the decline in ^{15}N enrichment from the consumption and mineralization processes, but still not accounting for remineralization. Symbols are defined in Table 1.

$$\frac{d\,AL}{d\,t} = @\,m - c\,\frac{d\,AL}{d\,AT}$$

Then

$$m = \frac{(AT2 - AT1)}{t}\,\frac{\log ((AL2/AT2)-@\ /(AL1/AT1)-@)}{\log (\ AT2\ /\ AT1)}$$

These equations can be applied to $^{15}NO_3^-$ as well as $^{15}NH_4^+$ additions (Schimel *et al.*, 1989), allowing calculation of both gross mineralization and nitrification rates. A calculation method allowing calculation of gross nitrification rates where only $^{15}NH_4^+$ is added has also been developed (Wessel and Tietema, 1992).

Kirkham and Bartholomew (1955) developed a second mathematical framework allowing for nitrogen mineralizing at natural abundance and possible remineralization of added labelled nitrogen, by estimation of the interacting organic nitrogen pool. However, this model was developed for a simple system of two pools with mass conservation assumed and it cannot be corrected for losses to the NH_4^+ pool other than by immobilization to organic nitrogen.

Numerical solutions of the differential equations have also been developed, where numerical simulation by use of non-linear curve fitting, uses the measured ^{15}N abundances in the mineral N pool to fit the gross transformation rates and the size of the initial organic nitrogen pool involved (Myrold and Tiedje, 1986; Barraclough and Smith, 1987; Bjarnason, 1988). The advantages of numerical solutions are that they can be applied to any set of differential equations and the solution procedure remains the same, irrespective of the chosen set of rates, pools and other conditions (Wessel and Tietema, 1992). However, a high degree of replication is required to fit a solution with any degree of certainty and analytical models offer a quick way to calculate gross rates, so long as their assumptions have not been violated.

A study was carried out on three soil types to investigate the potential problems in applying the method used to measure gross mineralization rates described by Davidson *et al.* (1991). Concentrations of NO_3^- and NH_4^+ in injected and uninjected cores were compared to establish if the addition of $^{15}NH_4^+$ solution provided a stimulus to mineralization.

Incubations were carried out for increasing periods to determine an appropriate length of field incubation under Scottish temperature regimes. Observations were made of reminer-alization on one soil type, whilst the effect of soil moisture potential on mineralization was observed in intact cores of the other soils. Some preliminary work was carried out to establish an appropriate injection methodology with minimum disturbance to the core, and to determine the variability of immediate NH_4^+ fixation within a soil type. A further experiment attempted to assess the replication necessary to allow estimates of the mean mineral nitrogen pool size.

MATERIALS AND METHODS

Core sampling and core preparation

Intact soil cores, 54 mm in diameter and 20 cm deep, were sampled inside PVC sleeves using a specially designed corer at three sites on the Bush Estate, 15 km south of Edinburgh, in winter 1991-1992. Properties of the soils used in the study are presented in Table 2. The cores from the Beechgrove site were roughly crumbled and the sward torn into pieces in an attempt to simulate ploughing. The 'ploughed' soil was then packed back into the PVC sleeve with the sward distributed randomly throughout. The core was pushed out of the liner into a polyester sock to enable good moisture equilibriation during incubation. The cores from the Glencorse and No. 3 sites were left intact for the incubations. A further 100 samples were taken from the Beechgrove site, three days after ploughing in the spring, using an Dutch auger, to allow assessment of the spatial vari-ability of mineral nitrogen at the site at a time when mineralization measurements in the field were likely to be carried out.

Table 2. Properties of the soils used (0-20cm).

Site	Glencorse	No. 3 field	Beechgrove
Topsoil texture	clay loam	sandy loam	sandy loam
O.M. %	5.0	4.0	4.8
pH	6.4	6.3	6.0
Crop	cereals	cereals	pasture
Soil series	Winton	Macmerry	Winton

Core Injection

Several preliminary tests were carried out using an iodine-green dye solution to assess the most suitable method of injection and appropriate points of injection into the core. A visual assessment of the distribution of the injected solution was made by slicing the core horizontally at 2 cm intervals and studying the cross-sections. Preliminary extractions were carried out on twenty cores from the Beechgrove site to establish how much $^{15}NH_4^+$ should be added to each core to give an NH_4^+ pool with an enrichment of approximately 25 atom%. Following this work, five ml (measured gravimetrically) of 0.17 g l^{-1} $(^{15}NH_4)_2SO_4$ solution of 99.2 atom% enrichment, containing 190.06 µg ^{15}N, was injected into the core at five points using 1 ml syringes, which penetrated approximately 3 cm into the core. As

the solution was injected the needle was slowly withdrawn to enhance the distribution of solution through the core.

Core incubation

The cores from the Beechgrove site were incubated in sand tanks at a range of constant temperatures (4, 10, 14 °C), chosen to reflect the seasonal range of temperatures in Scotland, for a number of incubation periods (1, 2, 4, 7, 11, 14 and 18 days). The tanks were filled with 32 cm depth of coarse sand, which had been saturated and left to drain to give a water table 1 cm above the base of the tank and 11 cm below the base of the cores. This water level was maintained using a simple gravity fed constant head device, and enabled the cores to be held at constant moisture potential through the incubation period. The cores were randomly divided into three groups and allowed to equilibriate for four days at each temperature before any cores were injected.

Cores of the Beechgrove soil were placed into the sand tanks into auger holes of an appropriate size and the sand was tamped to ensure a good core-sand contact. For each temperature and each incubation period, three cores were injected with $(^{15}NH_4)_2SO_4$ solution and four replicate cores were used for assessment of net mineralization rates. The position of the cores was randomized in the tank, with cores 4 cm apart. Core temperatures were measured on each sampling date, using a temperature probe inserted through the centre of each core, prior to its removal from the tank. Gravimetric moisture contents were calculated for each core after removal, by oven drying at 105 °C for 24 hours.

The cores from the Glencorse and No. 3 sites were incubated at room temperature, approximately 18 °C, at a range of matric suctions (300, 100, 10 and 1 kPa) for three periods of time (7, 18, and 40 days). The 300 kPa and 100 kPa moisture potentials were obtained using a pressure membrane apparatus. The 10 kPa moisture potential was set up using a tension tank and this apparatus was modified to provide the 1 kPa potential. Four replicate cores for each matric tension and time period were injected with $(^{15}NH_4)_2SO_4$ solution, after equilibriation at the appropriate moisture potential. The gravimetric moisture contents of the cores were measured at the end of each time period.

Nitrogen analysis

Available NH_4^+ and NO_3^- were determined by extracting soil using 1 M KCl in a 1:5 soil:solution ratio. Extracts were filtered through Whatman 42 filter paper and NO_3^--N and NH_4^+-N determined by continuous flow analysis (Best, 1976; Crooke and Simpson, 1970). Four cores from each temperature or moisture potential were extracted at the beginning of the incubations. Analysis of injected cores for $^{15}NH_4^+$ enrichment was carried out by steam distillation followed by mass spectrometric determination as described by Hauck (1982). Where NH_4^+-N concentrations in the extracts were < 500 μg, a carrier solution containing 1 mg N as NH_4^+ was added prior to steam distillation to ensure that enough N was present to allow determination of the ^{15}N abundance.

To allow for fixation of the NH_4^+ in the soil, a recovery factor was calculated by extracting the NH_4^+ pool of the soil 15 minutes after injection and determining its ^{15}N enrichment (Davidson *et al.*, 1991). The recovery was calculated as the proportion of the added $^{15}NH_4^+$, 190.06 μg ^{15}N per core, which could be extracted from the soil in the NH_4^+ pool using KCl. This was carried out for the Beechgrove and Glencorse soils. A recovery factor was also calculated for the No. 3 and Glencorse soils where extractions took place

within twenty four hours of injection, and a comparable 24 hour recovery factor could be calculated from the incubations with the Beechgrove soil. No direct measurements of NH_4^+ clay fixation were made, and the precise clay mineralogy was difficult to estimate from the literature since the glacial till from which the soils are derived is very variable.

Mathematical models

The analytical model developed by Blackburn (1979) was used to calculate the gross mineralization and consumption rates for the system, where remineralization could be neglected. As no value for the natural ^{15}N enrichment of the active organic pool was available, a natural abundance of 0.3663 atom% was used. This may have led to slight underestimates of the rates of gross mineralization.

Statistical methods

Differences between treatments were compared using oneway Anova procedures and injected and uninjected cores compared using t-tests. All analyses were carrried out using the MINITAB stastistics package.

RESULTS AND DISCUSSION

Injection method

Preliminary tests revealed that one large injection often led to macropore flow out of the core, whilst several smaller injections of smaller volumes gave a better distribution of solution. It was therefore decided that five injections of 1 ml should be made to minimize leaching straight through macropores and maximise the proportion of the core volume receiving the solution. One injection was made centrally at the top of the core followed by four injections into the sides at approximately 5, 9, 13 and 17 cm from the top of the core. Further tests on four cores using this injection procedure found that 27 % of the cross-sections showed no visible signs of dye and there were clear signs of preferential flow of the solution along macropores.

Recovery

After 15 minutes, only 61.4 % on average of the $^{15}NH_4^+$ injected into the Beechgrove soil was recovered in a KCl extract, i.e. the mean recovery factor was 0.614 (S.E. 0.0366, 12 replicates). In the Glencorse soil, the corresponding recovery factor was 0.42. The processes leading to this disappearance did not seem to be highly spatially variable for any one soil series, but recovery was significantly different between soils ($P < 0.05$). The recovery factor was significantly ($P < 0.001$) affected by soil moisture (Figure 1), with the values decreasing in the drier soils ($r = 0.854$; $df = 22$). The relationship seemed to hold over a relatively wide moisture range, irrespective of soil texture. Injected solution was subject to greater soil aggregate suction in the drier soils, hence in these soils the solution may have penetrated further into the soil aggregates and possibly encountered more fixation/consumption sites in the same time period.

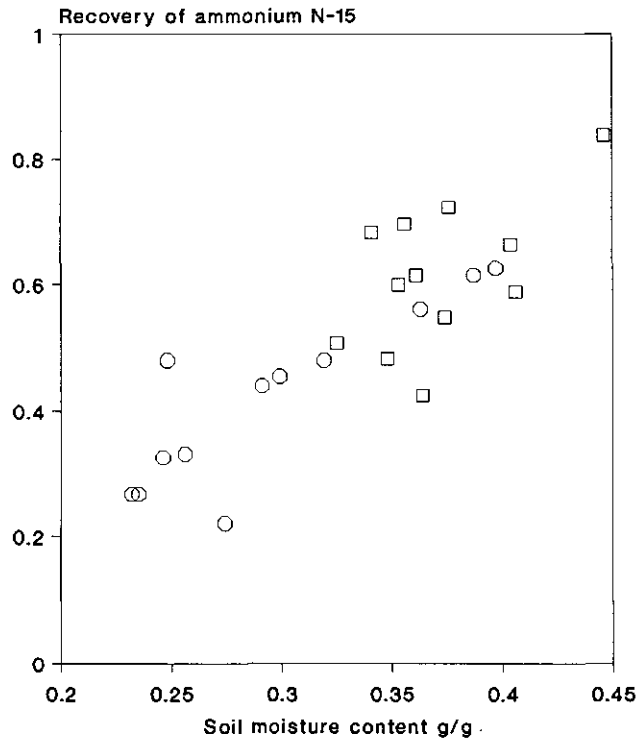

Figure 1. The recovery factor 15 minutes after injection plotted against soil moisture content (g g^{-1}) for two sites: Glencorse (clay loam) (Graph symbol, o) and Beechgrove (sandy loam) (Graph symbol, □). r = 0.854 df = 22.

Table 3. Mean recovery factors for $^{15}NH_4^+$ in the three soils, measured after 15 minutes and 24 hours. 18°C was room temperature.

Site	Time (hours)				
	0.25			24	
	Temperature (°C)				
	18	4	10	14	18
Beechgrove	0.61	0.076	0.054	0.13	N.D.
Glencorse	0.37	N.D.	N.D.	N.D.	0.17
No. 3	N.D.	N.D.	N.D.	N.D.	0.34

N.D. = not determined.

Recovery measurements made within 24 hours of injection for No. 3 field and the Glencorse site showed that the recovery in No. 3 soil was higher than that in Glencorse soil (Table 3). Using the data from extractions of the NH_4^+ pool made on the Beechgrove soils after 24 hours incubation, compared to the amounts of ^{15}N injected, very low 24 hour recoveries were calculated, which were not significantly different between incubation temperatures (Drury and Beauchamp, 1991).

Fixation of NH_4^+ by clays (Drury and Beauchamp, 1991) or organic matter (Foster *et al.*, 1985) are possible mechanisms to explain the rapid removal of $^{15}NH_4^+$ from the extractable pool after addition. Davidson *et al.* (1991) did not observe any significant effect of sterilization on the process and suggested that fixation by vermiculite and other 2:1 clays is the cause. Shen *et al.* (1984) observed that immediately after addition of $^{15}NH_4^+$, 1-5 % of the labelled N was found in the "fixed fraction", extracted by hypobromite.

It is assumed that after adjustment of fixed NH_4^+, equilibriation of the added and exchangeable NH_4^+ rapidly occurs to give a homogeneous exchangeable NH_4^+ pool, which can be simply described in terms of its size and ^{15}N enrichment. The injection methodology should therefore be optimized to give as close to uniform distribution of added ^{15}N as is possible. However, any injection procedure will introduce liquid preferentially into the macropores. Thus the injected NH_4^+ is spatially separated from much of the natural solution, exchangeable and fixed NH_4^+. Given the low effective diffusion coefficient for NH_4^+ in soils (Barber, 1984), it has been calculated that on average in a moist soil NH_4^+ or K^+ will diffuse about 0.13 cm in a day (Wild, 1981). The complex spatial distribution of production, consumption and fixation sites for NH_4^+ (Drury *et al.*, 1991) means that $^{15}NH_4^+$ may well not reach many of the microsites during the initial incubation period and hence it would be liable to a different consumption rate than the natural NH_4^+. If preferential consumption of $^{15}NH_4^+$ is occurring shortly after injection, then overestimates of gross mineralization rates will be made, if that period is included in the calculations.

Davidson *et al.* (1991) observed that there was no difference in the amount of ^{15}N extracted from the sterilized soils at 15 minutes or 24 hours, and therefore suggested that the abiotic reaction is completed very quickly. However, this is contrary to the results of Drury and Beauchamp (1991), who observed that fixation of $^{15}NH_4^+$ continued for at least 3 days. Schimel *et al.* (1989) measured similar consumption rates in intact and mixed cores and suggest that this indicates that the distribution of $^{15}NH_4^+$ was adequately uniform in the intact cores. Although it is not clear what processes are contributing to the rapid fall in the extractable pool of $^{15}NH_4^+$, it would seem more appropriate to allow at least 24 hours for the soil and added NH_4^+ to come into equilibrium before an initial measurement of the ^{15}N content of the NH_4^+ pool is made (Barraclough, pers. comm.). However, other workers only incubate cores for a 24-26 hour period to measure mineralization rates (Davidson *et al.*, 1991; Ambus *et al.*, 1992). Where preliminary tests are carried out on a soil then the addition of ^{15}N can be increased to allow for the $^{15}NH_4^+$ likely to be 'lost' in the first 24 hours, so that the enrichment of the pool does not approach background too quickly. Further work needs to be done to assess the speed with which an injected solution will diffuse throughout the soil pore system in soils of different structures and textures, perhaps using fluorescent dyes. Diffusion models (Darrah *et al.*, 1983) may also be used to assess how rapidly equilibrium between the pools of added and soil NH_4^+ may be attained.

Remineralization

One of the assumptions necessary for the use of most analytical solutions of pool dilution measurements is that enriched NH_4^+ immobilized in microbial tissue during the incubation is not remineralized (Kirkham and Bartholomew, 1954; Nishio *et al.*, 1985). An increase in the size of the ^{15}N content of the NH_4^+ pool strongly suggests that remineralization is occurring (Bjarnason, 1988) and if this is not taken into account in the mathematical framework used, negative gross immobilization rates may result (McTaggart, 1992).

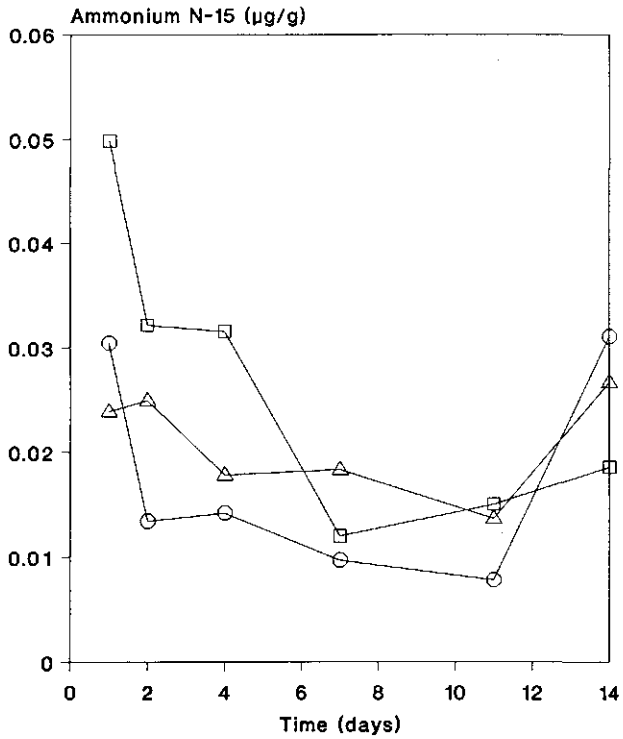

Figure 2. The change in $^{15}NH_4^+$ pool size (µg N g^{-1}) with length of incubation using soil from the Beechgrove site at three incubation temperatures: 4 (o), 10 (Δ), and 14 °C (□).

The incubations of Beechgrove soil cores showed an increase in the ^{15}N content of the NH_4^+ pool after seven days at 14 °C (Figure 2) , whilst at the lower temperatures, where slower turnover might be expected, evidence of remineralization appeared later in the incubations. Incubations should not therefore be carried out for longer than a week (Bristow *et al.*, 1987; Bjarnason, 1988), although at soil temperatures greater than 14 °C, this period might need to be further reduced to minimize the effect of remineralization. Remineralization is likely to be occurring before it is indicated by the increase in size of the $^{15}NH_4^+$ pool and will therefore decrease the decline in ^{15}N abundance and lead to underestimates of the gross rate of mineralization (Wessel and Tietema, 1992). Only by using models which take account of remineralization (mostly numerical) throughout the incubations (Myrold and Tiedje, 1986; Bjarnason, 1988) can gross rates of mineralization be found, where turnover rates are high and remineralization is significant from early in the incubations.

Release of $^{15}NH_4^+$, which has disappeared early in the experiment, can also lead to underestimates of the gross rates of mineralization. Fixed NH_4^+ is only released very slowly and when the solution activity of NH_4^+ is very low (Pasricha, 1976). Shen *et al.* (1984) observed that $^{15}NH_4^+$ fixed immediately after addition was released slowly during an incubation of 20 days with unfumigated soils, but remained constant or increased slightly when previously fumigated soils were incubated. In short-term experiments release of NH_4^+ from sites, where it has been selectively fixed, is unlikely to significantly influence the change in ^{15}N enrichment of the NH_4^+ pool.

Spatial variability of soil mineral nitrogen

Shortly after ploughing at the Beechgrove site in spring, NO_3^- levels were very low and at the bottom of the detectable range. NH_4^+ levels were higher, ranging from 1-7.5 μg N g^{-1} and showing a log-normal distribution (Macduff and White, 1984; Figure 3). The geometric mean was 2.52 μg N g^{-1} and the coefficient of variability was 60 %. Random groups of ten cores selected from the 100 cores sampled indicated that the mean was estimated within its true 95 % confidence interval on 6 out of 10 occasions (Figure 4). The problem of spatial variability of the inorganic nitrogen pool is well known and large samples need to be taken to allow the population mean to be accurately estimated, at this site 40 cores. The sampling of a large number of cores at the beginning and end of the field incubations would allow better estimation of the size of the mineral nitrogen pool, though if cores were covered to prevent leaching the sampling area would also have to be covered.

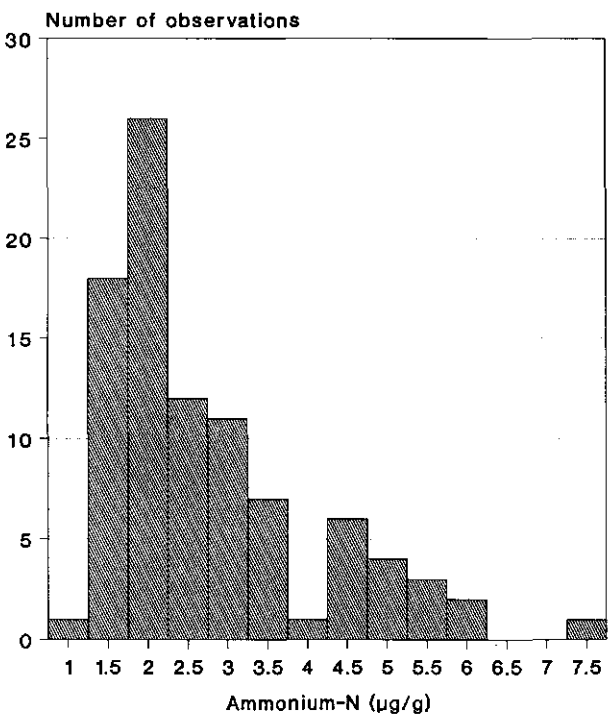

Figure 3. The frequency distribution for NH_4^+ concentration (μg N g^{-1}) determined for 100 cores from the Beechgrove site after spring ploughing.

This study of spatial variability was carried out after the core incubations were completed and indicated that the number of replicates in the experiment was far too low to allow reliable estimation of the mineral N contents of the population of cores at any sampling time. Wessel and Tietema (1992) carried out a separate experiment with a larger number of replicates to follow the changes in the mineral nitrogen pool as a part of their pool dilution experiments. However, practical considerations: initial core sampling;

injection time; available space for the incubation tanks; and analysis of mineral N in the cores; limited the number of replicates that could be included in the core incubations to three injected cores and four cores for assessment of net mineralization. This still resulted in 21 extractions, with 1 l of KCl per extraction, being carried out on each of the sampling days, which was approaching the maximum capacity of the laboratory at that time.

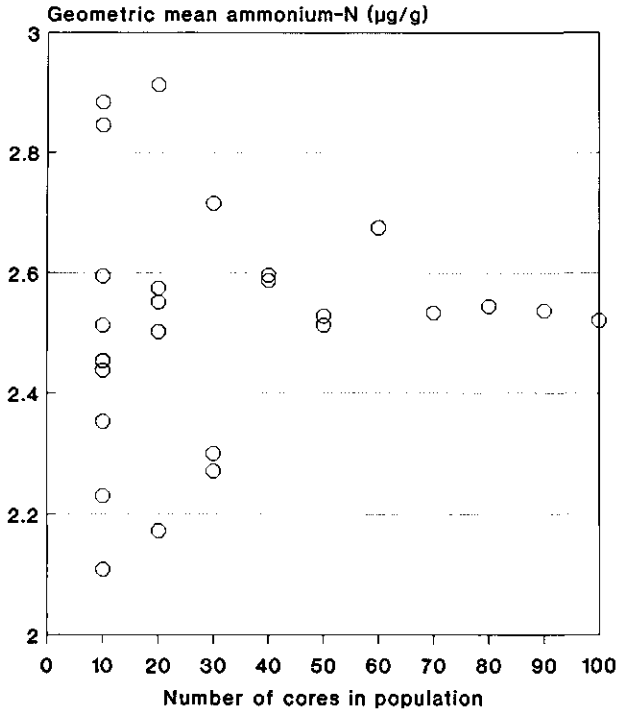

Figure 4. The geometric means for populations of different numbers of cores selected randomly from the 100 cores sampled at the Beechgrove site after spring ploughing against number of cores in the population.

Spatial variability of the soil mineral nitrogen pool and rates of transformation processes (Drury *et al.*, 1991) will lead to spatial variability of the ^{15}N enrichment of the NH_4^+ pool, even where the application is uniform (Davidson *et al.*, 1991). Where such variability is random, small but non-significant errors are introduced to the mineralization rates calculated, though spatially biased distribution of injected $^{15}NH_4^+$, e.g. with respect to depth, should be avoided. Davidson *et al.* (1991) showed by simulation that where less than 70% of mineralizing-immobilizing microsites received ^{15}N as a result of the injection, mineralization rates would be significantly underestimated.

Net mineralization rates

There were no significant differences in NO_3^- and NH_4^+ concentrations in injected and uninjected cores, indicating that injections of small amounts of high enrichment NH_4^+ solution in the experiment did affect rates of net mineralization. This is in accordance with

previous studies showing that net mineralization was unaffected or reduced following additions of inorganic nitrogen (Shen *et al.*, 1984). It was considered by Jenkinson *et al.* (1985) that only in exceptional circumstances (e.g. following recent additions of high C:N ratio residues or when pH is affected by fertilization) would addition of nitrogen affect net mineralization. In the Beechgrove soil, seven replicate cores were therefore used to give the NO_3^- and NH_4^+ concentrations for each temperature, at the end of each incubation period. NH_4^+ and NO_3^- concentrations were widely different between cores and even with seven replicates the standard error of the mean was large (Table 4). This was confirmed by the study carried out in the field after ploughing. In experiments with all the soils, the variability of the NH_4^+ and NO_3^- concentrations within treatments was often more significant than the difference between treatments and this affected the calculation of the net mineralization rates. The net rates of mineralization varied between 3.48 μg N g^{-1} day^{-1} and -1.23 μg N g^{-1} day^{-1}. No significant effects of increasing temperature or moisture on rates of net mineralization were seen, but the No. 3 soil showed more rapid rates of net mineralization (0.25 μg N g^{-1} day^{-1}) than Glencorse (0.13 μg N g^{-1} day^{-1}).

Since estimates of NH_4^+ pool sizes are needed for the calculation of gross mineralization rates, such variability introduces a large degree of uncertainty into the calculation of gross, as well as net, mineralization rates.

Gross mineralization and consumption rates

The estimates of gross mineralization rates in all the soils are similar to those observed by Davidson *et al.* (1991) in grassland soil. Estimates made in forest floor litters give much higher mineralization and consumption rates (Davidson *et al.*, 1991; Wessel and Tietema, 1992). There was no significant effect of increasing moisture potential in the No. 3 soil, with mineralization and consumption rates being estimated at 0.9 μg N g^{-1} day^{-1}. In the Glencorse soil, no significant effect of moisture potential was seen for consumption rates (0.75 μg N g^{-1} day^{-1}). However, mineralization rates increased significantly with decreasing moisture potential from 0.9 μg N g^{-1} day^{-1} at 300 kPa to 1.8 μg N g^{-1} day^{-1} at 10 kPa. In the Beechgrove soil, estimates of gross mineralization rates were made with reference both to day 0 (with a correction for 15 minute recovery) and to day 1 (Table 5). Decreasing rates were seen with increasing incubation period if day 0 was used. However, this trend almost disappeared if concentrations after 1 day were used as the initial conditions. This seems to indicate that it may not be remineralization leading to the fall in gross mineralization rates with time (Wessel and Tietema, 1992) but that some preferential consumption of $^{15}NH_4^+$ is occurring before equilibrium is established. Gross mineralization rates can also be calculated using the previous sampling date to give the new initial values, however, this provides little extra information; the use of measurements from day 1 gives the average of the other combinations. The spatial variability of the soil mineral N pool introduces problems in the calculation of precise gross mineralization rates (as indicated earlier). Mineralization and NH_4^+ consumption rates determined in intact cores indicate a very rapid turnover of the active fraction of the soil organic nitrogen (Ambus *et al.*, 1992).

Table 4. The concentrations of NO_3^- and NH_4^+ in the cores from the Beechgrove site at the end of each incubation period. Values are means of seven replicates with standard errors of the means given in brackets.

Incubation temperature (°C)	Time (days)	NH_4^+ (µg N g^{-1})	NO_3^- (µg N g^{-1})
4	0	0.96 (0.19)	7.05 (1.23)
	1	1.85 (0.26)	9.64 (1.09)
	2	2.01 (0.45)	8.99 (0.89)
	4	3.04 (1.07)	9.34 (0.76)
	7	2.04 (0.27)	10.09 (1.06)
	11	1.81 (0.12)	12.81 (1.00)
	14	5.61 (1.11)	12.45 (1.68)
	18	3.00 (1.04)	11.12 (1.53)
10	0	1.75 (0.18)	5.78 (3.12)
	1	2.60 (0.39)	3.70 (0.53)
	2	2.92 (0.41)	4.59 (1.80)
	4	3.32 (0.71)	3.91 (0.55)
	7	5.14 (0.63)	3.30 (0.48)
	11	3.51 (0.85)	3.46 (0.75)
	14	3.93 (1.45)	10.58 (1.78)
	18	2.62 (0.77)	6.87 (1.85)
14	0	3.51 (1.12)	3.84 (0.78)
	1	4.72 (1.23)	4.24 (1.63)
	2	4.01 (0.93)	5.51 (2.58)
	4	4.35 (1.45)	7.32 (2.78)
	7	2.89 (0.96)	5.00 (0.89)
	11	2.44 (0.68)	4.02 (1.00)
	14	3.25 (1.00)	7.75 (1.57)
	18	2.70 (0.83)	18.38 (3.84)

Table 5. Gross mineralization (m) and consumption (c) rates for the Beechgrove soil for the first seven days calculated with t = 0 and t = 1 as the initial values.

Incubation temperature	Day	m (µg N g^{-1} day^{-1})		c (µg N g^{-1} day^{-1})	
		To t = 0	To t = 1	To t = 0	To t = 1
4 °C	1	4.35		3.67	
	2	3.42	2.91	3.00	2.74
	4	2.82	2.28	2.33	1.85
	7	1.26	0.86	1.13	3.56
10 °C	1	7.30		6.62	
	2	4.01	0.37	3.50	1.89
	4	2.89	1.23	2.53	0.98
	7	2.20	1.20	1.87	0.93
14 °C	1	9.69		8.62	
	2	5.40	2.10	5.22	1.41
	4	2.98	1.01	2.80	1.14
	7	2.52	1.90	2.63	2.20

CONCLUSIONS

The use of pool dilution experiments seems to provide a method for measurement of the gross rates of some of the simultaneous and often opposing processes occurring in soils. However, the soil internal nitrogen cycle model proposed by Jansson (1958) does not always fit all the observed results (Myrold and Tiedje, 1986; Drury *et al.*, 1991). Pool dilution experiments may well pose as many questions as they answer about the interlocking processes occurring in the soil. Use of the method must be accompanied by a check that the underlying assumptions are valid and experiments must be designed to minimize error multiplication factors (Wessel and Tietema, 1992). Most information may be obtained, where the fate of ^{15}N added is also determined at the end of the incubation period.

Optimizing the injection procedure for the soil type on which mineralization measurements are to be carried out can help to ensure that a homogeneous NH_4^+ pool is achieved as rapidly as possible. The injection procedure should be checked for each soil to be studied using dyes and/or modelling. Even where uniform addition of $^{15}NH_4^+$ can be achieved, slow equilibriation of NH_4^+ between the fixed and exchangeable pools, limited by diffusion, will also lead to a pool dilution effect and added $^{15}NH_4^+$ and natural NH_4^+ may be subject to different consumption rates initially. While this problem is exacerbated by non-uniform additions in intact cores, it may also be a problem in laboratory incubations. Short-term recovery analysis to correct for the amount of $^{15}NH_4^+$ becoming irrecoverable is necessary (Davidson *et al.*, 1991). However, from our results it would seem more appropriate to determine the initial NH_4^+ pool size and enrichment at least 24 hours after injection. It is also important that the ^{15}N natural abundance in the soil organic N, or active pool if possible, is also determined at the study site.

Davidson *et al.* (1991) suggest that estimates of rates are most accurate where drops in the enrichment of the pool are large over the incubation period, but the final ^{15}N enrichment should not approach too closely to background (Wessel and Tietema, 1992). Larger additions of $^{15}NH_4^+$ would help to achieve these aims, especially in soils where recovery values are low, but care must be taken as the system may be dramatically altered if substrate is added to a substrate-limited process.

Application of analytical solutions to calculate gross rates means that incubations should be completed before recycling becomes significant. Recycling was observed after seven days in the experiment with the Beechgrove soil. However, remineralization may become significant much earlier. The length of incubations should therefore be limited to approximately one week after injection, where soils temperatures do not exceed 14 °C. This limits the use of analytical solutions to incubations carried out over very short periods, once an initial equilibriation has been carried out. The use of numerical solutions may remove this restriction. However, numerical models may need to be expanded to take NH_4^+ fixation into account.

The measurement of gross mineralization rates can however only produce estimates of these rates especially where measurements are made *in situ*. The spatial variability of mineral nitrogen in soils leads to a measurement of initial and final mineral nitrogen pools with significant errors attached, which are multiplied in the calculation of gross mineralization rates. The use of a parallel experiment using a large number of uninjected cores over the same or a longer time period than the injected cores, allows an increase in the accuracy with which pool sizes can be measured.

The use of pool dilution techniques may on many occasions seem to have more problems than advantages. However, the continued sensible use and continual reassess-

ment of these procedures can lead us further in our understanding of the processes controlling the supply of mineral nitrogen from soils in forms available to plants.

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Nitrogen fluxes in a cropped sandy and a loamy soil measured by sequential coring

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Abstract

During a three year period N fluxes in the plough layer (0-20 cm) were measured in a coarse sandy soil and a sandy loam soil using *in situ* core incubations. N mineralization, nitrification and plant uptake plus leaching from the plough layer were calculated for treatments with barley and a catch crop. Due to high variability no statistically significant differences were observed between treatments, but temporal variations in N fluxes were reflected by the technique. Rates of N mineralization in the sandy loam soil were 184, 146, and 198 kg N^{-1} yr^{-1}, respectively. In the coarse sandy soil the corresponding values were 65, 60, and 70 kg N^{-1} yr^{-1} in treatments fertilized with 60 kg N^{-1} yr^{-1}, and 93, 73, and 87 kg N^{-1} in treatments receiving 120 kg N^{-1} yr^{-1}.

INTRODUCTION

Nitrogen availability in agricultural soils is determined by fertilization and by N mineralization. It has proved difficult to measure mineralization rates in the field because of the lack of suitable technique. However, a sequential *in situ* coring technique has been developed (Adams and Attiwill, 1986; Raison *et al.*, 1987) for measuring N mineralization, as well as other N fluxes such as nitrification, leaching, and plant uptake. The technique has been used to study N fluxes in forest soils (Adams and Attiwill, 1986; Raison *et al.*, 1987; Whynot and Weetman, 1991), peat soil (Williams and Wheatley, 1992), grassland soils (Hatch *et al.*, 1990, 1991), and fertilized agricultural soils (Debosz and Vinther, 1989; Boone, 1990; Mazzarino *et al.*, 1991; Debosz *et al.*, 1991; Debosz, 1994), and has shown promising results concerning mineralization measurements in these ecosystems.

The main objectives of this study were to: (i) estimate N fluxes in an agricultural cropping system and (ii) further evaluate the technique for use in agricultural systems.

MATERIALS AND METHODS

Measurements of the N fluxes were carried out during the years 1988-1991 on two soil types in Denmark, a coarse sandy soil in Jyndevad and a sandy loam soil in Ødum. The

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following treatments were included: (i) spring barley without a catch crop, (ii) spring barley with a catch crop (Ryegrass, *Lolium perenne* L.), ploughed under in the autumn and (iii) spring barley with a catch crop, ploughed under in the following spring just before sowing the next crop. In Jyndevad two fertilization levels were included corresponding to 60 and 120 kg N ha^{-1} yr^{-1}. The fertilizer was calcium-ammonium-nitrate containing ammonium and nitrate in the ratio 1:1. In Ødum there was only one level of fertilization (130 kg N ha^{-1} yr^{-1}), and the fertilizer was applied as NPK (21-4-10) also containing ammonium and nitrate in the ratio 1:1. The field experiments were established according to a split-plot design with four replications (blocks).

The technique as described by Raison et al. (1987) was used. At the start of each incubation period, soil samples were collected and PVC-tubes with a diameter of 35 mm were inserted 20 cm into the soil and equipped with a lid to prevent leaching of nitrogen during the incubation period. At the end of the incubation period, which lasted from one to two months depending of the time of the year, the soil from the tubes was collected, and a second incubation was started. The number of replicates and analyses per treatment are shown in Table 1.

Table 1. Number of samples, replications and analyses per treatment.

	No. of samples per plot	No. of analyses per plot*	No. of replicates (blocks)	No. of analyses per treatment
Bulk soil:				
Jyndevad	10	1	4	4
Ødum	10	1	4	4
Incubated soil:				
Jyndevad	6	2	4	8
Ødum	10	4	4	16

* Soil in tubes (incubated soil) were pooled and divided into 2 and 4 samples, respectively.

The difference in the concentration of inorganic nitrogen in the bulk soil before incubation ($N_{b(0)}$) and at the end of incubation ($N_{b(t)}$), as well as the content of inorganic nitrogen in the *in situ* incubated tubes ($N_{c(t)}$), was used to calculate N fluxes (Figure 1).

Net mineralization	$= N_{min}$	$= (N_{c(t)} - N_{b(0)}) + N_{den}$
Net nitrification	$= N_{nitr}$	$= (NO_{3c(t)} - NO_{3b(0)}) + N_{den}$
Plant uptake plus leaching	$= N_{plupt+leach}$	$= N_{c(t)} - N_{b(t)}$

The denitrification loss (N_{den}) has been measured (Vinther, 1992), and was in the coarse sandy soil less than 1 kg N ha^{-1} yr^{-1}, and in the sandy loam soil 14, 9 and 14 kg N ha^{-1} yr^{-1}, respectively, during the three years of investigation.

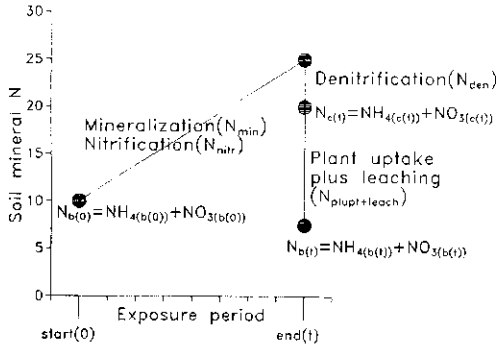

Figure 1. Scheme used to estimate N fluxes during one incubation period.

Inorganic nitrogen in the soil samples was extracted by shaking for 1 h with 2 M KCl in a soil:liquid ratio of 1:2. Concentrations of extracted ammonium and nitrate were determined by colorimetric methods adapted to an automatic flow injection system (FIAstar®, Tecator, Höganäs, Sweden). The content of nitrogen was calculated on dry soil basis. Moisture content was determined gravimetrically on subsamples dried to constant weight at 105 °C.

Statistical calculations were performed using SAS-procedures (SAS Institute Inc., 1989). The General Linear Model (GLM) procedure was used for analysis of variance to determine statistically significant differences between treatments. The number of analyses (samples) required to estimate inorganic N pools to within a 10 or 20 % standard error of the mean were calculated for each treatment based on the formula of Whynot and Weetman (1991):

$N = t^2 S^2 / E^2$, where N = no. of analyses per treatment, t = Student's t for a given probability, S = variance, and E = precision desired. The number of analyses (N) was then correlated with the months in which the samples were collected in order to evaluate how fertilization and plant growth affects the number of analyses necessary to obtain a given accuracy rate.

RESULTS AND DISCUSSION

No significant differences between treatments were found for either mineralization (Table 2), nitrification or "loss" (plant uptake + leaching), when testing individual soils. Only the effects of major components were significant, e.g. soil type ($P < 0.05$), level of fertilization ($P < 0.001$) and the time of the year, at which incubations took place ($P < 0.001$). The interactions between treatments and incubation period were also non-significant, indicating that there were no periods during the year where significant differences between treatment could be found. Any real treatment effects that may have occurred were obscured by high variability. An analysis of the number of samples required to

Table 2. Summary of analysis of variance on calculated N-mineralization.

	Degrees of freedom	F-value
soil	1	7.80 *
N level	2	16.20 ***
residual	654	
total	657	
Jyndevad:		
N level	1	15.60 ***
residual	429	
total	430	
60-N:		
incub. period (ip)	17	2.60 **
block	3	1.50 ns
treatment	2	0.10 ns
ip * treatment	34	1.20 ns
residual	159	
total	215	
120-N:		
incub. period (ip)	17	12.20 ***
block	3	0.50 ns
treatment	2	1.40 ns
ip * treatment	34	1.30 ns
residual	159	
total	215	
Ødum:		
incub. period (ip)	18	4.56 ***
block	3	0.41 ns
treatment	2	1.45 ns
ip * treatment	36	0.80 ns
residual	168	
total	227	

* probability of arising by chance is less than 0.05
** probability of arising by chance is less than 0.001
*** probability of arising by chance is less than 0.0001
ns non significant

estimate inorganic N pools with 10 % precision showed that up to 150 samples were needed, as compared to the 8 or 16 used in the two soils, respectively (Table 1). The number of samples required depended on the period of the year where incubations took place. In Figure 2, the number of samples needed to obtain 20 % precision is presented. This shows a need to sample more intensively after fertilization and in the growing period, than in the autumn and winter periods. Similarly, Whynot and Weetman (1991) concluded that due to high spatial variability in inorganic pools following fertilization, significant differences could not be found between the calculated flux rates. Due to the

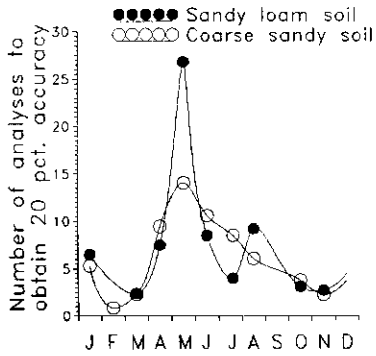

Figure 2. Number of samples required to estimate pools of inorganic N with 20 % precision.

high spatial variability, and consequently no differences between treatments, the N fluxes presented below were calculated as means of treatments.

Monthly fluxes of nitrogen showed similar trends for mineralization, nitrification and plant uptake + leaching; increasing rates during the spring and summer followed by decreasing rates during the autumn and winter periods. Figure 3 shows the monthly N-mineralization rates. Highest mineralization rates were measured in the periods after fertilizer application, at the same time as the content of inorganic nitrogen dropped to background level, and during the growing season. The mineralization rates measured during the growing periods in the sandy loam soil were ranging from 0.3 to 1.5 kg N ha^{-1} d^{-1}, whereas the rates during the winter periods were in the range from 0.1 to 0.5 kg N ha^{-1} d^{-1}. The corresponding values in the coarse sandy soil were 0.15 - 0.5 kg N ha^{-1} d^{-1} and 0.03 - 0.17 kg N ha^{-1} d^{-1}, respectively. Debosz *et al.* (1991), also using the *in situ* core technique, measured N mineralization rates from 0.02 to 1.9 kg N ha^{-1} d^{-1} in a sandy loam soil in Denmark to which pig slurry has been applied. At the same location as in the present investigation (coarse sandy soil in Jyndevad) Debosz *et al.* (1991) measured rates from 0.1 to 1.3 kg N ha^{-1} d^{-1}, which corresponds well with the present results. In grass and grass/clover swards in S.W. England, Hatch *et al.* (1990) found daily mineralization rates ranging from 0.02 to 1.90 kg N ha^{-1}. This is slightly lower than those found by Hatch *et al.* (1991), who measured overall mean daily rates from 1.7-2.3 kg N ha^{-1} d^{-1} in grass swards.

The higher N mineralization rates during the growing periods can be attributed to different factors such as soil temperature, the *in situ* core technique itself, and re-mineralization of microbially immobilized fertilizer nitrogen, or most likely to a combination of these factors.

Firstly, soil temperatures in Denmark are during the months from March to July increasing from 3-5°C to 15-20°C, which partly explains the increasing N mineralization rates during this period. The relationship between temperature and N mineralization is generally described with a Q_{10} of approximately 2 (Stanford *et al.*, 1973; Addiscott, 1983; Kladivko and Keeney, 1987).

Secondly, the method itself may contribute to higher mineralization rates during the growing season by disturbing the natural water balance in the incubated soil and by

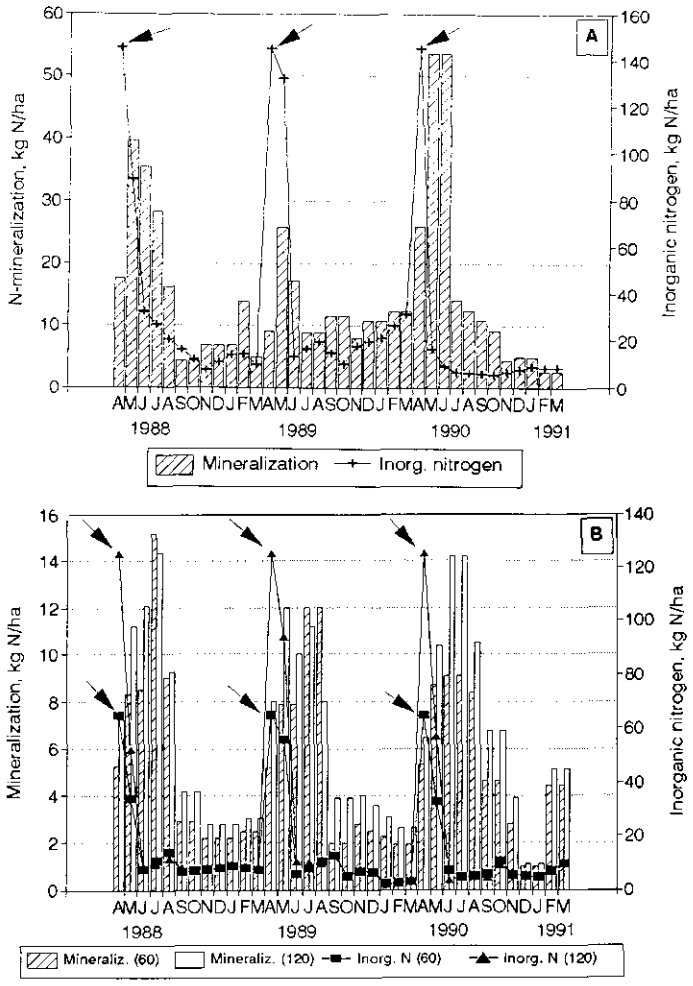

Figure 3. Mineralization rates on a monthly basis in the sandy loam soil (A) and the coarse sandy soil (B). Arrows indicate application time and these values of inorganic N were not measured.

severing roots when inserting the tubes into the soil or by isolating roots from the incubated soil.

Mineralization rates are known to be sensitive to the content of soil water (Cassman and Munns, 1980; Myers *et al.*, 1982). By protecting soil in the covered tubes from rain and by preventing plants from taking up soil water from the incubated soil, the moisture content is likely to be different from that of the bulk soil. In the present investigation, the difference between soil moisture content inside and outside the tubes on most occasions were statistically non-significant. However, moisture contents in soil incubated in tubes

were during the growing season consistently higher than the moisture contents in the bulk soils, with a few exceptions where sampling was done shortly after rain showers. Maximum difference was observed once where samples were collected while it was raining following a dry period. This resulted in a 6 % higher soil moisture content in the bulk soil than in the incubated soil. During the winter period (October to March) the difference was never above 1 %. Similarly, Whynot and Weetman (1991), who used the coring technique, and Redman *et al.* (1989) incubating their soils in plastic bags, observed that the soil moisture contents were higher in the incubated soils than in the bulk soils during the growing period. The slightly higher moisture content inside the tubes may also contribute to higher mineralization rates during these periods.

The problems as a consequence of isolating roots from the incubated soil or root severing have been discussed with somewhat contradictory conlusions (e.g. Raison *et al.*, 1987; Rees, 1989; Hatch *et al.*, 1990). Incubating soil in isolation from plant roots often results in a rapid increase in mineral N, and immobilization of mineralized N, especially in soils with high microbial activity (Rees, 1989). In that case an underestimation of the mineralization rates by the *in situ* core method can be the consequence. Mineralization rates obtained by the method may also be overestimated through decomposition of excised roots and the release of inorganic nitrogen (Hatch *et al.*, 1990). On the other hand, Raison *et al.* (1987) noted the inclusion of excised roots might result in increased N immobilization and therefore underestimate mineralization. During the first two months after fertilizer application a considerable increase in the mineralization rates were observed (Figure 3), which partly can be explained by the factors described above. But also re-mineralization of microbially immobilized fertilizer nitrogen may contribute to the higher rates in this period. Whynot and Weetman (1991) found that even during the period immediately following fertilization, net mineralization occurred in all fertilized plots.

The accumulated N fluxes expressed as annual fluxes are shown in Table 3. During the three years of investigation, the annual N mineralization was 184, 146 and 198 kg N ha^{-1}, respectively, in the sandy loam soil. The corresponding values for nitrification were 179, 178 and 195 kg N ha^{-1}, showing that all mineralized nitrogen was also nitrified. In the coarse sandy soil the corresponding values for N mineralization were 65, 60, and 70 kg N ha^{-1} yr^{-1} in treatments fertilized with 60 kg N ha^{-1} yr^{-1}, and 93, 73, and 87 kg N ha^{-1} yr^{-1} in treatments receiving 120 kg N ha^{-1} yr^{-1}. In the coarse sandy soil nitrification accounted for 97-146 % of the mineralization. The amount of nitrogen which was nitrified was generally higher than the amount mineralized. This was probably due to the fact that 50 % of the fertilizer was in ammonium form, and could therefore also contribute to the nitrification. At both locations, but especially at the sandy loam soil, the total mineralization was lower in the second year than in the first and third year. This was most likely due to the climatic conditions. The second year of investigation (1989) was very dry. The precipitation was at the sandy loam location only 75 % of normal, whereas the precipitation at the coarse sand location was 91 % of normal, resulting in only a minor reduction in mineralization (Table 3).

Table 3. Annual fluxes of inorganic nitrogen (kg N ha^{-1}) in the plough layer (0-20 cm) of a sandy loam soil and a coarse sandy soil. Bracketed values represent standard error of mean of the three treatments.

Period	N input/output	Soil type		
		Sandy loam	Coarse sand	
1988-89:	Input			
	Deposition*	21	21	21
	Fertilization	130	60	120
	Mineralization	184(18)	65(7)	93(4)
	Output			
	Uptake+leaching	317(30)	139(11)	269(48)
	Denitrification**	14	1	1
	Input - output	4	6	-36
1989-90:	Input			
	Deposition*	21	21	21
	Fertilization	130	60	120
	Mineralization	146(6)	60(3)	73(9)
	Output			
	Uptake+leaching	305(10)	154(4)	250(8)
	Denitrification**	9	1	1
	Input - output	-17	-14	-37
1990-91:	Input			
	Deposition*	21	21	21
	Fertilization	130	60	120
	Mineralization	198(23)	70(18)	87(19)
	Output			
	Uptake+leaching	302(10)	107(10)	198(49)
	Denitrification**	14	1	1
	Input - output	33	43	29

* from Asman and Runge (1991), ** from Vinther (1992).

In order to estimate *in situ* mineralization, which is highly dependent upon environmental conditions, it is important that measurements are carried out under conditions which are as close as possible to the natural conditions. By using the *in situ* core method, soil disturbance and compaction are minimal, and the method provides a relatively rapid and convenient means of estimating the N fluxes. Results showed that seasonal and annual fluctuations were positively correlated to temperature and precipitation, respectively. The major disadvantages of the method seems to coincide with fertilizer application and plant growth, and are connected to the different conditions, which exist inside and outside the incubated cores.

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Investigation of N mineralization immobilization dynamics in blanket peat to optimize the N economy of improved grass pasture

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Abstract

The spring application of fertilizer nitrogen often fails to stimulate the growth of grass in improved grass pastures on blanket peat. The quantity of N available for plant uptake will be influenced by the mineralization/immobilization dynamics of the microbial biomass. A field experiment was set up to investigate the extent of net N mineralization and immobilization throughout the year.

The seasonal pattern of net N mineralization and immobilization was determined by *in situ* incubation. Fertilizer N was applied as ammonium nitrate (250 kg N ha^{-1}) split over a spring and summer application.

In fertilized plots, net N immobilization predominated in spring while over summer, net mineralization proceeded, releasing N for plant uptake.

Poor plant responses to fertilizer N in spring may be due to the occurrence of N immobilization, limiting the quantity of available N for plant uptake. This aspect was investigated further in a series of laboratory microcosm experiments using intact cores removed from the field site. Fertilizer N was applied as ammonium nitrate (150 kg N ha^{-1}) to the cores. Initial experiments showed that although immobilization of N into the microbial biomass was occurring (20 % of applied N appeared to be incorporated into the biomass), it was not to such an extent that plant growth would be N limited. Microbial immobilization was enhanced by lowering the water table level suggesting the degree to which the microbial biomass competes with grass roots for fertilizer N is strongly dependent on a combination of climatic and edaphic factors.

INTRODUCTION

Low productivity of upland grass pastures may be improved by drainage, liming and the application of fertilizers (Frame *et al.*, 1985). However, fertilizer application in spring often fails to stimulate grass growth giving low fertilizer use efficiencies (Rangeley, 1988).

The dynamics of the microbial biomass play a major role in determining the supply of nutrients, particularly N, for plant uptake. Mineralization and immobilization of N occur

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simultaneously. In peat, as in other soil systems, it is the net production of mineral N which will determine whether N is available for plant uptake.

Microbial immobilization has been shown to be a rapid and important process in newly-sown grassland systems (Nannipieri *et al.*, 1985). A substantial proportion of applied N may be found in the microbial biomass; amounting to approximately 20 % in experiments by Ledgard *et al.* (1988) following a late winter application of fertilizer to a grass-clover pasture.

The extent of competition for available N between plant roots and the microbial biomass will depend on a combination of climatic and edaphic factors. Conditions in spring may be such that microbial immobilization of N is favoured, resulting in competition for available N and restricted plant growth.

A field experiment was set up to investigate the extent of net mineralization and immobilization throughout the year on an improved grass pasture. Microcosm experiments were then designed to examine in more detail the factors contributing to the net N immobilization that occurs at this site in spring.

MATERIALS AND METHODS

Site description

The field site is an improved grass pasture on blanket peat in Sutherland, Northern Scotland. The site is 200 m above sea level with a mean daily temperature of 8 °C and an average rainfall of about 850 mm per year. Previous vegetation at the site consisted of *Trichophorum caespitosum* L. Hartm., *Eriophorum vaginatum* L., *Molinia caerulea* L. Moench and *Sphagnum* spp. In 1982, the site was screw-levelled (vegetation cut and re-distributed), limed with ground limestone (1 t ha^{-1}) and reseeded with a mixture of *Phleum pratense* L. (Timothy), *Lolium perenne* L. (ryegrass), *Poa trivialis* L. and *P. pratensis* L. (rough and smooth-stalked meadow grass) and *Trifolium repens* L. (white clover).

In 1985, at the start of the experiment, the vegetation consisted mainly of rough and smooth-stalked meadow grass with some white clover.

Field experiment

Plots (2 m by 2 m) enclosed to prevent grazing by sheep, were fertilized with NPK as detailed in Williams and Wheatley (1992). The plots were sampled at approximately monthly intervals between March and November over three years. Sampling consisted of extracting cores from the peat, removing the surface vegetation, wrapping the cores in polythene and replacing in the peat, for a one month *in situ* incubation. On the next sampling date, fresh and incubated cores were removed to the laboratory for analysis.

Differences in mineral N content at the end of the incubation compared to that of fresh samples taken at the start of the incubation were used to calculate mean daily rates of net mineralization or immobilization for the period.

Microcosm experiment

Intact cores (7.6 cm diameter, 19.5 cm long) with vegetation were removed from the peat and re-established under constant water table level in the glasshouse. Fertilizer N as

ammonium nitrate (rate equivalent to 150 kg N ha^{-1}) was applied to the cores as a solution.

Water table depth was varied by standing the cores in beakers containing different volumes of water. Harvest occurred 10 days after fertilizer addition and plant N, biomass N and available N concentrations were determined.

A number of cores were amended with glucose to investigate whether microbial immobilization of N was enhanced by the presence of available C. The glucose solution (rate equivalent to 150 kg C ha^{-1}) was injected into the cores at two depths ensuring an even distribution of C within the top 0-5 cm. This was repeated at five-day intervals, for four applications in total, allowing the continuous presence of a readily available carbon source. Harvest occurred on two separate dates, 21 days and 40 days after the application of fertilizer.

RESULTS

Field experiment

During spring 1985 and 1986, the net change in the mineral N content was negative (Figure 1), suggesting immobilization of N. Increases in the mineral N content occurred over the summer, in two years out of three, releasing N for plant uptake. In 1987, immobilization of N continued from spring into the summer months. An increasing proportion of mineral N (mainly ammonium-N) was incorporated into the microbial biomass over the three years.

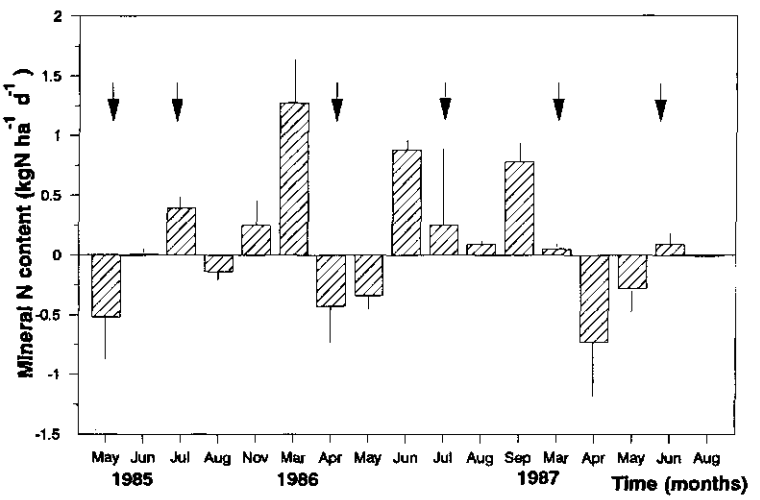

Figure 1. Changes in mineral N content (kg N ha^{-1} d^{-1}) in peat fertilized with NPK (arrows). Bars show standard errors.

Microcosm experiment

The figures given in Tables 1 and 2 represent differences between the fertilized and unfertilized cores (or treated and untreated cores). Not all the N found in these pools will have been derived directly from the application of fertilizer.

Table 1. Partitioning of N into the microbial biomass and above-ground herbage at different water table levels, 10 days after fertilizer N application. Values represent differences between fertilized and unfertilized cores (n = 5). Figures in parentheses are standard errors.

Water table level below surface	Biomass N (kg N ha^{-1})	Plant offtake (kg N ha^{-1})
5 cm	20 (± 29)	31 (± 6)
15 cm	38 (± 13)	34 (± 6)

Table 1 illustrates the partitioning of N into the microbial biomass and plant herbage at two different water table levels. At the lower water table level, there was an increased diversion of N into the microbial biomass although no differences in the amount found in plant offtake could be seen.

Table 2. Partitioning of N into the microbial biomass and above-ground herbage, with and without carbon addition, 20 days after fertilizer N application. Values represent differences between fertilized and unfertilized cores (n = 5). Figures in parentheses are standard errors.

Treatment	Biomass N (kg N ha^{-1})	Plant offtake (kg N ha^{-1})
N	15 (± 8)	69 (± 13)
C + N	13 (± 11)	53 (± 5)

The addition of carbon to the cores did not appear to stimulate microbial immobilization of N (Table 2) although a decrease in plant N offtake values was observed.

DISCUSSION

The balance of available N varies throughout the year. The release of N via mineralization results in N being available for plant uptake over the summer months, although, immobilization which generally occurs in spring, may be limiting early plant growth. The early application of fertilizer to upland, improved pastures is important in providing a good sward. Problems of immobilization of available N into the microbial biomass need to be investigated further to manage these systems more effectively.

Low temperatures, characteristic of spring, generally increase the proportion of applied N immobilized (Ledgard *et al.*, 1989) and so the optimum time of fertilizer application will

become increasingly important to predict for upland areas (Frame *et al.*, 1985).

By lowering the water table level in peat, more N was diverted into the microbial biomass. Increased amounts of potentially available nutrients, particularly N (Williams and Wheatley, 1988), and greater microbial activity generally occur under drier conditions. Plant growth and root development are also improved by aeration, although this may be a more long-term process.

Providing the microbial biomass with a readily available supply of carbon (added as glucose in this study or via rhizodeposition in the field) may enhance immobilization of N. At sampling, however, no increases in the proportion of N immobilized were detected. This probably reflects the relative short-term nature of N-processing by the microbial biomass in peat (harvest occurred 20 days after the application of fertilizer). Immobilization of N can occur very rapidly (Bristow *et al.*, 1987) and consequently, the sampling regime may not have been coupled with immobilization dynamics. There was no evidence of gaseous loss of applied N in this study, although the denitrification potential at this site is high (Wheatley and Williams, 1989).

In conclusion, field investigation of an improved grass pasture on blanket peat demonstrated immobilization of N in spring with mineralization predominating over the summer, releasing N for plant uptake. Use of laboratory microcosms identified that lowering the water table level caused a greater diversion of N into the microbial biomass. The extent of competition for available N between plant roots and the microbial biomass in peat was affected by the amount of water-logging at the site, which arose from a combination of climatic and edaphic factors.

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A modified Stanford and Smith method for the study of the mineralization of nitrogen from organic materials

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Abstract

A modified Stanford and Smith method for the determination of potentially mineralizable organic nitrogen in soil was studied to determine the suitability of this method as a testing procedure for the mineralization of nitrogen from different organic materials (animal manure, earthworm casting, leather meal, lactic casein). The various organic materials were applied to five different Italian soils and the mineralizable nitrogen was determined periodically during a 12-week incubation period. The results show that this method is suitable for the study of the mineralization of nitrogen from the various organic materials applied to the soil.

INTRODUCTION

Organic fertilization is a very important agricultural practice in Italy, where the humus depletion is about 1.5 % per year (Benedetti, 1984) (the humus content of Italian soils is on average 1.5 %), to preserve fertility of soils.

Furthermore, the mineralization of organic nitrogen is an important factor when determining the appropriate rate of organic application to crop land.

One of the most important problems in soil science today is the evaluation of the potentially mineraliazable nitrogen arising from the application to the soil of organic materials.

In order to evaluate the mineralization of organic nitrogen, good progress was made by Stanford and Smith (1972) who developed a method based on leaching the nitrogen mineralized, at prefixed intervals, in order to simulate plant uptake. Later this method was used to measure the mineralization of sewage sludge, animal manure and compost (Magdoff and Amadon, 1980; Parker and Sommers, 1983; Carloni *et al.*, 1984; Voos and Sabey, 1987; Chae and Tabatabai, 1986).

A number of chemical methods have been used to evaluate the available nitrogen coming from the mineralization of organic matter of soil (Keeney and Nelson, 1982).

Such methods give little information on the evolution of the organic matter turnover. Biological methods are more suitable for this purpose. Among these, the methods based

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on incubation and leaching of mineralized nitrogen at a prefixed time (Stanford and Smith 1972) described the dynamics of mineralization better than the methods based on incubation with final extraction of mineralized nitrogen (Bremner, 1965). In fact, in the second procedure immobilization of mineralized nitrogen can occur (Keeney and Nelson, 1982).

The objective of the research reported in the present paper was to determine the suitability of the modified Stanford and Smith method (Benedetti, 1983; Benedetti and Dell'Abate, 1987) as a testing procedure for the potential mineralization of nitrogen from different organic materials in soil to have information regarding the cumulative amounts of nitrogen release and the pattern of N release applied to soil.

MATERIALS AND METHODS

The trials were carried out using five Italian soils (1, 2, 3, 4 and 5) from some experimental fields of the Institute for Plant Nutrition (ISNP) located in Central and Northern Italy. These soil have different chemical, physical and biological characteristics (Table 1).

Table 1. Chemical, physical and biological characteristics of the five soils used in this study. (Values refer to dry matter at 105 °C.)

Sample	Tor Mancina Monterotondo (1)	Roma (2)	Mantova (3)	Paliano (4)	Città di Castello (5)
Sand (%)	22.0	88.6	13.9	24.0	59.2
Silt (%)	54.0	2.1	60.1	37.5	14.3
Clay (%)	24.0	9.3	26.0	38.5	26.5
Field capacity (%; pF = 2.5)	29.8	34.5	24.5	27.6	18.0
pH (H_2O 1:2.5)	7.8	7.5	8.1	5.9	7.9
Organic C (%; Springer-Klee)	0.70	2.44	1.20	1.07	1.18
Organic matter (%; Cx1.724)	1.21	4.21	2.10	1.85	2.03
C/N	5.4	13.9	15.0	8.9	9.0
Total N (%; Kjeldhal)	0.13	0.18	0.08	0.12	0.13
NO_3^--N (kg ha^{-1}; Bremner)	7.1	73.0	42.0	7.8	11.1
NH_4^+-N (kg ha^{-1}; Bremner)	5.8	7.0	5.6	trace	9.0
Respiration rate (ppm CO_2 per 1000g soil)	1014	1373	822	432	280

The source and selected general characteristics of the organic materials studied are shown in Table 2.

They included cattle and sheep manure, pig slurry, the earthworm castings from the same manure and slurry furnished by the Italian Association of Earthworm Farmers (Ass. It. A. L.), hydrolyzed leather meal (a commercial Italian organic fertilizer) and lactic casein.

Table 2. Chemical characteristics of the organic amendments.

	Ash (%)	pH	Org. C (%)	Tot. N (%)	C/N	Org. matter (from ASH) (%)	Available nitrogen (Bremner) NO_3^-N (mg kg^{-1})	NH_4^+N (mg kg^{-1})	$(NO_3^-+NH_4^+)$ –N (mg kg^{-1})
Cattle manure	33.6	8.9	28.2	2.5	11.2	66.4	534	7	541
Sheep manure	34.9	8.8	31.3	3.1	10.1	65.1	2611	11	2622
Pig slurry	11.7	6.6	43.1	3.1	13.9	88.3	45	162	207
Cattle casting	64.9	6.4	18.4	1.9	9.7	35.1	1982	9	1991
Sheep casting	44.2	8.6	29	3.1	9.4	55.8	1245	18	2163
Pig casting	31.3	5.8	33.8	3	11.3	68.7	2919	78	2997
Leather meal	12.3	--	43.2	13.3	3.2	87.7	210	771	981

The mineralized organic nitrogen was measured following the method proposed by Stanford and Smith (1972) and partially modified by Benedetti (1983). Specifically, 50 g of soil, air-dried and sieved to pass a 2-mm screen were mixed with quartz sand in a 1:1 ratio (particle size of the sand 0.2-0.8 mm) and treated with the organic materials at a rate of 250 mg N per kg of soil. The soils prepared in this way were incubated in three replicates in a Buchner funnel (outer diameter 13 cm), at 60 % WHC (pF 2.5) at 30 °C for different periods of time. Initially, the total incubation period was 30 weeks, but in the later experiments reported here this time was reduced to 12 weeks. The mineral nitrogen in the soil was leached before incubation by adding 900 ml $CaSO_4$ solution and 100 ml of a nitrogen-free (N minus) nutrient solution (0.002 M $CaSO_4$ *2 H_2O, 0.05 M $Ca(H_2PO_4)$, 0.0025 M K_2SO_4, 0.002 M $MgSO_4$). The NO_3-N and NH_4-N were measured periodically throughout the incubation period using an Autoanalyzer Technicon II.

The humification indices according to the Sequi's method (1986) to describe the course of the humification level after composting were used for cattle and sheep manure and pig slurry, and earthworm castings. Sequi's method consists of extraction from samples of organic materials by a solution of NaOH 0.1 M + $Na_4P_2O_7$ 0.1 M and subsequent chromatographic separation of humic and fulvic acids on polyvinylpirrolidon (PVP) column. The quantities of humic Acids (HA), Fulvic Acids (FA), Total Organic Carbon (TOC) and Total Extractable Carbon (TEC) were determined. As a result:

DH = (HA + FA)/TEC*100

HR = (HA + FA)/TOC*100

The mineralization of organic nitrogen was studied, either using the same soil (i) treated with different organic materials, or using different soils (ii) with the same organic material (hydrolyzed leather meal).

RESULTS AND DISCUSSION

The values of mineralization of different organic materials added to soil 2 are reported in Table 3.The trend of mineralization of the same organic material (hydrolyzed leather meal) added in the five different soils is shown in Figure 1.

Table 3. Mineralization of different organic materials in the same soil (Rome) expressed as a percentage of the nitrogen added. The values, without soil mineralization, refer to dry matter at 105 °C.

Treatment		Time in weeks			
		2	4	8	12
Cattle manure	min %	16.5	27.7	21.1	29.7
	Tot. %	16.5	44.2	65.3	95.0
Sheep manure	min %	0.0	0.0	0.0	3.1
	Tot. %	0.0	0.0	0.0	3.1
Pig slurry	min %	26.8	14.6	33.8	11.0
	Tot. %	26.8	41.4	75.2	86.2
Cattle casting	min %	31.2	6.7	0.4	0.0
	Tot. %	31.2	37.9	38.3	38.3
Sheep casting	min %	0.0	0.0	4.7	0.0
	Tot. %	0.0	0.0	4.7	4.7
Pig casting	min %	35.6	5.6	19.4	0.0
	Tot. %	35.6	41.2	60.6	60.6
Leather meal	min %	71.0	14.0	13.0	2.0
	Tot. %	71.0	86.0	98.0	100.0
Lactic casein	min %	65.0	9.0	4.0	2.0
	Tot. %	65.0	74.0	78.0	80.0

The results obtained show that the method of Stanford and Smith (1972) partially modified by Benedetti (1983) is suitable to describe the cumulative curves of mineralization of organic materials added to the soil. Indeed, the same soil fertilized with different organic materials showed different rates of release of mineralized nitrogen. In particular, over a 12-week incubation period, the cattle manure and its earthworm castings mineralised 95 % and 38 % of nitrogen respectively, while, in the same period, the sheep manure and its earthworm castings gave values for the mineralization of nitrogen which were nearly the same and much lower than those of the cattle manure (3.1 % and 4.7 %, respectively). This result is indicative that the method can also provide some information about the different humification indexes. Indeed, as previously reported (Benedetti, 1992) cattle manure has lower humification indices than that of its earthworm castings (HR = 25.5 vs. 34.2; DH = 83.7 vs 86.3), while there is little difference in the humification index of sheep manure and its earthworm castings (HR = 33.6 and 35.2 respectively; DH = 81.1 vs 88.9). The pig slurry has HR = 18.3 and DH = 70.5, while earthworm casting has HR = 21.6 and DH = 81.1.

Figure 1 also shows that the mineralization of the same material is a function of the biological fertility of the soil (Benedetti, 1983; Benedetti and Rossi, 1988). In agreement with the values of biological fertility expressed by respiration rate (Freytag 1969; Saive, 1972) (Table 1) over the 12-week incubation period, the different soils treated with the same material (hydrolized leather meal) released 100.0 %, 54.0 %, 53.6 %, 36.1 % and 28.0 %, respectively, of the nitrogen added.

The method also provides an indication of the evolution over time of the release of nitrogen in the soil. This aspect is very important in order to rationalize the amounts of organic materials applied as a function of the real demand of crops. It was found that in all soils, the hydrolyzed leather meal released more than 50 % of the total nitrogen added during the first two weeks of incubation, while the other products used in this study

showed different trends. In some cases, a constant increase in the amount of nitrogen released over time was found (pig slurry, cattle manure), while in others mineralization began only after eight weeks of incubation (sheep manure and its earthworm castings). The trends found for the mineralization of the lactic casein, earthworm castings from the cattle manure and the pig slurry were similar to that of the hydrolyzed leather meal.

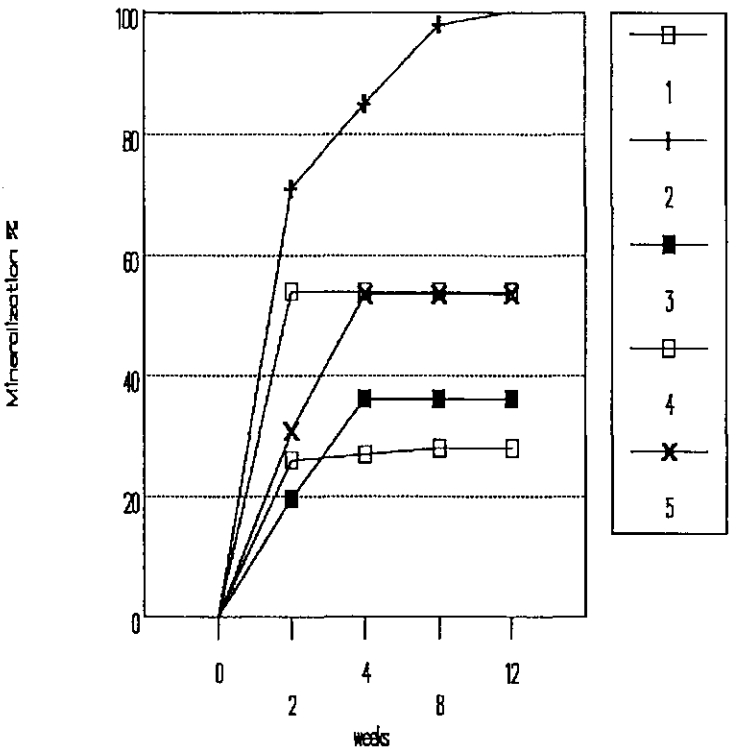

Figure 1. Amounts of N released as a percentage of total N added as hydrolyzed leather meal to different soils.

CONCLUSIONS

The results obtained in this study clearly show that the method of Stanford and Smith (1972) partially modified by Benedetti (1983) can be successfully used to study the organic nitrogen mineralization of organic materials applied to the soil. These results are in good agreement with the data referred to by other authors regarding the use of Stanford and Smith's method to predict the mineralization of sewage sludge (Magdoff and Amadon, 1980; Chae and Tabatabai, 1985; Parker and Sommers, 1993). It should be emphasized, however, that the data reported here were all obtained in a controlled environment and they represent the potential mineralization of the organic materials. It is still very difficult to relate such results obtained in the laboratory to results that can be expected under field conditions. In this regard, preliminary studies carried out on the mineralization of hydrolyzed leather meal in the Mitscherlich pots have shown no substantial differences in the

trend of mineralization rate from the laboratory data (Benedetti *et al.*, 1991; Figliolia *et al.*, 1992). It would seem likely, however, that such agreement can be found only under climatic conditions as those of the Mediterranean region where the soil temperature is on average rather high during all seasons of the year.

This method should be compared with plant uptake, but it is able to give good information regarding the behavior of different organic materials after their application to crop land.

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Effects of agricultural practices on mineralization kinetics of organic nitrogen

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Abstract

Different treatments have been performed on an Italian soil. They were cultivation, liming, and manure fertilization. The determination of the potentially mineralizable nitrogen according to the technique of Stanford and Smith was carried out on soil samples taken from the plots with the different treatments in order to evaluate their effect on the minaralization kinetics. Results show that first-order kinetics can be used to describe only nitrogen mineralization of soils at equilibrium conditions, i.e., without any treatment.

INTRODUCTION

The increased environmental awareness of recent years needs primarily to involve the control and use of mineral and organic fertilizers. To accomplish this successfully, an in-depth understanding of the problem is necessary, i.e. the rate of nitrogen supply by the soil to crops is yet to be accomplished.

It is important to be able to predict the quantity of potentially mineralizable nitrogen in soil to avoid excess nitrogen application.

Stanford and Smith (1972) and subsequently other authors (Addiscott, 1983; Beauchamp *et al.*, 1986; Deans *et al.*, 1986) have made valuable contributions to the study of this parameter, but many uncertainties still remain, both on the mineralization of organic nitrogen under field conditions and on the effects of agricultural practices and activities (Boone, 1990; Cabrera and Kissel, 1990; Hadas *et al.*, 1986).

It should be remembered that the Stanford and Smith method is based on leaching of the nitrogen mineralized at 35 °C at prefixed times, to simulate plant uptake.

The nitrogen mineralization was described by a first-order reaction according to the following equation:

$$Nt = No\ (1\text{-}e^{-kt}) \qquad (1)$$

where *Nt* is the nitrogen mineralized in the period considered, *No is* the potentially mineralizable nitrogen, *K* the velocity constant in $week^{-1}$.

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Stanford and Smith attributed the same K value (0.054 ± 0.009) $week^{-1}$ to all soils. In addition, in order to transfer laboratory values to field conditions, Stanford and Smith correlated K with the temperature of the soil. They demonstrated that for each increase of 10 °C (in the range from 5 °C - 35 °C) the value of K doubles (Q_{10} for soil N mineralization = 2) according to the relationship expressed by the Arrhenius equation:

$$\log K = 7{,}71 - 2758\ (1/T) \qquad (2)$$

Nevertheless, different values for Q_{10}, ranging from 1.7 to 3, have been found by other authors (Ross and Bridger, 1980; Tabatabai and Al-Khafaji, 1980).

Furthermore, Stanford and Epstein (1974) found that nitrogen mineralization is a function of the moisture content of the soil and in order to transfer the laboratory results to field conditions (N_c), it is necessary to correct the laboratory N_o values using the equation:

$$N_c = N_o\, K\, C_m \qquad (3)$$

where K is the rate constant at the established temperature and C_m is a coefficient determined by the ratio between the moisture contents of the soil under field and under laboratory conditions.

This method, tested in a controlled environment (Stanford *et al.*, 1973), has not been sufficiently checked in the field (Smith *et al.*, 1977; Westerman, 1980; Benedetti, 1984). Recent studies have shown that Stanford and Smith's method gives values which are too high (Cabrera and Kissel, 1988), attributed to many causes such as the use in the laboratory of disturbed samples of soils (Marion *et al.*, 1981; Smith *et al.*, 1982; Griffin and Laine, 1983; Frankenbergen, 1985).

In particular, Hadas *et al.* (1989) pointed out that the mineralization in field is 13 ÷ 26 % lower than the calculated values.

It is very important to study the mineralization rates of added and natural soil organic matter in order to evaluate and predict with reasonable accuracy the nutrient availability and losses in soil; in fact, only through the determination of the kinetics of soil nutrients in different pedoclimatic environments a good management of fertilizer applications is possible to be accomplished.

Study of organic nitrogen mineralization interactions with tillage, cropping, fertilizers, liming and irrigation practices is needed to establish crop N requirement and to determine management practices that promote the efficient use of N. Some studies were carried out by El-Haris *et al.* (1983) and Boone *et al.* (1990) but we have few studies in this regard on mediterranean soil.

The objective of this research was to evaluate the effects that agricultural practices can have on N_o (potentially mineralizable nitrogen) and K (rate constant) of some Italian soils.

MATERIALS AND METHODS

The research was carried out on one arable soil from the Institute for Plant Nutrition's (ISNP) experimental field located in Paliano (Frosinone), in Central Italy. The soil samples were taken at depths of 0-30 and 30-60 cm in order to study the variability of No in the profile. The experiments were carried out on a field with a surface area of 1 ha, subdivided into plots of 250 m^2 each.

This subacid soil, formed from reddish ash which is volcanic in nature and affected by advanced pedogenesis processes of the piroclastic flow of a leucytic nature, a vacuoler lithoid with a ashy-micropomiceous matryx has been assigned to Class 2 of the USBR Land Classification system and is identified by the symbol 2s/X 1_i m_1.

On the basis of the analysis, which was performed using the methods employed by the SISS (Società Italiana di Scienza del Suolo), the soil was proved rich in organic matter and exchangeable K_2O being supplied with available P_2O_5 at a median level.

For the purpose of research the following treatments were established:

PLOT A: without fertilization and cultivated with durum wheat c.v. Valnova.

PLOT B: without fertilization and cultivated with durum wheat c.v. Trinacria.

PLOT C: limed without fertilization and cultivated with durum wheat c.v. Valnova. A rise in pH from 5.6 to 6.2 occurred after a treatment performed three months before cultivation with 5 t of $Ca(OH)_2$ per ha.

PLOT D: cultivated with durum wheat c.v. Valnova and treated with 100 t of cattle manure per ha.

All soil samples were taken before sowing and after harvesting. Chemical and biological analyses were carried out on samples obtained by mixing three different subsamples of soil for each plot.

The method proposed by Stanford and Smith (1972) and subsequently further developed by Benedetti (1983) was used to evaluate the potentially mineralizable nitrogen. In particular the mineral nitrogen in the soil was leached before incubation by adding 900 ml of $CaSO_4$ solution and 100 ml of nutritive solution "N minus" (0.002 M $CaSO_4$*2 H_2O, 0.05 M $Ca(H_2PO_4)$, 0.0025 M K_2SO_4, 0.002 M $MgSO_4$).

Subsequently the NO_3^--N and NH_4^+-N concentrations were measured periodically (2, 4, 8, 12, 16, 22 and 30 weeks).

The values of No and K were calculated using a Non-linear Least Squares method based on minimizing the sum of square deviations (Σd_i^2) between the experimental data and the theoretical data calculated by the equation:

$$\Sigma d_i^2 = (1/n) \{\Sigma [Nt_i - N_o (1 - \exp(-kt_i)]^2\}$$

where Nt_i is the experimental value of the mineralized nitrogen in the time range (0-t_i) and n is the number of experimental points used to plot the curve.

RESULTS AND DISCUSSION

Figures 1 and 2 show the theoretical and experimental values of nitrogen mineralized for the soil samples. The experimental data only correspond with the theoretical curves before sowing for PLOT A and B and it is therefore impossible to plot theoretical curves after harvesting. PLOT D (treated with manure 100 t ha^{-1}) is equally impossible to predict and does not lend itself to the plotting of theoretical curves that correspond with the experimental data. For an accurate mathematical description of the nitrogen mineralization of these soils it is necessary to resort to higher order reactions.

It was not possible to draw a curve for soil samples of PLOT A and PLOT B after cultivation due to a change in concavity. This tendency can also be seen for soil samples from PLOT C treated with $Ca(OH)_2$ and for PLOT D, treated with cattle manure, but in this case only before cultivation.

The nitrogen mineralization curves of soil samples from PLOT A and PLOT D at the dif-

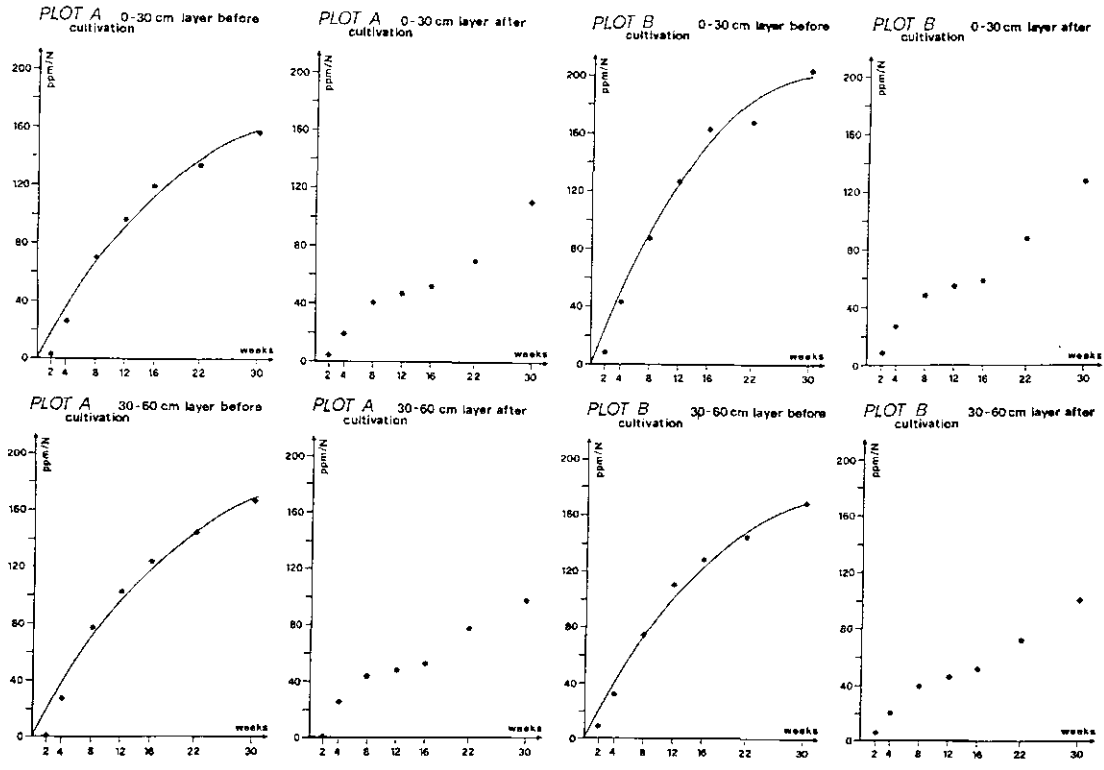

Figure 1. Theoretical and experimental values of nitrogen mineralized for the soil samples of PLOT A and B.

ferent depths of 0-30 and 30-60 cm do not show major variances, thereby showing how deep the surface layers of many Italian soils are.

The results reported here show that first-order kinetics can be used to describe the nitrogen mineralization levels in agricultural soils under equilibrium conditions, i. e., when there are no distortions caused by human activity.

The value of K is typical of each soil (Benedetti and Sebastiani, in press); the average value of K found in this study using only one soil and calculated by the non-linear least squares (NLLS) method of Smith *et al.* (1982) does, however, not vary seriously from the value proposed by Stanford and Smith for each soil 0.054 ±0.009.

The N_0 and K for PLOT A, before cultivation are: 203 and 0.052 (0-30 cm depth); 224 and 0.048 (30-60 cm depth), respectively. The values for PLOT B, before cultivation, are 266 and 0.054 (0-30 cm depth); 210 and 0.056 (30-60 cm depth). The values for PLOT D after cultivation are 135 and 0.068 (0-30 cm depth); 135 and 0.054 (30-60 cm depth).

Results for soil samples after cultivation are different, not according to first-order but possibly to higher order reactions.

The validity of Stanford's original single exponential model has been questioned because several sources of organic N undergo mineralization simultaneously at different rates (Skjemstad *et al.*, 1988).The two-pool models, with one pool mineralizing rapidly and

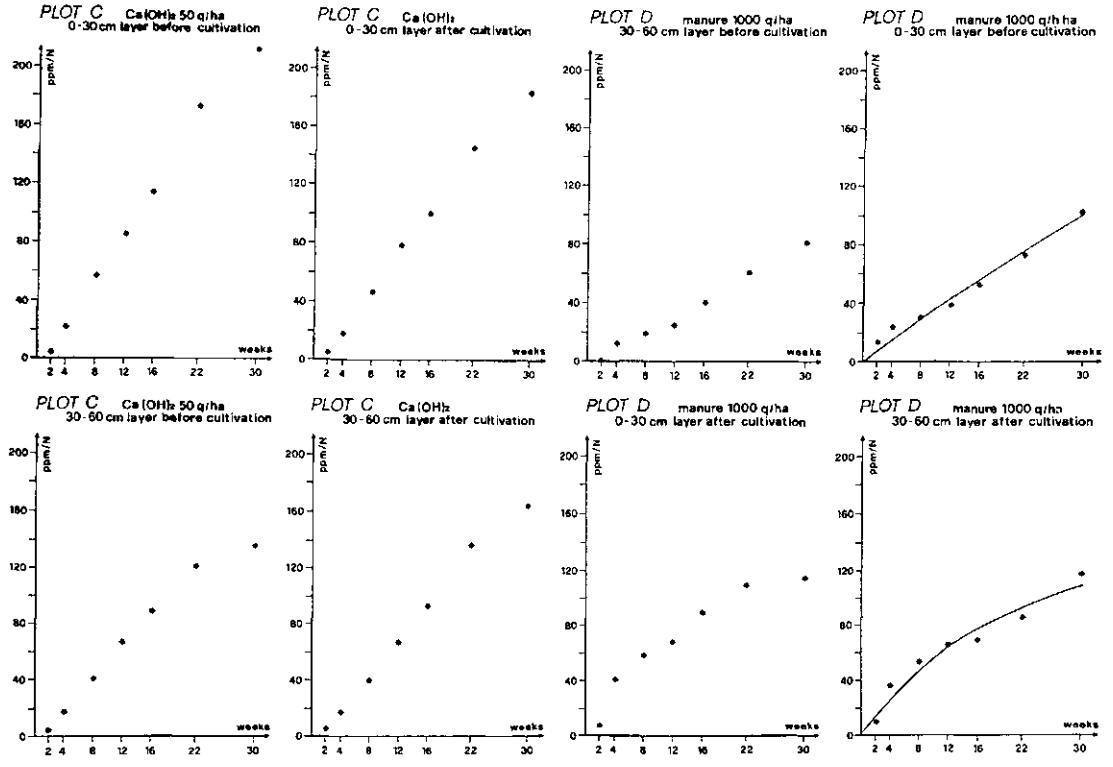

Figure 2. Theoretical and experimental values of nitrogen mineralized for the soil samples of PLOT C and D.

the other slowly, are assumed to fit the data better in many cases. The rapidly decomposing pool is thought to be largely composed of soil microbial biomass killed by soil handling and pre-treatment, especially air-drying.

Not all studies of N mineralization show a curvilinear relationship between the amount of N mineralized and time. Where a curvilinear relationship exists, a hyperbolic equation may fit the data just as well as a first-order reaction, but gives very different estimates for N_0 and half-life of mineralization.

Furthermore, liming also strongly influenced the trend of the mineralization rate. In fact, the effect of lime on N mineralization may also be a result of changes in the microbial populations and their activity. Black (1968) suggested that the effect of liming on N mineralization in acid soil was due to increased susceptibility of the organic N to mineralization. In this case we have two mineralization pools of nitrogen and the time of soil sampling between sowing and harvesting was not sufficient to re-establish the equilibrium conditions after liming.

We believe the extrapolation of the N_0 values to field conditions requires a mathematical approach that accounts for the varying forces involved in nitrogen mineralization (e.g. fertilization, crop systems, irrigation, etc.) (Tombesi *et al.*, 1989), more than the effects of moisture and temperature (although these parameters must always be considered). It will be possible to quantify the increase and decrease of the mineralization rate only by carrying out experiments in the field using labeled nitrogen.

ACKNOWLEDGEMENTS

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Microbial grazer populations in a ^{15}N labelled organic residue and the uptake of residue N by wheat

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Abstract

The aim of this study was to determine the extent and timing of mineral N release from a localized source of decomposing residue (hotspot) and its subsequent uptake by a growing plant, in relation to changes in the populations of microbial-feeding nematodes and protozoa. Dried and ground ryegrass labelled with ^{15}N was used to create a hotspot at a depth of 30 cm in a column of clay-loam soil. Wheat plants grown in the columns were harvested after 10, 16, 22, 28 and 34 days and analyzed for total N and ^{15}N content, along with simultaneous measurements of soil mineral N and microfauna. There was a rapid increase in microfauna and mineral N, indicating rapid mineralization activity. A total of 25 % of the N initially in the residue was present in the wheat plants after 34 days. The pattern of residue N uptake followed a sigmoidal pattern, with 62 % of residue N uptake occurring between days 16-22. The importance of synchronization between mineralization and plant N uptake is discussed.

INTRODUCTION

A feature of organic residues added to soil is their heterogeneous distribution, with localized concentrations of organic material surrounded by bulk soil containing little or no fresh residues. Studies of decomposition and decomposer activity on such localized 'hotspots' of residue material have demonstrated distinct successions of activity and populations (Christensen, S. *et al.*, 1992; Griffiths and Caul, 1993; Griffiths *et al.*, 1993). It is possible that the mineralization and turnover of N is greatest in the periods of maximum activity, as hotspots have been shown to have a short-lived period of denitrification activity (Henriksen and Larsen, 1991). The activity of decomposer microorganisms can be monitored by observing the population changes of microbial-feeding nematodes and protozoa. These organisms are the major consumers of bacteria and fungi in soil and can be used as indicators of microbial productivity (Sohlenius, 1990; Christensen, H. *et al.*, 1992) as well as being directly involved in the mineralization of N (Griffiths, 1989). The aim of this study was to determine the extent and timing of N release from a residue hotspot

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and its subsequent uptake by a growing plant, in relation to the changes in populations of nematodes and protozoa in the hotspot.

MATERIALS AND METHODS

A clay-loam soil of the Carpow Association (Laing, 1976) was collected from fallow following potatoes, sieved through a 4-mm mesh and stored at 15 °C for 3 weeks. The soil was fertilized with P (14.5 μg g^{-1}, equivalent to 36 kg ha^{-1}) and K (27.7 μg g^{-1} and 69 kg ha^{-1}) before use. Perennial ryegrass (*Lolium perenne* L. cv. Aurora) was labelled with ^{15}N by growing it from seed in aerated nutrient solution (Hewitt, 1966) made with 99 atom % ^{15}N as $^{15}NH_4$ $^{15}NO_3$ (Europa Scientific Ltd, Crewe, UK). Plants (shoots plus roots) were harvested after 3-4 weeks growth, washed, dried at 60 °C and coarsely ground in a hammer mill. The experimental plants were grown in lengths of electrical conduit (Marshall-Tufflex Ltd, Hastings, UK) (Robinson, 1991), which are essentially long (115.5 cm) tubes with a narrow cross-sectional area (4.6 x 2 cm) with removable front cover giving access to whole soil. Tubes were filled with 1.485 kg stored field soil (1.3 kg dry weight) and packed to give a uniform bulk density between tubes. A 2 cm wide band of soil was removed from 30 cm below the soil surface and replaced with the experimental hotspot, a mixture of 15 g soil and 0.75 g ground grass residue. Control tubes, without hotspot material, were made by removing the 2 cm band of soil but replacing it with an equivalent weight of mixed stored soil. Five replicates of hotspot material were sampled immediately after preparation as detailed below. One pre-germinated seedling of spring wheat (*Triticum aestivum* L. cv. Tonic) was then planted 30 cm above the hotspot. The tubes were placed upright in a growth chamber maintained at 15 °C with a 16:8 h day:night regime. Plants were watered to a gravimetric water content of 22 % twice a week by removing the front face of the tube and spraying distilled water over the exposed soil surface.

Five replicate hotspot tubes were sampled 10, 16, 22, 28 and 34 days after planting. The hotspot (including wheat roots) was removed, as was a 2 cm wide band of soil 5 cm above and 5 cm below the hotspot. Roots were removed from the samples and a representative 4 g was taken from each sample and extracted for NH_4^+ and NO_3^- as described previously (Wheatley *et al.*, 1991). The hotspot soil was further sampled, and 4 g shaken in 40 mls Neff's amoeba saline (Page, 1967) for 10 min. Protozoa were enumerated by most-probable-number (Darbyshire *et al.*, 1974) in which 4 x 0.1 ml aliquots were serially diluted 3-fold in 10 % v/v nutrient broth (Oxoid) in Neff's amoeba saline, and microscopically checked after 7, 14 and 21 days incubation at 15 °C. Nematodes were counted following flotation in colloidal silica (Griffiths *et al.*, 1990). Remaining soil was used for a dry weight determination (105 °C). Shoots and roots (washed from the soil remaining in the column) were dried at 60 °C, weighed and analyzed for total N and ^{15}N by continuous-flow ratio mass spectrometry (Tracermass, Europa Scientific Ltd). Data were analyzed by standard analysis of variance procedures using the Rothamsted GENSTAT program.

RESULTS

The ryegrass residue material contained 2.48 (± 0.08, standard error) % N, 22.16 (± 0.31) atom % ^{15}N, thus the hotspot contained 18.6 mg N and 4350 μg ^{15}N, and the atom % ^{15}N

of the wheat seeds was 0.37. The growth and N/^{15}N content of the wheat plants grown in both hotspot and control tubes, together with the calculated amount and percentage of residue N taken up by the wheat, is given in Table 1.

Table 1. The dry weight (shoot + root), total N and ^{15}N content of wheat plants grown through a localized source of ^{15}N labelled organic residue, the amount of wheat-N derived from the residue, and the percentage of residue N taken up by the wheat (n = 5).

		DAYS					
		10	16	22	28	34	Control[1] 34
Dry wt.	mg	67	154	389	990	1912	1831
	± s.e.	7	14	6	50	183	153
Total N	mg	2.09	5.08	12.53	21.34	29.08	26.59
	± s.e.	0.14	0.37	0.59	1.20	0.83	0.93
Excess[3] ^{15}N	µg	9	116	821	1090	1203	N.D.[2]
	± s.e.	1	15	73	16	45	-
N from[3] residue	mg	0	0.4	3.3	4.1	4.7	-
	± s.e.	0	0.1	0.3	0.1	0.2	-
% residue[3] N in plant		0	2.2	17.8	22.1	25.3	-

[1] Values from control tubes with no added hotspot.
[2] N.D. None detected above background.
[3] Calculated according to: excess ^{15}N = (total ^{15}N content of shoot + root) - (mass % ^{15}N of seeds x total N content of shoot + root x 0.01); N from residue = initial total N content of residue x % residue N in plant x 0.01; % residue N in plant = (excess ^{15}N/initial ^{15}N in residue) x 100.

In only one of the five replicate tubes taken after 10 days had the roots reached the hotspot, whereas after 16 days roots had grown through the hotspot in all the tubes. The concentration of soil mineral N (NH_4^+ and NO_3^-) in the hotspot, compared with soil above and below the hotspot, was increased initially and there was a large increase up to day 10 before concentrations declined to the same values as the surrounding soil after 28 days (Figure 1).

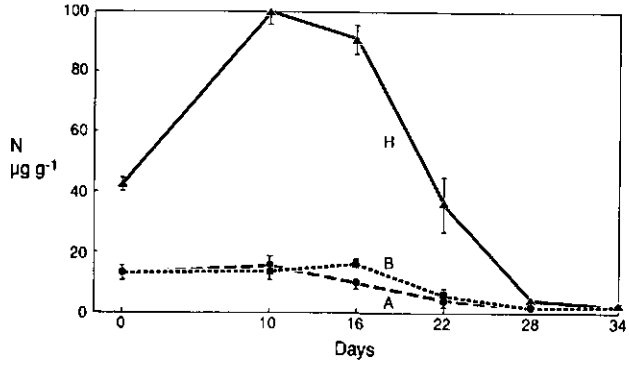

Figure 1. The concentration (mean ± s.e.) of soil mineral N (NH_4^+ + NO_3^-, µg g^{-1}) in a hotspot of decomposing organic residue (H) and in bulk soil 5 cm above (A) and below (B) the hotspot (n = 5).

There were large increases in the numbers of nematodes, flagellates and amoebae after 10 days, compared with the start, the initial populations being 30 (± 3), 13729 (± 3121), 2110 (± 738) g^{-1} hotspot soil respectively (Figure 2).

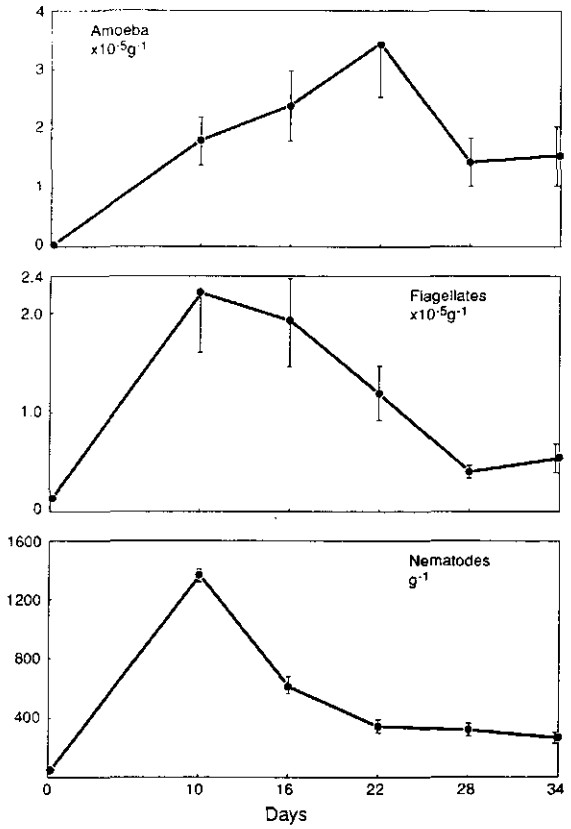

Figure 2. The populations (mean ± s.e.) of amoebae, flagellates and nematodes in a hotspot of decomposing organic residue (n = 5).

Nematodes and flagellates showed a significant ($P \leq 0.01$) decline after 10 days, whereas the numbers of amoebae did not did not differ significantly between days 10-34.

DISCUSSION

The percentage of residue N taken up by the wheat plants (25 %, Table 1), is within the range of values reported by other workers for ^{15}N labelled residues of similar N content, i.e. 17-25 % (Müller and Sundman, 1988) and 10-34 % (reviewed by Ta and Farris, 1990). It is likely that the final residue N uptake in this experiment would have been slightly greater than 25 %, as the plants were only 34 days old, but as the rate of residue N uptake had levelled off the increase would have been marginal. It has been concluded that only rarely will more than 25 % of residue N become available to field grown plants over the first 1-2 years, and much less each succeeding year (Vallis, 1983). The majority of residue N, therefore, enters the soil organic matter pool (Seligman *et al.*, 1986), and

mineral soil has a strong affinity for residue N (Müller and Sundman, 1988).

The sigmoidal pattern of ^{15}N uptake observed in the wheat plants cannot be demonstrated in those studies which only sample plant N at the end of the growing period, but the studies that have sampled periodically throughout the growing season show a similar pattern of N uptake, in both pot (Moore, 1974) and field experiments (Vallis, 1983; Bremer and Van Kessel, 1992). This pattern of N uptake implies a period of rapid N mineralization, resulting in plant available N and plant uptake. Large and rapid increases in microbial biomass have been observed following the addition of residues with both low (Ocio *et al.*, 1991) and high N contents (Zaccheo *et al.*, 1993). Thus, the micro-organisms responsible for mineralization respond rapidly to the addition of residue and the extent of N mineralization then depends on well defined relationships between the C:N ratios of substrate and decomposers (Bartholomew, 1965; Russell, 1977). In this study there was an increased concentration of mineral N in hotspot soil, compared with the surrounding soil, already present immediately following residue addition. Similar increases following the addition of ryegrass residues were attributed to the high mineral N content of solution grown plants (Zaccheo *et al.*, 1993).

The timing of N mineralization will be important in determining to what extent mineralized N can be taken up by plants. If residue N is only available for a limited period, as suggested by the fact that after 22 days the plants were still taking up N but the availability of ^{15}N was much reduced then the synchronization of the presence of plant roots, plant N demand and residue N availability would be necessary for maximum plant N benefit. Similarly, Bremer and Van Kessel (1992) noted that the timing of incorporation and sowing can alter the N benefit of green manure for field grown crops, although Müller and Sundman (1988) stated that the low availability of residue N to a subsequent crop was not due to a lack of synchronization of high N release and high N demand. Lack of synchronization would increase the potential for N losses from the system, such as leaching and denitrification, and the incorporation of mineralized N into other pools.

The bacterial-feeding microfauna (nematodes and protozoa) reached equivalent population densities and showed the same temporal variations in hotspots of ryegrass residue incubated in the absence of growing plants (Griffiths and Caul, 1993). Previous studies with hotspots of ryegrass residue (Griffiths and Caul, 1993) and barley root residue (Christensen, S. *et al.*, 1992) indicate that there would have been no increase in micro-faunal populations in the soil 5 cm above or below the hotspot. The patterns of soil mineral N and microfaunal activity indicate that N mineralization was occurring before and soon after the roots entered the hotspot around day 10. Large populations of microfauna indicate considerable bacterial productivity (Christensen, S. *et al.*, 1992) and N cycling (Griffiths, 1986; Griffiths and Caul, 1993). This activity would decline with time, as indicated by the reducing numbers of nematodes and protozoa. Roots present while mineralization is actively proceeding may have access to more N than roots with access only to the net pool of mineralized N. Such a close coupling between microfaunal activity and plant N uptake has been shown by Clarholm (1989) for field-grown plants. The results of this study emphasize the importance of synchronization between the N mineralizing activity of residues and plant uptake ability (a combination of root presence and N demand).

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Effects of soil compaction on N mineralization and microbial C and N: field measurements and laboratory simulation

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Abstract

Effects of soil compaction on net nitrogen mineralization and microbial biomass dynamics were studied both in the field and in a laboratory simulation experiment. Soils were silty clay loams in either a permanent pasture (4.6 % C) or a 28 y cropped site (2.1 % C). In the field study compaction treatments were performed by five passes of a tractor. Soil dry bulk densities only increased significantly in the permanent pasture site. However, CO_2 flux from the soil surface decreased substantially after compaction on both sites and a highly reduced air permeability caused by deformation of the topsoil was a possible reason for this. Oxygen diffusion rates at 5 and 10 cm depth only changed significantly following compaction on the 28 y cropped site. Nitrogen mineralization measurements by the in situ core technique were found to be problematic as high soil moisture contents during a large part of the incubation period may have led to some denitrification. The microbial biomass levels did not change significantly over time in response to the compaction treatment. In the laboratory experiment, coarsely sieved soils from the two sites were compacted into cores of different bulk densities and equilibrated to different soil water potentials before incubating at 25° C for 21 d. Basal respiration was positively correlated with soil moisture levels. Net nitrogen mineralization showed a similar pattern only for the well aerated, low density permanent pasture cores. In 28 y cropped and in high density permanent pasture cores apparent net N mineralization reached a maximum at -10 kPa. The microbial biomass estimates decreased from the initial level during incubation, most in the samples with high moisture, if measured by fumigation-extraction, but increased from the initial level and were relatively unaffected by moisture if measur-ed by the substrate-induced respiration method.

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INTRODUCTION

On heavier-textured soils compaction can be a major limitation to crop production (Soane, 1990). In such soils the resistance to compaction is particularly influenced by the strength and proportion of soil macro-aggregates (Sparling *et al.*, 1992). The proportion of stable macro-aggregates decreases (Tisdall and Oades, 1982) when native soils or permanent pastures are cultivated. This is as a result of a marked decline in organic carbon content and microbial biomass. The microbial biomass in soil has a rapid turnover and thus plays a major role in nutrient transformations (Doran, 1987). The loss of nitrogen via denitrification is also known to increase upon compaction (Bakken *et al.*, 1987; Douglas, 1992; Torbert and Wood, 1992), but how the mineralization-immobilization turnover is affected by soil compaction has only been addressed by a few authors (Landina and Klevenskaya, 1984; Dick *et al.*, 1988; Van der Linden *et al.*, 1989; Kaiser *et al.*, 1990). The objective of this study was to:

a) Assess the effects of soil compaction in the field on soil physical properties, net nitrogen mineralization, soil microbial biomass and activity on two similar soils of different cropping history.
b) Assess the changes in turnover of nitrogen and microbial biomass caused by soil compaction and different soil moisture levels under laboratory simulation of field conditions with the same soils as in a).

MATERIALS AND METHODS

Soils were silty clay loams (clay 36 %, silt 58 %, sand 6 %) of the Manawatu district, New Zealand from a field cropped continuously with cereals for 28 years using conventional tillage (2.1 % C) and from a permanent pasture (4.6 % C). Further details of the soil types can be found in Sparling *et al.* (1992).

Field experiment: Compaction treatments were carried out with five passes of a tractor (4,880 kg total weight). Soil bulk densities before and after the compaction treatment were measured i) in 5-cm depth segments to a depth of 30 cm with a Troxler 3440 soil moisture-density gauge (gamma-ray device), and ii) by taking cores (60 mm diameter) in 10-cm depth segments. Only results from the latter are given in this paper. These cores were equilibrated to different water potentials on ceramic tension plates and the mean effective pore diameter and hence the pore size distribution calculated from the water potential - moisture content relation according to Papendick and Campbell (1981), using a particle density of 2.64 t m^{-3} to calculate total pore space from the dry bulk density. Air permeability was measured on the cores using a flow-rate air permeameter (Eijkelkamp, The Netherlands). Basal soil CO_2 flux from the soil surface was measured in the field immediately after compaction in both compacted and non-compacted areas using the static chamber technique with ten replicate field respirometers (Tate *et al.*, 1993). Oxygen-diffusion rates (O.D.R.) were measured concurrently using ten replicate platinum O.D.R. electrodes (Stolzy and Letey, 1964) inserted to depths of 5 and 10 cm, respectively. Nitrogen mineralization was measured using an in situ method (Raison *et al.*, 1987) with eight replicate cores per treatment, inserted into 15 cm depth and covered to avoid leaching. The cores were incubated in the field for two subsequent incubation periods (28+28 d for 28 y cropped and 21+21 d for permanent pasture). Before analysis soils were sieved (< 5.6 mm). Inorganic-N was measured using colorimetric methods on an AutoAnalyzer. Microbial biomass was estimated using both fumigation-extraction (FE) (Vance *et al.*,

1987) and substrate-induced respiration (SIR) (West and Sparling, 1986). Biomass C (μg C g^{-1} soil) was calculated as extractable C-flush / 0.33 (Ross, 1990) or 50 * S.I.R. rate (μl CO_2 g^{-1} soil h^{-1}) (Sparling and West, 1990). Biomass N (μg N g^{-1} soil) was calculated as extractable ninhydrin-reactive N-flush / 0.20 (Joergensen and Brookes, 1990).

Laboratory experiment: Moist and coarsely sieved (< 5.6 mm) soils from 0-15 cm of both of the above described sites were compacted into 60 mm (d) x 25 mm (h) cores by mini Proctor compaction to two different bulk densities, Low density (1.07 and 0.88 t m^{-3} for 28 y cropped and permanent pasture, respectively) resembling a cultivated situation, and High density (1.30 and 1.15 t m^{-3} for 28 y cropped and permanent pasture, respectively) resembling the compacted field density. The water contents of the cores were adjusted on tension plates to -1, -5, -10 and -100 kPa, and the retention curve used to calculated the pore size distribution etc. as mentioned above. The percentage of water-filled pore space (WFPS) was calculated as (Volume of water-filled pores) * 100 / (Total pore volume). Air permeability of the cores was then determined as described above and the cores incubated at 25 °C for 21 days. Before analysis the soil from the cores was not sieved again but the compacted soil cores were disintegrated. Net N mineralization was estimated from the increase in inorganic-N during the incubation period, C mineralization was measured by absorbtion of the evolved CO_2 in alkali. Microbial biomass C was estimated as described above before soils were compacted and after the incubation period.

Statistical comparisons were done by either Students t-test or analysis of variance.

RESULTS

The mean dry bulk density in 0-10 cm depth in the field did not increase significantly upon the compaction treatment in the 28 y cropped site (Table 1), but increased significantly ($P < 0.001$) in the permanent pasture. The CO_2 flux from the soil surface following compaction was substantially lowered (by 57-69 % relative to non-compacted soil, $P < 0.001$, Table 1) in both soils. This was accompanied by a large decrease in the air permeability of the 0-10 cm layer ($P < 0.05$, Table 1). However, oxygen-diffusion rates at 5 and 10 cm depth did only decrease significantly following the compaction treatment in the 28 y cropped site ($P < 0,05$, Table 1).

Table 1. Mean values of dry bulk density, CO_2 flux rate, O_2-diffusion rate and air permeability for 28 y cropped and permanent pasture soils, before and after field compaction. Means ± SE (n = 6 for dry bulk density and air permeability, n = 10 for others).

		28 y cropped		Permanent pasture	
Parameter	Compaction:	Before	After	Before	After
Dry bulk density (t m^{-3}), 0-10 cm		1.30 ± 0.02	1.34 ± 0.02	1.00 ± 0.03	1.17 ± 0.01
CO_2-flux rate (mg C m^{-2} h^{-1})		60 ± 7	19 ± 1	148 ± 11	64 ± 4
O_2 diffusion rate at 5 cm		17 ± 3	9 ± 2	36 ± 3	31 ± 3
" (10^{-8} g O_2 cm^{-2} min^{-1}) at 10 cm		19 ± 9	11 ± 2	35 ± 2	35 ± 3
Air permeability[a] (10^{-11} m^2) 0-10 cm		3.5 ± 1.6	0.25 ± 0.07	1.1 ± 0.4	0.02 ± 0.06

[a] Air permeability values > $4 \cdot 10^{-11}$ m^2 are considered to be non-limiting to plant growth, while values < $0.1 \cdot 10^{-11}$ m^2 are considered to be completely limiting to plant growth (B. Kroesbergen, pers. comm.)

The compaction treatment reduced mainly the intermediate (3-30 μm) and micro (0.2-3 μm) pores (Figure 1a). Macro pores (> 30 μm) initially only comprised ca. 5 % of total soil volume at both sites and only decreased slightly upon compaction. Inorganic nitrogen content of covered *in situ* cores (Figure 2) in the 28 y cropped site showed no significant differences between treatments. However, denitrification losses from the covered cores may have occured as there was a decrease in inorg.-N content between day 28 and 56, where wet conditions prevailed. In the permanent pasture the apparent net N mineralization rates were only significantly lower in the compacted treatment between day 21 and 42 ($P < 0.01$), but whether this was due to lower mineralization or increased denitrification can not be elucidated. Microbial biomass C and N (Table 2) did not change significantly over time in compacted or uncompacted treatments.

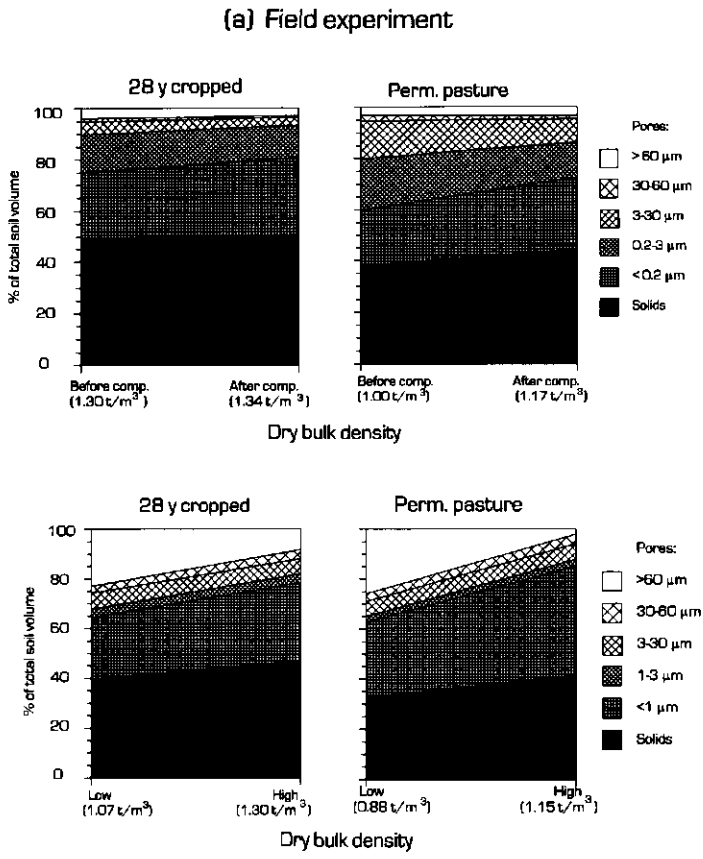

Figure 1. Pore size distribution as a percentage of total soil volume for different dry bulk densities of 28 y cropped soil and permanent pasture soil in (a) field experiment and (b) laboratory experiment. Note different lower limit for micro pores in (a) and (b).

Table 2. Microbial biomass C and N as affected by compaction in the field study. Means ± SE. (SIR $n = 4$, FE $n = 3$).

			Biomass C (µg g^{-1} soil)		Biomass N (µg g^{-1} soil)
	Time	Compacted	SIR	FE	FE-ninhydrin
Cropped	0 d		387 ± 24	328 ± 24	51 ± 3
	28 d	No	316 ± 8	340 ± 44	46 ± 2
	28 d	Yes	309 ± 14	421 ± 48	43 ± 10
Pasture	0 d		696 ± 33	807 ± 14	129 ± 2
	21 d	No	618 ± 25	841 ± 23	110 ± 12
	21 d	Yes	691 ± 11	862 ± 23	135 ± 9

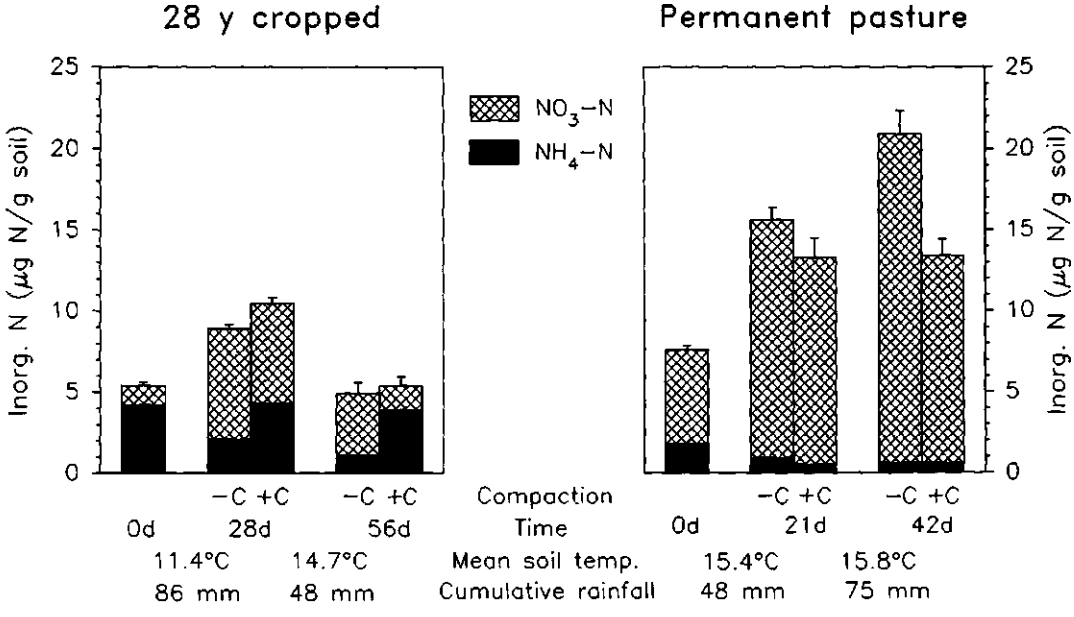

Figure 2. Inorganic-N content of *in situ* cores as a function of incubation time and compaction treatment (+C: compacted, -C: noncompacted). Mean soil temperature at 10 cm depth and cumulative rainfall given for the respective incubation period. Bars indicate SE (n = 8).

Table 3. Water-filled pore space (WFPS, % of total pore space) and air permeability (10^{-11} m^2) of soil cores as affected by bulk density and water potential in the laboratory study (n = 6).

		28 y cropped			Permanent pasture	
Water pot. (kPa)	Density (t m^{-3})	WFPS (%)	Air perm.[a] (10^{-11} m^2)	Density (t m^{-3})	WFPS (%)	Air perm. (10^{-11} m^2)
-1	1.07	79	2.9	0.88	78	3.3
-5		62	3.7		62	8.5
-10		57	15		57	11
-100		47	18		48	17
-1	1.30	100	< 0.02	1.15	100	< 0.02
-5		84	0.18		97	0.22
-10		78	0.50		90	0.49
-100		66	3.4		79	8.8

[a] See table 1 for limits.

When only compacted to the low density the coarsely sieved soils used in the laboratory experiment naturally had a much larger proportion of macro pores (> 60 µm, Figure 1b) than in its natural state (Figure 1a). Compaction treatments in the laboratory experiment mainly decreased the proportion of these macro pores, while coarse, intermediate and micro (30-60, 3-30 and 1-3 µm, respectively) pores remained unchanged. The chosen range of water potentials and bulk densities yielded water-filled pore spaces (WFPS %) between ca. 50 and 100 % and air permeability values in the full range from totally limiting to non-limiting for plant growth (Table 3). Carbon mineralization at 25 °C was generally positively correlated with soil moisture levels (Figure 3). Net nitrogen mineralization showed a similar pattern only for the well aerated, low density permanent pasture cores with a C:N-mineralization ratio of approximately 25. However, in most of the other cores apparent net N mineralization rates showed a different pattern from that of C mineralization, with maximum rates occuring at -10 kPa. Apparent net N mineralization rate in the high density, -1 kPa permanent pasture cores was very low, possibly due to increases in denitrification activity. The microbial biomass C levels decreased following compaction and during the incubation period if measured by FE ($P < 0.01$, Table 4), most in the samples with high moisture. However, if determined by SIR, the biomass C estimates increased significantly following compaction and incubation ($P < 0.05$ for 28 y cropped soil and $P < 0.01$ for permanent pasture). SIR biomass C seemed unaffected by density and moisture in the 28 y cropped soil whereas it was slightly lowered by high density in the permanent pasture soil ($P < 0.05$) .

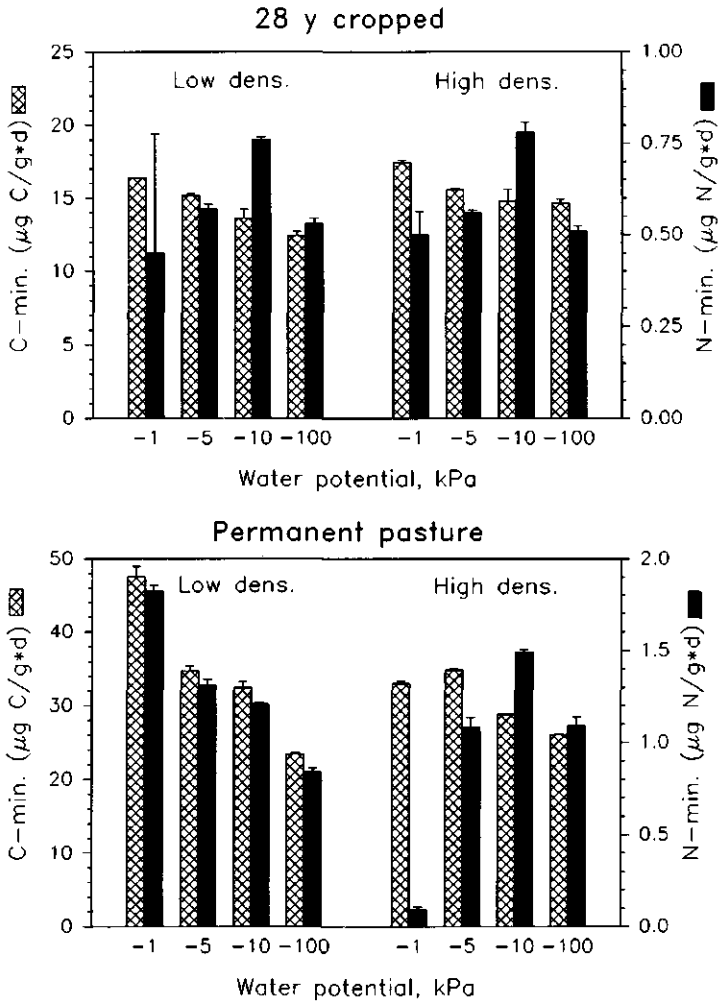

Figure 3. C and N mineralization rates as affected by soil water potentials and dry bulk density. Low density (1.07 and 0.88 t m^{-3} for 28 y cropped and permanent pasture, respectively) resembles a cultivated situation and High density (1.30 and 1.15 t m^{-3} for 28 y cropped and permanent pasture, respectively) resembles compacted field density. Bars indicate SE (n = 2).

Table 4. Microbial biomass C (μ g^{-1} soil) as affected by bulk density and three of the water potentials during 21 d incubation at 25 °C in the laboratory study. Means ± SE (SIR n = 4, FE n = 3).

	28 y cropped			Permanent pasture		
Water pot. (kPa)	Bulk.dens. (t m^{-3})	SIR	FE	Bulk.dens. (t m^{-3})	SIR	FE
Initial[a]	-	244 ± 18	414 ± 57	-	534 ± 14	1202 ± 30
-1	1.07	369 ± 20	151 ± 21	0.88	837 ± 25	507 ± 6
-10		316 ± 11	177 ± 10		861 ± 43	601 ± 10
-100		357 ± 22	315 ± 13		858 ± 21	1021 ± 14
-1	1.30	291 ± 20	198 ± 22	1.15	733 ± 24	52 ± 6
-10		352 ± 14	192 ± 31		743 ± 21	632 ± 35
-100		384 ± 12	342 ± 19		762 ± 12	721 ± 39

[a] Initial values measured before soils were compacted and saturated for moisture adjustment.

DISCUSSION

The lack of any significant increases in dry bulk densities upon the compaction treatment for the 28 y cropped site was probably due to two factors: i) the fairly high soil moisture content (32 % w/w at 0-15 cm depth) near the plastic limit of the soil at the time of compaction, ii) the soil had a relatively high density and a quite deteriorated physical structure even before the compaction treatment, which was probably the result of many years of continuous cereal cropping with inappropriate soil tillage practices. The compaction energy input to the soil rather resulted in a deformation of the topsoil with some redistribution of pore space to smaller pore size classes at both sites. In addition to this a smearing of continuous pores in the uppermost layer of the soil could also have contributed to the reduced air permeability. The large decrease in CO_2 flux from the surface of the compacted areas was probably caused by this reduction in air permeability and not by substrate or oxygen limitations to the soil respiration. This can be concluded for at least the permanent pasture site, because there the oxygen diffusion rates in lower-lying layers did not change significantly after compaction.

The inherent problems of nitrogen mineralization measurements by the *in situ* technique (alteration of microclimate, accumulation of moisture, denitrification losses, the absence of plant roots, etc.) have previously been discussed by Adams *et al.* (1989) and Sierra (1992). With this particular soil type and under the prevailing weather conditions of high rainfall in the present study the technique proved to be problematic because negative apparent net N mineralization rates were determined at both sites. High soil moisture contents during a large part of the incubation period possibly led to N losses via denitrification in the 28 y cropped site, and maybe also to some degree in the permanent pasture site. Lower air permeability in the compacted area of the pasture probably caused somewhat anaerobic conditions, which was indicated by a more blueish color of the soil at the 42-day sampling. If this caused an enhancement of denitrification, the measured N mineralization rates in the compacted area would seem lower than the actual values.

In contrast to the field experiment the compaction treatment in the laboratory experiment mostly decreased the proportion of macro pores. The volume of coarse,

intermediate and micro pores, remained relatively unchanged, and therefore the related change in bulk density would not have affected the microbes or their grazing by the micro-fauna directly (Van der Linden *et al.*, 1989). However, the changes in water-filled pore space and the air permeability would be expected to have great impact on the microbial activity (Linn and Doran, 1984; Kaiser *et al.*, 1990). It is not surprising that the C and the N mineralization of the well aerated, low density permanent pasture soil tended to be highest for the highest soil moisture levels, as this would promote a higher level of exoenzymes and greater solubilization of soil organic matter (Asmar *et al.*, 1994) with a relatively constant C:N-ratio. On the other hand, when the high soil moisture is associated with low air permeability as in the high density, -1 and -5 kPa cores of both soils, this could result in partly anaerobic conditions and N loss via denitrification (Bakken *et al.*, 1987; Torbert and Wood, 1992). This is the most probable explanation for the difference beween the two densities of the permanent pasture soil. It is surprising, however, that the N mineralization pattern of the low and high density 28 y cropped soil does not differ more, as there are very large differences in air permeabilities between the two densities. Although it was coarsely sieved, the 28 y cropped soil could still have retained some relatively dense aggregates smaller than 5.6 mm in which partly anaerobic conditions could prevail at high moisture content in spite of a relative high air permeability of the whole soil core. However, this explanation remains speculative as no measurements to confirm it has been performed.

Both the field and the laboratory experiments illustrated the importance of quantifying denitrification losses when studying nitrogen turnover in compacted soils. In a similar laboratory experiment Torbert and Wood (1992) successfully quantified denitrification losses by calculating a mass balance of applied ^{15}N-labelled NH_4NO_3. This approach could possibly also be applied in future field studies with the in situ coring method.

It is surprising that the microbial biomass C estimates by the FE and the SIR method differ so substantially. The effeciency of the fumigation procedure with relatively wet samples in the FE method could be questioned, but fumigation effeciency has normally proven to be unafffected by high moisture (D.J. Ross, pers. comm.), whereas it is commonly known to be affected by moisture below 40 % of water holding capacity (Sparling and West, 1989). Even if the -1 kPa samples are omitted there was still a decrease in FE biomass C levels following the compaction and the largest decrease occurred when the subsequent incubation period was performed at high moisture content. As mentioned earlier, the compaction treatment did not affect the microbial habitat directly but only through changing aeration status at similar water potentials and it seems unlikely that this change in aeration status would cause the decrease in FE biomass C from the initial sieved soils to the compacted soils. With increasing level of compaction Van der Linden *et al.* (1989) also found decreases in microbial biomass as estimated by FE. Both their and the current laboratory experiment were conducted at higher than normal field temperatures and this could be a possible explanation why the microbial biomass decreased in the laboratory and not in the field experiment.

However, the SIR biomass C estimates showed an increase following compaction and subsequent incubation. This is completely opposite to the FE biomss C estimates, although the SIR biomass C estimates were also lower at the high density as compared to the low density. The SIR method for measurement of microbial biomass C has been interpreted more as a measure of active than total microbial biomass by some authors (Van de Werf and Verstraete, 1987; Wardle and Parkinson, 1990; Hassink *et al.*, 1991). In the present study it could be proposed that this increased SIR response of the compacted and incubated samples could indicate a change in population structure and metabolic activity

of the microbial community under the high moisture conditions. However, as long as more detailed studies into these effects on the microbial biomass have not been made, no final conclusions on the differences in FE and SIR biomass C estimates can be made.

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Soil microbial and *in situ* nitrogen mineralization after 20 years of different nitrogen fertilization and forage cropping systems

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Abstract

Soil microbial biomass and N mineralization were measured after 20 years of treatments, namely: 1. cropping systems: annual forages (AF), rotation of temporary leys (TL), permanent grassland (PG); 2. fertilization: no N fertilization (C), mineral N (N), slurry (S), both (NS). After the cultivation period, the soils were ploughed and kept bare for leaching during three years. The microbial biomass was measured in three consecutive years in spring. The cumulated leached NO_3-N gave an estimation of annual N mineralization. A significant effect of all treatments was found on N mineralization and biomass-C. Grassland duration and previous slurry application increased the two variables. Previous mineral N applications had no effect on N mineralization and decreased biomass-C. The relationship between N mineralization and microbial biomass-C was closer in the 2nd and 3d year (r^2 = 0.92 and 0.86 respectively; n = 24) than in the first year (r^2 = 0.63; n = 24) due to different behaviour of TL and PG treatments. In cropping systems including grasslands, a labile pool could operate together with microbial biomass to determine soil N mineralization.

INTRODUCTION

Nitrogen mineralization in soil is the result of microbial activity. The measurement of the microbial biomass pool could give a rapid diagnosis of the N mineralization potential of soils if its activity was only related to its size. Several models of soil N cycle are based on such a proportionality.

However, the same models assume that the level of soil microbial biomass is determined by the input of metabolizable residues or the level of other active N pools in the soil. So the actual knowledge represents N mineralization as a dual result of microbial biomass and a metabolizable N pool.

This paper focuses on the relation between microbial biomass and N mineralization in order to check the reliability of a simple correlation, including the effect of different managements on the same soil.

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MATERIALS AND METHODS

The long-term lysimeter experiment of Theix (Puy de Dôme) was used to study the correlation between soil biomass and N mineralization. An initial sandy soil (16.1 % C, C/N 9.8) was differentiated by 20 years of treatments including three forage cropping systems and four N-fertilizer managements with two lysimeters per treatment: annual forages (AF), rotation of temporary leys (*Festuca arundinacea*, 4-5 years) with cereals (2-3 years) (TL) and permanent grassland (PG); no N fertilization (C), mineral N dressings at a mean level of 300 kg N ha^{-1} y^{-1} (N), cattle-slurry application at a rather low mean level of 80 kg N ha^{-1} y^{-1} (S), and both (NS). In order to minimize the border effects, each treatment occured in the lysimeter (3 m^2) and in a surrounding area of 16 m^2. After the cultivation period, the soils were ploughed in April and kept bare during three years.

Drainage was maintained by summer irrigation throughout the three years and the cumulated leaching of NO_3-N was measured at two-week intervals. The level and frequency of irrigations was 57 mm in three times in 1988, 190 mm in nine times in 1989 and 153 mm in nine times in 1990. The microbial biomass was measured by the fumigation/extraction method (Chaussod *et al.*, 1988; Wu *et al.*, 1990) in three consecutive springs, at the beginning of the growing period. The measurements were made in the most active layer of the soil profile (first 15 cm). The results are expressed in mg extractable biomass (mg C flush kg^{-1}). The mean annual temperatures were 8.65, 9.61 and 9.49 °C for the three consecutive years.

RESULTS

N mineralization

NO_3-N concentrations at the bottom of the lysimeters showed marked seasonal patterns with maximum values in winter and minimum values at the end of July (Loiseau *et al.*, 1990[a]). This resulted probably from a delay period of six months for the mineralized nitrogen to be recovered at a depth of 80 cm (Figure 1). So the successive annual leachings were calculated from August to August (Figure 2).

Total leaching of three years decreased from PG to AF with respectively 903, 681 and 435 kg N ha^{-1} (average of four fertilizer treatments). Previous applications of mineral N showed no mean effect on subsequent N mineralization, but slurry application increased N leaching from 609 to 735 kg N ha^{-1} (three years, average of three cropping systems and two levels of mineral N).

N leaching decreased with time: 264, 236 and 172 kg N ha^{-1} y^{-1}, respectively, for the three successive years as a mean of all treatments. Interactions took place: 1. between time and cropping system, with a more important annual decrease after PG than after TL (-40 and -29 %, respectively); 2. between year and fertilization, with more annual decrease after previous applications of slurry or mineral N than after control (particularly, mineral N caused a small increase in N mineralization in the first year and a small decrease in subsequent years); 3. between cropping system and fertilization, with more N leached, resulting from previous slurry applications on cultivated cropping systems (+52 kg N $ha^{-1}y^{-1}$) than on permanent grassland (+19 kg N ha^{-1} y^{-1}).

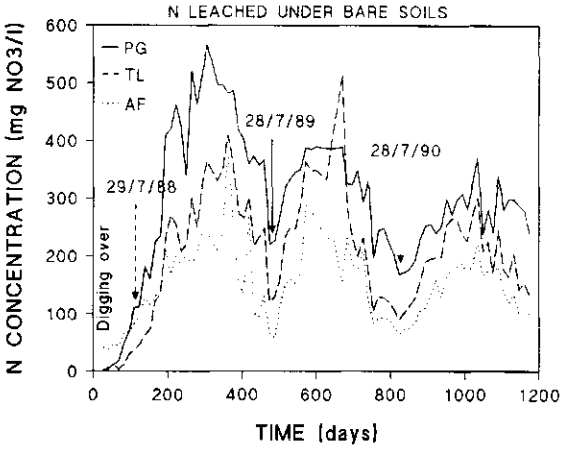

Figure 1. NO_3 concentrations in leachates during the first three years in fallow after various cropping systems. PG: permanent grasslands, TL: temporary leys, AF: annual forages. Mean of four fertilizer treatments and two replicates.

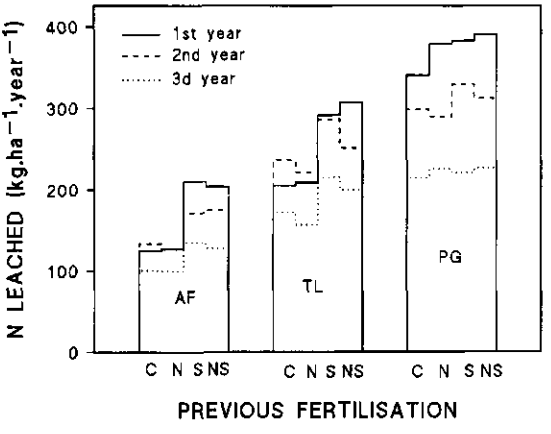

Figure 2. Annual leaching under bare soils in relation to previous management. AF, TL, PG: see Figure 1; C: control, N: mineral fertilizer, S: slurry, NS: mineral fertilizer and slurry.

Microbial biomass

The effect of the previous cropping system and fertilization regime on biomass-C was highly significant ($P < 0.0001$). Just after ploughing the level of extractable biomass-C was 102, 212 and 231 mg C kg^{-1} for AF, TL and PG, respectively (Figure 3). The previous slurry applications influenced the biomass more than did the previous mineral-N fertilization. Slurry enhanced biomass-C by 22 % and mineral N fertilization decreased biomass-C by 10 % (average of three years). No interaction occured between the two fertilizer treatments, but a highly significant interaction took place between previous cropping system and mineral N application, biomass-C being more decreased by previous N application after TL than after PG or AF.

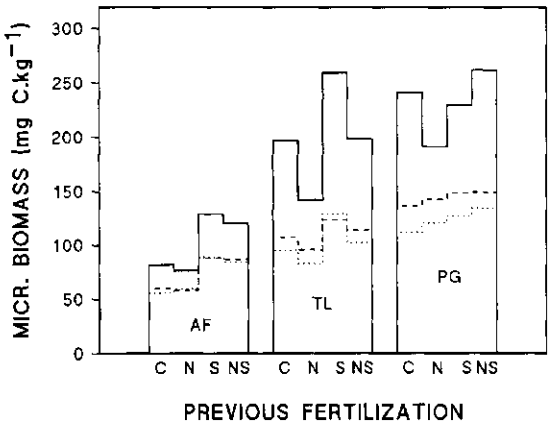

Figure 3. Microbial biomass (mg C-flush kg^{-1}) in relation to previous management. See figure 1 and 2 for legends.

In the third year, the mean biomass was 72, 102 and 123 mg C kg^{-1} for AF, TL and PG, respectively. Biomass decreased with time, with a mean of 181 mg C kg^{-1} just after ploughing, 109 one year later and 99 two years after ploughing. So the decrease was fast in the first year and tended to reach a new equilibrium asymptotically. The relative decrease differed according to the cropping system, being high for previous systems including grasslands (respectively -47 and -52 % for PG and TL for two years), and moderate after annual forages (-29 %). However, two years after ploughing, the microbial biomass was still at a higher level after grasslands than annual forage. A second interaction took place between time and previous mineral applications. The negative effect of mineral N was limited to the first year. The positive effect of slurry, however, was observed each year.

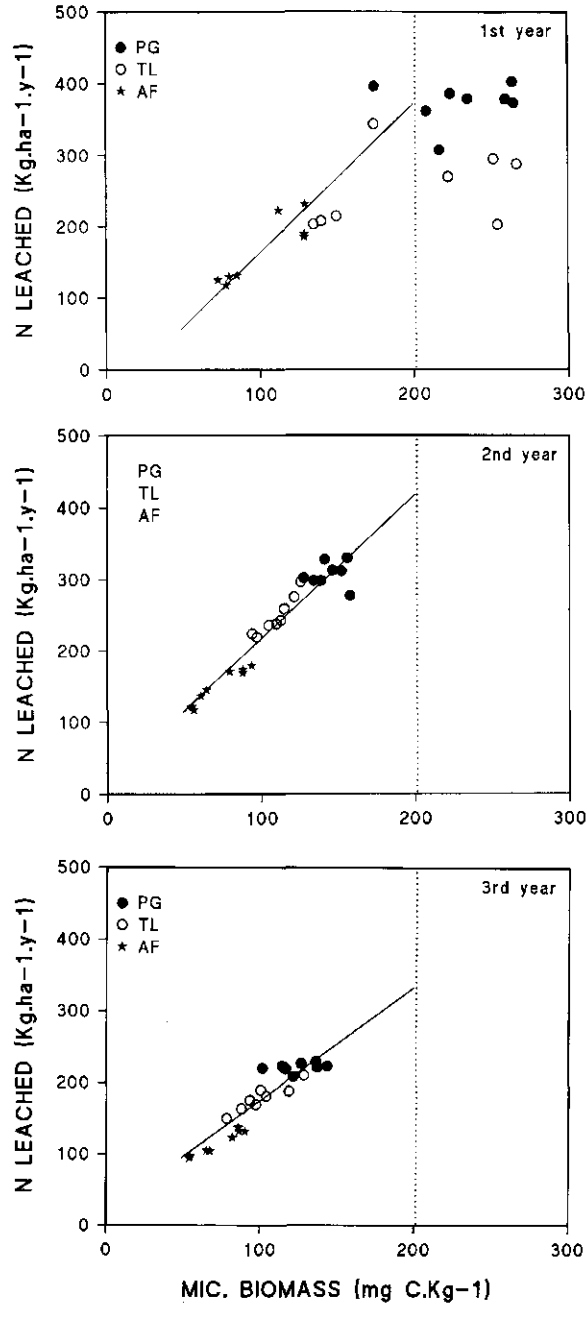

Figure 4. Correlation between microbial biomass and N mineralization estimated by *in situ* annual leaching under bare soil.

Correlation between microbial biomass and N mineralization

The regression was examined between N leaching at the bottom of the lysimeter and biomass C measured in the first 15 cm of the soils. The regression coefficient is only an indicator of the role of biomass activity in N mineralization, i.e. the amount of mineralized N per unit of microbial biomass. Within-year correlations showed that the intercept was not significantly different from 0 (Table 1), so that N mineralization was directly proportional to biomass-C.

In the first year after ploughing, N mineralization was poorly related to biomass for grassland treatments but the correlation was improved when taking in account only levels of biomass lower than 200 mg kg^{-1} C (Figure 4; Table 1). The correlations were better for years 2 and 3, when biomass had decreased (Table 1). For biomass C not exceeding 200 mg kg^{-1}, the mean ratio between mineralized N and biomass-C was 2.0 +/- 0.3. Within year and permanent grasslands, the mineralized N was never related to microbial biomass.

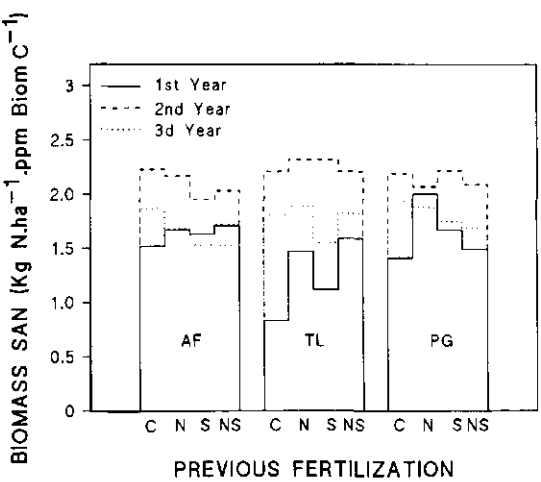

Figure 5. Index of microbial activity for N mineralization in relation to previous management (kg N leached ha^{-1} y^{-1} mg C kg $biomass^{-1}$).

As the regression line of N mineralization and biomass-C came through 0, a direct calculation was made of the ratio between the two variables (Figure 5). The mean ratio of 1.81 +/- 0.5 was influenced by year, with an increase from 1.51 to 2.17 from the first to the second year and a drop to 1.74 in the 3rd year (95% confidence interval 0.16). S or N showed no mean effect, but some significant interactions: a positive effect of N was limited to the first year and to the TL cropping system; a negative effect of S occurred only in the 3rd year as a mean of all cropping systems.

Table 1. Correlation between N mineralization (NMin) and biomass-C (CBiom). Linear regression: NMin = a + b CBiom. Values and standard error for the parameters a and b.

Year	a		b		n	r^2
	mean	SE	mean	SE		
All	79.9	14.6	1.11	O.10	72	0.63
All (1)	- 9.2	15.5	1.99	0.14	60	0.78
1988-89	72.1	38.3	1.06	0.20	24	0.57
1988-89 (1)	-58.3	40.2	2.20	0.32	12	0.83
1989-90	9.2	14.5	2.08	0.13	24	0.92
1990-91	9.5	14.6	1.64	0.14	24	0.86

(1) Biomass-C less than 200 mg kg^{-1}

DISCUSSION

Biomass-C was only measured in the first 15 cm of soils in order not to disturb N transfer in the lysimeters, but measured N leaching was the result of soil N mineralization in 80-cm deep soils. The regression coefficient proposed between total N mineralization and biomass-C is only an approximation of biomass activity for N mineralization. The comparison of regression coefficients implies the hypothesis of similar biomass profiles according to the different treatments. A correct expression of the microbial activity would involve further work on the distribution of biomass in the soil profile (Hassink *et al.*, 1991).

As biomass was measured only three times, it was impossible to give reliable values for the decay rate of the microbial biomass and mineralizable organic N pool. Jenkinson and Ladd (1981), and Chaussod *et al.* (1988) estimated the turnover time of microbial biomass to be 1-2 years. The results indicate that 30 % of the microbial biomass was supported by the organic matter fraction which is decomposed within a year. In grasslands, this fraction appeared to support about 50 % of the biomass.

From the annual mineralization rates, it is obvious that the soil accumulated different levels of organic reserves by stabilization of organic C and N from plant and microbial origin. Campbell *et al.* (1991) established that rotations including grasslands accumulated more potentially mineralizable N as compared with unfertilized crops. This appears to be also valid at similar levels of N inputs.

Taking all dates, cropping systems and fertilizations, a positive correlation is obtained on the whole between N mineralization and biomass-C, mainly due to a positive effect of previous grassland or slurry applications together on final available N, N mineralization and microbial biomass.

The size of the microbial biomass is affected by an equilibrium between its C requirements for maintenance and the nutrient supplying capacity of the soil. Other factors may play a role, such as grazing pressure due to microbivores, and soil texture, which may result in protection of microbes in small pores against predation. In our case, only variations of soil structure could be involved.

The labile organic pool proceeds from exudates, decomposing plant material and partly from older, more or less stabilized soil organic matter. Another part of the labile pool is the result of microbial death and activity ("humads", Molina *et al.*, 1983). Nitrogen is mineralized as a by-product of microbial activity (Parnas, 1975).

Poor correlations within cropping systems were found in the first year after PG and TL, when biomass levels are high, and still in the 2nd and 3rd year after PG. In the first year, points under the global regression line suggest soil conditions proper to microbial proliferation, protection or reduced activity. The additionnal biomass above 200 mg kg^{-1} seems to be linked to the presence of decomposing root material and N accumulation in the soil during grassland life (Loiseau *et al.*, 1990[b]). This is in accordance with Hassink *et al.* (1991) assuming that biomass-C represents a greater part of total soil C in systems with larger organic inputs, and with Sparling (1992), who noticed a higher "microbial quotient" (i.e. proportion of biomass-C/organic-C) under pasture than under annual crops.

In subsequent years, the grassland points came back on the regression line. The correlation within cropping systems between biomass-C and N mineralization was restored in TL but not in PG. This suggests that, in the case of grasslands, other important factors are involved in N mineralization. Three hypotheses could be set out. 1. Denitrification could occur more intensively after grasslands because of the higher organic matter and nitrate contents. However, the sandy soil allows good conditions for soil oxygenation. 2. More protection of microbial biomass could occur in microsites under PG in relation with the previous absence of tillage, and protection would set against activity. 3. Microbial specific activity could be limited by another factor like the pool size or the accessibility of readily decomposable organic matter.

Better correlations within cropping systems occur onwards from the 1st year for AF and from the 2nd year for TL. In these cases, microbial biomass seems to be a good index of N mineralization, both for cropping systems or fertilizations. This does not mean that a labile N pool is not involved in microbial activity, but microbial biomass alone is a sufficient index for predicting N mineralization. This would be the case if the labile pool was or became proportional to the microbial biomass. Understanding the role of microbial biomass in N mineralization requires to measure and understand the relationships between biomass and the active N pool ("humads" or "pool II", McGill *et al.*, 1981; Molina *et al.*, 1983; "active pool", Parton *et al.*, 1987), in order to study its covariation with biomass and give a multivariable explanation of N mineralization.

N mineralization is enhanced by moderate applications of slurry, but not by larger mineral N applications. So, intensification of forage production by mineral dressings does not necessarily increase N availability through increased returns of dead material. In the same manner, Hassink and Neeteson (1991) found that N accumulation under mown grasslands on a sandy soil was independant of the levels of mineral N fertilization between 250 and 750 kg N ha^{-1} y^{-1}. On the other hand, mineral N applications decrease biomass-C (1st year) and increase its activity for net N mineralization (TL, 1st year). This is associated with a lowering of the C/N ratio of the labile organic pool (data not presented), and a possible decrease of the microbial N immobilization rate.

The year-effect on the regression coefficients results from immediate climatic conditions or/and from a cumulative effect of the incubation time. As temperature increases N mineralization (Malkomes, 1991) in an exponential way (Honeycut *et al.*, 1991), the

correction of the regression coefficients for temperature shows a decrease with time at the constant mean temperature of 9 °C: 2.14 for the first year, 1.17 for year 2 and 1.06 for year 3. This decrease appears to be more pronounced than the decrease in biomass. The especially higher value in the first year could be attributed to an effect of ploughing on soil mixing and aeration, dissapearing in time by earth packing.

CONCLUSION

The microbial biomass in the first 15 cm of soils gives a better prediction of N mineralization in bare soils after annual crops than after grassland. More research has to be carried out on an easily mineralizable organic pool, which may operate together with microbial biomass to determine soil N mineralization.

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Temporary microbial immobilization of nitrogen in an arable loess soil

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Abstract

The variation of soil microbial biomass N in an arable soil in the southern Niedersachsen loess region was studied during spring and summer 1991. Microbial immobilization and remobilization after application of mineral N were investigated on wheat cropped and fallow microplots. ^{15}N was used to examine the fate of fertilizer N. Biomass N was determined by the Fumigation-Extraction-Method (FEM). During the period of observation the amount of biomass N varied between 40 and 90 µg g^{-1} soil (180-400 kg [ha 30cm]$^{-1}$). Little difference was detected between the cropped plots. In the fallow plots microbial biomass N decreased in summer. After the application of N-fertilizer a N mineralization flush was observed. ^{15}N measurements indicate that up to about 60 % of fertilizer N is incorporated into the microbial biomass within one week and is released afterwards within two to six weeks.

INTRODUCTION

The microbial biomass of a soil is an important factor in the N cycle of soils. On the one hand it is responsible for the decomposition of organic matter in the soil. On the other hand the microbial biomass itself can incorporate or release nitrogen during periods of growth or decay. Therefore, the mineral N pool of the soil is not only affected by the mineralization potential which is satisfactorily described by first-order kinetics (Nuske and Richter, 1981) but also by the population dynamics of the microbial biomass.

Variations in microbial C and N under a spring barley crop were reported by Ritz and Robinson (1988). Carter and Rennie (1984) observed microbial N immobilization associated with root development. Seasonal fluctuations in soil microbial biomass may also be caused by soil temperature and moisture (Campbell and Biederbeck, 1976; Malkomes, 1991). The incorporation of plant residues of different composition may affect the amount of microbial biomass for about one year (Bremer and Van Kessel, 1992). Straw incorporation results in immobilization of mineral N for several months (Nieder and Richter, 1986). Decreases in the amount of mineral N which can not be explained by plant uptake or leaching have been observed especially in spring, after N-application (Kersebaum and Richter, 1991). The authors attribute this to microbial immobilization.

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The objective of this work was to determine the influence of plants and of N-fertilizer on dynamics of microbial biomass N during spring and summer.

MATERIALS AND METHODS

Site description

The experiment was carried out on a loess derived luvisol with approximatly 21.7 % clay (< 0.002 mm), 1.5 % organic C, 0.16 % total N and pH(CaCl) 6.8. The soil had been in agricultural use for at least 30 years in a cereal - sugar beet - rotation.

Treatments

The experiment included three treatments with three replications each: (1) fertilized, $(NH_4)_2SO_4$ equivalent to 140 kg N ha^{-1} divided over two applications (18.03 and 30.04), equivalent to 70 kg N ha^{-1} each), (2) unfertilized, (3) fallow, unfertilized. (1) and (2) were cropped with winter wheat, the preceding crop was also winter wheat.

Plastic tubes of 30 cm length and 25 cm diameter were rammed into the soil of treatment (1) plots in March 1991 to border the area for ^{15}N application. Labelled nitrogen (60 % enrichment) was applied in the amount described above. Samples were taken from the arable layer (30 cm).

Analytical methods

Mineral N was determined with a continuous-flow autoanalyzer (CHEMLAB instruments) after extraction with 1M potassium chloride. The soil:extractant ratio was 1:4. Biomass N was determined after fumigation with chloroform and extraction with 1M potassium sulfate, followed by Kjeldahl digestion and steam distillation of the extracts (Brookes *et al.*, 1985). NO_3^- was reduced prior to Kjeldahl digestion (Pruden *et al.*, 1985). A conversion factor (K_{EN}) of 2.22 was used to calculate microbial biomass N as suggested by Jenkinson (1988). K_{EN} was not used to calculate ^{15}N labelled biomass N and incorporated fertilizer N, for it would result in recovery rates higher than 100 %.

For determination of ^{15}N, all the soil of one of the plastic tube was removed and mixed thoroughly on each sampling date. About 1000 g were taken to the laboratory for analysis. Samples were treated as described above. ^{15}N was determined using a JASCO emission spectrometer.

RESULTS AND DISCUSSION

There is little difference in the amounts of microbial biomass N between the plots for the first two months, although nitrogen has been applied to the fertilized plots at the end of March (Figure 1). This suggests that the differences between the plots observed later in the year are due to the treatments and not to spatial variability.

The amounts of microbial N begin to differ around mid April. In spite of changes with time, the cropped plots show similar amounts of microbial N, while microbial N in the fallow plots has a different course with a decrease in July and August. Since the mineral N content in the upper 30 cm of the fallow plot has increased to about 16 µg g^{-1} (72 kg ha^{-1}), there is no deficiency of available N. Gravimetric water content is highest in the fallow plots, remaining above 18 %. It seems therefore, that by the middle of July the carbon supply becomes a limiting factor for microbial growth in the fallow plots.

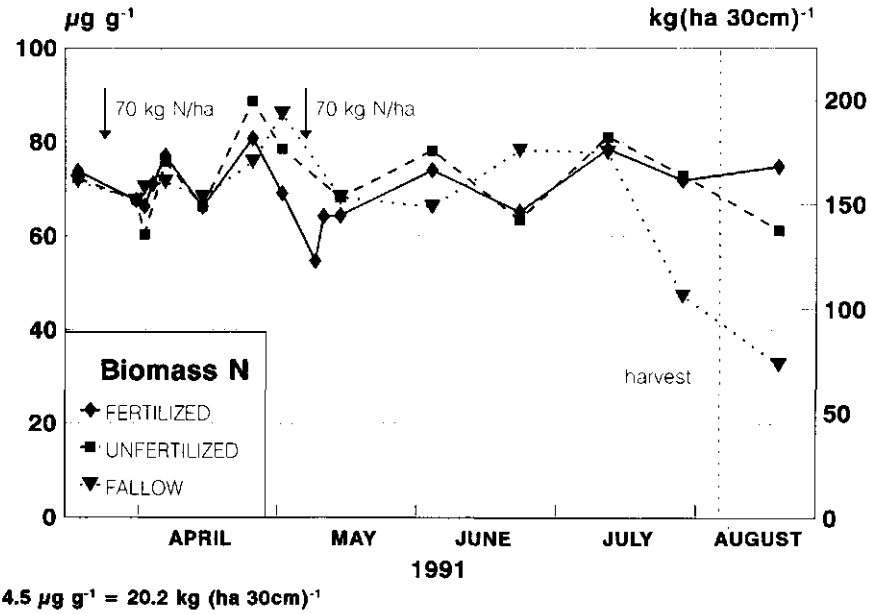

Figure 1. Plant and fertilizer effects on soil microbial biomass N.

Until mid April, the amount of mineral N increases in all plots, even in periods when microbial biomass increases too. This indicates that the N content of the mineralized organic matter is higher than the demand of the microorganisms. In this period, the N supply of the microbial biomass can therefore not be considered as a limiting factor.

Comparison of fertilized and unfertilized plots suggests that there is little effect of fertilizer N on microbial biomass N. There is an increase in microbial N after both applications of N, but this increase is also observed in the unfertilized plots.

The ^{15}N data, however, demonstrate that approximately 8 µg g^{-1} (36 kg ha^{-1}) and 6 µg g^{-1} (27 kg ha^{-1}) of fertilizer N are incorporated into the microbial biomass after the first and second application of fertilizer, respectively (Figure 2). As explained above, the amount of biomass N was calculated without using k_{EN}. Therefore, the actual amount of incorporated fertilizer N might be even higher. It seems that recently incorporated mineral N is easy to extract after fumigation compared to older and presumably more structured microbial cell material. Azam *et al.* (1989) showed that succession of the microbial population after addition of ammonium and glucose causes different extractabilities of microbial N. After both applications, fertilizer N is incorporated into the microbial biomass, but simultaneously soil N is mineralized. According to the literature on "mineralization-immobilization-turnover" (MIT) (Jansson, 1958; Molina *et al.*, 1990) there is no direct incorporation of organic N into the microbial biomass. The data shown above can be explained using the assumption of MIT. After fertilizer application, the NH_4^+ pool consists mainly of fertilizer N. Soil-derived and fertilizer NH_4^+ are incorporated into the microbial biomass in proportion to their concentration in the soil. Therefore, incorporated NH_4^+ consists mainly of fertilizer N. To satisfy their C demand, microorganisms mineralize organic compounds and release N to the soil.

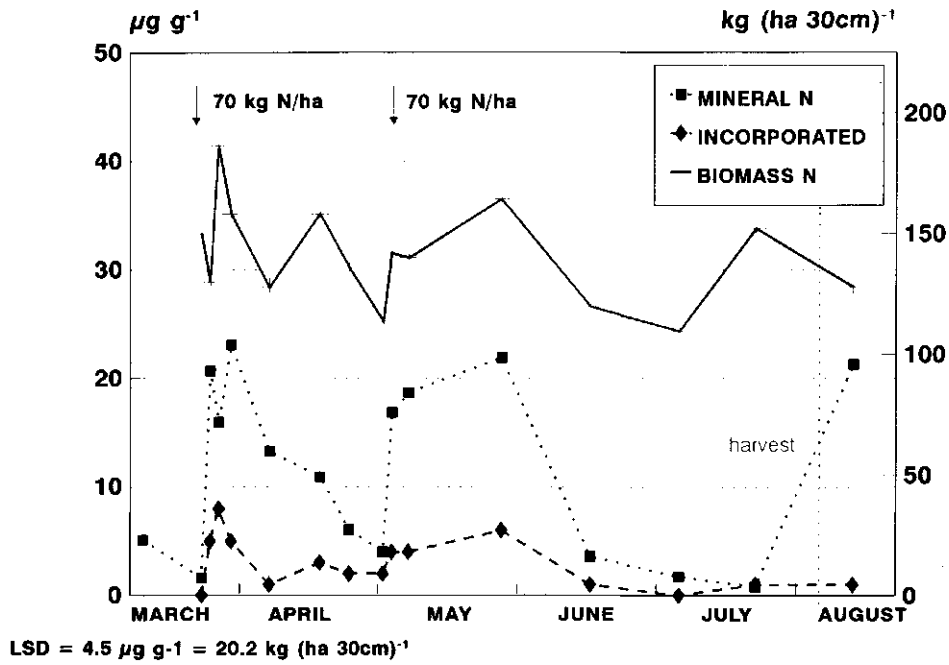

Figure 2. N uptake by microbial biomass after fertilization: ^{15}N experiment.

Nielsen and Jensen (1986) reported that 12 days after the application of NH_4^+ between 60 and 80 % of the applied fertilizer (between 30 and 150 kg N ha^{-1}) had "disappeared" from the soil solution. Plant uptake was small and gaseous losses were neglegible. Therefore, the authors attribute the "dissapearance" of N to microbial immobilization. In a laboratory experiment Recous *et al.* (1990) measured immobilization of only about 5 µg g^{-1} NH_4^+-N soil 5 days after application of 100 µg N g^{-1} soil. However, when glucose was added to the soil, about 25 µg N g^{-1} soil was immobilized within 4 days. This suggests that in the presence of easily available carbon high amounts of NH_4^+ can be immobilized. The data presented show immobilization rates between 2 and 4 µg g^{-1} day^{-1}. They are similar to those reported by Nielsen and Jensen (1986) for higher N applications and within the range of those reported by Recous *et al.* (1990).

Most of the ^{15}N incorporated after the first application is released from the microbial biomass within about two weeks, either to the mineral N pool or to the organic N pool. At this time, a decrease in microbial biomass N is observed in all plots, indicating conditions unfavorable for microbial maintainance.

After the second application, fertilizer N is again incorporated into the microbial biomass, but to a smaller extent. In the beginning of May plants are in a phase of exponential growth. Therefore, roots take up a larger amount of nitrogen than a month before. The release of N from the microbial biomass takes about twice as long as after the first application. During May microbial biomass N increases slightly in all cropped plots contrary to the period after the first application (Figure 1). Easily decomposable carbon from root exudates and decayed roots may have supported microbial growth and maintainance in this period.

The presented results suggest that after N fertilization part of the applied N is incorporated into the microbial biomass even if net immobilization is not observed. Net immobilization might occur if the N content of soil organic matter is a limiting factor for microbial growth.

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Suitability of microbial biomass as indicator for the N mineralization capacity of soils: influence of immobilizing conditions

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Abstract

A 3-year pot experiment was carried out to examine the suitability and limitations of microbial biomass measurements as indicator for the N mineralization capacity of soils. After stimulating microbial growth by supplying different amounts of carbon and nitrogen to the soil, biomass N content was monitored. As indices for the mineralization capacity of the soils under study N_{min} accumulation (autumn/ winter) and N uptake by plants (spring/summer), respectively, were measured and correlated to the microbial biomass values.

Simultaneous addition of carbon and nitrogen resulted in a considerable increase of microbial biomass. At the end of the 3-year experiment biomass declined to a soil typical level and only small differences were measured between the several treatments. Not utilized fertilizer N in the exclusively anorganic supplied treatments and extreme immobilizing conditions in the soils which had received carbon and nitrogen in a 50/1 ratio are reasons for unsatisfactory correlations between biomass content and N_{min} accumulation and plant N uptake, respectively. After the turnover milieu has reached again an equilibrium stage biomass measurements seem to be a suitable tool for estimation of the mineralization capacity of soils.

INTRODUCTION

A substantial improvement in adjusting N fertilization to the nitrogen requirements of agricultural crops compared to farmers' practice was attained by considering the amount of mineral nitrogen (N_{min} = NO_3-N + NH_4-N) in the rooting zone at the beginning of the growing season. Using this so-called "N_{min}-method" according to Wehrmann and Scharpf (1979) the first fertilizer rate in spring is calculated from a crop specific "N_{min} target value" minus the measured N_{min} content in the 0-90 cm layer. N mineralization during the vegetation period, which might differ enormously, is only considered on an average. Several methods have been proposed to estimate the amount of easily mineralizable nitrogen for the calculation of fertilizer recommendations: incubation experiments, measurement of

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microbial biomass, and chemical extraction procedures in order to determine an easily decomposable soil nitrogen fraction (for details see reviews of Keeney, 1982; Olfs and Werner, 1994; Stanford, 1982).

In the last decade, measurement of microbial biomass in soils has gained greater importance. This is due to the role of the biomass as an important N pool on the one hand and to its function as transformer on the other hand (Anderson and Domsch, 1980; Jenkinson and Ladd, 1981). Jansson and Persson (1982) have summarized this idea in their "Mineralization-Immobilization-Turnover" concept. Although it is generally accepted that microbial bound nitrogen represents a soil N fraction with a high mineralization rate (Chichester et al., 1975; Myrold, 1987; Schnürer and Rosswall, 1987) less information is available on the possibilities to use biomass determinations for the improvement of N fertilizer recommendations.

Therefore, our investigations were focused on the question whether biomass measurements are a generally suitable indicator for the N mineralization capacity of soils and if any limitations have to be regarded.

MATERIALS AND METHODS

A pot experiment was carried out with a luvisol derived from loess (C_t= 1.4 %, N_t= 0.14 %, pH ($CaCl_2$) = 5.9, 7.4 % sand, 75.7 % silt and 16.9 % clay) for 3 vegetation periods (March 1987 - August 1989). At the beginning of the experiment microbial activity was stimulated by adding different amounts of carbon (as cellulose or ground grass-clover) in combination with mineral nitrogen (as calcium nitrate) into the soil (Table 1).

Table 1. Experimental design.

Treatment code	Addition (g per pot)		C/N ratio
	N	C	
N0	-	-	-
N1	0.6	-	-
N2	1.2	-	-
N4	2.4	-	-
N1-25	0.6	15	25/1
N2-25*	1.2	30	25/1
N4-25*	2.4	60	25/1
N1-50	0.6	30	50/1
N4-50	2.4	120	50/1
N4-GC	2.4	as grass-clover	-
N2+N2-GC	2.4	50 % as grass-clover	-

* Treatment with ^{15}N application

In two treatments (N2-25 and N4-25, respectively) ^{15}N labelled fertilizer (10 atom-% ^{15}N; Icon Services Inc., New Jersey, USA) was used. Each pot was filled with 9.5 kg air-dried soil and stored in a greenhouse without temperature control (minimum -2 °C, maximum 42 °C). Once per day during the experimental period soil moisture was adjusted to 50 % of water holding capacity. At the beginning of the second and third year (i.e. after 42 and 100 weeks) the soil was dried at 40 °C in a drying cabinet, the soil material (including plant roots) of each treatment was pooled, thoroughly mixed and distributed again.

Because of the four soil samplings in the first year (one pot in week 1 and 4, 3 pots in week 13, 5 pots in week 22) and at the start of the second vegetation period in week 42 (3 and 5 pots, respectively), only 8.5 kg soil remained per pot (with 8 replicates). In the second year soil samples were taken 62 and 70 weeks after the start of the experiment (4 pots per date). In the third vegetation period pots were filled with 9.0 kg soil (6 replicates) and sampled in week 108 and 123, respectively.

Eight weeks after the start of the experiment maize (*Zea mays* cv. Alize) was sown and above-ground plant parts were harvested in week 13 and 22 (3 and 5 replicates, respectively). In the second year, wheat (*Triticum aestivum* cv. Star; sown in week 50, harvested in week 70) and in the third period grass (*Lolium multiflorum italicum* cv. Turilo; sown in week 108, harvested in week 113, 118, and 123, respectively) was planted (for more details see Olfs, 1992).

Plant material was analyzed in a nitrogen analyzer (NA 1500, Carlo Erba Strumentazione, Milano, Italy) after drying (105 °C, 24 h) and grinding. The content of mineral nitrogen (N_{min} = NO_3-N + NH_4-N) was determined in a continuous flow analyzer (Technicon AA II, Bran & Lübbe, Hamburg, Germany) after extracting 50 g moist soil with 200 ml K_2SO_4 solution (10 g l^{-1}). ^{15}N in the N_{min} extracts was analyzed using an automatic continuous-flow isotope ratio mass spectrometer (Roboprep-CN + Tracermass, Europe Scientific Ltd., Crewe, U.K.) after applying a modified "diffusion method" (Olfs *et al.*, 1992) for sample preparation. Biomass was measured according to the "substrate induced respiration" (SIR) method (Anderson and Domsch, 1978) via O_2 uptake (Sapromat, Fa. Voith, Heidenheim, Germany) and biomass C was calculated from the equation mg O_2 h^{-1} kg^{-1} soil * 28 = mg biomass C kg^{-1} soil (Beck, 1984). Assuming a C/N ratio of 10/1 for the microorganisms biomass C values were converted into biomass N.

Differences in N_{min}, microbial biomass N and N uptake by plants between treatments were analyzed using the procedure "*One-Way Tuckey HSD Test*" and regression analysis was done with the method "*Regression*" of the software package "SPSS PC+" (SPSS Inc., Chicago, USA). A statistical analysis for biomass values was not possible for soil samples from week 1 and 4 (sampling without replications) and from week 108 and 123 (replicates pooled before analysis), respectively.

RESULTS

For biomass N content in the control treatment (N0) and in treatments exclusively supplied with nitrogen (N1, N2, and N4, respectively) only slight fluctuations were measured in the 3-year experiment (Table 2).

Simultaneous application of nitrogen and carbon increased microbial biomass N in the soil already after one week. Up to 190 mg N kg^{-1} soil (= 75 % of the supplied nitrogen) was incorporated in the additionally biomass build up. In week 13 and 22, respectively, significantly higher biomass values were found for all treatments with N and C addition (excepting N1-25) compared with the biomass content of the control. Although biomass values for treatments with C and N application declined more or less pronounced in the second and third year, differences for treatments without C additions could be determined.

Soil N_{min} did not change according to an uniform pattern during the experiment (Table 3). In week 13 very low N_{min} levels were found for N0, N1-50 and N4-50.

Table 2. Microbial biomass N (mg kg^{-1}) after differentiated C/N supply in week 0 during the 3-year pot experiment.

Treatment code	Week							
	1	4	13	22	62	70	108	123
N0	16.52	23.80	26.04	23.80	24.92	19.60	17.36	22.40
N1	16.80	15.40	22.12	24.08	22.96	20.72	19.88	21.84
N2	16.80	10.36	22.68	27.16	26.60	26.88	21.00	23.80
N4	16.80	11.20	23.24	37.52	30.52	31.64	25.76	26.32
N1-25	86.52	47.32	40.88	30.80	28.00	23.52	22.40	25.48
N2-25	84.56	115.08	61.32	43.12	29.68	28.28	23.24	28.28
N4-25	103.32	193.48	88.20	64.40	41.72	38.08	28.00	32.76
N1-50	85.96	44.80	72.52	35.84	30.52	23.80	20.16	25.20
N4-50	164.92	124.04	108.36	80.08	55.16	37.80	31.08	33.60
N4-GC	205.80	62.72	60.76	52.92	41.16	41.72	30.80	30.24
N2+N2-GC	87.08	49.28	47.60	54.04	37.24	40.88	32.48	31.08
LSD 5 %	*	*	28.00	10.92	5.32	3.36	*	*

* Statistical analysis not possible (soil sampling or analysis without replication).
For treatment description see Table 1.
LSD 5 % = least significant difference between treatments at the 5 % level.

Treatments without C addition always showed higher N_{min} contents than the corresponding treatments. N_{min} values declined markedly for all treatments until week 22. Mineral N in week 42 for pots harvested after 13 weeks ranged from 51 to 369 mg kg^{-1} and showed a comparable order as the values in week 13. Contrasting results were obtained for pots harvested after 22 weeks (higher N_{min} levels for treatments with N and C application). Fluctuations in N_{min} were less noticeable from week 70 onwards to soil sampling in week 123, but N4-50 always had significantly higher N_{min} contents.

Table 3. Soil N_{min} (mg kg^{-1}) after C/N supply in week 0 during the 3-year pot experiment.

Treatment code	Week						
	13	22	42[a]	42[b]	70	108	123
N0	3.0	2.3	51.5	38.4	4.2	14.8	1.8
N1	27.6	2.0	95.8	32.2	3.9	13.3	2.0
N2	97.0	1.8	175.6	37.9	3.1	15.5	2.0
N4	208.5	0.9	369.5	44.7	2.8	14.9	2.0
N1-25	22.5	3.1	99.7	53.0	4.5	18.8	2.1
N2-25	41.6	3.5	127.2	76.3	4.3	19.9	1.9
N4-25	98.8	3.8	215.2	127.5	5.6	23.8	2.0
N1-50	13.0	2.5	73.4	49.5	4.1	16.8	2.0
N4-50	13.0	2.4	51.5	66.8	7.0	27.3	2.6
N4-GC	23.0	3.8	177.3	115.1	4.2	19.4	2.0
N2+N2-GC	92.2	2.9	253.1	99.3	3.5	17.7	1.9
LSD 5 %	44.6	1.2	93.5	33.3	1.2	3.1	0.4

[a] pots harvested after 13 weeks
[b] pots harvested after 22 weeks
For treatment description see Table 1.
LSD 5 % = least significant difference between treatments at the 5 % level.

Details about the origin of mineral N in the soil in spring 1988 and 1989 (42 weeks, and 108 weeks after the start of the experiment, respectively) can be deduced from ^{15}N fertilized treatments (N2-25, and N4-25, respectively). At the beginning of the second vegetation period (week 42) the portion of N_{min} derived from fertilizer is 2 to 3 times higher in pots harvested after 13 weeks than after 22 weeks (Table 4).

Table 4. Differentiation of N_{min} in soil N and fertilizer N (mg N kg^{-1} ; fertilization with 10 atom-% ^{15}N labelled calcium nitrate).

Date*	Treatment	Soil N	Fertilizer N
I	N2-25	75	86
	N4-25	61	176
II	N2-25	49	31
	N4-25	49	69
III	N2-25	14	5
	N4-25	14	9

*I = N_{min} 42 nd week after harvest in week 13;
II = N_{min} 42 nd week after harvest in week 22;
III = N_{min} 108 th week after harvest in week 70.
For treatment description see Table 1.

After exhaustion of the N_{min} pool by plants (pots harvested after 22 weeks) subsequent mineralization of native soil N is similar for both treatments (about 49 mg kg^{-1} up to week 42, and additionally 14 mg kg^{-1} up to week 108, respectively) but in spring 1988 (week 42), and 1989 (week 108), respectively, for treatment N4-25 more labelled nitrogen was found in the N_{min} pool than for treatment N2-25 (69 versus 31 mg kg^{-1}, and 9 versus 5 mg kg^{-1}, respectively).

Correlation coefficients were calculated for the relationship between biomass values and N_{min} accumulation in the soil to assess the suitability of biomass measurements for the estimation of N mineralization. No relationship was found between biomass content after the first harvest in week 13 and the amount of N_{min} in these soils after 42 weeks. Significant correlations ($r^{n=11}$ = 0.70* and $r^{n=10}$ = 0.96** excluding N4-50, respectively) appeared for the relation biomass in week 22 versus content of mineral N after a 20-week mineralization period (N_{min} week 42). Values for biomass in autumn 1988 (week 70) and N_{min} in the following spring (week 108) showed a close, but not significant relationship ($r^{n=11}$ = 0.62).

As second indicator for the actual N mineralization N uptake of plant was measured. The amount of mineral N in the soil (i.e. plant available N) is quasi detected as a "sum graph" so that short-term, more or less coincidental fluctuations are unimportant. For treatments with exclusive inorganic N fertilization (N1, N2, and N4, respectively) the amount of applied N was the dominating factor in the first year (Table 5).

Application of N in combination with C resulted in lower values for N uptake (e.g. N1-25 compared to N1). Treatments with high rates of C supply (e.g. N4-50) showed a very low N uptake as can be seen from a comparison with the data obtained for the unfertilized control. In 1988 only small differences were found in N uptake between treatments with the same level of N supply (except N4-50). In the third experimental period N uptake was higher for treatments with simultaneous supply of N and C.

Table 5. N uptake by plants (mg kg^{-1}) after differentiated C/N supply in week 0 during the 3-year pot experiment.

Treatment code	1987 Week 13	1987 Week 22	1988	1989
N0	7.58	9.47	15.41	18.78
N1	31.47	56.74	23.06	16.78
N2	36.74	91.37	37.18	19.33
N4	56.42	196.84	60.24	18.67
N1-25	14.63	26.74	24.24	20.67
N2-25	21.47	45.47	35.29	24.00
N4-25	24.11	84.74	57.53	29.56
N1-50	6.84	10.32	25.06	21.44
N4-50	5.89	8.42	44.94	34.78
N4-GC	46.42	85.68	54.24	24.11
N2+N2-GC	46.00	124.32	57.29	23.00
LSD 5 %	11.58	20.73	3.53	1.86

For treatment description see Table 1
LSD 5 % = least significant difference between treatments at the 5 % level

In the second and third vegetation period acceptable correlations were found between biomass content and N uptake ($r^{n=11}$ [1988] = 0.60, and $r^{n=11}$ [1989] = 0.67, respectively). Calculating the relationship between biomass and N uptake in 1988 without treatment N4-50 resulted in a better correlation ($r^{n=10}$ [1988] = 0.79*).

DISCUSSION

The rapid increase in the amount of microbial biomass N in the first experimental year (week 1 - week 22) depended mainly on the simultaneous supply of N and available carbon. Biomass growth after carbon and nitrogen addition was reported by many other authors (e.g. Azam *et al.*, 1989; Voroney and Paul, 1984). The decrease in biomass N observed for treatments exclusively fertilized with anorganic N until the 4^{th} week may be due to enhanced mineralization after the rewetting of dry soil at the start of the experiment (e.g. Ahmad *et al.*, 1973; Scherer *et al.*, 1992; Stevenson, 1956). On the other hand it is also possible that the high nitrate concentration itself stressed the micro-organisms (Fog, 1988; Kowalenko *et al.*, 1978). The significantly higher contents of biomass N in treatment N4 compared to the control soil at the end of the first vegetation period (week 22) could be due to stimulated microbial activity after increased C input via plant roots. The declining values for biomass N during the 3-year period can be explained by the fact that mineralized nitrogen, at least in part, is removed from the soil by plant uptake.

The suitability of biomass measurements for the estimation of net N mineralization can be assessed through the relation to N_{min} accumulation in the soil after a sufficient period under favorable conditions for mineralization. Furthermore no changes in the N_{min} pool due to plant uptake should occur. In our 3-year pot experiment the periods after harvest of maize and wheat, respectively, until the following spring met this requirements. A relationship between biomass N and N_{min} accumulation only existed if no residual fertilizer N (e.g. for treatments N1, N2, and N4, respectively) and no immobilization milieu (e.g. N4-50) were observed. Because after harvest in week 22 no more mineral N was

found in the soil, N_{min} found in the soil in the following spring must originate from mineralization of microbial immobilized N. Most of this organically bound nitrogen probably belongs to the newly immobilized biomass N (= NIB-N; He *et al.*, 1988a). He *et al.* (1988b) found a higher extractability ratio for the NIB pool indicating that this soil N fraction is reasonably easier decomposed than other organic N fractions. Furthermore, the results from Azam *et al.* (1989) and Nicolardot (1988) show that mineralization of NIB-N that has developed after stimulating microbial growth by adding carbon and nitrogen to the soil even exceeds that of "native biomass N". The higher amounts of labelled N_{min} for treatment N4-25 compared to N2-25 in spring 1988 and 1989 confirm that newly immobilized nitrogen has been re-mineralized to a greater extent than native soil N during autumn and winter under the favorable conditions in the greenhouse.

The results for the relationship between biomass and N uptake by plants can be explained in a similar way. For treatments N1, N2, and N4, respectively, N mineralization is unimportant for the N requirements of plants because of the fertilizer application. On the other hand, microorganisms presumably were superior to the plants in assimilating the mineral nitrogen from the soil solution under immobilizing conditions.

Overall, the relationship between biomass and N_{min} accumulation on one hand, and N uptake by plants, respectively, on the other hand obtained in our study confirm that measurement of microbial biomass in principle represents a suitable index for short-term mineralizable nitrogen in soils. Moreover, significant correlations between microbial biomass and several N indices determined with simple extraction procedures have been reported (Kohl and Werner, 1987; Olfs and Werner, 1992; Rheinbaben, 1988). As mentioned earlier, this similarity may be due, at least in part, to a pronounced extractability of microbial bound nitrogen.

Nevertheless, the turnover milieu of the soil is the deciding factor. Under immobilizing conditions (e.g. after application of microbial utilizable carbon) all N_{min} in soil (from previous mineralization or fertilizer application, respectively) and all nitrogen mineralized in future will immediately be immobilized by the microbial biomass until the surplus of decomposable C is consumed. Under such circumstances it is not possible to estimate the amount of plant-available nitrogen from biomass data because no net mineralization occurs. Under field conditions such a situation may be important after straw incorporation. For the estimation of the N mineralization potential of soils it seems to be necessary not only to determine the pool size (i.e. the amount of microbial biomass nitrogen) but to measure the microbial activity (i.e. the intensity of the turnover process) as well (Olfs *et al.*, 1990).

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Characteristics and effects of organic matter on Belgian loamy soils: a reference system

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Abstract

In the context of loamy soils with low organic matter (O.M.) content and extreme sensitivity to sealing, a reference system has been progressively developed through different case studies of farms and experiments which take into account soil types and their uses, the technical orientations of agrosystems, and the incorporated substances. The chemical, physical and physico-chemical impacts of soil O.M. are mainly evaluated by O.M. fractionation and measurements of structural stability and cation exchange capacity. Positive relations are assessed between:

- **O.M. contents and long-term structural stability,**
- **highly biodegradable substances and short-term structural stability,**
- **fibrous substances, free O.M. content and more polymerized, complexed and condensed linked O.M.,**
- **residues from sugar factories (which probably influence the behaviour of associated O.M.) and structural stability.**

Hypotheses of morphodynamic effects and of complexed O.M. incidence on lower (relative to clay and O.M. percentage) cation exchange capacity are raised. The broad lines for a future programme are suggested.

INTRODUCTION

Evolving from mixed agriculture towards industrial crops, soil organic matter (O.M.) content has dropped while intensive livestock breeding farms are confronted with surplus substances.

Moreover, an increasing number of by-products from urban and industrial activities seek potential fields of application in agriculture (sludge from water-treatment plants, residues from paper-mills, effluents from food-processing industries).

Finally, the recent guidelines from the Common Agricultural Policy of the European Community on set-aside, production diversification and alternative land-use provide new opportunities such as cultivated fallow, organic farming, afforestation (Bock and Rondeux, 1990).

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Therefore, confronted with trends where more pressure is put on soil in terms of crop production, recycling of residues and water filtration, the Belgian Institute for the Promotion of Scientific Research in Industry and Agriculture (I.R.S.I.A. – I.W.O.N.L.) in 1989 funded a Research Committee on Soil O.M. which includes five teams.

In this context, which gives rise to new technical strategies for the use of organic substances respecting soil conservation and the environment, the objectives of this team focus on humification processes and their physical, chemical and physico-chemical consequences in agriculture on loamy soils of Central Belgium; a region where the low O.M. content (0.8 to 1.2 % of total organic carbon) and the extreme sensitivity of soils to sealing are well-known (Bollinne, 1982; Bock and Hebberechts, 1987). The latter induces infiltration refusal leading to erosion risks.

The first two steps of the above-mentioned programme on soil O.M. are currently in progress and involve:

- establishing a system of reference which is inspired not only by the necessity to address the diversity of the issues but also to evaluate the sensitivity of selected analyses,
- seasonal monitoring of incorporated substances mainly to characterize the maturation processes.

MATERIALS AND METHODS

The originality of the approach for these first two steps stems more from the sampling concepts and the complementary of arguments than from the novelty of a particular technique (Avril *et al.*, 1986; Bock and Delecour, 1989).

The objects studied concern soil typologies in their morphosequential relations, technico-economic orientations of the agrosystem in the case of farms or research orientations in the case of field experiments, correlative management of organic applications and functions attributed to soil O.M.

Soil typologies, distributions and uses are obtained by reference to the Belgian Soil Map surveyed at 1/5,000 and edited in colour at 1/20,000 (Anonymous[(1)]), by locating farm plots on photo-mosaic at 1/10,000 (Anonymous[(2)]) and by field surveys. Presently, the two main mapping series of this gently undulating loamy area (loess) were considered as follows, in their representative sequence:

- on plateaux and slopes, silty loamy soil (favourable natural drainage), with B textural horizon (richer in clay) i.e. Aba, leached brown soil, (pale) Udalf,
- in valleys and depressions, soil on silty loam (favourable natural drainage) without profile development i.e. Abp, undeveloped soil of colluvial origin, Udordhent.

The agricultural situations studied regard farms which conduct intensive 3-year rotations or organic farming as well as experiments dealing with management of harvest residues and applications in classical rotation, green manure comparisons or research of non exclusive but more biological solutions.

The land use and substances studied concern crops and meadows, mustard, vetch and rye-grass, manure, slurry and composed products.

The laboratory method applied to identify the impact of these organic substances on soil properties involve:

- the quantitative evaluation of soil O.M. by hot mineralization of total organic carbon and by 'total' nitrogen measurement,

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(1) (2) references and dates in relation to the sheet used and its issue.

- its qualitative characterization through fractionation (carbon and nitrogen) which results in the differentiation between free and linked substances and within the latter between fulvic and humic acids (both subdivided into sodium pyrophosphate and sodium hydroxide extracts) and humins according to the methods of Monnier *et al* (1962) densimetric and Duchaufour-Jacquin's (1967) chemical separations,
- its physical implication on soil structure and its maintenance by measuring structural stability according to Henin's method (1958) which involves three comparative pretreatments, using water as reference, alcohol as protective porosity film and thus as resistance formula against aggregate break-up, benzene as product discriminating between the relative presence or absence of O.M. and the reaction to moisture,
- its influence on soil physico-chemical properties by measuring cation exchange capacity with 1.0 M ammonium acetate at pH 7,
- finally, its role in providing nutrients by measuring bases and phosphorus availability with 0.5 M ammonium acetate + EDTA at pH 4.65.

Furthermore, particle size analysis (in 9 fractions and with a chain hydrometer), measurements of pH-H_2O and normal pH-KCl at 2/5 and, if necessary, titration of total carbonates are performed.

Composite type sampling with a tubular auger comprises a minimum of 25 takings per ha, real number and depths being adapted to situations.

It soon becomes apparent that it is sufficient to consider the surface sample only (0-20 cm maximum).

RESULTS

The long-term trial on organic matter performed for over 35 years by the Station of Crop Production Technology of the Research Centre in Agronomy of Gembloux (C.R.A.Gx – Ministry of Agriculture) shows (Roisin *et al.,* 1991) that at the end of a 3-4-year rotation the organic status of treatments 'manure' (40 t/3 years), 'harvest residues + vetch/3 years' and 'residues + pig slurry (40 t/3 years) + mustard/3 years + residues from sugar factories (30 to 40 t/3 rotations)' is fairly similar and 15 to 20 % higher than that of a reference which with 0.75 % of total organic carbon should represent the steady state of the intensive system. However, we notice that the treatment 'organic matter + residues from sugar factory' i.e. + $CaCO_3$ offers the best results for structural stability. In addition, referring to natural calcareous mediums, it is interesting to observe that this treatment, as opposed to the others, shows a relative reduction in humins and an increase in organic acids when measurements are replicated from spring to summer.

The characterization of plots under organic farming (Dumoulin's farm) gives a perfect answer to the specificity of:

- land use, i.e. higher O.M. contents for a meadow than for crops; O.M. (without forgetting the effect of cover) which being clearly more polymerized, induces better structural stability,
- age of system conversion, i.e. higher O.M. contents for a 15-year plot than for an 8-year plot and for a 5-year plot leading to better structural stabilities,
- soil type under crops, i.e. higher clay and O.M. contents for 'Aba' than for 'Abp'; however if in the first case, structural stability and resistance to sealing are greater, better polymerized organic acids in the second case (associated with a higher percentage of stable aggregates with benzene) suggest a more stable behaviour of what is called soil humus. This difference (compared to the homogeneity under

meadows), suggests a morphodynamic groove or the ploughing of the Bt horizon in plateau soils as well as (colluvial!) quality compensations through the sequence.

The comparison of organic applications in organic market gardening plots (trial of the Interuniversity Research Group in Organic Farming based in Gembloux) demonstrates after 3 years of conversion that most parameters respond better to brushwood compost and manure than to slurry, which gives answers close to or lower than those of the reference.

The comparison of ploughed-under green manure (trial of the Station of Agricultural Physics and Chemistry – C.R.A.Gx) illustrates that (under equivalent conditions) a 'rye-grass' treatment conserves more free O.M. than a 'mustard' treatment while offering more polymerized and condensed linked O.M.; both treatments giving on the other hand the same level of complexion.

The incorporation of green manures (vetch, mustard) in autumn and their trimestrial monitoring (trial of the Station for Agricultural Physics and Chemistry – C.R.A.Gx) under a sugar beet cultivation shows:

- an important mineralization of free O.M. during winter and proportionally more humified and condensed substances for vetch,
- a vertical homogenization probably due to the sugar beet sowing and a predominance of non-extractable O.M. in the beginning of summer (covering crops) positively influencing structural stability with vetch and the reference 'only incorporated straw of the previous harvest',
- a state of less polymerization, complexion and condensation of organic acids in autumn (10 months later),
- all this corresponds to a better final status in total O.M. and principally linked with mustard than with vetch close to the reference and a higher cation exchange capacity in both the latter which infers more a freeing of charge rather than a real increase.

DISCUSSION

These different case studies highlight the following generalities:

- the content of colloidal compounds (% clay and/or total O.M.) conditions structural stability and resistance to sealing; the best identified situations concern meadows, plateau type soil, systems with regular O.M. applications or using fibrous substances (manure versus slurry),
- this structural stability seems to be enhanced during a cultural season by incorporated vetch,
- fibrous substances, as well as meadows or ploughed-under rye-grass, favor the duality of free O.M. in higher proportions and of more polymerized, complexed and condensed organic acids; duality which probably ensures the constancy of processes,
- the greater part of polymerized and condensed organic acids (in relation with a higher percentage of stable aggregates in benzene) in a colluvial type soil suggests some attention to morphodynamism,
- a treatment with residues from sugar factories enhances the structural stability and probably influences the behavior of associated O.M.,
- cation exchange capacity is positively increased by the clay and O.M. contents but would be limited by the degree of complexation.

Moreover, this first approach emphasizes the need:

- to initiate a more systematic inventory under real conditions and to follow experiments or conversions which tend towards a steady state. This programme will be

continued with similar acquisition conditions and well-targeted parameters in order to build a predictive model on valid data. These conditions should remain focused on soil type, the agrosystem and its history, the type and characterization of ploughed-under substances, especially C/N ratio of lignin (Merckx R. – K.U.L., pers. commun.), dates of incorporation and sampling every year or every rotation with an evident preference for the (critical) end of winter and the option for composite taking at only one depth. The parameters should still be those cited in this paper but O.M. fractionation should exclude sodium pyrophosphate extraction; moreover, structural stability monitoring should devote more attention to surface state;

- to define more accurately the relations through pot experiments limiting temporo-spatial variations while remaining close to field truths, in particular specific root decomposition, synergic action of carbonated applications, impact of faunal activity, behavior of less classical substances through short-term observations;
- to achieve better data acquisition concerning soil organic status including phosphorus, and its effects on mineral nitrogen profile, hydraulic parameters or even on soil solution and phreatic water quality.

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Mineral nitrogen in an oxisol from the Brazilian Cerrados in the presence of *Brachiaria* spp.

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Abstract

The effects of commercial varieties of *Brachiaria decumbens*, *B. humidicola*, and *B. brizantha* on nitrogen mineralization and nitrification were examined in a Dark Red Latosol from the Cerrados region of Central Brazil. *B. decumbens* responded most rapidly to the addition of fertilizer N. *B. brizantha* also absorbed the fertilizer N rapidly but grew more slowly. By contrast, *B. humidicola* showed a slower response to the fertilizer addition, both in N uptake and growth. The concentrations of mineral N in the soil under both *B. decumbens* and *B. brizantha* indicated that most of the fertilizer N remaining in the soil was quickly immobilized, as also indicated by the results of incubated soil core samples. On the other hand, the soil under *B. humidicola* showed strong mineralization of native organic N between three and six days after the addition of N in the presence of the plants, but not during the incubation. In this species there was also a clear indication of early nitrification, whereas in the soil under the other two species the build up of the nitrifier population seemed to be delayed. It is concluded that the species studied present different rates of N uptake and assimilation and stimulate microorganisms differently in their rhizospheres, which leads to contrasting transformations on the soil mineral N pool. The observed patterns of mineralization/immobilization and nitrification changed so quickly that analysis of the effects of plants on soil N transformations based on a few harvests or over long time intervals can be misleading.

INTRODUCTION

Nitrogen fertilizer is rarely added to pastures in the Brazilian Cerrados area and the use of pasture legumes is not common. Consequently, the grasses are dependent on the soil available N, relying on an efficient natural recycling of N.

There are some studies suggesting that plants could stimulate net mineralization of soil organic N, through the exudation of carbon compounds by the roots (Wheatley *et al.*, 1990; Whipps and Lynch, 1985). A species with such a potential would thus have an advantage in obtaining otherwise limiting soil N, as in the Cerrados soils. The significance of such a process is still controversial and, as stated by Griffiths and Robinson (1992) it is

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unlikely a plant would obtain any benefit from such a process, since the theoretical gains would be small.

In this paper we present the results of an experiment conducted with three commercial varieties of *Brachiaria* spp., the most common grasses used on the Brazilian Cerrados area. When these species are introduced together with P fertilization pastures are initially productive, but productivity gradually declines - a process known as pasture degradation. Rates of pasture degradation appear to be greater under *B. humidicola* than under *Brachiaria* species.

The objectives of the experiment were to examine the response of the plants to N fertilizer (added as ammonium-N) and the transformations which occur in the soil mineral N pool following the N addition among the three species.

MATERIAL AND METHODS

Sixty pots containing 300 g of a clay Cerrados soil (dark-red Latosol with 70 % clay, 15 % sand, 0.12 % N, pH 5.1) collected from an area under native pasture near Brasilia, Brazil, were prepared for this experiment. Each pot received a basic fertilization of P, K, Ca, Mg, B, Zn and Mo. The soil was moistened to 30 % moisture content (weight basis) and incubated for two weeks in a growth-chamber, at 25 °C and 12 h of light per day. Settings were kept constant throughout the experimental period, the moisture being corrected daily. After two weeks three seedlings of the Brazilian commercial varieties of either *B. decumbens*, *B. humidicola*, or *B. brizantha*, pre-germinated in perlite, were transplanted to each pot, with twenty replications for each species.

Sixty days after planting, four pots of each species were harvested and a solution containing 45 mg of N as $(NH_4)_2SO_4$ enriched with 4.524 atom % ^{15}N excess was added to the remaining pots. Thereafter four replications of each species were harvested 3, 6, 12, and 24 days after the N addition. At the time of the harvest a soil core was carefully extracted (using a PVC cylinder of 25 mm diameter and 150 mm height), and incubated aerobically for 7 days before mineral N extraction. At the same time, soil samples were collected around the hole made by the cylinder for a direct extraction of the mineral N, and measurements of soil moisture, soil pH, soil total N, and soil ^{15}N enrichment.

The soil mineral N was extracted by transferring 30 g of soil to a flask, adding 150 ml of a 1M KCl solution, and shaking the mixture for two hours. The solution was then filtered using glass-fibre filter paper (previously washed with KCl). Ammonium-N was determined by the salicylate method, and nitrate-N was determined by an automatic colorimetric method, as described by Keeney and Nelson (1982). The total soil mineral N was considered as the sum of both ammonium-N and nitrate-N fractions.

Two subsamples of the air-dried soil were directly analyzed for total N content and ^{15}N enrichment using a CN auto-analyzer (Roboprep CN, Europa Scientific Instruments) linked to a Micromass 622 mass spectrometer (VG Isogas). After soil sampling the shoots and roots were separately collected, dried for 3 days at 65 °C, weighed and ground on a roller mill and directly analyzed for N content and ^{15}N enrichment, as described above.

RESULTS AND DISCUSSION

The shoot dry weight of *B. decumbens* increased linearly from the day of the N addition, whilst there was a measurable increase in *B. humidicola* only after six days (Figure 1).

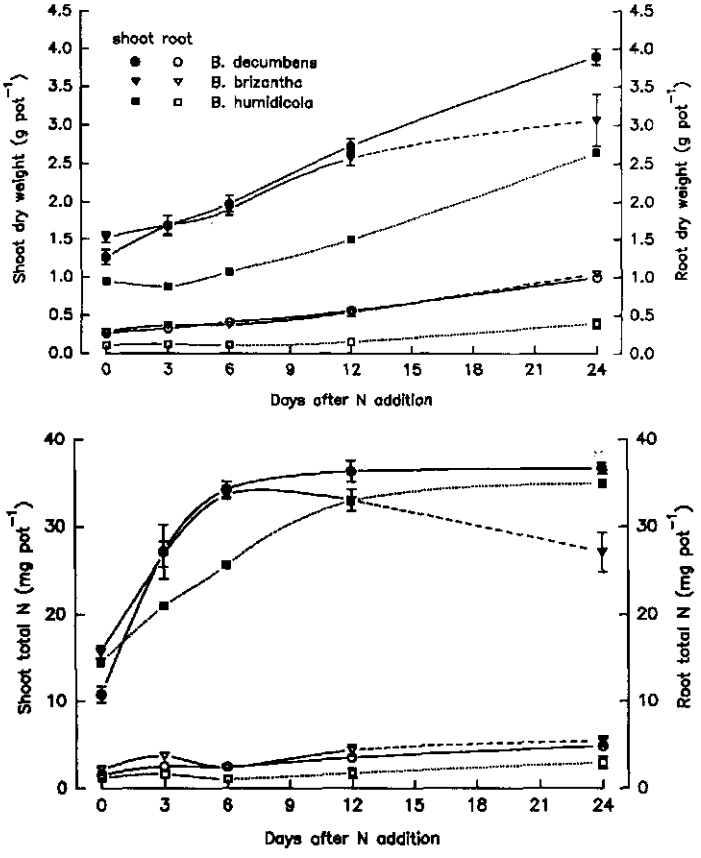

Figure 1. Dry matter (g pot^{-1}) and total N (mg pot^{-1}) of shoots and roots of *B. decumbens*, *B. humidicola*, and *B. brizantha* (n = 4 ± SEM).

B. brizantha increased dry weight at a similar rate to *B. decumbens* over the first four harvests, but growth rate was reduced between the last two harvests. Root growth response was delayed in relation to shoot growth in all species in the first two harvests, but thereafter showed a tendency to increase more strongly.

B. decumbens and *B. brizantha* were initially much more aggressive than *B. humidicola* with respect to fertilizer N uptake and their N contents increased sharply after the fertilizer addition. The N content of *B. decumbens* then remained constant over the last three harvests, whilst that of *B. brizantha* decreased. Root N contents were more similar between species but again *B. humidicola* showed the smallest increase in N content.

The species also showed a different behaviour regarding the uptake of native soil N. *B. humidicola* took up N mainly from the fertilizer during the experimental period, maintaining a constant N content derived from the soil (Table 1). *B. brizantha* also used mainly fertilizer N, reducing its content of N derived from the soil by the last harvest. *B. decumbens*, constantly increased the uptake of N from the soil during the experiment, suggesting a stimulatory effect of the fertilizer, probably due to a priming effect.

Table 1. Plant N derived from the applied fertilizer (45 mg pot^{-1} applied at day 0) or from the native soil N (mg pot^{-1}) and total recovery of the applied N in the plant-soil system (n = 4 ± SEM).

	Days after N addition				
	0	3	6	12	24
Plant N derived from the fertilizer N (mg pot^{-1})					
B. decumbens		15.8 ± 2.5	21.0 ± 0.6	21.2 ± 0.8	20.6 ± 0.5
B. humidicola		5.6 ± 1.1	9.3 ± 1.3	16.8 ± 0.9	19.0 ± 1.8
B. brizantha		11.8 ± 0.6	18.6 ± 0.9	21.1 ± 0.4	17.7 ± 1.6
Plant N derived from the soil (mg pot^{-1})					
B. decumbens	12.4 ± 0.8	13.9 ± 1.3	15.8 ± 0.2	18.7 ± 0.4	20.6 ± 0.5
B. humidicola	15.7 ± 1.1	17.0 ± 1.3	17.4 ± 1.4	17.9 ± 1.8	18.9 ± 0.9
B. brizantha	19.8 ± 0.7	20.4 ± 0.9	19.8 ± 0.6	18.0 ± 1.1	16.1 ± 0.8
Total plant and soil N derived from the fertilizer N (mg pot^{-1})					
B. decumbens		45.2 ± 2.5	44.2 ± 0.6	41.4 ± 1.2	41.2 ± 0.6
B. humidicola		41.6 ± 0.8	41.4 ± 0.8	41.5 ± 1.5	40.5 ± 0.9
B. brizantha		44.3 ± 0.6	43.1 ± 0.9	42.6 ± 0.6	40.1 ± 1.7

The balance between the N inputs to the system (applied N plus initial mineral N) and the N outputs in the following harvest (total N accumulated in the plants plus soil mineral N extracted) provides a reliable indication of the transformations occurring in the soil mineral N pool. This balance can be made for each period between two consecutive harvests, considering as inputs the concentration of soil mineral N extracted in the previous harvest, and as outputs the variation in plant N content during the same period. If the balance is positive it is due to *de novo* N production, as a result of native organic N mineralization; if the balance is negative it would be the result of mineral N immobilization. To obtain comparable values the results were divided by the length of the period between harvests.

Making this calculation based on the total increase in plant N uptake (N from the soil plus N from the fertilizer - Table 1) and total soil mineral N (sum of the ammonium-N and nitrate-N concentrations at the harvests - Table 2), a negative balance was recorded for *B. decumbens* in the first two harvests (-5.5 and -0.5 mg N pot^{-1} day^{-1}). In the last two harvest a small positive balance was observed (0.3 and 0.1 mg N pot^{-1} day^{-1}). A similar result was observed for *B. brizantha*, indicating that immobilization was the main pattern of N transformation in the soil under these two species, which was confirmed by the results of incubated soils samples (Table 2).

For *B. humidicola* a different picture was obtained. The balance was negative for the first period (-1.9 mg N pot^{-1} day^{-1}), but highly positive in the following interval (9.5 mg N pot^{-1} day^{-1}), indicating a strong mineralization of the soil organic N from day 3 to 6. The occurrence of mineralization was confirmed by the increase in the ammonium-N fraction. During the following periods the balance was negative (-5.2 and -0.8 mg N pot^{-1} day^{-1}), indicating dominance of immobilization, following the pattern of the other two species.

There was a small increase in the nitrate-N concentrations at the two final harvests of *B. brizantha* and more significantly in the two intermediary harvests of *B. humidicola*, suggesting that nitrification was active in the presence of the plants. No significant variations were observed under *B. decumbens*. Since this species was the only one actively taking N from the soil as well as the fertilizer N, it could indeed include any nitrate-N

Table 2. Concentrations of ammonium-N and nitrate-N (mg pot^{-1}) at harvest or after seven days of incubation of the soil under *B. decumbens*, *B. humidicola*, and *B. brizantha* (n = 4 ± SEM).

	Days after N addition				
	0	3	6	12	24
B. decumbens					
NH_4 at harvest	0.4 ± 0.0	11.5 ± 2.8	3.2 ± 0.8	0.8 ± 0.1	1.4 ± 0.1
NH_4 after incubation	0.7 ± 0.1	13.0 ± 3.1	2.5 ± 0.4	2.7 ± 0.2	1.0 ± 0.1
Variation	0.3	1.5	-0.7	0.9	-0.4
NO_3 at harvest	0.7 ± 0.1	1.5 ± 0.1	1.0 ± 0.2	1.5 ± 0.1	1.2 ± 0.1
NO_3 after incubation	0.1 ± 0.0	0.8 ± 0.1	2.8 ± 0.1	1.7 ± 0.1	1.0 ± 0.1
Variation	-0.6	-0.7	1.8	0.2	-0.2
B. humidicola					
NH_4 at harvest	0.5 ± 0.1	32.7 ± 0.2	51.1 ± 0.1	14.9 ± 0.3	1.7 ± 0.3
NH_4 after incubation	0.7 ± 0.1	15.5 ± 0.6	11.0 ± 0.1	13.4 ± 0.7	0.6 ± 0.1
Variation	0.2	-17.1	-40.1	-1.5	-1.1
NO_3 at harvest	0.8 ± 0.1	1.0 ± 0.2	6.7 ± 1.4	3.7 ± 0.3	1.3 ± 0.1
NO_3 after incubation	0.9 ± 0.1	16.7 ± 0.6	8.3 ± 1.4	6.5 ± 0.4	1.2 ± 0.1
Variation	0.1	15.7	1.6	2.8	-0.1
B. brizantha					
NH_4 at harvest	0.5 ± 0.1	17.3 ± 3.1	3.0 ± 0.6	2.0 ± 0.1	1.7 ± 0.3
NH_4 after incubation	0.7 ± 0.1	16.2 ± 2.4	3.2 ± 0.6	3.6 ± 0.2	0.8 ± 0.1
Variation	0.2	-1.1	0.2	1.6	-0.9
NO_3 at harvest	1.0 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	1.6 ± 0.1	1.2 ± 0.1
NO_3 after incubation	0.1 ± 0.0	0.8 ± 0.1	2.9 ± 0.3	2.4 ± 0.2	1.1 ± 0.1
Variation	-0.9	0.4	2.2	0.8	-0.1

Variation in NH_4 (or NO_3) = concentration after incubation - concentration at harvest.

formed, thus not accumulating enough nitrate-N to detect nitrification.

Considering the ammonium-N and nitrate-N fractions in the soil after seven days of incubation it can be seen that in the soil taken from *B. humidicola* nitrification was stimulated (Table 2). This was indicated by the increase of nitrate-N during the incubation period at all harvests except the last one, where the amount of ammonium-N in the soil at the harvest was perhaps not enough to stimulate nitrification. The high rate of nitrification under *B. humidicola* at the harvest on day 3 seems to be due, at least in part, to the slow uptake of N by the plant, thus leaving large concentrations of ammonium in the soil. It is known that nitrification is inducible by substrate availability (Prosser, 1986). At the same time the nitrifiers seemed to be particularly active in that period, since a proportional concentration of the ammonium-N that disappeared was recovered as nitrate-N. At the next harvest the nitrifiers seemed to have lost the competition for the available ammonium-N, since the nitrate production was now less than 5 % of the ammonium available at the beginning of the incubation.

In the soil under the other two species, which were initially very aggressive in N uptake, less ammonium-N was available, but the amounts present should still have been enough to induce nitrification. However, there were no clear signs of early nitrification (day 3) under these species. Some nitrate was formed at later harvests (6 and 12 days) perhaps

suggesting that the build-up of the nitrifier population was delayed under these species.

In the harvest at day 3 there was complete recovery of the applied N in the *B. decumbens* and *B. brizantha* plant and soil systems (Table 1), with some losses recorded under *B. humidicola*. In the following harvests around 10 % of the applied N was missing from the plant-soil system. Since the moisture was strictly controlled losses from leaching could be disregarded; ammonia volatilization probably did not occur either, since the soil became acidic as a result of the N uptake (data not shown). Some of the lost N probably would be found in the very small roots, which are impossible to collect, and which were excluded from the soil analysis. However, this may not fully account for all the missing N, and it is probable that the main losses were the result of denitrification. Denitrification as the main pathway of N losses could explain the early lower total fertilizer recovery of *B. humidicola* (Table 1). This species had a much higher initial nitrification than the other ones, hence more nitrate-N available for denitrification. This could also explain the low recovery of nitrate-N observed in soil under this species in the field when compared with other *Brachiaria* spp., as reported by Sylvester-Bradley *et al.* (1985).

Based on the overall results, it can be concluded that the species studied presented a different pattern of N uptake and assimilation, mainly related to the speed of such processes. With that they indirectly influenced the soil N dynamics, as differences in the available soil mineral N stimulate the growth of soil microorganisms differently. It is possible that the species studied excrete different amounts of substrate, or support a distinct microflora in their rhizosphere, since a different pattern of mineralization/ immobilization of the organic soil N was observed among the species tested. The variations observed in the ammonium-N and nitrate-N both in the presence of active plants or in soil incubated in the absence of the plants are complex and analysis of the effects of plant on mineral N transformations based on a few harvests or over long time intervals can be misleading.

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Does phosphorus supply enhance soil-N mineralization in Brazilian pastures?

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Abstract

Two *Brachiaria decumbens* paddocks on a sandy soil Entisol were compared to investigate whether degradation of tropical pastures may be due to phosphorus deficiency limiting plant growth and/or soil-N mineralization processes. Grass dry matter (168 vs. 130 kg ha^{-1} $week^{-1}$, wet season) and litter production (130 vs. 86 kg ha^{-1} $week^{-1}$) as well as standing root biomass (3744 vs. 1683 kg ha^{-1}, 0-60 cm) were considerably greater in the P fertilized (100 kg P_2O_5 ha^{-1}) plot. *In situ* soil-N mineralization measurements indicated substantially greater rates of N release under the P fertilized pasture only at the beginning of the dry season. Soil mineral-N amounts present during the wet season were higher in the unfertilized plot, presumably due to the smaller N demand of P deficient plants.

In a laboratory incubation experiment, testing three acidic soils of varying texture, P supply increased the amount of soil-N mineralized with time especially in the more clayey, P-fixing soils. The effect was associated with slight increases in soil pH, probably due to a decrease in charge of (Al- and Fe-) hydroxide complexes. Biomass-N was not significantly affected by P supply but biomass efficiency (mineralized-N per unit biomass-N) increased with P supply in the clayey soils.

Our results suggest that in poor tropical sandy soils under grass pastures, the addition of P stimulated plant N uptake primarily by alleviation of plant P deficiency and more efficient N cycling rather than by a direct effect on soil-N mineralization.

INTRODUCTION

In Brazil approximately 200 million hectares of land are dedicated to pastures mainly for beef production. The nutrients which are most limiting in these tropical soils are nitrogen and phosphorus. Previous research performed at the Centro Nacional de Pesquisa de Gado de Corte (CNPGC), Campo Grande, Brazil indicated that in some cases decline in the productivity of ageing grass pastures may be temporarily alleviated solely by phosphorus (P) supply (Schunke *et al.*, 1991). Improved cattle weight gains with phosphorus fertilization were associated with a greater amount of forage on offer and an increased

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nitrogen (N) concentration in the grass tissue. Increases in plant N uptake after P fertilization could be due to the increased demand for N by the grass after P stress was reduced. However in grasses, an increased N demand would not account for the observed increase in N concentration as the latter is often inversely related to dry matter production when no N fertilizer is applied (Coates *et al.*, 1990). In tropical legumes, N tissue concentrations increase by alleviating severe P deficiency due to improved N_2 fixation (Cadisch *et al.*, 1993). In pure grass pastures potentially an enhanced supply of soil-N in response to P addition could be due to increased soil-N mineralization as i) the soil microbial biomass may be limited by phosphorus or ii) an improved litter input quality/quantity in P fertilized pastures stimulates decomposition. Positive effects of soil-P fertility on soil-N mineralization and/or nitrification have been observed by other researchers (Hue and Adams, 1984). Alternatively, an improved plant N nutrition could be due to an enhanced scavenging of N as a result of a better root development in the P fertilized plants.

The objective of the research carried out at CNPGC was to test the plant N demand versus the N supply hypothesis by comparing the effect of P fertilization on soil mineral-N contents, *in situ* mineralization of soil organic-N and root development. In order to compare the effect of P supply on mineralization in a broader range of soils with different P fixation abilities, an incubation experiment was performed in a controlled environment using soils with increasing clay contents. The laboratory experiment also provided incubation conditions with the absence of living roots and litter inputs and was further used to test the hypothesis that microbial biomass efficiency is limited by P.

MATERIALS AND METHODS

Field experiment

Field measurements of soil-N mineralization were performed in two established grazed *Brachiaria decumbens* plots in Campo Grande, Mato Grosso do Sul, Brazil. One of the plots had received 100 kg P_2O_5 ha^{-1} in December 1990 as single superphosphate and the other plot was left unfertilized. Soil (Quartz Psamment) characteristics were: 91% sand and 6% clay, pH 5.2 (H_2O), 0.04% total N (Kjeldahl) and 2.7 mg kg^{-1} P (Mehlich), soil bulk density 1.1 kg dm^{-3}. Average annual rainfall is 1455 mm and annual mean temperature 22.8 °C. Dry matter production was measured from November 1991 to March 1992 at monthly intervals as the difference between dry matter on offer and that accumulated in cages (20 cages per paddock with 1 m^2 harvest area) used for animal exclusion. Litter production was estimated by collecting litter accumulated during monthly intervals in 20 fixed quadrats per paddock of 1.0 x 0.5 m.

The *in situ* soil core technique for evaluating mineralization of soil N was adapted from that of Raison *et al.* (1987). PVC cores of 7.5 cm diameter were driven into the soil to 60 cm depth. In order to avoid compaction wider cores (7.5 cm) were used compared to those proposed by Raison *et al.* (1987) as well as performing evaluations when soil was not too dry; these measurements proved to be satisfactory for this very sandy soil. One core was immediately removed for the evaluation of the initial content of mineral-N; the other one was capped to avoid leaching and left incubating *in situ* for a period of two weeks.

Thereafter, the capped core was removed to assess the accumulated mineral-N since, due to the cutting of roots no plant N uptake had taken place during that time. The soil in the columns was separated into four different depths (but only the combined data are presented here) and mineral-N was extracted using a solution of 1M KCl. Ammonium-N and nitrate-N were analyzed by steam distillation for the first two evaluations and thereafter by colorimetric flow injection techniques (Alves *et al.*, 1992). The difference in mineral-N between the two cylinders was the calculated net N mineralization. Twelve columns per plot arranged in four replicates of three columns were used. Four evaluations were performed in 1991/92 on 10-26.6.91, 7-20.11.91, 20.11-4.12.91 and 25.2.-11.3.92. At the first harvest root mass recovered in the column was evaluated.

Laboratory incubations

Samples of 50 g of three acidic soils: i) a sandy soil (Quartz Psamment) with 91% sand, 6% clay and 0.04% total N (Kjeldahl); ii) a sandy clay loam soil (dark-red Latosol) with 50% sand, 35% clay and 0.08% N; and iii) a clay soil (dark-red Latosol) with 70% clay, 15% sand and 0.12% N were mixed with sand (1:2) and incubated in columns (3 cm diameter) at 25 °C. At the beginning and after 7, 21, 39, 48, 62, 88, 116 and 152 days of incubation the soils were leached with 100 ml (in 20 ml increments) of a solution containing either no P or 1mM P as KH_2PO_4. Additionally, all solutions contained 1mM K (KCl or KH_2PO_4), 1mM Mg ($MgSO_4$) and 1mM Ca ($CaCl_2$) (Cassman and Munns, 1980) adjusted to pH 5.0 (with HCl). This (minus N) macro nutrient solution avoids the use of a separate extraction with $CaCl_2$ and the subsequent addition of a nutrient solution to replace leached nutrients as proposed by Stanford and Smith (1972). Ammonium-N and nitrate-N were analyzed by colorimetric methods with an autoanalyzer.

After the incubation period soil microbial biomass was estimated by 24 h chloroform fumigation and direct 0.5 M K_2SO_4 extraction (Brookes *et al.*, 1985). Extracts were analyzed for total-N (Kjeldahl) or ninhydrin-reactive N (amino acids and NH_4^+).

RESULTS

Field experiment

Dry matter production of *Brachiaria decumbens* and litter deposition rates were considerably greater in the paddock receiving 100 kg P_2O_5 ha^{-1} compared to the unfertilized plot (Table 1). There was also more plant material on offer in the pasture supplied with phosphorus despite the more intense grazing pressure in that treatment (14 compared with 11 animals per paddock). The increased plant material on offer may also partly explain the greater amounts of litter present during the wet season as well as the greater litter deposition rates in the fertilized plot. Phosphorus application doubled root mass throughout the soil depth investigated (0-60 cm) indicating that a considerable amount of photosynthates was translocated to below-ground parts of *B. decumbens* plants. A profile wall study (made in May 1992) confirmed the above results with

Table 1. Effect of phosphorus fertilization on standing plant biomass and on shoot dry matter and litter production of B. decumbens on a sandy soil, Campo Grande, Brazil, during the wet season. Average values from November 1991 to April 1992 except for root mass (0-60 cm) which was evaluated on June 10, 1991. (Values in brackets are average SE of means (n = 20/12)).

	Without P supply	With 100 kg P_2O_5 ha^{-1}
Standing biomass:	------ kg ha^{-1} ------	
Shoot dry matter on offer	1217 (65)	2487 (201)
Litter present	973 (99)	1535 (139)
Root mass	1851 (345)	3744 (525)
Production rates:	------ kg ha^{-1} $week^{-1}$ ------	
Shoot dry matter production	130 (16)	168 (19)
Litter deposition	86 (10)	130 (11)

P fertilized plants having a much denser rooting system in the upper soil layers. This study also showed that roots of *B. decumbens* penetrated to more than 2 m soil depth in this sandy soil, regardless of phosphorus supply.

In situ soil-N mineralization and mineral-N contents

Net soil nitrogen mineralization occurred during the period between June 10-26, 1991 (Figure 1). Mineralization per gram of soil was greater at larger soil depths (not presented) presumably due to drier soil conditions in the top layers as the experiment entered the dry season. Phosphorus supply doubled the amount of N mineralized in the 0-60 cm soil layer during this incubation period. The positive effect of P on soil-N mineralization was independent of soil depth. The amount of mineral soil-N present at the end of this measurement period was greater in the P fertilized plot. In contrast, during the wet season consistently more mineral-N was available in the unfertilized plot compared to the fertilized one.

In situ mineralization during the period November 7-20, 1991 was much less than during the previous evaluation and in fact immobilization predominated in the unfertilized plot. The plant nitrogen uptake calculated using the method of Raison *et al.* (1987) with an additional inserted uncapped cylinder was higher in the phosphorus plots by about 2 kg N ha^{-1} $week^{-1}$. However, this was not due to increased N mineralization during that period but to a depletion of the soil-N pool available initially (Figure 1) and less leaching losses occurring as indicated by the above calculations.

Immediately after the previous evaluation new cylinders were inserted to start a new evaluation period (November 20 - December 4, 1991). Contrary to the previous observation a strong net mineralization occurred during this period (Figure 1). This difference could not be attributed to better moisture conditions as the gravimetric soil water content of the covered cylinder at the end of the incubation period varied between 50-70 % of field capacity for the period November 7-20 and 30-50 % for the period November 20 -

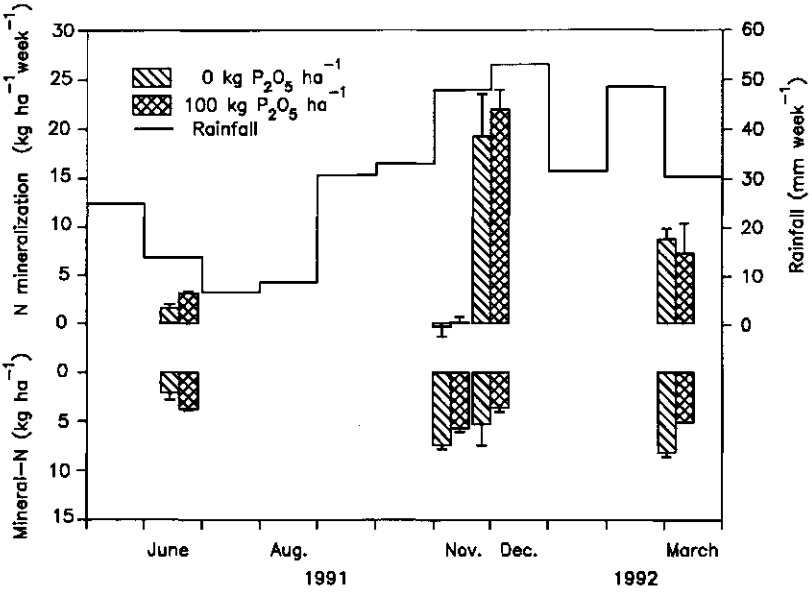

Figure 1. Rates of in situ soil-N mineralization, rainfall distribution and amounts of mineral-N present at the end (June evaluation) or at the beginning of the incubation periods under an unfertilized or fertilized (100 kg P_2O_5 ha^{-1}) *B. decumbens* grazed paddock. (Vertical bars are SE of means (n = 12)).

December 4, 1991. As in the evaluation of June, phosphorus supply tended to increase the amount of soil-N mineralized in all soil depths but differences appeared not to be significant. Mineralization during the period February 25 to March 11, 1992 was intermediate compared to the previous two observations. No significant effect of phosphorus was observed due to the large variation encountered especially in the P fertilized plot.

Leaching tube experiment

Soil pH increased slightly during the incubation period in the heavier soils especially when phosphorus was added with the leaching solution (Table 2). The sandy soil retained about half of the applied P in the leaching solution at the beginning but very little by the end of the experiment. In contrast, in the clay soil hardly any P could be detected in the leachates. The increased P adsorption ability with increased clay content is probably due to the coating of clay particles with positive charged (aluminium- and iron-) hydroxide

Table 2. Soil pH (1:3 = soil:water) at the beginning and end of the laboratory experiment and phosphorus concentration in leachates (average or at day (d) 7, 39 and 116 for the P treatment) of three soils as affected by P concentration in the leaching solution (0 or 1 mM P).

	Soil pH			P concentration in leachates (mM P)			
	Initial	End		0 mM P	1 mM P		
		0 mM P	1 mM P		7 d	39 d	116 d
Sandy soil	5.37	5.18	5.30	<0.01	0.48	0.46	0.86
Sandy clay	4.93	5.16	5.56	<0.01	<0.01	<0.01	0.28
Clay soil	5.04	5.13	5.53	<0.01	<0.01	<0.01	0.08

complexes. The increase in soil pH due to P supply could be due to a decreased hydroxide activity by reacting directly with P or to reversible HPO_4^- exchange on the hydroxide sites against OH^- ions.

Phosphorus supply increased the amount of N mineralized with time especially in the more P fixing clayey soils (Figure 2). The more pronounced effect of P on mineralization at later leachings could be related to the saturation of P fixing sites in the soils, and thus a higher P concentration in the soil solution, or to the slightly increased pH.

Soil biomass-N was not significantly increased by P supply in all three soils at the end of the incubation period (Table 3). However, microbial biomass of the heavier soils was more efficient in soil-N mineralization when supplied with P than without P addition (Table 3). Biomass efficiency was superior in the sandy soil and was not affected by the amount of P supplied. The two tested biomass-N analysis methods correlated well (Kjeldahl-N = 3.2 x (Ninhydrin-N), R^2 = 0.97).

Table 3. Biomass-N (extracted-N, without using correction factors) in three tropical soils after 152 days incubation in leaching tubes as affected by phosphorus concentration (0 or 1 mM P) in the leaching solution and assay method. (SED = standard error of difference).

	Biomass-N (µg N g^{-1} soil)				Biomass efficiency[1] (day^{-1})	
	Ninhydrin assay		Kjeldahl-N			
	0 mM P	1 mM P	0 mM P	1 mM P	0 mM P	1 mM P
Sandy soil	2.08	2.16	5.97	6.65	0.080	0.077
Sandy clay	3.11	3.64	10.15	10.81	0.049	0.062
Clay soil	5.89	6.06	19.74	20.65	0.020	0.044
SED	0.33		1.76		0.004	

[1] Biomass efficiency = N-Mineralization $rate_{116\ to\ 152\ days}$ / Biomass-$N_{(Ninhydrin)}$

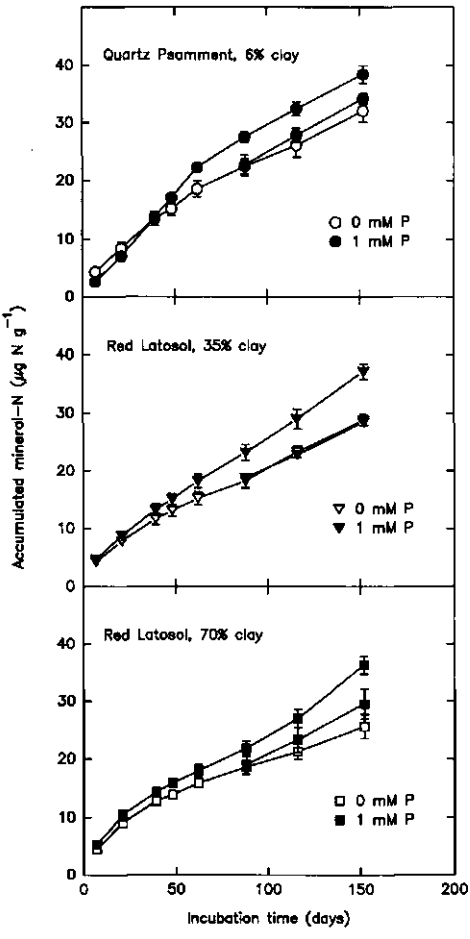

Figure 2. Accumulated mineralized soil-N (NH_4^+-N+NO_3^--N) in three soils as affected by P concentration (0 or 1 mM P used at start or after 88 days) in leaching solution. (Vertical bars are SE of means (n = 6/3)).

DISCUSSION

A strong stimulation of P fertilization on *in situ* soil-N mineralization was observed only at the beginning of the dry season when plant demand for N was presumably small from both fertilized and unfertilized plots. The low plant N demand at that time and the enhanced soil-N mineralization thus resulted in the observed increased soil mineral-N content in the P fertilized pasture compared to the control. During the most productive plant growth phase, the wet season, no strong positive effect of P fertilization on soil-N mineralization was apparent. The productivity of P fertilized *B. decumbens* plants

increased thus primarily due to an alleviation of P deficiency rather than due to an enhanced soil-N supply. The greater growth rate led to an enhanced N demand and thus increased N accumulation in P fertilized pastures as observed previously (Schunke *et al.*, 1991). An elevated N demand during the wet season was also indicated by the consistently smaller amounts of mineral-N present in P fertilized pastures compared to the control plots during that growth period. In the unfertilized plots, P deficient plants were not able to take advantage of the available soil-N in the same way as P fertilized plants, leaving more mineral-N free which could be lost by leaching. The less efficient use of mineral-N by P deficient plants could theoretically be due to the lower root mass and thus a less efficient soil volume exploration. An improved N scavenging by the strongly increased root system could also partly explain the previously observed increased N tissue concentration with P fertilization on the same site (Schunke *et al.*, 1991). However, the critical root length densities (Van Noordwijk and De Willigen, 1991), even for NH_4^+ uptake, are probably much lower than the ones present in this study as suggested by the large root mass even in unfertilized plots. The observed standing root mass values are equivalent to the respective aboveground dry matter production rates during the wet season and thus represent an important input of soil carbon when an approximately yearly root turnover is assumed (Chacon *et al.*, 1991).

In the laboratory incubation assay, without the presence of roots and litter inputs, the addition of P to the soil increased N mineralization, especially after a few weeks. Improved mineralization in glasshouse incubation studies due to a combined P, K and S fertilization of previously unfertilized temperate pasture soil samples was also obtained by Ross and Bridger (1978). Similarly Munevar and Wollum (1977) suggested that available P was a rate-limiting factor for organic matter mineralization in Colombian Andepts. The present data suggest however, that the initial increase in mineralized soil-N solely due to P supply may be too small to be adequately estimated within a 14 day *in situ* incubation period given the large variability in the field. This may be the reason why the P effect was often small in the field. Competition between plant roots and microbial biomass for uptake of available soil-P appeared to have no significant effect on mineralization in the field. The variable results of the *in situ* mineralization studies further suggest that other temporal factors such as soil moisture and litter turnover may enhance or mask effects of P. Generally, *in situ* mineralization showed a similar pattern of fluctuation as rainfall, except for the early November evaluation where a rapid change from immobilization to net mineralization was observed. This difference could not be explained by changes in soil moisture nor by plant growth. Litter decomposition data (not presented) indicated a very intensive microbial activity during this time which, together with possible changes in litter quality (lignin and C:N ratio), may partly explain the fluctuation in mineralization observed. While field N mineralization rates were normally lower than the potential rates established in the laboratory experiment (13/17 kg N ha^{-1} $week^{-1}$ between day 7-21) they were higher during the December evaluation further suggesting a direct influence of decaying plant material. Alternatively, the immobilization could be due to cutting of young roots in November assuming that there was an increase in root growth after the dry period. This strong variation of *in situ* mineralization suggests that unless we understand fully temporal changes in litter and root turnover rates any predictions of field N supply on the basis of laboratory data will be very unreliable.

Microbial biomass-N was not significantly affected by fertilizer treatments suggesting that microbial growth was not limited by P deficiency. It appears that soil biomass activity rather then the amount of microbial biomass would be a better indicator of the effect of P on soil-N mineralization since biomass efficiency was improved with P supply. It would

therefore be advisable to include soil respiration measurements in further experiments. The lower biomass efficiency in the clayey soils without P supply could be associated with P limitations for soil microbial turnover in these high P fixing soils as well with changes in soil pH. Improved biomass efficiency with P addition reflected well the greater N mineralization towards the later harvests in the clayey soils. Biomass-N values were comparatively small compared to published values in the literature (Amato and Ladd, 1988). This could be due to the substrate limitations towards the end of the incubation or due to the more acidic soils used here. Both ninhydrin and Kjeldahl methods for the estimation of microbial biomass yielded similar conclusions. The mean ratio of biomass Kjeldahl-N to ninhydrin-N of 3.2 was lower than that of 5.1 reported by Amato and Ladd (1988).

Even if P addition stimulated N mineralization on some occasions, the primary reason for pasture degradation in this sandy soil appeared to be P limitation for plant growth. P deficiency also increased the risk of N losses by leaching since more mineral-N was present in the soil during the wet season. The addition of P to pure grass pastures resulted in a temporary increase in productivity, a higher N demand and more N cycled through the different pools of the soil-plant-animal system. Whereas a greater litter production and a more dense root system in P fertilized plots allowed an more efficient recycling of N, increases in stocking rates, due to increased pasture productivity, may shift N losses from leaching to atmospheric losses (ammonia volatilization and denitrification of animal excreta-N). This fact emphasises the importance of measuring N losses, especially from urine and dung patches. Thus until we replace lost N, e.g. by introducing N_2 fixing legumes (Cadisch *et al.*, 1994), and adopt adequate stocking rates soil N exhaustion in the long-term may be equal or even accelerated by P fertilization.

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A comparison between an organic matter dynamics model and a food web model simulating nitrogen mineralization in agro-ecosystems

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Abstract

Crop growth will depend increasingly on the mineralization of nitrogen (N) in agricultural practices in which the use of inorganic fertilizer is reduced in favor of the use of organic manure. Simulation models have been developed to simulate the (dynamics of) N mineralization. One class of models describes N mineralization in relation to the decomposition of organic matter, making a distinction in the quality of the chemical components available as substrate for soil microbes. Another class of models describes N mineralization in relation to the abundance and activity of soil organisms, especially the decomposers.

In the present paper we describe examples of each of these two types of models. As a case-study, we use the models to simulate the dynamics in N mineralization in a reduced input (integrated) and a high input (conventional) arable farming system. Although the simulated rates fitted in part the observed rates, output of neither model matched the observed N dynamics over the entire year. The results also indicated that both models suffer from the same drawbacks, i.e. an inadequate description on the composition and utilization of the organic matter that serves as substrate for the soil microbes.

INTRODUCTION

Plant growth will depend increasingly on the mineralization of nitrogen (N) in agricultural practices in which the use of inorganic fertilizer is reduced in favor of the use of organic manure. Mineralization of N in soil is a biological process, associated with decomposition of organic compounds. Since carbon (C) is energy-carrier in heterotrophic processes, the N cycle is connected with the C cycle.

Models have been developed that simulate the N cycle in relation to decomposition of organic matter (Van Veen *et al.*, 1984). In these models the decomposition of organic matter is simulated by defining different fractions of organic matter, each with a specific quality as substrate for soil biota. The organic input is divided into a labile, easily

In: J.J. Neeteson and J. Hassink (eds.), 1994. Nitrogen mineralization in agricultural soils; proc. symp. held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993. AB-DLO Thema's, AB-DLO, Haren, pp. 207-219.

decomposable fraction, and a less easily decomposable, structural fraction. The soil organic matter is divided into a fraction microbial biomass, an active fraction, and a stabile fraction. These 'functional' components are defined on the basis of their chemical structure, not on their availability as substrate for microbes. It has, however, been assumed that the availability as substrate for the microbial population does not only depend on the chemical characteristics, but also on the location in the soil (Van Veen and Kuikman, 1990). Organic material in small pores may be protected against degradation by microbes. This concept of 'physical protection' has been taken into account in recent models on soil organic matter dynamics (Verberne *et al.*, 1990). Within these models both 'dead' organic matter as well as living biomass is divided into a physically protected and a physically non-protected fraction. This distinction is based on recent experiments showing that soil structure and texture may significantly affect the microbial turnover rates (Van Veen and Kuikman, 1990). These differences in turnover rates are thought to be related to the availability of substrate to the microbes and the availability of the microbes to microbivorous fauna. The concept of physical protection therefore implies that the turnover of microbes may depend on both their natural growth rates and on their trophic interactions with other soil organisms. In the present study, a simplified model of the organic matter dynamics was used, constructed by Van Faassen and Lebbink (1994). This model included four pools: decomposable plant material, resistant plant material, physically protected soil organic matter ('young' humus) and the soil microbial biomass.

Another class of models has been developed which simulate C and N flux rates in relation to the abundance and activity of the soil organisms constituting the soil food web (Hunt *et al.*, 1987; De Ruiter *et al.*, 1993[a]). In these models organisms are classified as functional groups according to food choice and life-history parameters (Moore *et al.*, 1988). Food web models take explicitly the contribution of the soil fauna into account, because experiments have shown that the fauna may contribute considerably to mineralization processes (Coleman *et al.*, 1983; Verhoef and Brussaard, 1990; Woods *et al.*, 1982). Consumption rates among the groups of organisms are calculated based on biomass and turnover rates. Subsequently, N mineralization rates are derived from consumption rates using information on energy conversion efficiencies and C:N ratios of the organisms.

In the present paper we will give an example of each of these two types of models. As a case-study the models will be used to simulate N mineralization in two different arable farming systems. These arable farming systems were studied as part of the "Dutch Programme on Soil Ecology of Arable Farming Systems" (Brussaard *et al.*, 1988), in which a form of integrated arable farming was compared with a conventional farming system. Integrated farming differed from conventional farming by (1) relatively high input of organic manure instead of inorganic fertilizer, (2) reduced soil tillage, and (3) reduced use of pesticides and no soil fumigation. These two systems were practiced on the Lovinkhoeve experimental farm (Marknesse, Noordoostpolder, NL). Both systems consisted of a four-year crop rotation with winter wheat, sugar beet, spring barley and potato (Kooistra *et al.*, 1989). The data presented in this paper are from the 1989/90 winter wheat crop.

MATERIALS AND METHODS

Site description

The test site is located at the Lovinkhoeve Experimental Farm in the Noordoostpolder

(Marknesse, the Netherlands). The soil is a calcareous silt loam with pH-KCl 7.5, reclaimed in 1942. Annual precipitation at the site is usually between 600 and 950 mm. The results presented in this paper are from two management practices: integrated and conventional. Integrated differs from the conventional practice in reduction of N-fertilizer application to 50-65% of the recommended rates in the conventional practice and integration of the use of fertilizers and manure, a reduction of pesticide application and a reduction of soil tillage (conventional: 20-25 cm plough, integrated: 12-15 cm cultivator, no inversion of the top soil). The organic matter content of the upper 25 cm of the conventional plot is 2.1%; on the integrated plot it is 2.7%. Since 1985, the four-year crop rotation on both plots has been the same: winter wheat, sugar beet, barley and potatoes. The data presented here are from the 1990 winter wheat crop. A full description of the site and management practices is given by Kooistra *et al.* (1989).

The organic matter dynamics model

The organic matter dynamics model is a modification of the model by Jenkinson and Rayner (1977). Carbon and nitrogen turnover in soil are described by coupling the biodegradation of organic matter to the production of microbial biomass. The model distinguishes four pools of organic matter, each with its own first-order rate of degradation and efficiency of respiration, biomass formation and transition to other organic matter pools (Figure 1).

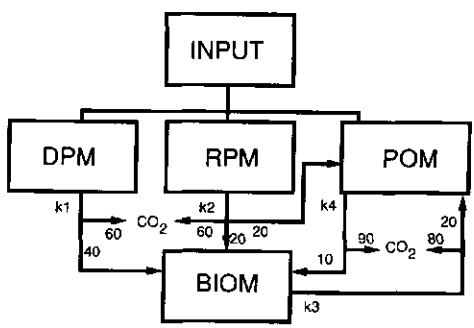

Figure 1. Diagram of the soil organic matter model (Van Faassen and Lebbink, 1994). DPM: decomposable plant material; RPM: resistant, slowly decomposable plant material; POM: physically protected organic material; BIOM: biomass. Numbers indicate fractions (%) of the flux rates. k_i: degradation coefficients (see also Table 1).

The large pool of old soil organic matter was considered as inert, restricting the model to 'young' humus, referred to as physically protected organic matter (POM). Within the crop residues added to the soil, the model distinguishes easily decomposable plant material (DPM) and resistent plant material (RPM). Microbial biomass in soil (BIOM) is the fourth pool.

Degradation of DPM and POM leads to the production of CO_2 and BIOM, while degradation of RPM and BIOM also results in POM:

$$\frac{d[DPM]}{dt} = -k_1\,[DPM] \quad (1)$$

$$\frac{d[RPM]}{dt} = -k_2\,[RPM] \quad (2)$$

$$\frac{d[POM]}{dt} = -k_4\,[POM] + 0.2\,k_2\,[RPM] + 0.2\,k_3\,[BIOM] \quad (3)$$

$$\frac{d[BIOM]}{dt} = -k_3\,[BIOM] + 0.4\,k_1\,[DPM] + 0.2\,k_2\,[RPM] + 0.1\,k_4\,[POM] \quad (4)$$

where [DPM], [RPM], [POM], and [BIOM] are the amounts (kg C ha^{-1}), and k_1, k_2, k_3, and k_4 the degradation coefficient of decomposable plant material, resistant plant material, physically protected organic matter and biomass, respectively. N mineralization is calculated from the N released from decomposed organic matter inputs minus N incorporated in products:

$$N_{min_i} = \left(\frac{1}{CN_i} - \sum_{j=1}^{4} \frac{e_{ij}}{CN_j}\right) k_i\,[PS_i] \quad (5)$$

where $Nmin_i$: N mineralization rate (kg ha^{-1} yr^{-1}) due to the degradation of organic matter pool *i*; CN_i: C:N ratio of pool *i*; CN_j: C:N ratio of pool *j*; e_{ij}: fraction of the carbon going from pool *i* to pool *j*; k_i: degradation coefficient of pool *i* (yr^{-1}); $[PS]_i$: size of pool *i* (kg C ha^{-1}). The model used a time step of one month in calculating the sizes of the pools.

The food web model

The food web of the Lovinkhoeve was constructed by assembling species into functional groups defined mainly by food choice (Moore *et al.*, 1988), but also by differences in growth rate and mode of feeding (Figure 2). Earthworms were only found in the integrated field. The model (sensu Hunt *et al.*, 1987) derives N mineralization from feeding rates following a scheme (Figure 3) in which the feeding rate, i.e. the rate at which material is taken from an energy source, is split into a rate at which organic material is returned to the environment (i.e. into detritus) in the form of faeces or prey residues, a rate at which material is incorporated into the biomass of the consumer, and a rate at which material is released in inorganic form.

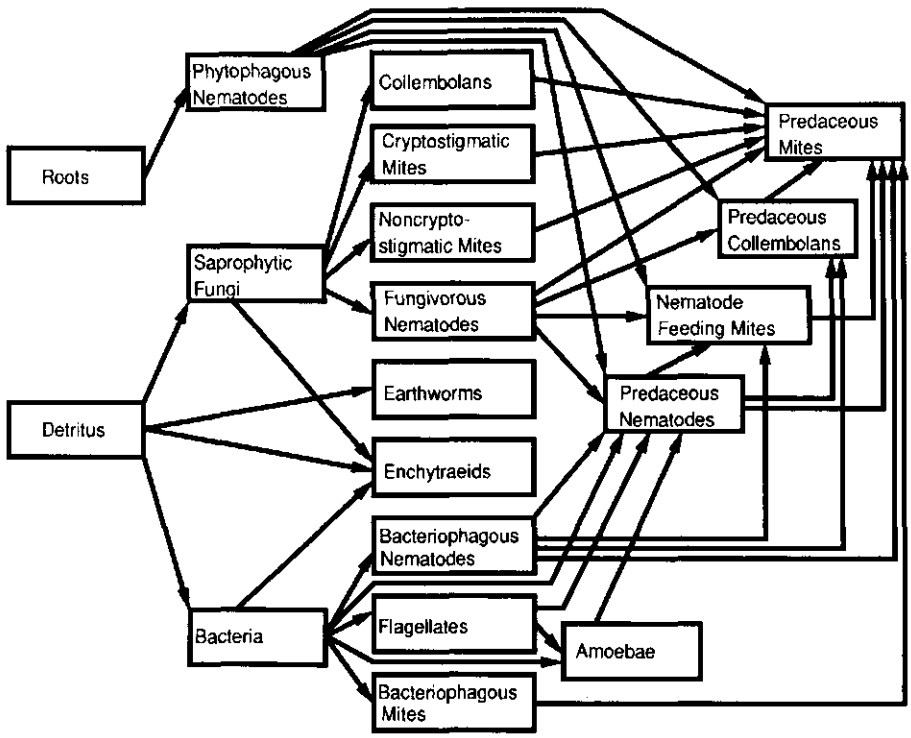

Figure 2. Diagram of the food web model (De Ruiter *et al.*, 1993). Arrows indicate flux rates (consumption rates). Earthworms were only present in the integrated plot.

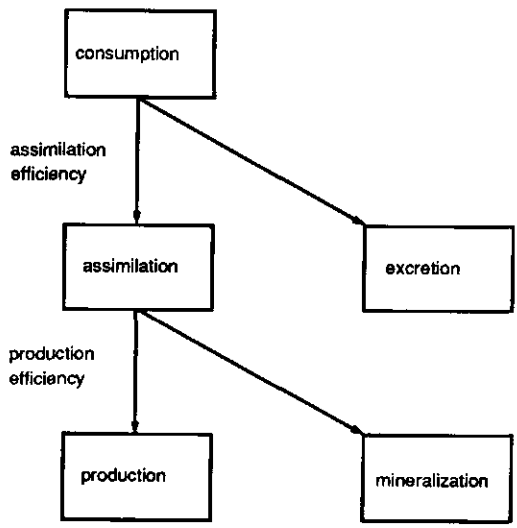

Figure 3. Scheme relating consumption, excretion (of organic material) and mineralization.

Average annual feeding rates of the functional groups are calculated using the steady-state assumption, i.e. the production rate of a group balances the rate at which material is lost through natural death and predation:

$$F = \frac{D_{nat}\ B + P}{e_{ass}\ e_{prod}} \qquad (6)$$

where F: feeding rate (kg C ha^{-1} yr^{-1}); $Dnat$: specific natural death rate ($year^{-1}$); B: biomass (kg C ha^{-1}); P: death rate due to predation (kg C ha^{-1} yr^{-1}); e_{ass}: assimilated carbon per unit consumed carbon; e_{prod}: biomass production per unit assimilated carbon.

Simulation of the dynamics of C and N flows within a year required that the biomass dynamics were incorporated in the model. This was done by adding the rate of change in biomass of a functional group to the rate of material loss (O'Neill, 1969):

$$F = \frac{D_{nat}\ B + P + \Delta B/\Delta t}{e_{ass}\ e_{prod}} \qquad (7)$$

where ΔB: change in biomass between two sampling dates (kg C ha^{-1}), Δt: time interval between two sampling dates (wk), and other terms as in equation (6).

If a predator was considered to feed on more than one prey type, then both the preference of the predator for a given prey and the relative abundances of the prey types were taken into account:

$$F_{ij} = \frac{w_{ij}\ B_i}{\sum_{i=1}^{n} w_{ij}\ B_i}\ F_j \qquad (8)$$

where F_{ij}: feeding rate of group j on group i (kg C ha^{-1} wk^{-1}); w_{ij}: preference of j for prey i relative to other prey types and n: number of functional groups in the web; F_j: total feeding rate of group j.

N mineralization was calculated per trophic interaction depending on feeding rate, assimilation efficiency, production efficiency and the C:N ratios of food and consumer:

$$N_{min} = e_{ass_i}\left(\frac{1}{CN_i} - \frac{e_{prod_j}}{CN_j}\right) F_{ij} \qquad (9)$$

where N_{min}: N mineralization rate resulting from a trophic interaction (kg N ha^{-1} wk^{-1}); CN_i: C:N ratio of prey; CN_j: C:N ratio of predator.

The calculations start with the feeding rates of the top predators of which only natural death reduces the biomass. The predatory losses in the groups of one trophic level lower are calculated from the feeding rates of the top predators. These losses are added to the non-predatory losses in order to calculate the feeding rates of the groups at this level. All feeding rates are subsequently calculated throughout the food web, working back to the primary consumers, i.e. microorganisms and saprotrophs.

Parameter values

Initial pool sizes and time and amounts of additions of organic matter (residues, manure) in the organic matter model are given in Table 1.

Table 1. Initial pool sizes (kg C ha^{-1}), C:N ratios, and input amounts for the organic matter pools in the organic matter model. Initial pool sizes were the steady-state values based on organic matter inputs and degradation coefficients. DPM: decomposable plant material; RPM: resistant, slowly decomposable plant material; POM: physically protected organic matter; BIOM: biomass; k: degradation coefficients ($year^{-1}$) for a temperature of 10 °C; INPUT: organic matter input (kg C ha^{-1}). IF: integrated farming; CF: conventional farming.

	Initial pool size		C:N ratio	k	INPUT	
	IF	CF			IF	CF
DPM	64	66	17	4.2	1280[1]	800[2]
RPM	363	215	80	1.8	1000[1]	360[2]
POM	14144	3328	12	0.06	1320[1]	
BIOM	468	398	4	1.8		

[1] given at day 1990: 235
[2] given at day 1990: 335

The initial pool sizes were obtained by calculating the steady-state pool sizes based on inputs and degradation over a complete four-year crop rotation (Van Faassen and Lebbink, 1994). Degradation rate constants were based on empirical humification coefficients that give the mass fractions of organic matter inputs still present in soil one year after its addition and includes turnover products formed from this input (Van Faassen and Lebbink, 1994). Each crop residue was distributed over DPM and RPM and organic manures over DPM, RPM and POM; this was done in such a way that their summed turnover calculated by the model fits with the given humification coefficients of 0.2 for young plant materials, 0.35 for straw, stubble and roots and 0.50-0.85 for organic manures. Each organic matter pool was assigned its own C:N ratio and utilization efficiency by the microbes (Figure 1). The parameter values used led to N immobilization with the degradation of DPM and RPM, and to N mineralization with the degradation of POM and BIOM.

The food web model requires biomass estimates, specific death rates, preference weighing factors, assimilation efficiencies, production efficiencies, C:N ratios and soil temperature. Biomass dynamics of the functional groups were from Zwart *et al.* (1994). Methods used to sample and calculate biomass of the organisms is described by De Ruiter *et al.* (1993[a]). Bacteria and protozoa were determined every three weeks. Nematodes, microarthropods, enchytraeids and earthworms were counted every six weeks. Bacteria and protozoa were also determined one and two weeks after day 235 when crop residues were added and soil fumigation (only at conventional) and tillage took place. To reduce strong temporal variation in the biomass data, the model calculations were based on three-point moving averages (TMA) of the biomass of the populations. In the present study we used two sets of physiological parameter values (Table 2).

Table 2. Physiological parameter values and biomass estimates (kg C ha^{-1}) for the functional groups the food web model. e_{ass}: assimilation efficiency; e_{prod}: production efficiency; D_{nat}: specific natural death rate ($year^{-1}$) for temperature of 10 °C; IF: integrated farming; CF: conventional farming.

	C:N ratio	e_{ass}	e_{prod}	D_{nat}	Biomass IF	CF
Microbes						
Bacteria	4	1.00	0.30/0.40[1]	1.20/0.50	245	228
Fungi	10	1.00	0.30	1.20	3.27	2.12
Protozoa						
Amoebae	7	0.95	0.40/0.30	6.00/10.0	18.9	11.5
Flagellates	7	0.95	0.40/0.30	6.00/10.0	0.63	0.53
Nematodes						
Herbivores	10	0.25	0.37	1.08	0.35	0.19
Bacteriovores	10	0.60	0.37	2.68	0.36	0.30
Fungivores	10	0.38	0.37	1.92	0.13	0.08
Predators	10	0.50	0.37	3.00	0.06	0.06
Microarthropods						
Predatory Mites	8	0.60	0.35	1.84	0.08	0.06
Nematophagous Mites	8	0.90	0.35	1.84	0.006	0.004
Cryptostigmatic Mites	8	0.50	0.35	1.20	0.003	0.007
Non-Cryptostigmatic Mites	8	0.50	0.35	1.84	0.04	0.02
Bacteriovorous Mites	8	0.50	0.35	1.84	0.0003	0.001
Fungivorous Collembola	8	0.50	0.35	1.84	0.38	0.47
Predatory Collembola	8	0.50	0.35	1.84	0.008	0.03
Annelids						
Enchytraeids	5	0.25	0.40	5.00	0.21	0.43
Earthworms	5	0.25	0.40	2.40	63.5	-

[1] The two values refer to food web simulation I and II, respectively (see text)

The first set (Food Web Simulation I) was chosen similar to the set used by Hunt *et al.* (1987); for these values it was found that the annual C and N mineralization could be simulated close to observed rates (De Ruiter *et al.*, 1993[b]). However, the dynamics in N mineralization could not satisfactorily be simulated using these values, especially not the periods of N immobilization (De Ruiter *et al.*, 1994). Therefore, we used a second parameter set (Food Web Simulation II) in which the C:N ratio of the substrate for bacteria was increased (from 10 to 12), and in which the production efficiency and natural death rates of the bacteria and protozoa were adjusted as to obtain a closer fit between the simulated and observed dynamics in N mineralization (Table 2). The food web model calculated with a time step of one week (using the mean temperature per week). Biomass estimates per week were obtained by linear intrapolation between the adjacent TMA's.

Temperature fluctuations throughout the year were taken into account in both models. The organic matter dynamics model related the rate constants with monthly average soil temperature. The food web model related the specific death rates of the soil organisms to temperature using a Q_{10} of 3 similar to the value used by Johnsson *et al.* (1987). Effects of fluctuations in soil moisture content were not accounted for in either of the models.

Observed *in situ* N mineralization rates was determined by inserting cores into the field for six weeks and then analyzing changes in mineral N over time, according to Raison *et al.* (1987). The incubations were started every three weeks (Bloem *et al.*, 1994).

RESULTS

The observed N dynamics showed an average net N mineralization, including short periods of N immobilization, especially in the conventional farming system (Figure 4a).

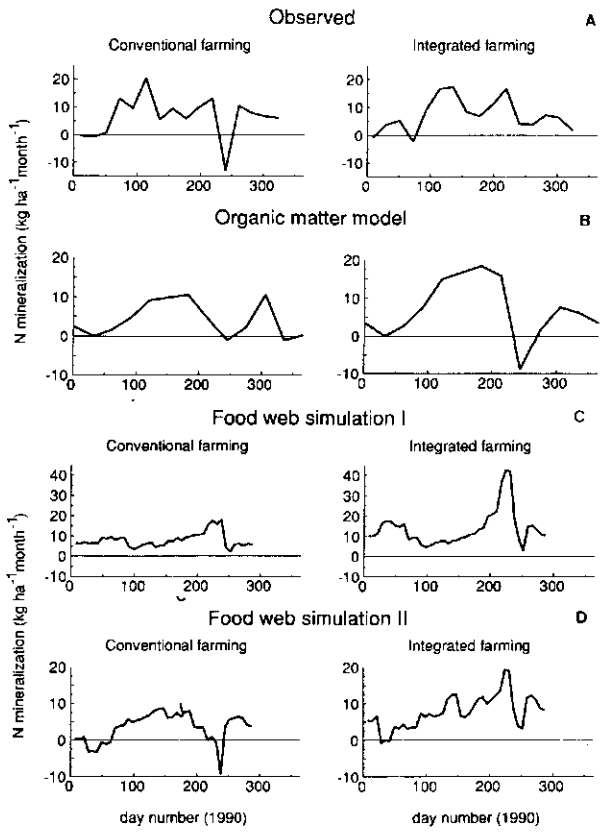

Figure 4. Observed and simulated dynamics in N mineralization/immobilization rates (kg ha^{-1} $month^{-1}$). A. In situ observed rates. B. Rates according to the soil organic matter model. C. Rates according to food web simulation I (using physiological parameter values from Hunt et al. (1987), see text). D. Rates according to food web simulation II (using adjusted parameter values, according to De Ruiter et al. (1994), see text).

This period of immobilization was directly after day 235 when soil tillage took place with the addition of crop residues and soil fumigation. In the integrated plot also soil tillage and addition of crop residues took place at day 235, but this was followed only by a decrease in N mineralization and not by N immobilization. The observed annual N mineralization accounted for 70 and 100 kg ha^{-1} $year^{-1}$ for the conventional and integrated practice respectively (Figure 5).

The soil organic matter model simulated N dynamics including periods of N mineralization and N immobilization (Figure 4b). In the conventional practice after the addition crop residues (day 235) and of green manure (day 335) the model predicted declines in N mineralization including short periods of slight immobilization. In the

integrated practice, the model predicted N immobilization after the addition of crop residues and champost (at day 235), whereas this immobilization was not observed.

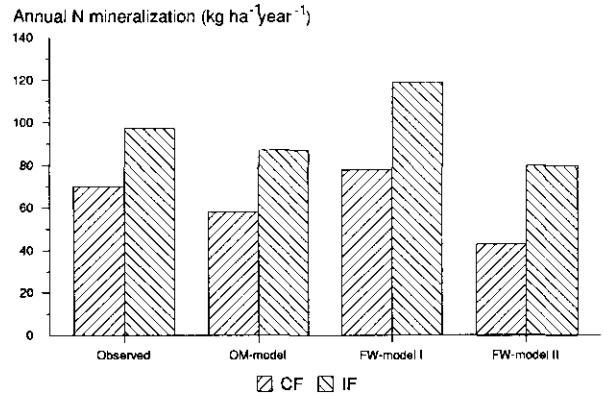

Figure 5. Observed and simulated annual N mineralization rates (kg ha^{-1} $year^{-1}$). Observed: In situ observed rates. OM: Rates according to the soil organic matter model. FW-model I: Rates according to food web simulation I (using physiological parameter values from Hunt et al. (1987), see text). FW-model II: Rates according to food web simulation II (using adjusted parameter values, according to De Ruiter et al. (1994), see text). CF = conventional farming; IF = integrated farming.

The annual N mineralization as predicted by the soil organic model accounted for 60 and 90 kg ha^{-1} $year^{-1}$ in the conventional and integrated practice respectively, which was close to the observed annual N mineralization rates (Figure 5).

The food web simulation I, using the physiological parameters according to Hunt *et al.* (1987) resulted in net N mineralization over the entire year and no N immobilization in either farming system (Figure 4c). After soil tillage, addition of crop residues and soil fumigation (only at the conventional plot), the N mineralization rates decreased as a consequence of decreasing biomass of microbes and protozoa, the most abundant faunal group (Zwart *et al.*, 1994). The food web model using this parameter set simulated annual N mineralization rate somewhat higher than the observed rates: 80 and 120 kg ha^{-1} $year^{-1}$ for the conventional and integrated practice, respectively (Figure 5).

The food web simulation II, using adjusted values for the C:N ratio of the substrate for bacteria and death rates and production efficiencies of the bacteria and protozoa (De Ruiter *et al.*, 1994; Table 2) showed a period of N immobilization in the conventional practice, whereas for the integrated practice, the model simulated net N mineralization for the entire year (Figure 4d). These patterns are in agreement with the observed patterns in N dynamics (Figure 4a). The period of N immobilization resulted from relatively high bacterial growth rates after day 235, leading to immobilization combined with relatively low growth rate of the protozoa (Bloem *et al.*, 1994). The food web simulation II simulated annual N mineralization rates lower than the observed rates: 40 and 70 kg ha^{-1} $year^{-1}$ for the conventional and integrated practice, respectively (Figure 5).

DISCUSSION

The results presented in this paper show the outcome of two different models simulating the annual N mineralization rates and the dynamics in N mineralization/immobilization rates. Neither model simulated both annual rates and N dynamics close to the observations. The soil organic matter model simulated annual rates matching the observed rates, but did not predict N immobilization in the conventional practice as was observed. The fact that no N immobilization was observed in the integrated practice after the addition of the crop residues might have been due to differences in organic matter additions between the practices: in integrated farming consisting of wheat roots, stubble and spent mushroom compost and in conventional farming of a green manure crop (mustard) together with residues from the wheat crop. It might also have been due to the high N mineralization by the soil fauna, especially the protozoa. The food web simulation did explicitly take this N mineralization into account, which led to N mineralization rates according to both food web simulations. This might be therefore one of the drawbacks of the soil organic matter model, i.e. that all soil biota are aggregated into one pool.

The food web simulation I also simulated annual N mineralization rates relatively close to the observed rates, but predicted net N mineralization over the entire year in both farming systems, whereas immobilization was observed at the conventional practice. The absence of N immobilization according to food web simulation I was due to the fact that consumption of all groups of organisms (microbes and fauna) led to net N mineralization over the entire year. The declines in N mineralization in both plots were due to the decreased numbers of microbes and fauna directly after day 235 when crop residues were added and the soil was fumigated (the latter only at the conventional plot). The simulated annual N mineralization rates were slightly higher than the observed rates.

The food web simulation II used adjusted parameter values leading to N immobilization by bacteria. This adjustment was based on the findings of Bloem *et al.* (1994), i.e. that periods of rapid microbial growth were accompanied by N immobilization rather than N mineralization, possibly due a relatively high production efficiency of microbes during such rapid growth. A period of rapid growth occurred following the decline in biomass at day 235 when crop residues were added and the soil was fumigated (only at the conventional plot). The dynamics in N mineralization/immobilization according to the food web simulation II were as follows. At the conventional plot, microbes showed high growth rates after their decline at day 235, but faunal densities were also low due to the soil fumigation, which in combination led to a net N immobilization. At the integrated plot, where faunal densities were not affected by soil fumigation, the N immobilization by the bacteria was obscured by the relatively high N mineralization by the fauna, which led to a net N mineralization. However, the fact that in food web simulation II bacteria always immobilize N resulted in simulated annual N mineralization rates which were low compared to the observed N mineralization rates.

The two food web simulations made clear that using constant parameter values for a complete year will not lead to an adequate simulation of the dynamics in N mineralization/immobilization rates. Probably, bacteria mineralize and immobilize N during different periods of the year, depending on the availability and constitution of their substrate. These aspects of microbial substrate form the starting point of the simulation of N mineralization/immobilization rates by the soil organic matter model. It seems therefore the obvious next step to develop a model in which both the dynamics of the soil organic matter and the dynamics of the soil biota are integrated, and the present results suggest that such a model might be able to simulate both annual N mineralization

rates and dynamics in N mineralization/immobilization satisfactorily. However, the models are not only different with respect to which processes are included in the model description, but differ also with respect to the nature of their input data and their applicability. The organic matter model requires information on the amount and constitution of the organic matter added to the soil and aims to simulate N flux rates based on planned aspects of management practices with respect to crop residues (amounts, timing, placement, quality), and in the future also with respect to soil tillage, and soil fumigation. The organic matter model may therefore serve to simulate future N dynamics, having a predictive value. The food web model, requiring data on the dynamics of the soil biota, is only able to calculate N mineralization rates during the period of observation, and has therefore primarily an explanatory value. A model including both the dynamics in soil organic matter and the dynamics of the soil biota needs both kinds of input data and might improve the explanatory value of the model. Such an integrated model might eventually lead to a predictive model that includes the dynamics in soil biota in the model as well as the dynamics in soil organic matter, i.e. the assumed responses of organic matter and soil biota to the planned management regimes.

Finally, both kinds of models are simplified descriptions of the soil processes involved. Further improvements can be obtained by making them more realistic, for example by including abiotic factors like humidity and drought, which might affect considerably the degradation of organic matter as well as the growth rates of the soil organisms (Bloem *et al.*, 1992).

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A model approach to simulate C and N transformations through microbial biomass

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Abstract

A model is presented which simulates C and N transformation based on the population dynamics of two fractions of microbial biomass. The distinction between the two fractions refers to the autochthonous and zymogenous microbial populations. The results of the model were compared with data from the literature. An example demonstrates the possibility of combining the model with a nonlinear regression program to estimate model parameters.

INTRODUCTION

Since about 20 years efforts have been made to develop models describing nitrogen dynamics (Beek and Frissel, 1973). Some of these models use a chemical distinction of the soil organic matter to characterize different availabilities for decomposition (e.g. Van Veen, 1977), taking also into account the transformations of the carbon cycle through the microbial biomass. Other models regard soil organic matter to consist of a few pools of different decomposition patterns (e.g. Van Veen *et al.*, 1984; McGill *et al.*, 1981). Some models, mostly for practical purposes, are just regarding the transformations of nitrogen. They are often based on the concept of net mineralization using the potentially mineralizable nitrogen as proposed by Stanford and Smith (1972). In the field as well as in incubation experiments a distinction of potentially mineralizable nitrogen into two pools of readily and resistant decomposable nitrogen pools has proved to be a very useful concept, especially when fresh organic matter was added to soils (Molina *et al.*, 1980, Richter *et al.*, 1982).

Nevertheless De Willigen (1991) stated in his report on the comparison of 14 simulation models that the modelling of transformation processes of nitrogen in soil is still unsatisfactory especially regarding the short-term dynamics. Results reported by Neeteson *et al.* (1986) and Lochmann *et al.* (1989) indicate a very rapid disappearance of added mineral nitrogen fertilizer. Kersebaum and Richter (1991) observed on a field trial that the

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temporal increase of nitrogen in microbial biomass determined by the fumigation-extraction method was very similar to the overestimation of mineral nitrogen and plant nitrogen uptake of their model not including N immobilization.

Laboratory experiments of Azam *et al.* (1985) with labelled carbon and nitrogen showed a very rapid decline of mineral N and a corresponding incorporation into organic compounds followed by a fast remineralization of about 50 % of immobilized N. Cochran *et al.* (1988) found in an experiment with ^{14}C labelled straw and an application of unlabelled glucose that CO_2 evolution increased only due to the C consumption of glucose C while decomposition of straw carbon remained unchanged until mineral nitrogen is added. From these results they concluded a distinction of the microbial biomass analogous to the "zymogenous" and "autochthonous" biomass proposed by Winogradsky (1949).

In the this paper we present a model very similar to that of Cochran *et al.* (1988) assuming one pool of microbial biomass growing very fast on readily available carbon sources like glucose and another one which reacts slowly and consumes the more resistant fractions of carbon.

MODEL DESCRIPTION

Dynamics of microbial biomass

The model describes the growth of the two populations and their transformations of carbon and nitrogen by ordinary differential equations which are solved numerically using the classical Runge-Kutta-method (Press *et al.*, 1992).

The dynamics of the biomass pools are described by the following equations for the slow reacting "autochthonous" biomass pool (Cb_1):

$$\frac{d\ Cb_1}{dt} = V_1 - D_1 \tag{1}$$

where V_1 is the growth of the biomass pool Cb_1 (kg C * ha^{-1} * day^{-1}) described by

$$V_1 = r_{max1}(E) * f_1(C_r) * f_1(N_r) * Cb_1 \tag{2}$$

The equations for the fast reacting "zymogenous" biomass pool (Cb_2) are

$$\frac{d\ Cb_2}{dt} = V_2 - D_2 \tag{3}$$

with

$$V_2 = r_{max2}(E) * f_2(C_d) * f_2(N_d) * Cb_2 \tag{4}$$

The following notations hold:

Cb_i : microbial biomass C of fraction i (kg C * ha^{-1})

$r_{maxi}(E)$: pool specific maximum growth rate at specific environmental conditions E (e.g. moisture, temperature etc.) (day^{-1})

$f_i(C_x)$: Michaelis-Menten response of biomass fraction i to C pool x normalized to 1 (dimensionless)

$f_i(N_x)$: Michaelis-Menten response of biomass fraction i to N pool x normalized to 1 (dimensionless)

C_r,N_r : resistant organic carbon resp. nitrogen pool (kg C (N) * ha^{-1})

C_d,N_d : easily decomposable organic C resp. N pool (kg C (N) * ha^{-1})

D_i : nonlinear mortality rate of biomass pool i (kg C * ha^{-1} * day^{-1})

Saturation responses like the Michaelis-Menten forms are used as typical for biological processes. This form is very flexible because it approaches first-order reactions for low nutrient concentrations and pseudo zero-order kinetics for high nutrient concentrations. If one employs the following form of the nonlinear mortality rate

$$D_i = \mu_i * (1 + \alpha_i * Cb_i) * Cb_i \tag{5}$$

with μ_i the pool specific linear mortality coefficient (day^{-1}) and α_i the pool specific coefficient of crowding (kg biomass C^{-1}), the growth equations (1) and (3) are identical to the logistic growth equation under constant environmental conditions and stationary nutrient levels:

$$\frac{d\,Cb_i}{dt} = r_i * Cb_i * \left(1 - \frac{Cb_i}{K_i}\right) \tag{6}$$

with K_i the environmental capacity of fraction i (kg biomass C*ha^{-1}), which can be expressed as

$$K_i = \frac{r_i}{\mu_i * \alpha_i} \tag{7}$$

and

$$r_i = r_{max\,i}(E) * f_i(C_x) * f_i(N_x) - \mu_i \tag{8}$$

Because the coefficient of crowding α_i is difficult to estimate it is easier to derive this parameter from the environmental capacity, the growth rate and the linear mortality via equation (7).

At nutrient levels above the respective K_m values of the Michaelis Menten response functions K_i reflects a maximum capacity for the pool i under optimum conditions, which may be more handy to estimate.

Mass balance of abiotic carbon pools

Organic matter is divided into a passive and an active part from which only the active part

contributes to the C and N transformations. According to Jansson (1958) about 85 % of the organic pool is bound in the passive part. The active part of organic matter consists of two fractions: the easily decomposable fraction (C_d, N_d) consisting of the "metabolic" part of added organic material and dead microbial biomass, and more resistant pool (C_r, N_r) of structural compounds of plant residues, microbial biomass and the active part of soil organic matter. It is assumed that easily decomposable organic matter can only be used by the "zymogeneous" biomass and the more resistant fractions are decomposed by the "autochthonous" biomass.

The corresponding mass balances for the two carbon pools are

$$\frac{dC_r}{dt} = -V_1 * \frac{1}{\varepsilon_1} - Cb_1 * m_1 + (D_1 + D_2) * f \tag{9}$$

for the resistant organic C compounds

$$\frac{dC_d}{dt} = - V_2 * \frac{1}{\varepsilon_2} - Cb_2 * m_2 + (D_1 + D_2) * (1 - f) \tag{10}$$

for the readily available organic carbon with the notations:

ε_i : pool specific yield coefficient for carbon (dimensionless)
m_i : pool specific maintenance coefficient (day^{-1})
f : fraction of structural C compounds in dead microbial biomass (dimensionless).

When high amounts of readily decomposable carbon are available, e.g. after addition of glucose, the carbon use efficiency of the fast growing biomass may be reduced to balance the immobilization of nitrogen to the inorganic nitrogen actually available (Molina *et al.*, 1983).

The CO_2 release is given by:

$$\frac{d\,CO_2}{dt} = V_1 * (1-\varepsilon_1) + V_2 * (1-\varepsilon_2) + Cb_1 * m_1 + Cb_2 * m_2 \tag{11}$$

Mass balance of abiotic nitrogen pools

For the transformation of nitrogen it is assumed that it will be decomposed together with carbon in relation to the C/N ratio of the substrate. Therefore, the decomposition of the organic N pools is associated with the flow of carbon in the following way:

$$\frac{dN_r}{dt} = - (\frac{V_1}{\varepsilon_1} + Cb_1 * m_1) * \frac{N_r}{C_r} + (\frac{D_1}{CN_1} + \frac{D_2}{CN_2}) * f \tag{12}$$

$$\frac{dN_d}{dt} = -\left(\frac{V_2}{\varepsilon_2} + Cb_2 * m_2\right) * \frac{N_d}{C_d} + \left(\frac{D_1}{CN_1} + \frac{D_2}{CN_2}\right) * (1 - f) \quad (13)$$

The following notations hold:

CN_1 : C/N ratio of slow reacting biomass pool (kg C * kg N^{-1})

CN_2 : C/N ratio of fast reacting biomass pool (kg C * kg N^{-1})

The net mineralization/immobilization rate is calculated by summing up all N-consuming and N releasing terms:

$$\frac{dN_{min}}{dt} = V_1 * \left(\frac{N_r}{\varepsilon_1 * C_r} - \frac{1}{CN_1}\right) + V_2 * \left(\frac{N_d}{\varepsilon_2 * C_d} - \frac{1}{CN_2}\right) + Cb_1 * m_1 * \frac{N_r}{C_r} + Cb_2 * m_2 * \frac{N_d}{C_d} \quad (14)$$

where the term in parenthesis determines whether net mineralization or immobilization occurs. In this first version of the model mineral nitrogen is only regarded as ammonium.

BEHAVIOR OF THE MODEL

The model was first tested using data reported in a paper of Bjarnason (1987). In two incubation experiments the author added labelled mineral nitrogen to a sandy moraine loam soil containing 2.49 % C and 0.19 % N. The soil was amended at the beginning with glucose (4.67 mg C g^{-1} soil) resp. barley straw (4.32 mg C g^{-1} soil) and a NH_4NO_3 solution (100 g N μg^{-1} soil). In the glucose experiment the same amount of nitrogen was added after day 8, in the barley straw amended soil the ammonium nitrate solution was added after day 21. The time course of mineral nitrogen (NH_4^+ and NO_3^-) was measured at 20 °C and a moisture content of 40 % of the water holding capacity.

The parameters and initial conditions used in the model for both experiments are given in Table 1.

Parameters and pool sizes were set by varying them within the ranges found in the literature until a good fit for both data sets were reached.

Figure 1 shows a comparison between the measured and simulated mineral nitrogen in the soil following the addition of glucose. All values for C and N are calculated as kg ha^{-1} * 30 cm^{-1} assuming a bulk density of 1.5 g * cm^{-3}.

The glucose caused a very rapid growth of the biomass pool 2. The low C/N ratio of 5 assumed for the fast biomass fraction leads to a sharp decline of the mineral nitrogen by immobilization. After 8 days, the observed mineral nitrogen is depleted which is well fitted by the model. Growth of biomass pool 2 is now limited by the availability of mineral nitrogen. The second application of mineral nitrogen is followed by an increase of biomass pool 2 until the easily decomposable carbon is depleted at about day 12 when the population breaks down. Fifty five percent of the dead microbial biomass C is assumed to join the resistant fraction. Due to this, remineralization of the dead microbes is modelled to be less than 50 % of the immobilized mineral nitrogen. The high amount of C and N in the resistant fraction results in a slight immobilization caused by the net growth of the

Table 1: List of initial pool size values and parameters used for simulation

	experiment I (Bjarnason, 1987)	experiment II (Bjarnason, 1987)	experiment III (Smith et al, 1986)
pool sizes (kg ha^{-1} 30 cm^{-1})			
C_r (~ 11% of C_t)	12326	12326	4000
N_r	1026 (12% of N_t)	1026 (12% of N_t)	290 (10% of N_t)
C_d	150	150	225 (meas. H_2O sol. C)
N_d	30	30	25
biomass 1 (Cb_1)	2400 (~ 2% of C_t)	2400 (~ 2% of C_t)	1900
biomass 2 (Cb_2)	30	30	170
C_r added	0	9720	0
N_r added	0	162	0
C_d added	21015	9720	0
N_d added	0	81	0
mineral N added (day)	450 (0) / 450 (8)	450 (0) / 450 (21)	0
parameters			
$r_{max\ 1}$ (temperature)	0.14 day^{-1} (20 °C)	0.14 day^{-1} (20 °C)	0.054 day^{-1} (22 °C)
$r_{max\ 2}$	3.0 day^{-1} (20 °C)	3.0 day^{-1} (20 °C)	3.5 day^{-1} (22 °C)
C/N ratio biomass 1	10	10	10
C/N ratio biomass 2	5	5	5
capacity K_1	5000 kg C ha^{-1} 30cm^{-1}	5000 kg C ha^{-1} 30cm^{-1}	7770 kg C ha^{-1} 30cm^{-1}
capacity K_2	4000 kg C ha^{-1} 30cm^{-1}	4000 kg C ha^{-1} 30cm^{-1}	2000 kg C ha^{-1} 30cm^{-1}
lin. mortality coeff. μ_1	0.012 day^{-1}	0.012 day^{-1}	0.012 day^{-1}
lin. mortality coeff. μ_2	0.4 day^{-1}	0.4 day^{-1}	0.4 day^{-1}
maintainance coeff. m_1	0.006 day^{-1}	0.006 day^{-1}	0.006 day^{-1}
maintainance coeff. m_2	0.024 day^{-1}	0.024 day^{-1}	0.024 day^{-1}
Michaelis-Menten coefficient K_{Cr}	4500 kg C_r ha^{-1} 30 cm^{-1}	4500 kg C_r ha^{-1} 30 cm^{-1}	4500 kg C_r ha^{-1} 30 cm^{-1}
Michaelis Menten coefficient K_{Cd}	800 kg C_d ha^{-1} 30 cm^{-1}	800 kg Cd_d ha^{-1} 30 cm^{-1}	800 kg Cd_d ha^{-1} 30 cm^{-1}
Michaelis-Menten coefficient K_{Nr}	200 kg N_r ha^{-1} 30 cm^{-1}	200 kg N_r ha^{-1} 30 cm^{-1}	200 kg N_r ha^{-1} 30 cm^{-1}
Michaelis-Menten coefficient K_{Nd}	40 kg N_d ha^{-1} 30 cm^{-1}	40 kg N_d ha^{-1} 30 cm^{-1}	40 kg N_d ha^{-1} 30 cm^{-1}
carbon yield coeff. ϵi	0.6	0.6	0.6

slow reacting biomass pool between day 14 and day 30. This is also reflected by the measurements.

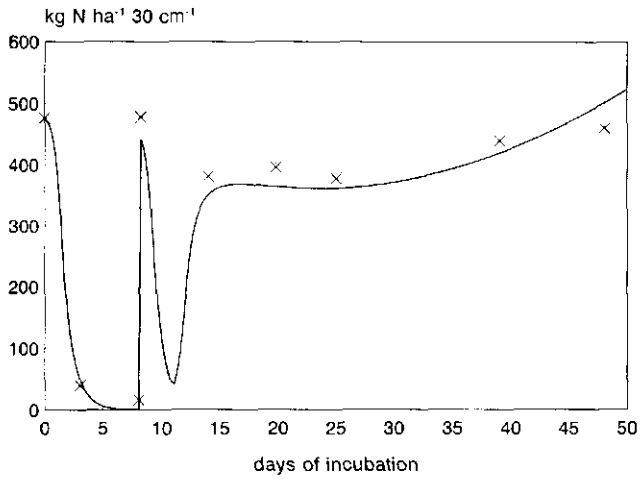

Figure 1. Simulated (——) and measured (*) time courses of mineral nitrogen (NH_4 and NO_3) following the addition of glucose (21 015 kg C ha^{-1} 30 cm^{-1} at day 0) and mineral nitrogen (450 kg N ha^{-1} 30 cm^{-1} at day 0 and day 8). The data are taken from Bjarnason (1987).

The same parameters are used for the second experiment where barley straw was added (Figure 2). It is assumed that half of the carbon added is partitioned to the readily available pool. Assuming a C/N ratio of 80 only 33 % of the nitrogen in the straw is calculated to be in the easily decomposable fraction.

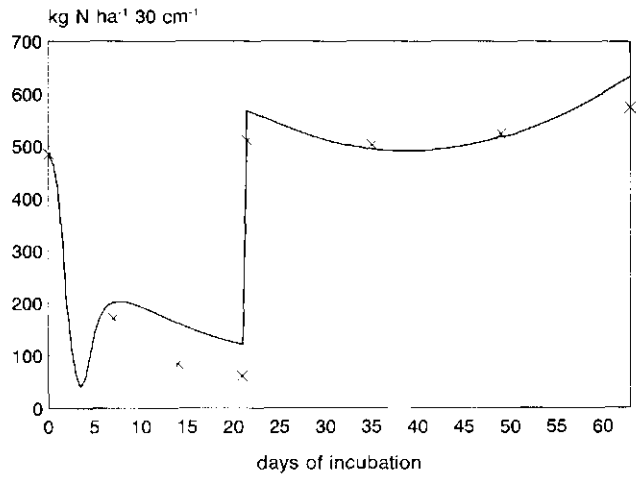

Figure 2. Simulated (——) and measured (*) time courses of mineral nitrogen (NH_4 and NO_3) following the addition of barley straw (19 440 kg C ha^{-1} 30 cm^{-1}) and mineral nitrogen (450 kg N ha^{-1} 30 cm^{-1} at day 0 and day 21). The data are taken from Bjarnason (1987).

For this experiment the model fits less well to the experimental data. The simulation predicts an immobilization of the mineral nitrogen until day 4 due to the growth of both biomass pools. The remineralization of recently immobilized nitrogen is relatively low due to the simultaneous immobilization by the biomass pool 2. To decide whether the predicted time course of mineral N is correct, more measured data are required during the first two weeks. The measurements indicate that the moderate decline of mineral nitrogen is probably because the microbes prefer ammonium instead of nitrate which is also described by Recous and Mary (1990). Nitrate is not calculated by the model, so this may cause a considerable error in the estimation of the parameters.

STATISTICAL PARAMETER ESTIMATION

A more efficient way to estimate parameters for a model is to link the model to observed data using statistical optimization procedures. Therefore, an experimental design is necessary where most of the simulated variables have been measured. A data set of a laboratory experiment of Smith *et al.* (1986) is used for a statistical estimation of model parameters as described by Richter and Söndgerath (1990). In this experiment the mineral nitrogen, the CO_2 evolution, the microbial biomass and soluble carbon during a 57 day incubation at 22 °C were measured simultaneously. In the statistical program package BMDP a derivative free nonlinear regression program is combined with a differential equation solver (Runge-Kutta-Fehlberg algorithm). Apart from some control statements in the BMDP control language and finding appropriate starting values the user has only to define his differential equations as FORTRAN statements.

Figure 3 shows the results obtained by employing this parameter identification procedure. Optimization is done for all four curves simultaneously.

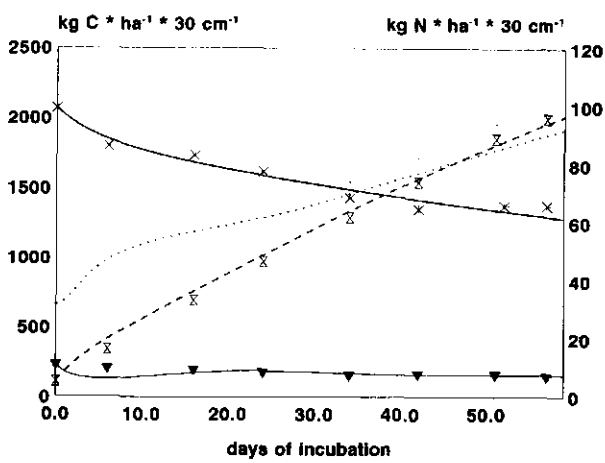

Figure 3. Simulation of the incubation experiment of Smith (1986). The model parameters were obtained by application of an estimation procedure for ordinary differential equations. Microbial biomass simulated (——) and measured (*), mineral nitrogen simulated (·····) and measured (+), CO_2 simulated (----) and measured (⧗), water soluble carbon simulated (——) and measured (▾).

Parameters used are given in Table 1. Maintenance coefficients were assumed in the order of magnitude reported in the literature (Smith *et al.*, 1986; Babiuk and Paul, 1970). K_m values of the Michaelis-Menten response functions were also kept constant. Estimation of K_m values is only feasible if data from a series of experiments with different applications of C and N sources are available. So the results of the parameter identification are to be regarded only as preliminary, because they are conditioned upon the choice of the fixed parameters.

Some remarks about the applicability of estimation methods in ordinary differential equations are in order:

i) Modern parameter estimation techniques provide the necessary link between simulation and statistics.
ii) The example has shown that the model is overparameterized with respect to the experimental data available. This is a common situation if experiments are not designed with the purpose of parameter estimation for a special model in mind.
iii) Parameter estimation in nonlinear ordinary differential equations demands a thorough experimental design. The design decides whether the mathematical problem involved is "well posed" or "ill conditioned".

There still exist high uncertainties concerning the pool sizes of active organic matter. Here, new experimental approaches are necessary to achieve unique results of the parameter estimation procedure and to proof the hypothesis of the model presented here as a reasonable explanation of experimental data.

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A simple statistical model for predicting N mineralization during soil incubation

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Abstract

The study was conducted to determine the factors affecting the rate of N mineralization in soils with different cropping and manuring histories. The statistical model for the prediction of N mineralization has generally available input variables. It utilizes soil texture and the history of soil management practices. The model is based on laboratory soil N mineralization measurements during four years of field experiments with winter wheat. Of the various cropping and manuring systems analyzed, previous crop exerted a highly significant influence on N mineralization. Manure amendment to the cover crop in the autumn was also highly significant for N mineralization. Previous crop manuring had no significant influence on N mineralization. The natural logarithm-transformed soil texture variable, ratio of sand to humus, was highly significant for N mineralization. The most appropriate factors for predicting N mineralization during soil incubation were previous crop, manuring of the cover crop nested within previous crops and soil texture.

INTRODUCTION

Determination of soil N availability for crops requires quantitative evaluation of the N mineralization potential in soil. Since the rates of soil N transformations are associated closely with chemical and physical properties of soil, these associations should be defined. Furthermore, the response of N mineralization to past N manuring and past cover cropping should be taken into consideration. The effects of soil characteristics (texture, type and amount of organic matter and pH) on biological activities in agricultural soils have been investigated (Jenkinson, 1977; Müller, 1988; Sauerbeck *et al.*, 1972; Sørensen, 1983; Szegi *et al.*, 1984). The aerobic incubation method has been used successfully to assess N availability in different soils (Bærug *et al.*, 1973; Keeney, 1982). General relationships are needed to predict the amount of N mineralized as a function of soil type as well as previous manuring and cropping histories.

The current study was conducted to predict N mineralized in soils during aerobic incubation from total soil N content, soil texture, previous crop and manuring history.

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Statistical models for prediction of N mineralization after different incubation periods were developed, based upon chemical and physical properties of soil and the history of soil management practices.

MATERIALS AND METHODS

Sites and sampling

Data were collected from 1988 to 1991 on farmers' fields with winter wheat as a cover crop. Fields (n = 59), representing major soil textural classes in Denmark (ranging from coarse sand to sandy loam) were included. The fields had different cropping and manuring histories. The previous year crops were corn, rape, or peas. Fields received different amounts (0-91 t ha^{-1} yr^{-1}) of liquid and solid pig and cattle manure, applied during the previous years as well as to the cover crop in the autumn. No manure was applied to the fields in the spring, when the experiments took place.

Soil sampling and preparation

Surface soil (0-25 cm) was sampled during each year in March. For each field, 15 cores were taken at random. The soil samples were bulked, thoroughly mixed and frozen. Five days before incubation, soil samples were thawed and dried at room temperature (18 °C) to a moisture content that allowed the soil to be sieved through a 2-mm screen. However, the moisture content was never below 50 percent of the field capacity (FC). After sieving, the water content was adjusted to 80 percent of FC and the soil was preincubated for 4 days at 20 °C. This procedure avoids the sudden flush of N mineralization which can occur when dried soil is remoistened (Beauchamp *et al.*, 1986).

Incubation method

The mineralization rate of organic N in the soil samples was determined in an aerobic incubation experiment. Soil samples (200 g) were weighed in polyethylene bags. Two subsamples were extracted immediately with 2 M KCl. The remainder of the subsamples were incubated at 20 °C with moisture lost during incubation being replenished weekly. After incubation periods of 28, 56 and 84 days in 1988-1991 and also 140 days in 1990 and 1991, two subsamples were extracted with 2M KCl. N mineralization in the soil was calculated as the difference in the concentration of inorganic N (NO_3^--N + NH_4^+-N) between the beginning and the end of the laboratory incubation. All soil N values refer to the sum of NO_3^--N and NH_4^+-N in kg ha^{-1} in the 0-25 cm layer.

Soil analysis

Concentration of NO_3^--N + NH_4^+-N in 2M KCl extracts (soil/solution ratio 1:2) of the soil samples were determined with a Technicon Autoanalyser (Henriksen and Selmer-Olsen, 1970). Total N was determined on a Tecator total-N analyzer using the Kjeldahl method. Total soil C was measured with a Leco combustion furnace (Tabatabai and Bremner, 1970). Particle size analysis was based on sieving and sedimentation.

Statistical analysis

The N mineralization data were analyzed using analysis of covariance to determine the significance of previous crop (PC), whether manure had been applied to the previous crop or not (MP) and the interaction between these effects for each incubation period. The statistical analyses were performed using SAS (GLM procedure, SAS Institute Inc., 1989 and MIXED procedure, SAS Institute Inc., 1992). Variation between years was included in the analysis as a random variable (η) assumed to be normally distributed. The linear effect of total soil N content (TN) was included as a covariate with one degree of freedom. The amount of manure to the cover crop was included as a covariate nested within previous crop (MC(PC)), with three degrees of freedom. The soil texture variables, proportions of humus, clay, silt, fine sand and sand, were analyzed as compositional data (Aitchison, 1986). After an additive natural log-ratio transformation, they were included in the statistical analysis as W_1, W_2, W_3 and W_4, each having one degree of freedom.

The following model was utilized:

$$\begin{aligned} N_{ijkl} &= \mu + PC_i + MP_j + PC{\cdot}MP_{ij} + a_i{\cdot}(MC(PC)_{k(i)} - \overline{MC(PC)_i}) \\ &\quad + b{\cdot}(TN_k - \overline{TN}) + c_1{\cdot}(W_{1k} - \overline{W_1}) + c_2{\cdot}(W_{2k} - \overline{W_2}) \\ &\quad + c_3{\cdot}(W_{3k} - \overline{W_3}) + c_4{\cdot}(W_{4k} - \overline{W_4}) + \eta_l + \varepsilon_{ijkl} \end{aligned} \qquad (1)$$

$$\Updownarrow$$

$$\begin{aligned} N_{ijkl} &= \gamma_{ij} + a_i{\cdot}MC(PC)_{k(i)} + b{\cdot}TN_k + c_1{\cdot}W_{1k} + c_2{\cdot}W_{2k} \\ &\quad + c_3{\cdot}W_{3k} + c_4{\cdot}W_{4k} + \eta_l + \varepsilon_{ijkl} \end{aligned}$$

with the indices

i = corn, rape, peas
j = without, with previous manuring
k = replications (fields) within i and j
l = year

where

W_1 = ln(clay/humus)
W_2 = ln(silt/humus)
W_3 = ln(fine sand/humus)
W_4 = ln(sand/humus)

and

N_{ijkl} is the N mineralization
μ is the general mean
PC_i is the fixed effect of previous crop of type i
MP_j is the fixed effect of previous manuring of type j
a_i, b, c_1, c_2, c_3, c_4 are regression coefficients corresponding to the covariates $MC(PC)_{k(i)}$, TN_k, W_{1k},W_{2k},W_{3k},W_{4k}
γ_{ij} is the general mean adjusted for the fixed effects and covariates
η_l is the random effect of the l'th year, $\eta_l \varepsilon NID(0,\sigma^2_{year})$
ε_{ijkl} is the random residual, $\varepsilon_{ijkl} \varepsilon NID(0,\sigma^2_{error})$
A bar above the variable indicates the mean of the variable.

A model for N mineralization was developed for each incubation period. The model was based on the analysis of covariance including those variables and covariates in model (1) that were significant at $p \leq 0.10$. The model parameters were estimated by least squares statistics.

The average prediction error for each incubation period was calculated as the square root of the averaged squared deviation between the observed and predicted value for each field.

The predicted value for each field was estimated by cross validation (Weisberg, 1985). In this procedure one observation was excluded and the model was fitted to the rest of the data. The excluded observation was then predicted using the fitted model. This procedure was repeated for each observation in turn.

RESULTS

Effects of previous crops, manuring, soil chemical characteristics and soil texture on N mineralization during soil incubation

Except for soil samples incubated for 28 days, the previous crop exerted a significant influence on N mineralization (Table 1). Manure amendments to the cover crop in the autumn, nested within previous crop, also were highly significant for N mineralization except for the incubation period of 28 days. The previous years' manuring and total soil N content

were not significant for N mineralization (Table 1). The variable W_4 = ln(sand/humus) was highly significant for N mineralization during all incubation periods. Variation between years was significant for all incubation periods except 140 days. Only two years were included in the analysis for that incubation period.

Table 1. Statistical significance of the covariates, effects of factors and interactions on N mineralization.

Source	Abbreviation	Significance of F-ratios [a] incubation period (days)			
		28	56	84	140[b]
Previous crop	PC	ns	**	***	**
Manure to previous crop	MP	ns	ns	ns	ns
Interaction PC and MP	PCxMP	ns	ns	ns	ns
Manure to cover crop nested PC	MC(PC)	ns	***	***	*
Total soil N	TN	ns	ns	ns	ns
ln (clay/humus)	W_1	*	ns	***	*
ln (silt/humus)	W_2	ns	ns	ns	*
ln (fine sand/humus)	W_3	*	ns	ns	ns
ln (sand/humus)	W_4	**	***	***	**
Year	η	***	*	***	ns
Coefficient of determination	R^2	0.46	0.53	0.62	0.68
Number of observations	n	59	53	53	23
Average N mineralization (kg N ha^{-1})		29.5	48.2	76.1	103.5
Experimental standard deviation (kg N ha^{-1})	s_ε	8.5	11.3	14.7	21.6
Standard deviation between years (kg N ha^{-1})	s_η	6.6	4.0	12.8	-
Average prediction error (kg N ha^{-1})	s_p	9.3	13.2	16.8	42.6

[a] Significance levels after removing the nonsignificant effects. *, **, *** significant at p = 0.10, 0.05, 0.01 levels, respectively; ns not significant.
[b] Analysis of the incubation period 140 days was based on the two years 1990 and 1991 only.

Modeling N mineralization

The prediction error was estimated for each incubation period (Table 1). The model included significant variables shown in Table 1. The prediction error for the first three incubation periods was of similar magnitude to the estimated experimental standard deviation, indicating that a good prediction of N mineralization can be expected. However, the model for N mineralization after 140 days of incubation had a prediction error that was about twice the experimental standard deviation. This high prediction error might be attributable to the limited number of observations. N mineralization predictions after 140 days of incubation (Table 1) are therefore expected to have large uncertainties.

Parameter estimates for three incubation periods, 28, 56 and 84 days, are shown in Table 2. The constant γ_{ij} describes the general mean adjusted for the fixed effect of the

Table 2. Parameter estimates of the model for prediction of N mineralization (kg N ha^{-1}) for three incubation periods. Included variables were significant at $p \leq 0.10$.

Days of incubation	Previous crop	Parameter estimated			
		γ_{ij}	c_1	c_3	c_4
28	all three	4.99	-4.18	6.71	5.03
		γ_{ij}	a_i	c_4	
56	corn	24.70	0.019	10.10	
	rape	28.71	0.145	10.10	
	peas	12.56	0.675	10.10	
		γ_{ij}	a_i	c_1	c_4
84	corn	39.52	0.706	-11.20	17.51
	rape	74.92	-0.432	-11.20	17.51
	peas	44.20	0.833	-11.20	17.51

γ_{ij} : Constant describing the average level combined with the effect of previous crop and covariates
a_i : Parameter corresponding to the covariate manure to cover crop for each previous crop $MC(PC)_{k(i)}$
c_1-c_4 : Parameters corresponding to the covariates, W_1-W_4

previous crop and the covariates. The regression coefficient a_i corresponds to the covariate manure to cover crop for each previous crop (MC(PC)) and c_1, c_3 and c_4 correspond to the covariates W_1, W_3 and W_4, respectively. For the incubation period 56 days the slope for the covariate manure to cover crop (a_i) was larger with peas as the previous crop than the slopes for the other previous crops, whereas the intercept (γ_{ij}) for peas was about half that of the intercepts for the two other previous crops. For incubation period 84 days the slope and the intercept for the previous crop corn and peas were about the same size. The intercept for the previous crop rape was about twice as large as the intercepts for the two other previous crops. The slope for the previous crop rape was negative.

As an example predictions of N mineralization after 56 days of incubation of two sample soil types are shown in Table 3. The natural log-ratio between sand and humus (W_4) was highest for the sandy loam soil, which increased the predicted N mineralization compared to the fine loamy soil. Increased amounts of manure to cover crop resulted in increased amounts of predicted N mineralization in both soils (Table 3). Especially with peas as the previous crop, N mineralization was highly influenced by the manure.

Table 3. Prediction of N mineralization for two soils for the incubation period of 56 days. Effect of previous crop, manure to cover crop and soil texture.

Previous crop	Manure to cover crop ($t\ ha^{-1}$)	Predicted N mineralization ($kg\ N\ ha^{-1}$) Soil texture	
		Fine loamy[a]	Sandy loam[b]
Corn	0	48.7	51.9
	15	49.0	52.2
	30	49.3	52.5
Rape	0	52.7	55.9
	15	54.9	58.1
	30	57.1	60.3
Peas	0	36.6	39.8
	15	46.7	49.9
	30	56.8	60.0

[a] Textural class - fine loamy, Typic Agrudalf, organic matter 3.1 %, clay 9.3 %
[b] Textural class - coarse sandy loam, Typic Haplumbrept, organic matter 2.5 %, clay 4.6 %

DISCUSSION

For soils incubated under uniform laboratory conditions (soil moisture, temperature and aeration), N mineralization rate was dependent on substrate quality and soil properties. Differences occurred in N mineralized during aerobic incubation of soils with different history of previous crops, manuring and soil properties made it possible to evaluate the effects of these characteristics on N mineralization.

This study demonstrated, in agreement with earlier incubation studies, a highly significant impact of soil cropping history and manuring on its N mineralization capacity. This also was observed by Beauchamp *et al.* (1986), Janzen and Radder (1989) and Vaidyanathan and Wilson (1992) but not by Bærug *et al.* (1973). Previous cropping practices had a significant effect on N mineralization, largely by their influence on indigenous organic matter quality (Janzen and Radder, 1989; Danso and Papastylianou, 1992).

Manure N retained in stalks, leaves and roots as well as that immobilized by soil microorganisms resulted in increased soil organic N and provided a residual N source for subsequent crops (El-Haris *et al.*, 1983). Thus, both previous crops and N in manure affected the quantity of mineralizable soil N available to the winter crop.

Several variables used in these models have been included in other models describing N mineralization. McCracken *et al.* (1989) similarly indicated that N mineralization was related to previous crops and manuring. The soil humus and texture factors were also significant in model development (Hansen *et al.*, 1990; Delphin, 1986; Freytag *et al.*, 1989). It has often been observed that N mineralization is slower in clay soils than in sandy soils (Debosz, 1994; Jenkinson, 1977; Hassink *et al.*, 1993).

Simplicity was an important prerequisite for this model development. The model therefore is not an exhaustive description of N mineralization. Rather, it can be used for prediction of N mineralization during soil incubation based on a few easily-measurable soil characteristics, previous crop and manure history. Although the model is simple, the prediction error was of similar magnitude to the estimated experimental standard deviation (except for the incubation period of 140 days), indicating that a good prediction can be expected.

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Models to predict nitrogen mineralization in soil

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Abstract

Modeling N mineralization in soil may serve both scientific and practical interests. Over the last fifty years, various models have been proposed to describe N mineralization. However, most of them have to be considered as research tools and few are proposed to predict N supply by soil under actual agro-ecological conditions. Development of models requires a strong cooperation between modelers and experimentalists; models have to be validated in the field and calibrated under various experimental conditions before their use as predicting tools.

WHY TO USE MODELS?

Nitrogen mineralization is one of the most important processes among N microbial transformations in soil. It influences N availability and N uptake by plants and may have consequences for N losses through runoff, leaching and denitrification. The respective importances of mineralization and humification processes are determined by the dynamics of soil organic matter. The soil N cycle is complex due to: (i) a synchronous occurring of different N microbial transformations (e.g. mineralization and immobilization or denitrification and immobilization); (ii) environmental influences (e.g. temperature, moisture, aeration) and agricultural practices. This complexity makes it necessary to analyze the N cycle in soil using mathematical or simulation models.

Models are of a scientific interest (Powlson, personal communication; Jenkinson and Smith, 1988). They can be considered as a tool for organizing knowledge. They allow to obtain a better conceptual understanding of complex problems and to test new concepts and hypothesis. They can be used to show gaps in present knowledge and promote new research strategies.

Models might also have a predictive value and can be useful to test changes and scenarios, for example, models to determine optimal strategies of N fertilization (Germon *et al.*, 1989; Jensen and Paustian, 1989; Neeteson *et al.*, 1989). On the other hand, expert systems for crop management (e.g. cotton, maize) which include both plant and soil simulation models provide insights about cropping systems (Kroll, 1992) and assist in the development of guidelines for best management practices.

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If models are a useful tool to understand and simulate complex transformations, they remain an imitation of a real system and cannot be a substitute for experiences and investigations.

WHICH KIND OF MODELS?

Since the forties different models have been published to describe or simulate N mineralization in soils (Tanji, 1982; Jenkinson, 1990; Molina *et al.*, 1994). Models differ in both type and complexity, but are characterized by the purpose for which they have been developed (de Willigen, 1991): empirical models (regression models) which fit a mathematical function to a set of data and mechanistic (deterministic) models which simulate one, several or all N transformations in soil. Mechanistic models include organic matter dynamics models (Molina *et al.*, 1994) and biological (e.g. food web) models which are concerned with N dynamics on a relatively short time scale (within years) or with annual N mineralization on a relatively small spatial scale (laboratory experiments, plots within fields...) (Hunt *et al.*, 1987; de Ruiter *et al.*, 1993).

Disadvantages of empirical models are their static nature: the re-calculation of coefficients and parameters is necessary if different sets of data are considered. By contrast, the dynamic nature of mechanistic models takes into account various conditions (e.g. environment, soil, practices) (Godwin and Jones, 1991) and integrate the notion of time.

The complexity of organic matter dynamics models depends on the number of organic functional pools considered. N mineralization has been described using one, two or several organic pools (Jenkinson, 1990). However, some models of decomposition (Bosatta and Agren, 1985) consider organic matter as a continuum. Pools are defined by their stability and position in the network (Molina *et al.*, 1994). The rates of C and/or N fluxes between the different pools has been described using first-order kinetics (Van Veen and Frissel, 1979) and/or Michaelis-Menten kinetics (Hunt *et al.*, 1985). While N mineralization has been simulated with one active pool (e.g. potentially mineralizable N proposed by Stanford and Smith in 1972), recent models are more complex and consider the role of the living component (e.g. microbial biomass) and the links between C and N transformations (McGill *et al.*, 1981; Molina *et al.*, 1983; van Veen *et al.*, 1984, Nicolardot *et al.*, 1994).

Most of the proposed models have to be considered as research tools and cannot be directly applied to answer practical problems. However, development of too simple models may induce the omission of important parameters and may present a limited interest if they cannot be used for various conditions.

LIMITATIONS OF MODELS

Modelers and users of models must be aware of the validity and limits of their tools. In preference model's components (e.g. parameters, coefficients, pools) must have a chemical, physical and biological meaning (Molina *et al.*, 1994).

The spatio-temporal scale for which the model has been designed has to be checked. Some models have been made to describe small-scale (e.g. incubation experiment) or large scale (e.g. regional models) systems. Another element of model's applicability is the resolution considered; some models properly describe long-term dynamics, but are unable to account for short-term evaluations (e.g. fast immobilization or re-mineralization).

N mineralization models have to be validated with field experiments under various soil and environmental conditions to be of practical use. Numerous field experimental data, especially if they include tracer data (e.g. ^{15}N), will allow to improve the validity and

performance of the model (Nicolardot and Molina, 1994). Finally, some soil models do not consider plant growth; their use to predict nitrogen mineralization under field conditions will require a link with plant and water balance models.

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The mineralization of N from finely or coarsely chopped crop residues: measurements and modeling

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Abstract

Sugar beet leaves, a mixture of sugar beet crowns and leaves, cabbage leaves or spinach leaves were either coarsely or finely chopped and mixed with either a sandy or clay soil and incubated at 20 °C under optimal moisture conditions for 24 weeks. The release of mineral N and the formation of microbial biomass were measured at intervals during the course of the experiment.

The release of mineral N was strongly dependent upon the amount of carbon and nitrogen added in the crop residues: spinach (C:N = 6) released most nitrogen and most quickly, cabbage leaves (C:N = 18) and the mixture of beet leaves and crowns (C:N = 42) released N more slowly. In most cases there was no significant difference in the decomposition rate of finely and coarsely chopped residues. An existing simulation model was able to simulate the mineralization or immobilization of N with the minimum of adjustment to its parameters using only information about the crop residues.

INTRODUCTION

Different crop residues decompose at different rates in soil depending upon their chemical composition. But it may be true that even the same residues decompose at different rates depending upon their physical state: how well they are mixed with soil for example. Large pieces of crop residue may not be broken down so quickly as small particles that lie more intimately in contact with soil. Finely divided residues may expose more of the soluble components within cells to bacteria outside. To test this we chopped residues either finely or coarsely, added them to soil and measured the rate at which they released nitrogen, comparing the results with simulations made with a computer simulation model of nitrogen turnover in soil which can take into account many of the chemical differences found between crop residues.

In: J.J. Neeteson and J. Hassink (eds.), 1994. Nitrogen mineralization in agricultural soils; proc. symp. held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993. AB-DLO Thema's, AB-DLO, Haren, pp. 245-253.

MATERIALS AND METHODS

Experimental

Beet leaves, including stems, were cut as closely as possible from the crowns; beet leaves, cabbage leaves and spinach leaves were first cut coarsely into pieces about 1.5 cm square and a sub-sample of each was more finely chopped in a food-processor. The entire beet crown sample was chopped finely in the food processor. Fresh crop residues (10 g) were added to moist soil (300 g) according to the following scheme: (i) & (ii) Finely or Coarsely chopped sugar beet leaves; (iii) & (iv) Finely or Coarsely chopped cabbage leaves; (v) & (vi) Finely or Coarsely chopped spinach leaves; (vii) & (viii) equal amounts of Finely chopped beet crowns with Finely or Coarsely chopped beet leaves; (ix) a control with no addition. In (vii) and (viii) note that a total of 20 g crop residues was added to soil. Table 1 shows the additions of N to soil from each crop residue.

Table 1. Initial composition of the added crop residues.

Soil	Residue ($\mu g\ g^{-1}$)	Organic N ($\mu g\ g^{-1}$)	NO_3-N	C:N[1]	Dry matter (%)
Sand	beet leaves	129	19.6	12	11.2
	beet crowns	36.9	11.8	69	21.5
	cabbage	71.7	14.7	18	9.71
	spinach	133	26.7	6	6.05
Clay	beet Leaves	133	20.3	12	11.2
	beet crowns	38.2	12.2	69	21.5
	cabbage	74.2	15.3	18	9.71
	spinach	138	27.7	6	6.05

[1] Assuming C 40 % of dry matter

The scheme was set up for two different soil types: a sandy soil from the Institute's experimental farm in Haren and a clay loam from the Dr. H.J. Lovinkhoeve experimental farm in Marknesse. Details of the soils are given in Table 2.

Table 2. Properties of the experimental soils.

			Particles					
Soil	C (%)	pH	<2μm	2-50μm	50-210μm	>210μm	N (%)	$CaCO_3$ (%)
Sand	1.66	7.16	4.4	2.6	77.6	15.4	0.106	0.6
Clay	1.87	6.98	48.4	24.5	26.0	1.1	0.168	0.4

Organic N in crop and soil was measured by the Kjeldahl method with the addition of salicylic acid and sodium thiosulphate to reduce nitrate; pH was measured in KCl. Pots were kept at a constant temperature of 20 °C and the moisture content kept at about 25 % (clay soil) or 20 % (sand soil) for 6 months. The entire contents of a pot were sacrificed for analysis after 1, 3, 6, 11, and 24 weeks. Sufficient pots were filled to allow two replicates to be sampled from each treatment and soil at each time. Ammonium and nitrate in soil extracts (1M KCl) were measured colorimetrically.

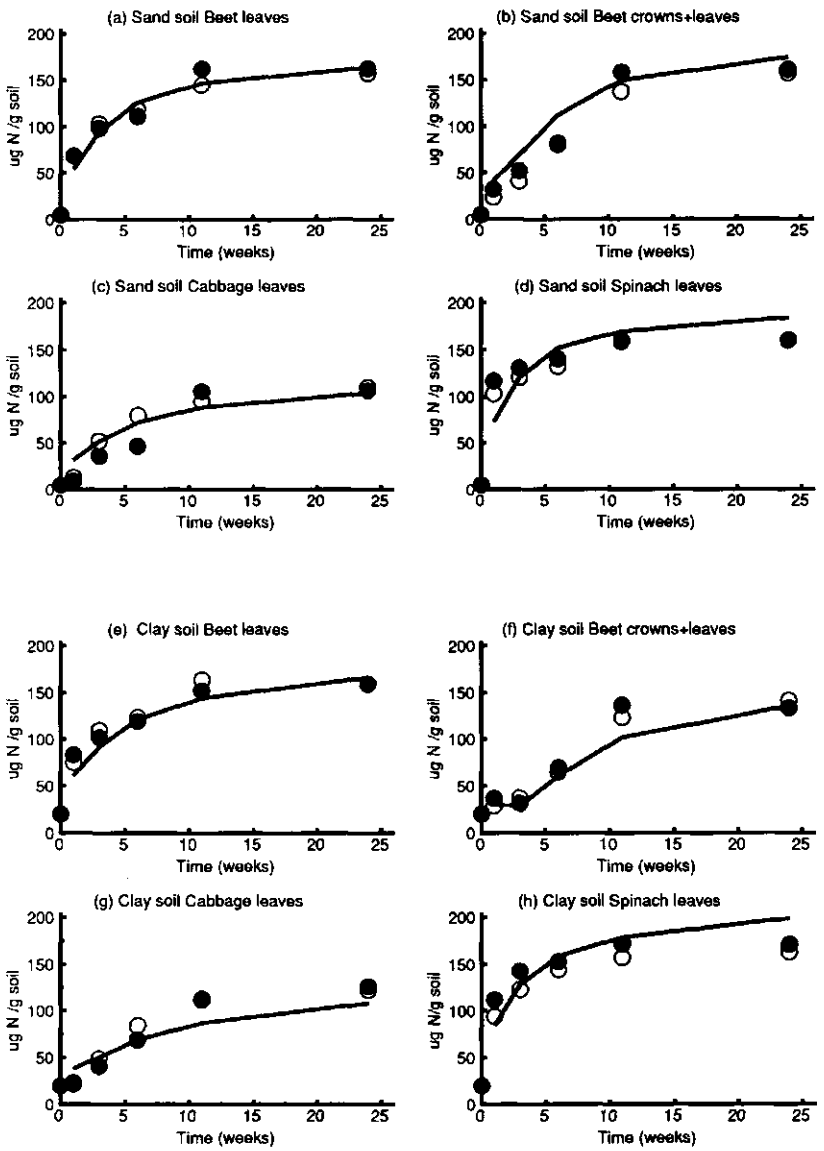

Figure 1. Mineral N found in soils with additions to sand soil (a-d) or clay soil (e-h) of: beet leaves (a,e), beet crowns + leaves (b,f), cabbage leaves (c,g), spinach leaves (d,h); open circles finely chopped, closed circles coarsely chopped, line model.

A sub-sample of 50 g of soil was taken for analysis of the carbon (Vance *et al.*, 1987) and nitrogen (Brookes *et al.*, 1985) in microbial biomass by Fumigation Extraction; crop samples were not removed from the soil prior to analysis. Biomass determinations, however, were unreplicated; a number of biomass C or N measurements were negative including some from the control soil (data not shown). Lignin, cellulose and hemi-cellulose in the original fresh crop residues were determined by the method of Van Soest (1963).

Statistical methods

We tested a null hypothesis that Finely and Coarsely chopped residues would mineralize at the same rate and then tested for deviations from this hypothesis with analysis of variance. The data are shown in Figure 1; there was no consistent difference between the experiments using coarse and finely chopped residues. The biomass data were very variable (see Figures 2 and 3) but nonetheless no significant effect of chopping could be found.

Accordingly the two experimental treatments (coarse and fine) may be used as replicates to test the computer model using a lack of fit test (Whitmore, 1991). In this test the null hypothesis is that the difference between model simulations and measurements is a non-significant component of the resultant residual sum of squares. The residuals are partitioned into one component due to random error in the data and another due to lack of fit of the model. The null hypothesis is rejected if lack of fit is found to be significantly greater than error using the F-test. Referring to Table 3 small results indicate a good fit, large values less good.

Table 3. Lack of fit between the model and measurements of mineral N, and biomass N and C.

Soil	Crop Residue	Mineral N	Biomass N	Biomass C
Sand	beet Leaves	3.7	2.5	1.2
	beet leaves + crowns	5.3*	3.0	7.9*
	cabbage	32.8***	1.8	1.4
	spinach	12.7**	11.2*	1.6
	combined	11.6***	1.93	1.98
Clay	beet leaves	8.0*	3.2	1.5
	beet leaves + crowns	21.7**	1.3	11.9
	cabbage	11.7**	4.4	1.6
	spinach	9.3**	21.1	3.4
	combined	11.2***	1.29	2.79*

* Significantly different by chance only 5 times in 100
** Significantly different by chance only once in 100
*** Significantly different by chance only once in 1000

The significance of the difference is indicated. This technique measures simultaneously: (i) deviations of the model significantly greater than the sum of the variances in all the measurements and (ii) bias in the model arising from (say) underprediction at the start of

an experiment and overprediction at the end. With good data it is a rather severe test for a model to pass because it tests whether the model and the data are identical; obviously they are not. For a discussion on this point see Whitmore (1991).

Modeling and model parameters

The organic matter turnover model of Bradbury *et al.* (1993) was used to estimate the decomposition and release of N from the crop residues. In this model organic matter in soil may belong to one of three compartments: fresh residues, microbial biomass or humified organic matter. Any organic carbon decomposing within the time-step of the model (1 week) may become either biomass (in proportion α), humus (in proportion β) or CO_2 (in proportion $1-\alpha-\beta$). Biomass and humus in the model have a C:N ratio which is fixed for the biomass at 8 in both soils but supplied from measurement for the humus and residues. Nitrogen flows follow those of carbon but whether any net mineralization occurs depends on the relative C:N ratios of the decomposing and resultant organic matter pools. To test the model the first-order decomposition rates, which were identical for both soils and all treatments, were: residues, 0.16 $week^{-1}$; biomass, 0.12 $week^{-1}$; humus, 0.00033 $week^{-1}$. In accordance with the formula given by Bradbury *et al.* (1993), $(\alpha+\beta)$ in the sandy soil was 0.32 but 0.426 in the clay soil. Decomposition is retarded if there is insufficient N available to meet the demand for incorporation of C into humus and biomass. Nitrate measured in the crop residues (Table 1) was assigned to the model's mineral N pool at the start of the simulations; note that the total N addition from residues is the sum of organic-N and nitrate-N (columns 3 and 4 in Table 1). The model makes no distinction between coarsely and finely chopped residues.

RESULTS AND DISCUSSION

Figures 1a-d (sandy soil) and 1e-h (clay) show the time course of the release of mineral N in the pot experiments. The mineralization of N from the crop residues was very similar in both soils and none of the experiments showed sign of net immobilization of N except the beet crowns and leaves mixture in the clay soil (Figure 1f). This treatment had the widest C:N ratio. The spinach residues released an enormous amount of N into the soil within 7 days and this must be caused at least in part by the large amount of nitrate present within the spinach leaves. Despite allocating all this nitrate directly to its mineral pool, the model underestimated the release of N from the spinach leaves. Clearly the organic residues in spinach are more readily decomposable than in the other crops.

The model fit by eye appears to be good in all cases but Table 3 shows that the ratio of lack of fit to error in many series was highly significant. Better fits might be obtained, especially to the cabbage leaves by optimizing parameters, but we have taken the view that the model, developed with data from long-term cereal experiments, should be tested in as nearly as possible its original state; crucially we wanted to see whether the same set of parameters could explain the mineralization of N from a range of different residues. Some of the reasons for the bias are explored at the end of this section. The bias accepted, 40 % of the simulations of mineral N were within 10 $\mu g\ N\ g^{-1}$ dry soil of the measurements and 80 % within 20 $\mu g\ N\ g^{-1}$.

Biomass carbon and nitrogen appear by eye to be not well estimated by the model (Figures 2 and 3). Interpretation is made difficult because no replicate determinations were made and because some data are negative (*e.g.* Figure 2d, 3e).

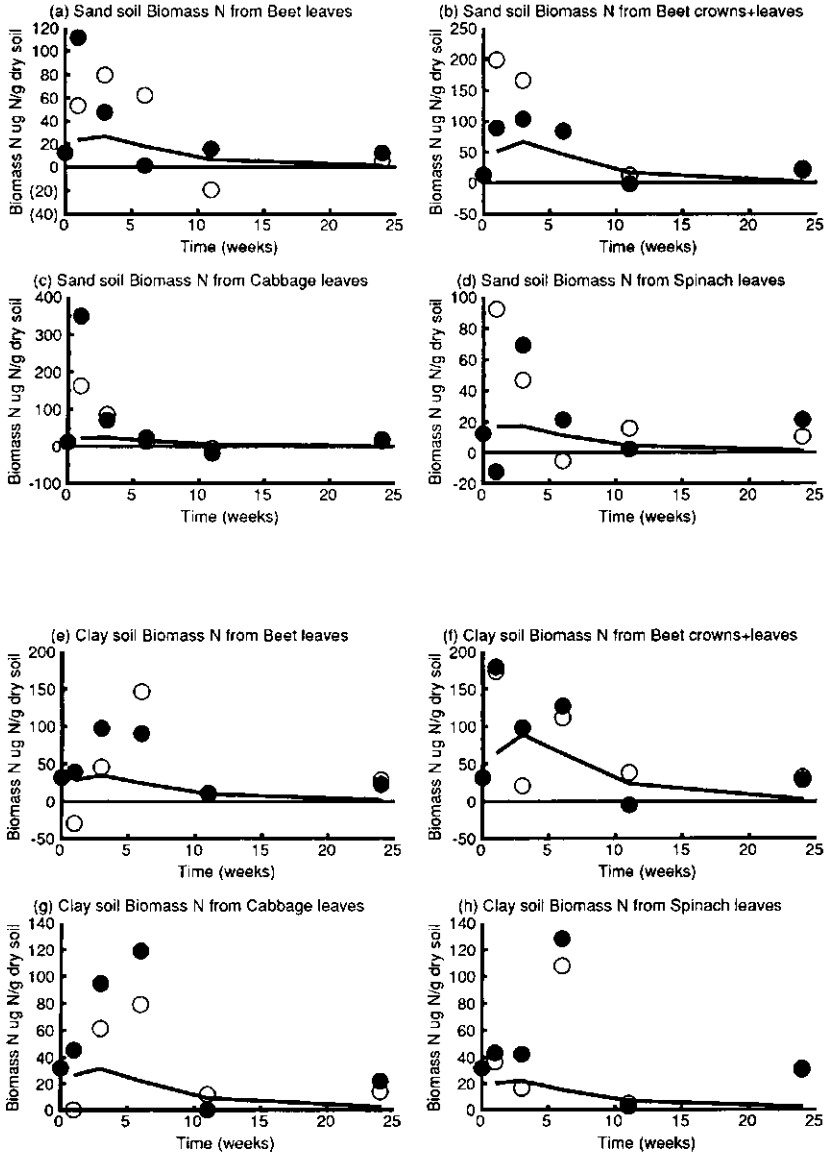

Figure 2. Biomass N found in soils with additions to sand soil (a-d) or clay soil (e-h) of beet leaves (a,e), beet crowns + leaves (b,f), cabbage leaves (c,g), spinach leaves (d,h); open circles finely chopped, closed circles coarsely chopped, line model.

No clear trends in the differences in the breakdown of coarsely or finely chopped residues appear in these data. The variation is large but appears to be random. Assuming that there is no systematic difference allows us to pool the data from the two treatments in

order to test the model but because the model is completely unable to simulate negative values these have been excluded. The lack of fit test suggests that the estimates made of biomass C and N with the model are usually within (a large) experimental error.

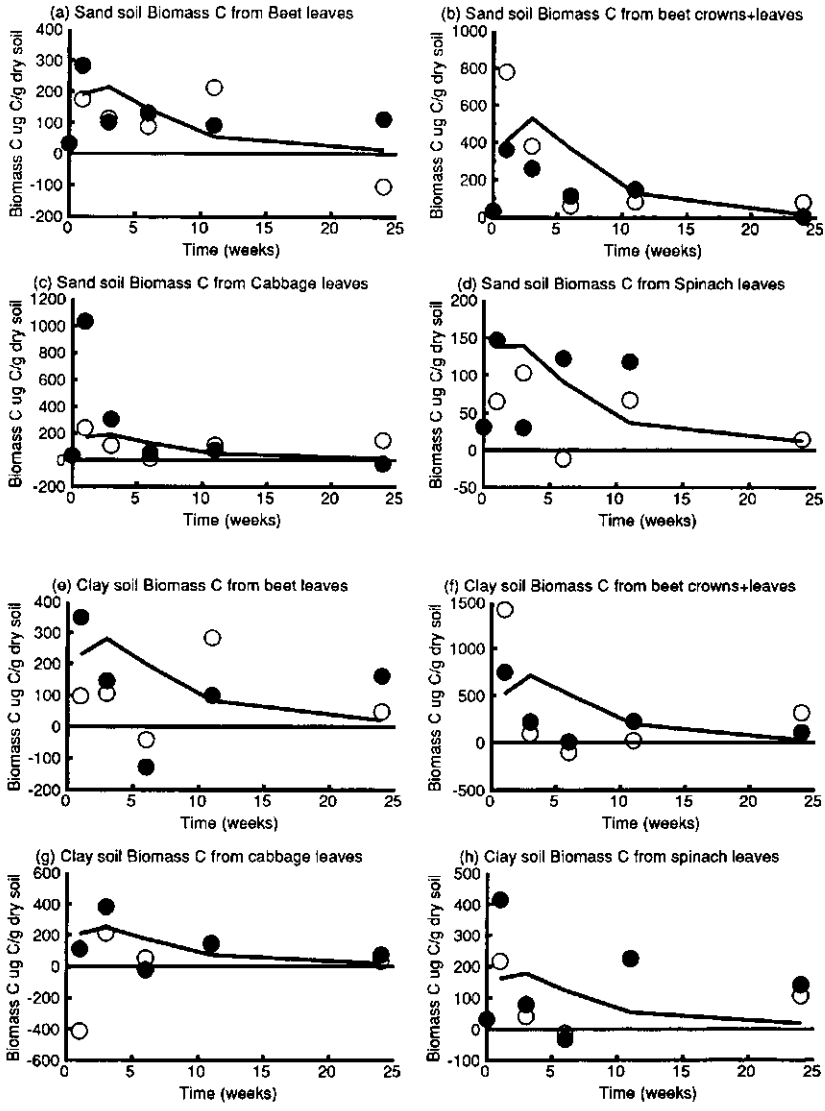

Figure 3. Biomass C found in soils with additions to sand soil (a-d) or clay soil (e-h) of: beet leaves (a,e), beet crowns + leaves (b,f), cabbage leaves (c,g), spinach leaves (d,h); open circles finely chopped, closed circles coarsely chopped, line model.

The size of this experimental error and the fact that it derives from pooled data do not allow firm conclusions to be reached, however. Some of the biomass N (but not C) measurements exceed the total addition (Figures 2b, c & g) yet the soils continue to mineralize N at the same time (Figures 1b, c & g). Background mineralization was about

0.3 μg N g^{-1} day^{-1} in both soils and cannot supply enough N to immobilize approximately ($\alpha+\beta$) of the added carbon, as required by the model, and yet mineralize N at the measured rate. There is clearly a discrepancy between the biomass N and mineral N measurements, so it is not surprising that the model appears to underestimate many of the biomass N data. Better fits might be obtained by reducing the C:N ratio of the biomass in the model or allowing it to vary. The measured C:N ratio of the biomass was on average 2.3 and varied very little from this value during the first 6 weeks of incubation. Reported C:N ratios for biomass are more often nearer 5 or 6 (*e.g.* Vanlauwe *et al.*, 1994) so it seems possible that some of the mineral N or residue N was interfering with the measurements of biomass N made here.

The amount of N added in residues (N_0) was found to account for 0 and 67.6 % of the variance in a regression on the amount of mineral N found in soil after 1 (N_1) and after 24 (N_{24}) weeks respectively. Adding in carbon as a term in a multivariate regression with N_0 improved the variance accounted for of N_1 to 86.6 % and for N_{24} to 91.4 %; the computer model presented here takes precisely these qualities into account. Adding in the cellulose content of the crop residues (Table 4) improved the prediction of N_1 to 97.7 % but no further improvement in the prediction of N_{24} could be obtained.

Table 4. Characterization of the crop residues.

Crop residue	Lignin		Cellulose		Hemi-cellulose	
	%	%N	%	%N	%	%N
Beet leaves	5.31	0.202	12.3	0.296	13.6	0.685
Beet crowns	1.11	0.0202	6.42	0.0557	5.52	0.123
Cabbage	4.13	0.113	14.2	0.161	5.19	0.256
Spinach	4.90	0.152	10.9	0.228	6.16	0.239

A very slightly less good relationship for N_1 was found by adding in a term for lignin in place of cellulose. These results suggest that provided the carbon content of the residues is similar as assumed here the C:N ratio plays a large part in determining whether N mineralizes or immobilizes immediately after addition of crop residues and that lignin and cellulose stabilize some of the crop residues against immediate breakdown. Including a recalcitrant fraction within crop residues in models (*e.g.* Verberne *et al.*, 1990) might improve the fit of our model with time where, as with the cabbage simulations, the model overpredicted at the start of the simulations but underpredicted at the end. Such bias in the model could also be removed by assuming the existence of a relatively small pool of active organic matter. Where N immobilizes at the start of an experiment (from say cabbage leaves) it could re-mineralize by the end, from a more active pool of dead organic matter than is currently present in the model. Interactions between active and less active organic matter may complicate the picture further (*e.g.* Vanlauwe *et al.*, 1994).

CONCLUSIONS

There appeared to be little or no difference in the rate at which nitrogen appears in soil from these crop residues whether they were finely or coarsely chopped. Chemical rather

than physical properties determined the rate of decomposition of these residues.

A computer model simulated the growth and N content of microbial biomass within (large) experimental error. The same model estimated the mineralization and immobilization of N well, with some bias but without the need to change any of the rate parameters controlling decomposition. More attention paid to recalcitrant fractions in crop residues and active (but not inert) fractions in soil might improve the model further but the current model is clearly able to simulate well the release of N from crop residues in soil.

ACKNOWLEDGEMENTS

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Modeling nitrogen dynamics in crop rotations in ecological agriculture

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Abstract

A model is described to calculate the availability of nitrogen in a crop rotation. It includes a simple water balance to estimate soil moisture and water leaching. Mineralization of initial soil organic matter and of the consecutive additions of crop residues and organic manure to the soil are calculated using Janssen's one parameter formula for the decomposition of organic matter (Janssen, 1984) with a correction for soil moisture and temperature. Crop uptake is based on actual yield data and derived amounts of crop residues and roots. Fixation by legume crops is estimated. Then a mineral nitrogen balance is made including mineralization, deposition, fixation, uptake and (leaching) loss.

The model is applied on 11-year data from a rotation on an experimental field of the Department of Ecological Agriculture of Wageningen Agricultural University.

According to the model enough N was released to supply the crops, measured mineral N (0-25 cm) in 1992 coincided well with model results in 6 of 7 plots and after 10 years no difference was found between the means of measured and calculated organic matter contents.

INTRODUCTION

In ecological agriculture no artificial N fertilizers are used and the availability of nitrogen at a certain moment is determined by farmer's decisions taken long before that moment. Many processes and factors determine how much nitrogen is released and which part is actually taken up or lost. Although many of these are largely understood, it is difficult on farm level to distinguish the influence of crop rotation, manuring and tillage on release, uptake and loss of nitrogen and to adapt successful strategies from one farm to specific conditions at other farms. This understanding and adaptation will be easier if the most important factors are integrated into a reliable calculating model. If the model is based on farm specific information and the calculated release and loss of nitrogen fit in the observed crop performance, the model can be used for evaluating and redesigning farm organization and management. If they do not, the model c.q. the concept, should be

In: J.J. Neeteson and J. Hassink (eds.), 1994. Nitrogen mineralization in agricultural soils; proc. symp. held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993. AB-DLO Thema's, AB-DLO, Haren, pp. 255-268.

reconsidered. In this paper we present the result of our effort to construct such a model. It is more extensively explained and discussed in a report by Habets and Oomen (1993).

MATERIALS AND METHODS

Overview of the calculations (Figure 1)

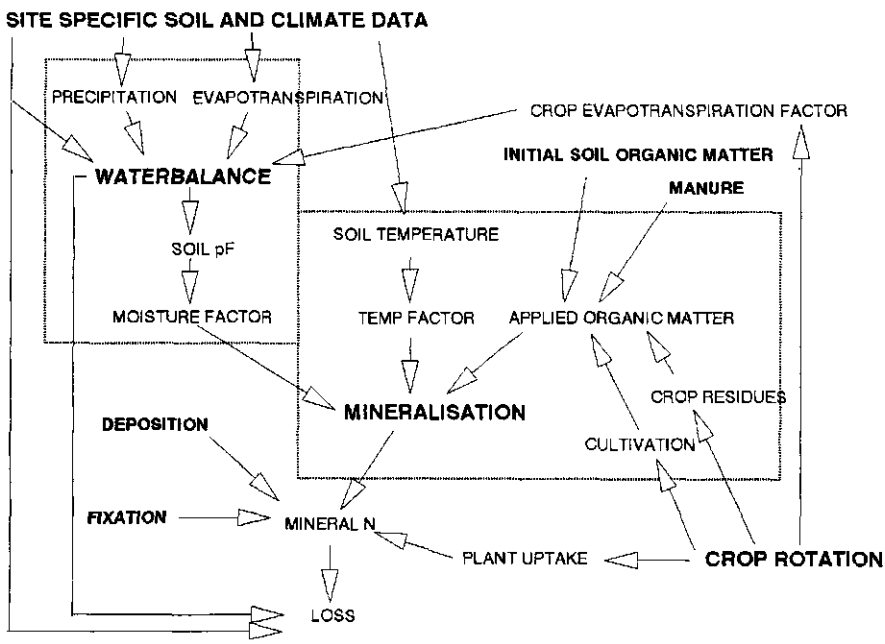


Figure 1. Schematic representation of information flow and calculating sequence in the N-DICEA model.

The Nitrogen-Dynamics model was built around the calculation of the mineralization of organic matter with the formula of Janssen (1984). A simple two-layer-model of soil was chosen. In the top layer tillage and application and mineralization of organic matter take place. The second layer is important for storage of water and nutrients. It reaches to where crops can take up water and nutrients. Most input data were derived from the farm administration and taken from the nearest weather station. Some soil parameters had to be estimated.

Water balance

The actual evapotranspiration is calculated using:

$$ET_{act} = ET_{pot} * f_{crop} * f_{pF} \quad (1)$$

ET_{act} = actual evapotranspiration
ET_{pot} = potential evapotranspiration

f_{crop} = correction factor for crop development stage
f_{pF} = correction factor for soil pF.

The potential evapotranspiration calculated according to Penman's formula is taken from the nearest weather station and the crop factor is taken from Hooghart (1987). A reduction factor for soil pF is based on the pF of the second layer: the evapotranspiration is reduced as soon as the pF of the second layer exceeds 2.7. The crops take water from both layers, 75 % of the maximal amount ($ET_{pot}*f_{crop}$) from the top layer as long its pF < 2.7. Extraction of water from the top layer decreases linearly between pF 2.7 and 4.2 from 100 % to 0 % of 0.75 * $ET_{pot}*f_{crop}$ (Van Huet, 1983). That of the second layer from 100 % to 0 % of ($ET_{pot}*f_{crop}$ – the extraction from top layer).

Precipitation is added to the top layer. After each time step the moisture content and pF are calculated for both layers using (Driessen, 1988):

$$\psi = e^{\sqrt{1/GAM*\ln(SMO/\theta)}} \quad (2)$$

$$pF = -\log(\psi) \quad (3)$$

ψ = matrix suction (cm)
θ = soil moisture content (m^3/m^3)
SMO = saturated soil moisture content (m^3/m^3)
GAM = texture specific constant

Water above field capacity present at the end of a time step is moved to the deeper layer. Water moved from the second layer is considered lost.

Decomposition of organic matter

The formula of Janssen (1984) is based on empirical data of Kortleven (1963), who studied the yearly decomposition of applied organic matter in bare soils. When *C* represents the total amount of organic carbon added to the soil, then the decomposition rate is:

$$\frac{dC}{dt} = -k*C \quad (4)$$

Janssen assumes a decreasing decay rate **k** for every organic application to the soil. How the decay rate decreases is characterized by the "apparent initial age" a of each kind of organic matter.

$$k = 2.82*(a+t)^{-1.6} \quad (5)$$

To get an impression of the mineralization within the year we chose a timestep of 10 days and added correction factors for temperature and soil moisture content.

$$C_t = C_0 * e^{4.7*[(a+fT*f\Theta*t)^{-0.5}-a^{-0.5}]} \quad (6)$$

C_0 = amount of added organic carbon [kg]
C_t = remaining amount of organic carbon at time t [kg]
fT = temperature correction factor [-]
fΘ = moisture correction factor [-]

Temperature correction factor

We assume that mineralization is mainly a biological process and decreases to nil at 0 °C. Therefore we correct for temperature by use of a modified Arrhenius approach (formula 7). Using the soil temperature data at 10 cm depth obtained from the weather station we matched the Arrhenius approach to the decomposition on a year-basis in a bare (continuously moist) soil according to Kortleven (1963) by changing the reference temperature and adding an extra constant:

$$fT = e^{-9000*(\frac{1}{T}-\frac{1}{282})}-0.349 \quad (7)$$

fT = temperature correction factor [-]
T = temperature [K]

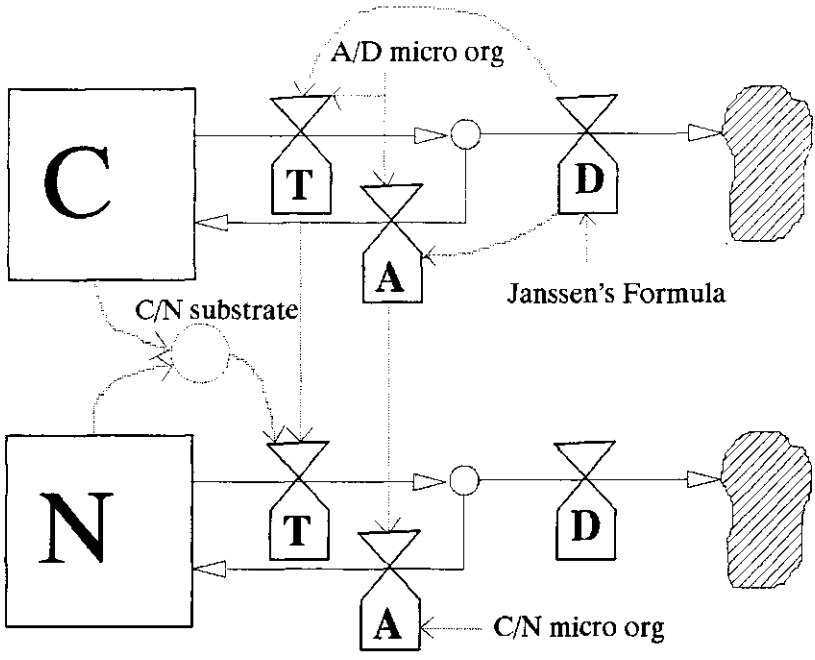
Moisture correction factor

The moisture correction factor for the mineralization rate according to Rijtema (1980) is equal to 1 up to pF 2.7 and then decreases linearly to 0 at pF 4.2.

Cultivation effect

Quantification of the effect of cultivation on mineralization is difficult because it seems to depend on so many complex factors like texture and soil condition during and after the cultivation, influenced by the weather, depth and type of cultivation. It is not yet clear what the origin of the effect is. Probably organic matter is gradually protected from decomposition by the formation of a more resistent skin and enclosure by soil particles. By cultivation, but also by drought and freezing a part of this protected organic matter can be freed (Haynes, 1986). In the model effects of plowing and seed bed preparation are included by taking a fixed fraction of the initial organic matter (450 kg) and adding it again with a lower apparent initial age (2.45 versus 24) each time the soil is cultivated. The tentative fixed amount is based on experiments with and without tillage in clay soils (Titulaer and Boone, 1984; Bakermans and De Wit, 1970).

Calculation of N-mineralization (Figure 2)



C=Carbon; N=Nitrogen; A=Assimilation; T=Turn-over; D=Dissimilation

Figure 2. Flow diagram regarding the relation between carbon and nitrogen mineralization.

In Figure 2 C and N are state variables, T, A and D are rate variables. Janssen's formula describes the rate of net dissimilation D of the organic matter. C is an amount of organic carbon. At the moment of application this is equal to the applied amount C_0. As mineralization proceeds the remaining carbon will contain less carbon in original organic matter and more in the form of decomposition and conversion products and biomass. The rate of consumption of organic carbon is indicated by T (turnover, Figure 2). Assimilation A indicates the part of the organic carbon that is, modified or not, reused as organic compound. Knowing D, the assimilation to dissimilation ratio (AD_{micro}) determines A and T.

$$A_C = AD_{micro} * D_C \tag{8}$$

$$T_C = (1 + AD_{micro}) * D_C \tag{9}$$

Assimilation of nitrogen is related to assimilation of carbon by the (assumed constant) C to N ratio of the microorganisms (CN_{micro}).

$$A_N = \frac{1}{CN_{micro}} * A_C \tag{10}$$

The turnover of nitrogen is related to the turnover of carbon by the (time depending) C to N ratio of the substrate C/N.

$$T_N = \frac{1}{C_t / N_t} * T_C \tag{11}$$

The difference between turnover and assimilation of nitrogen gives the mineralization of nitrogen.

$$D_N = T_N - A_N = [\frac{(1+AD_{micro})}{C_t / N_t} - \frac{AD_{micro}}{CN_{micro}}] * D_C \tag{12}$$

Substituting D_N = dN/dt, D_C = dC/dt and integrating with C_0 and N_0 as the initial amounts of carbon and nitrogen formula 13 gives a relation between C and N in the substrate, where C as a function of time was described earlier.

$$(\frac{1}{CN_{micro}} - \frac{1}{C/N}) = (\frac{1}{CN_{micro}} - \frac{1}{C_0/N_0}) * (\frac{C}{C_0})^{AD_{micro}} \tag{13}$$

The relation between the net dissimilation of carbon D_C and of nitrogen D_N (formula 14) shows that depending on C_0/N_0 of the substrate and C/N and A/D of the microorganisms, initially net mineralization or immobilization of nitrogen can occur.

$$D_N = [\frac{1}{CN_{micro}} - (AD_{micro}+1) * (\frac{1}{CN_{micro}} - \frac{1}{C_0/N_0}) * (\frac{C}{C_0})^{AD_{micro}}] * D_C \tag{14}$$

Nitrogen balance

A nitrogen balance for the mineral nitrogen was made using the calculated mineralization (initial organic matter, crop residues, manure), addition of chemical fertilizer and deposition as inputs. Plant uptake from the soil and losses are outputs.

For every type of crop a logistic nitrogen uptake curve is assumed from sowing date to harvest date. Nitrogen uptake from top- and second layer is based on equal proportionality for both layers to water uptake and mineral nitrogen concentration.

$$NUPT_1 = MINIMUM\left(\frac{NUPT_{pot}}{1+\frac{UPT_2}{UPT_1}*\frac{N_2}{N_1}*\frac{M_1}{M_2}}, N_{avail,1}\right) \quad (15)$$

From the second layer:

$$NUPT_2 = MINIMUM(NUPT_{pot} - NUPT_1, N_{avail,2}) \quad (16)$$

$NUPT_{1(2)}$ = N-uptake from layer 1(2) [kg/ha]
$NUPT_{pot}$ = potential N-uptake [kg/ha]
$UPT_{1(2)}$ = water uptake layer 1(2) [mm]
$N_{1(2)}$ = mineral N-stock layer 1(2) [kg/ha]
$M_{1(2)}$ = total water amount layer 1(2) [mm]
$N_{avail,1(2)}$ = available mineral nitrogen layer 1(2) [kg/ha].

Nitrogen fixation is not a mineral input to the soil. The nitrogen uptake from the soil is calculated as the nitrogen in the crop minus the nitrogen fixed. The fixation by legumes is found by trial and error in the following way: the legumes fix so much nitrogen that at the end of their growing season 10 kg N is left in the topsoil.

Losses are proportionate to the water outflow and the mineral nitrogen concentration in the layer. The N concentration is calculated after each time step by mixing the residual and added water. Water transport is not homogeneous through the soil, a larger part of the water will follow larger pores and cracks, while mineral nitrogen is also present in smaller pores. Therefore a "leaching" factor is introduced for every layer. This phenomenon is still under research and literature values are difficult to find. We estimated a leaching factor of 0.7 for the top layer and, assuming that the phenomenon is less important there, a leaching factor of 0.9 for the second layer. Denitrification is not included in the model as far as it is not proportional to the water outflow. The losses are calculated according: water outflow * nitrogen concentration * leaching factor.

Experimental field

The experimental field has a size of 2.3 ha divided into seven plots of about 0.34 ha. From 1982 on, data on fertilizer and manure application, important cultivation measures and crop yields have been recorded. Ecological agriculture was started in 1984. The soil consists of heavy clay and is well drained; there is never any water logging. The availability of phosphate is good to excellent, probably due to extensive manuring in the past. The availability of potassium is moderate, due to centuries of depletion. The availability of magnesium is good and pH is about 7. The manure was produced by five steers housed in a deep litter stable. The feed and about 50 % of the bedding material (straw) was produced on the experimental field.

Organic matter contents of all plots were measured in 1984 and 1993 according the wet oxidation method with $KMnO_4$. Yields and occasionally also nitrogen contents have

been measured. Distribution of dry matter over plant parts and nitrogen contents were based on relations found in experimental data in a similar situation (Verveda, 1984). In 1992 monthly samples of the topsoil for mineral nitrogen were taken, immediately dried at 40°C and analyzed later, for comparison with model results.

Sensitivity analysis

Not all data and parameters used in the model are accurately known. A sensitivity analysis was performed to examine the effect of possible deviations from real values on model results. It gives an impression of the spreading and the reliability of the results and indicates to which data most effort should be spent in order to find accurate values and figures. The average yearly mineralization and the average yearly loss were used as output variables.

RESULTS

Sensitivity analysis

Calculated average yearly mineralization is very sensitive to the choice of C/N ratio of the microorganisms and somewhat sensitive to the A/D ratio of the microorganisms. It is also relatively sensitive to the nitrogen content of the applied organic matter. Deviations in the apparent initial age have a proportional effect on mineralization. Differences in soil parameters and water uptake have little influence. Under Dutch circumstances reduction of mineralization by drought is seldom high enough to cause a significant difference in mineralization due to different soil types.

Calculated average yearly loss of nitrogen is sensitive to errors in estimating soil parameters, but less sensitive to errors in estimating the leaching factor. Parameters strongly influencing nitrogen mineralization also strongly influence nitrogen leaching. Distribution of water uptake from top and second layer has also a strong influence on leaching of nitrogen.

Organic matter development

The initial organic matter is added to the soil at the beginning of the calculation period. Decomposition then follows seasonal fluctuations. Crop residues are added every year and stable manure in some years. This results in a pattern of stepwise increase and gradual decrease of the total amount of soil organic carbon and nitrogen (Figure 3). On plot 5 the organic carbon level fluctuated around a level of about 50 000 kg ha^{-1}. The average of measured organic matter contents in 1984 (3.09 %) and 1993 (3.14 %) also showed no significant increase for the experimental field.

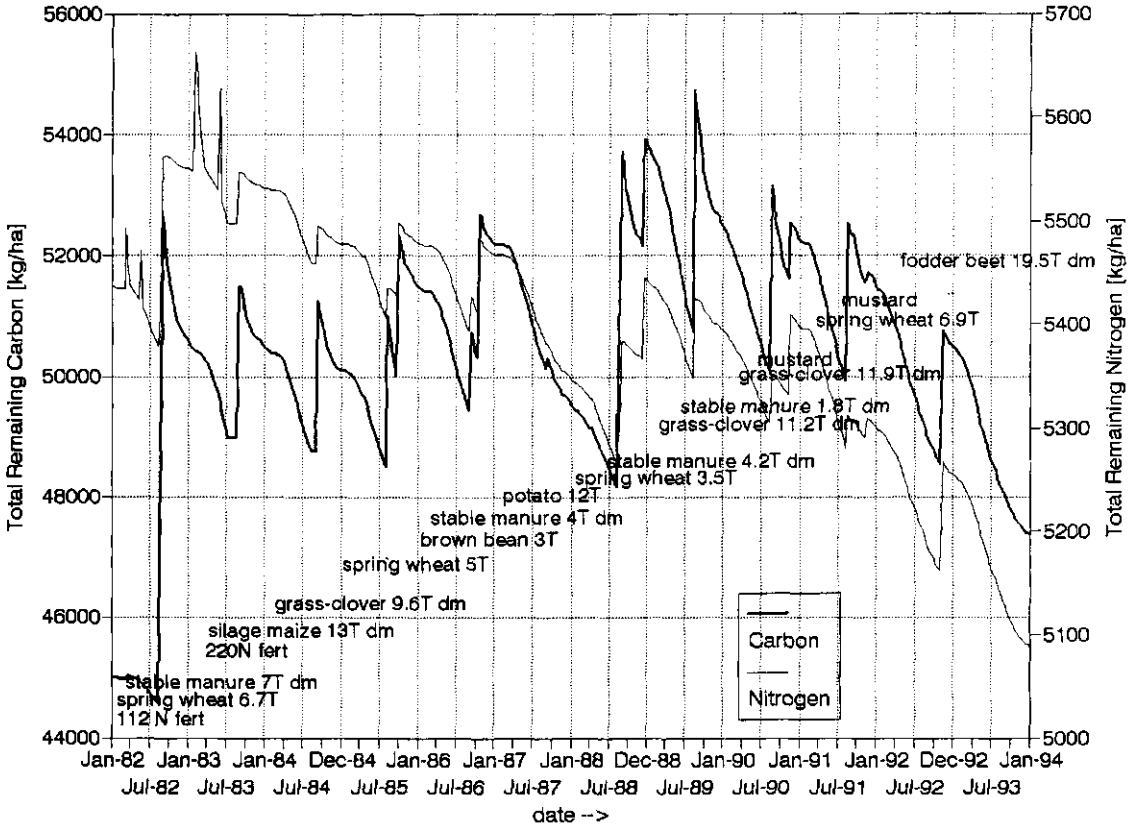


Figure 3. Total remaining soil organic carbon and organic nitrogen over 1982-1992 for plot 5.

Mineralization, uptake and loss of nitrogen

The amount of mineral nitrogen is equal to initial stock plus cumulative mineralization plus cumulative deposition minus cumulative loss minus cumulative plant uptake. This amount appeared to remain positive throughout most years for all the plots (Figure 4). This means that also according to the model the crops were able to find the nitrogen they actually took. It has to be remarked that the nitrogen in leguminous crops can come from either soil uptake or fixation as explained earlier. The cumulative available line shows a breakpoint after the conversion to ecological agriculture in 1984. After the conversion the line as a whole is less steep during grass-clover periods and steeper and more fluctuating during beans and root crops.

Details of available nitrogen, uptake and mineral nitrogen

Detailed results are shown for plot 5 in 1992 (Figure 5). On this plot fodder beets were grown. A very high yield of 19.5 ton dry matter of beet was reached. The uptake line touches the available line end of July. So, according to the model the real yield could not be reached, but deep rooting might have enabled fodder beets to take up water and

nitrogen from below the second layer. The model results are supported by what was observed in the field: in August the crop was lighter green turning to darker green again at the end of September, when mineralization exceeded uptake.

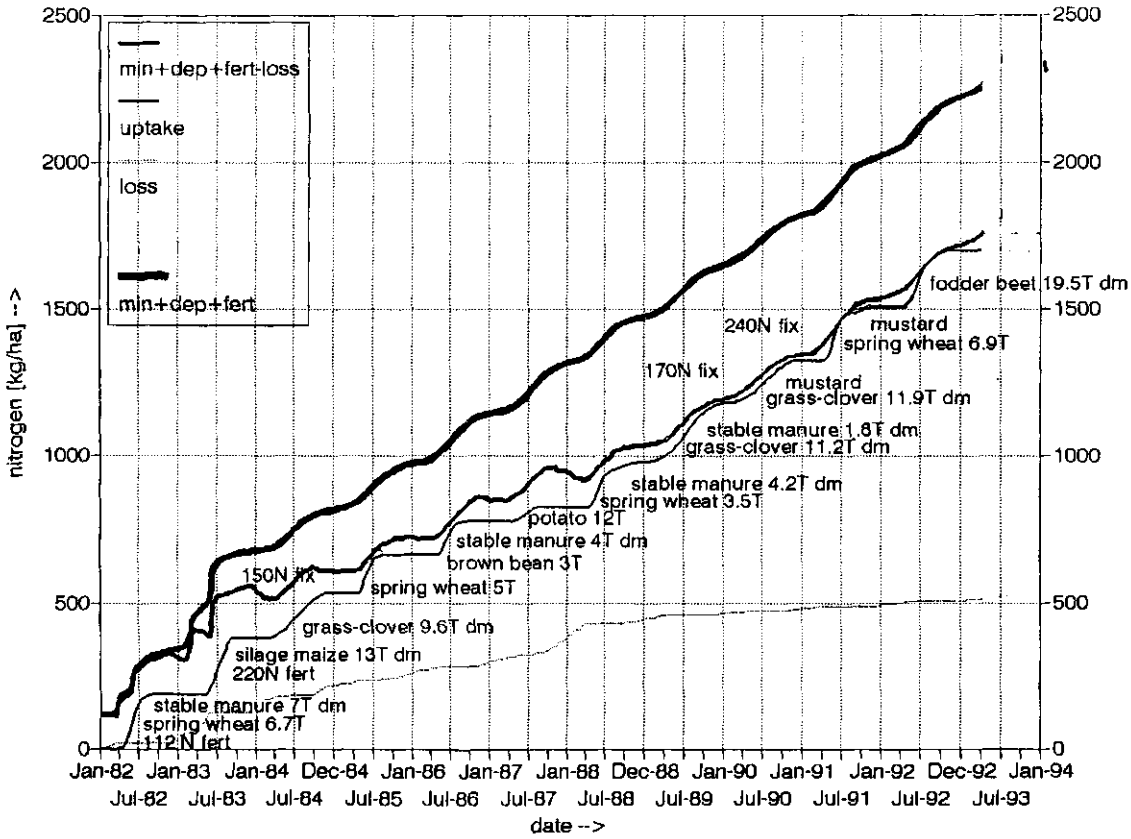


Figure 4. Cumulative availability and balance of mineral nitrogen over 1982-1992 for plot 5.

Comparison of calculated and measured values of N mineral

Measurements of the mineral nitrogen in the top layer coincide well with the model results for all seven plots except potatoes on plot no 2 (Figure 6).

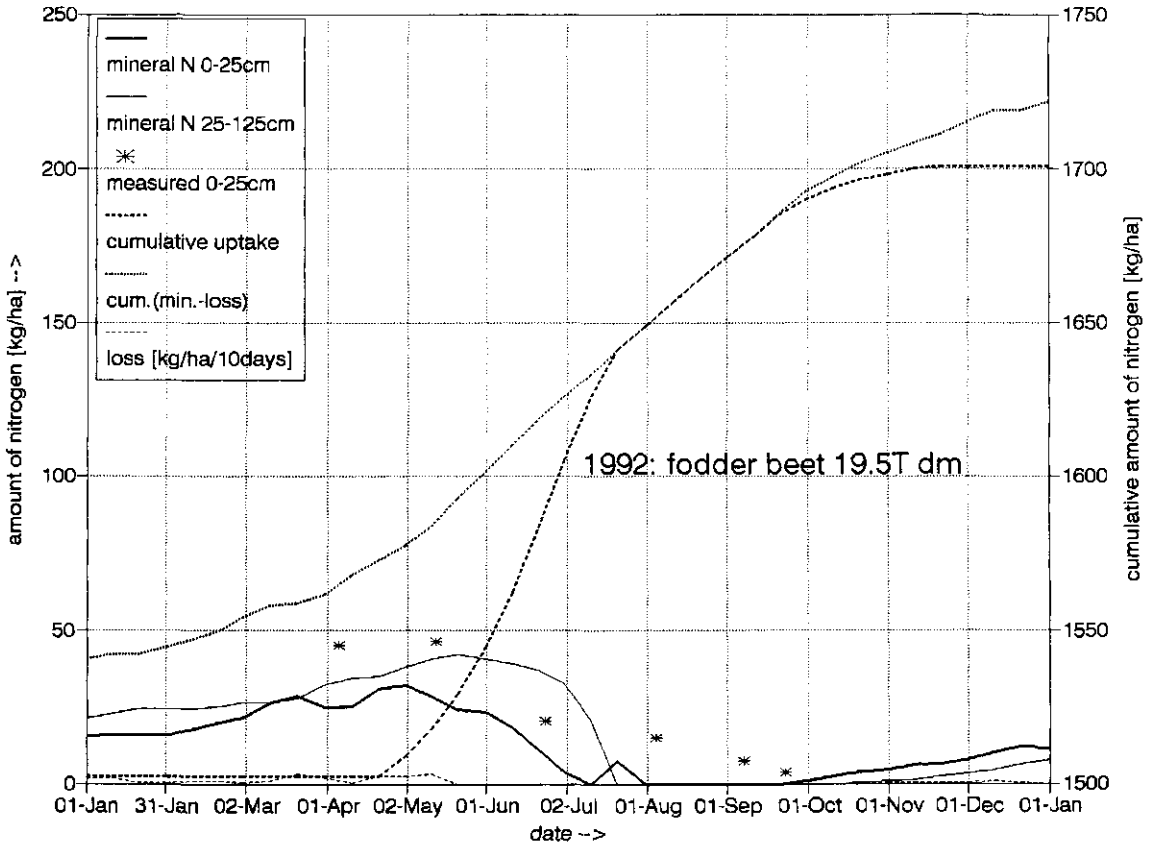


Figure 5. Detailed nitrogen availability and balance of mineral stock in 1992 for plot 5.

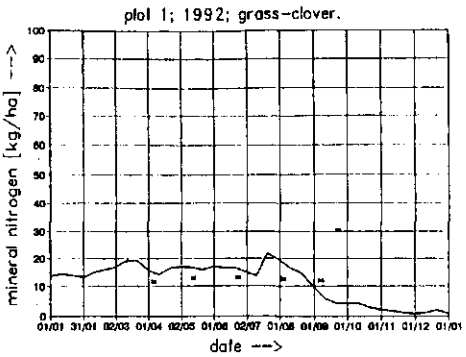
DISCUSSION

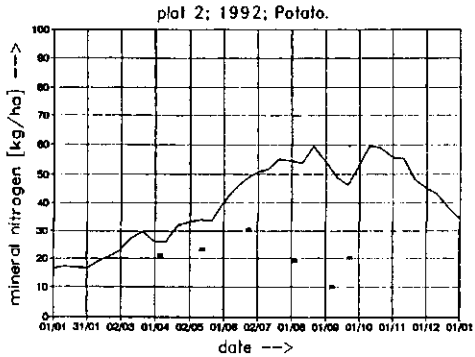
Validation of the calculating procedure

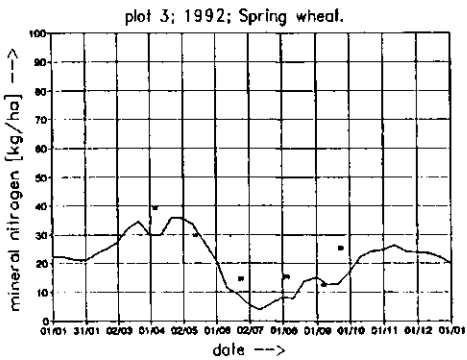
The results do not lead to a rejection of the calculating procedure: 1) according to the model the crops could nearly always find the nitrogen they actually found, 2) the measured values of the mineral nitrogen in the top layers coincided well with calculated values, and 3) neither the model nor the measurements indicate a change in organic matter content of the field.

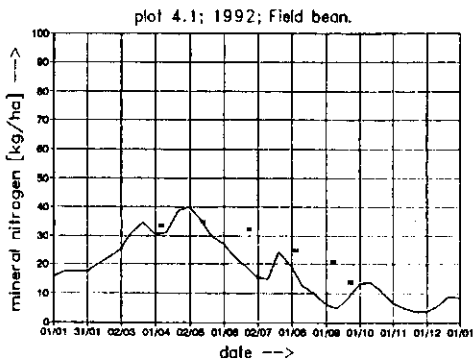
Critical notes to the procedure

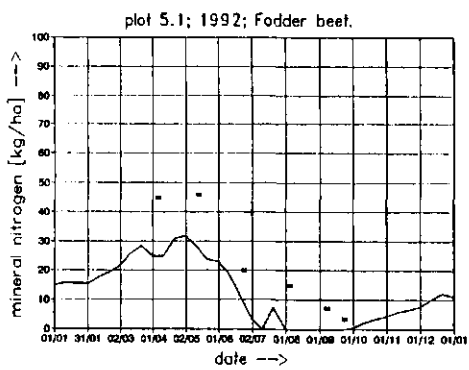
- Farmers have no data on amounts and nitrogen contents of crop residues. In the model these data are based on yield-related standard dry matter distributions and nitrogen contents for different crops originating from a limited amount of experimental values.

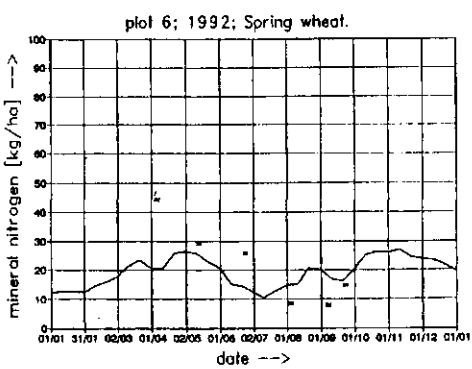












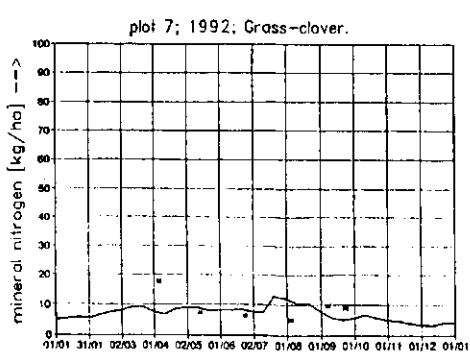


Figure 6. Comparison of calculated and measured values of N mineral in 1992.

- Values of the apparent initial age for different types of organic matter are not well known. Only distinction between a few groups of material is made.
- The model assumes a standard rooting pattern. In practice every crop may have a different rooting pattern and a different distribution of uptake from top and second layer. Shallow rooting crops probably will not take up all "available" nitrogen in the two layers, while deep rooting crops can retrieve nitrogen that was already leached according to the model.
- The way the influence of cultivation is incorporated in the model needs improvement. At the moment it is connected to the initial organic matter, of which a fixed fraction is rejuvenated. Probably after some years an important part comes from younger organic matter.
- The calculation of nitrogen mineralization from the mineralization of organic matter depends on the following assumptions:
  a) material is converted into new material with a constant C/N ratio of 10,
  b) within time steps nitrogen mineralization is proportional to mineralization of organic matter, after every time step the assimilated biomass is mixed homogeneously with the remaining organic matter, so that of all remaining fractions a proportionate part is decomposed. These assumptions are discutable in case of the calculation of the decomposition of fresh material just after application.

CONCLUSION

It seems that this extended and modified approach of Janssen can be used to describe nitrogen availability in a crop rotation on clay soils. This approach is based on information about cropping history, weather data and estimates of soil properties. Before using the model for evaluating and redesigning farm organisation and management it should be tested on more complete datasets.

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Mineralization of sugar beet and bean residues in laboratory incubations, comparison of measured and simulated results

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Abstract

A laboratory experiment was carried out to investigate the dynamics of N release from sugar beet and bean residues. The time course of N mineralization was initially described in terms of a single negative exponential function. This proved to be an unsatisfactory model to simulate the observed pattern of mineral N release. The application of sugar beet leaves with a C to N ratio of around 15 resulted in a N immobilization at the outset of the incubation while the application of bean leaves with a comparable C to N ratio resulted in a constant net N mineralization. The application of sugar beet roots with a C to N ratio of 40 gave an increase in N mineralization in excess of the amount organic N applied.

A carbon and nitrogen mineralization model was developed in which the N mineralization depended on the C mineralization, the C to N ratio of the organic material applied and the C to N ratio of the soil microbial biomass. The model simulated the N mineralization of different soils accurately when various forms of organic material, such as sugar beet leaves, bean leaves and roots, and mustard leaves, were added. The N mineralization of soil amended with sugar beet roots, however, was not satisfactorily simulated. Simulated values were much lower than measured ones. In order to increase the application possibilities of the model, further experimental evidence of some of the parameters used, such as a soil organic matter and the microbial biomass fractions, and a more dynamic microbial biomass is required.

INTRODUCTION

The timing and the magnitude of N mineralization from organic N in plant materials is still relatively unpredictable despite considerable research in this field. Analysis of N dynamics is important in relation to N demands by the crop and to the fate of organic and mineral N left in the field after harvesting. One of the important parameters which have to be

In: J.J. Neeteson and J. Hassink (eds.), 1994. Nitrogen mineralization in agricultural soils; proc. symp. held at the Institute for Soil Fertility Research, Haren, NL, 19-20 April 1993. AB-DLO Thema's, AB-DLO, Haren, pp. 269-274.

known to predict the N mineralization potential of farm wastes is the C to N ratio (Mellilo *et al.*, 1989). The objective of the present study was to develop mathematical functions describing N mineralization from crop residues. Therefore, an experiment was carried out in which sugar beet leaves showing a range of initial C to N ratios and obtained from field treatments which had received different applications of fertilizer N, were added to a sandy loam soil. Sugar beet roots and residues of beans were also used as experimental materials.

MATERIAL AND METHODS

Model structure

The model was based on models developed by Paul (1984) and Van Veen *et al.* (1985) and explained more in detail in earlier work (Dendooven, 1990). The model considered three main entities: applied organic material, soil organic matter and microbial biomass, each containing three fractions : active, resistant and stable. Sixty percent of the decomposed organic C (Van Veen *et al.*, 1985) was incorporated into the microbial biomass while the rest evolved as CO_2. The amount of mineralized N was the result of decomposed N (determined by the organic material C to N ratio) minus assimilated N (determined by the organic C to N ratio of the microbial biomass). Dying micro-organisms replenished soil organic matter which then became new substrate. The microbial biomass was supposed to be 3 % of the soil organic matter (Anderson and Domsch, 1989) with a C to N ratio of 8 (Jenkinson and Ladd, 1981). The size and turnover rates of the soil organic matter and the microbial biomass fractions were derived from the literature (Paul, 1984; Van Veen *et al.*, 1985).

Soil

Soil samples were collected on 17 October 1988 from the 0-30 cm of the Ter Munck soil nearby Louvain (B). The Ter Munck soil is a silt loam soil with a organic C and N content of 7.5 g kg^{-1} and 0.8 g kg^{-1} respectively. The inorganic fraction contained 119 g kg^{-1} clay, 765 g kg^{-1} loam and 116 g kg^{-1} sand.

Organic material

Crop residues of sugar beet and beans were collected in the field randomly. The material was chopped into pieces of approximately 5-10 mm square. Sugar beet leaves were obtained from 4 treatments with different levels of N fertilizer application which led to different C to N ratios in the residues. The organic N content was 24.9 g kg^{-1}, 20.9 g kg^{-1}, 15.5 g kg^{-1}, and 14.1 g kg^{-1} relative to the treatments. The organic N content of the sugar beet leaves was 11.9 g kg^{-1}. The organic N content of the bean leaves was 27.7 mg kg^{-1} and 8.5 g kg^{-1} for the bean roots. The organic C content of the crop residues was around 370 g kg^{-1}.

Experimental procedure

Sub-samples of 50 g soil (5 mm sieved) were thoroughly mixed with the crop residues and placed in a 200-ml beaker. The amount organic material applied was related to the amount of crop residues remaining on the field after harvest. The beaker was stored in a 1-l container with two beakers of 100 ml, one containing 20 ml of distilled water and the other 5 ml 0.5 M NaOH. The containers were airtight sealed and incubated at 25 °C. After specific time-intervals, three containers were selected at random for assays of nitrate and ammonium and for determination of CO_2 trapped in the 0.5 M NaOH. Assays for NO_3^- and NH_4^+ were obtained by shaking the soil samples for 30 min with 100 ml 1 M KCl. Extracts were filtered through a GFC Whatman filter paper and stored at -20 °C pending analysis. Ammonium and nitrate were measured on a Skalar automatic analyzer by the alkaline phenate/sodium nitroprusside and sulfanilamide/copper hydrazine method, respectively. CO_2 was determined titrimetrically with 0.05 M HCl.

Statistical analyses

Statistical analyses were carried out with the SAS statistical package (SAS Institute, 1988).

RESULTS

The application of sugar-beat leaves with a C to N ratio of 18.0, 24.2 and 26.6 caused a N immobilization within the first week followed by a net mineralization (Figure 1).

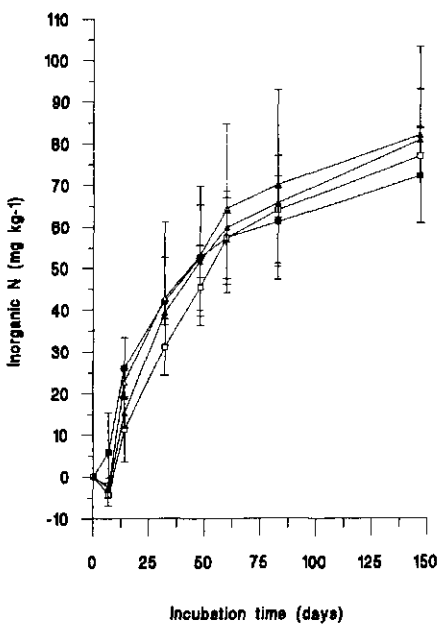

Figure 1. The N mineralization of the Ter Munck soil (mg N kg^{-1}) amended with sugar beet leaves with different C to N ratios : (Δ) C to N ratio of 18.0, (□) of 24.2, (▲) of 26.6 and (■) unamended soil. Bars indicate plus and minus standard deviations.

The N mineralization in the amended soil remained lower, although not significantly, than in the un- amended soil for the first 60 days. The application of bean leaves with a C to N ratio of 13.4 caused no N immobilization and the N mineralization was higher (not significantly) than in the unamended soil (Figure 2).

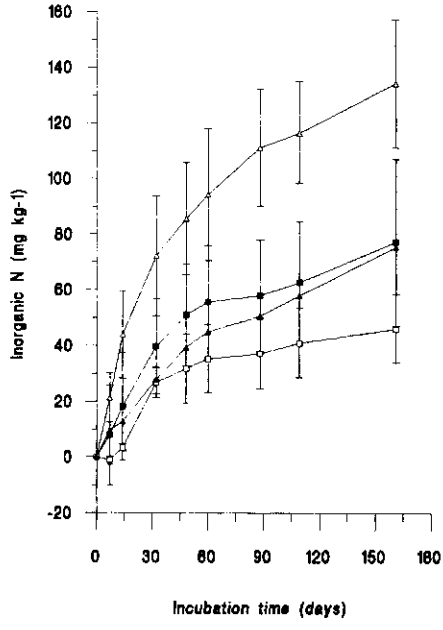

Figure 2. The N mineralization of the Ter Munck soil (mg N kg^{-1}) amended with crop residues of beans : (Δ) leaves, (■) a combination of bean leaves and roots, (□) bean roots and (▲) un-amended soil. Bars indicate plus and minus standard deviations.

The application of bean roots with a C to N ratio of 43.5 caused a reduction (not significantly different, however) in N mineralization as compared to the un-amended soil. The application of sugar beet roots with a C to N ratio of 64.6 caused an increase (significantly at a 0.05 level from day 28 onwards) in N mineralization (Figure 3). The increase was in excess of the organic N applied.

The percentage of organic N applied that was mineralized varied considerably and a relation with the C to N ratio of the organic material applied was not straightforward (Figure 4). Additionally, the percentages of the organic nitrogen applied that were mineralized varied according to the soil as 45.9 % was mineralized when sugar beet leaves were added to the Ter Munck soil and only 35.5 % when added to a soil with a comparable inorganic fraction but with a higher organic C and N content, i.e. 13.4 g kg^{-1} and 1.3 g kg^{-1}, respectively.

The model satisfactorily described the N mineralization of the Ter Munck soil amended with sugar beet leaves (Figure 3). A further proof of the applicability of the model was given by the possibility to simulate the N mineralization of mustard leaves (Figure 3).

The shortcomings of the model, however, were clearly illustrated when the N dynamics of sugar beet roots were simulated (Figure 3). The increase in N mineralization was not correctly simulated and largely underestimated, more than 150 mg N kg^{-1} after 56 days, the amount N mineralized.

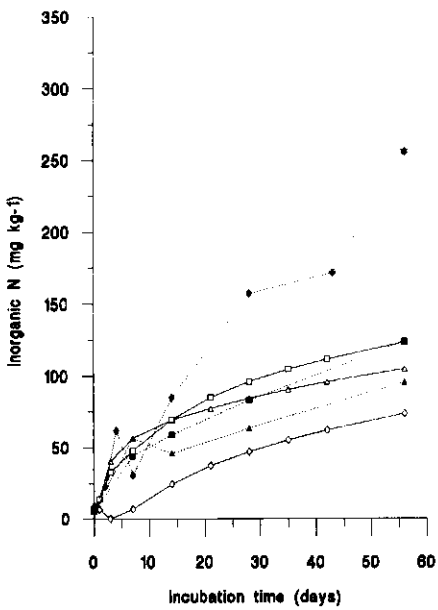

Figure 3. Measured and simulated N mineralization data following the application of mustard leaves, sugar beet leaves and sugar beet roots to the Ter Munck soil. (- ■ -) measured data following the application of sugar beet leaves, (- ▲ -) mustard leaves, (- ♦ -) and sugar beet roots, and (__□__) simulated values following the application of sugar beet leaves, (__Δ__) mustard leaves, (__◊__) and sugar beet root. Bars indicate plus and minus standard deviations.

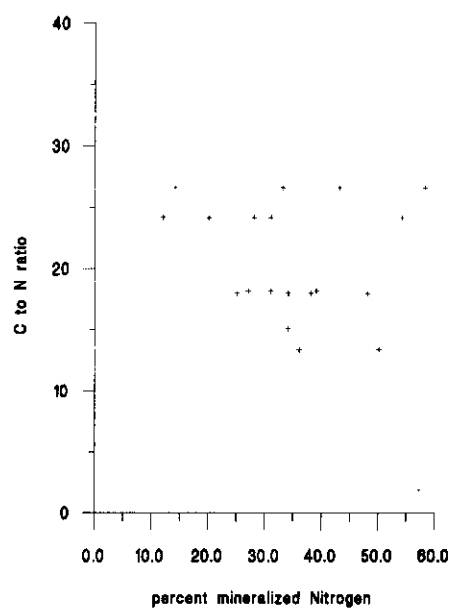

Figure 4. The relation between the C to N ratio of crop residues of sugar beet and beans added to the Ter Munck soil and the percentages of organic N mineralized.

DISCUSSION

It became apparent that a single function was not flexible enough to simulate the N mineralization of soils amended with crop residues of sugar beet and beans and that properties other than the total N content and the C to N ratio of the organic material applied had to be included in the model. Therefore, a carbon and nitrogen mineralization model was developed in which the N mineralization depended on the C mineralization, the C to N ratio of the organic material applied and the C to N ratio of the soil microbial biomass. The model permitted to simulate the N mineralization of different soils when various forms of organic material, such as sugar beet leaves, bean leaves and roots, and mustard leaves, were added. It also allowed to simulate the N mineralization after ^{14}C-labelled maize leaves were added to the Ter Munck soil (Dendooven *et al.*, 1990). The model failed, however, to simulate the N mineralization of the Ter Munck amended with sugar beet roots. It appeared that the model should also include a change in the C to N ratio of the microbial biomass and the efficiency for C when different forms of organic material were applied as considered in models developed by McGill *et al.* (1973) and Van Veen *et al.* (1985).

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