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A MODEL FOR CALCULATING NITROGEN FLUXES IN SOIL USING ^{15}N TRACING

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Summary—Published methods for calculating gross N rates differ in their assumptions and the method of calculation (algebrical equations or numerical methods). The calculation model presented here called FLUAZ considers the major N processes occurring in soil and enables testing of the importance of the various assumptions. It combines a numerical model for solving the mass balance equations and a non linear fitting program for optimizing the N rate parameters. It can be applied to a single or "paired" treatment(s) of an experiment in a bare soil. The model has been evaluated in two experiments made in the laboratory with wheat straw, each experiment involving two "paired" treatments. When FLUAZ was applied to the "paired" treatments, a good fit was obtained between the simulated and measured values of 10 variables (amount of NH_4^+ and NO_3^-, isotopic excess of NH_4^+, NO_3^- and organic N). This fit validated the compartmental model and enabled calculation of six N fluxes: mineralisation (m), ammonium immobilisation (i_a), nitrate immobilisation (i_n), nitrification (n), volatilisation (v) or denitrification (d) and remineralisation of recently immobilised N (r). Sensitivity analysis indicated that the classical assumptions of exclusive ammonium immobilisation ($i_n = 0$) and absence of N remineralisation ($r = 0$) had to be rejected. NH_4^+ immobilisation appeared to be dominant when ammonium and nitrate were both present, but was not exclusive: a Langmuir-type relationship could be established between the immobilisation ratio $i_a/(i_a + i_n)$ and the molar ratio of soil N concentrations $NH_4^+/(NH_4^+ + NO_3^-)$. Remineralisation of N occurred simultaneously with immobilisation during wheat straw decomposition and represented 7–18% of gross immobilisation. Taking into account small gaseous losses, volatilisation or denitrification, allowed a better fit to be obtained between observed and simulated N and ^{15}N pools. Nitrification was better described by first order than by zero order kinetics. The eventuality of direct assimilation of organic N by microbial biomass or N humification could not be determined but had no significant influence on the calculation of other fluxes. When FLUAZ was applied to a single treatment (NH_4^+ labelled), it also gave a good fit but only m, i ($= i_a + i_n$), n, v or d could be determined. The mineralisation and immobilisation rates were slightly lower than those found with the paired treatments: this difference was mainly due to the hypothesis $r = 0$ and disappeared when r was fixed at the value obtained with the paired treatments. The "apparent" immobilisation rates ($i - r$) were then similar. The model is very useful to test the consistency of measurements, estimate several N rates simultaneously and quantify the importance of various assumptions.

INTRODUCTION: REVIEW OF CALCULATION METHODS

Calculating the rates of gross N transformations in soil, particularly mineralisation and immobilisation, is essential to improve our understanding of N dynamics in soil and evaluate the concepts introduced in simulation models describing C and N transformations. However, the methods proposed in the literature to calculate gross N rates in soil using ^{15}N tracing differ by several aspects: the calculation method itself, the modelled system, the measured variables and the N rates which are determined. The main features of the methods dealing with N rates in aerobic soils (excluding marine sediments) are summarised in Table 1.

In the pioneer work of Kirkham and Bartholomew (1954), the system modelled considered only two pools, i.e. the mineral and organic N fractions. These authors showed that measuring mineral N and mineral ^{15}N vs time enabled determination of the mineralisation rate m, using the isotopic dilution principle. In this work, the immobilisation rate i was deduced from a N balance on the mineral N pool, assuming that no gaseous loss took place either as ammonia volatilisation or denitrification and that remineralisation (r) of recently-labelled immobilised N was negligible. In a second paper, Kirkham and Bartholomew (1955) removed this latter assumption and gave a new analytical formulation of their biphasic system to calculate both the mineralisation rate m (in fact rate $m + r$) and the immobilisation rate i, supposing either m and i constant with time or m and i varying as first order rates. The measurement of total organic N and organic ^{15}N is then required to use the equations derived by the

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Table 1. Comparison of various methods proposed to estimate N rates in soils using ^{15}N tracing

Reference	Method	System considered	Principle	Measurements	Rates assessed	Assumptions
Kirkham and Bartholomew (1954)	analytical	mineral N total organic N	isot. dilution N balance	mineral N and ^{15}N	m i	rates constant $r = 0$ $v = d = 0$
Kirkham and Bartholomew (1955)	analytical	mineral N total organic N	isot. exchange	mineral N and ^{15}N organic N and ^{15}N	$m + r$ i	rates constant or 1st order $v = d = 0$
Nishio *et al.* (1985)	analytical	ammonium nitrate	isot. dilution N balance N balance	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$	m n i_a	rates constant $r = 0$; $i_n = 0$ $v = d = 0$
Barraclough (1991); Davidson *et al.* (1990, 1991)	analytical paired exp.	ammonium nitrate	isot. dilution isot. dilution N balance	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$	m n i	rates constant $r = 0$ $v = d = 0$
Schimel *et al.* (1989); Ambus *et al.* (1992); Tietema and Wessel (1992)	analytical paired exp.	ammonium nitrate	isot. dilution isot. dilution	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$	m n i_a, i_n	rates constant $r = 0$ $v = d = 0$
Nishio, 1994	analytical paired exp.	ammonium nitrate total organic N	isot. dilution isot. dilution	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$	m n i_a $i_n + d$	rates constant $r = 0$ $v = 0$
Myrold and Tiedje (1986)	numerical paired exp.	ammonium nitrate active organic N	isot. exchange	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$ organic N and ^{15}N	$m + r$ n, n_h d	rates constant or 1st order $i_n = 0$ $v = 0$
Bjarnason (1988)	numerical paired exp.	ammonium nitrate active organic N	isot. exchange	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$ Organic N and ^{15}N	$m+r$ n i_a, i_n	rates constant or 1st order $v = d = 0$
Wessel and Tietema (1992)	numerical paired exp.	ammonium nitrate total organic N	isot. exchange	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$	$m + r$ n i_a, i_n	rates constant $v = d = 0$
Smith *et al.* (1994)	numerical	ammonium nitrate active organic N	isot. exchange	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$ organic N and ^{15}N	$m + r$ n i_a	rates constant $i_n = 0$ $v = d = 0$
This work	numerical paired exp.	ammonium nitrate biomass-N humus-N	isot. exchange	NH_4 and $^{15}NH_4$ NO_3 and $^{15}NO_3$ organic N and ^{15}N	m r n i_a, i_n v, d	rates constant or 1st order

Rate symbols: m is gross mineralisation, i gross immobilisation, i_a gross NH_4^+ immobilisation, i_n gross NO_3^- immobilisation, n autotrophic nitrification, n_h heterotrophic nitrification, r remineralisation, v volatilisation and d denitrification.

authors. The principle of the calculation in this second paper can be called "isotopic exchange" since it considers simultaneously the isotopic dilution of the mineral N pool, isotopic enrichment of the organic N pool and equilibration between the two pools. The first formula based on the isotopic dilution method is no longer valid. These two papers have given the basic concepts and ideas for calculating N rates using ^{15}N tracing. However, three main objections can be made to this work:

(i) the authors did not distinguish the ammonium and the nitrate fractions within the mineral pool. The distinction would not be necessary if both fractions were equally assimilated by soil microorganisms, but the ammonium preference in microbial metabolism has been clearly shown in several studies (e.g. Rice and Tiedje, 1989); considering separately of ammonium and nitrate in the modelled system is therefore essential (Schimel, 1996).

(ii) the organic N pool was supposed to be a homogeneous pool. The size of the pool which is the sink for immobilisation is very important in calculating N recycling rate. Several authors have indicated that the immobilising pool (called "active" organic pool) was smaller than the total organic pool (Myrold and Tiedje, 1986; Bjarnason, 1988; Smith *et al*., 1994). Indeed N immobilisation is an input rate for only a part of the microbial biomass which is itself a small proportion (3–10% according to the authors) of the total organic N.

(iii) small but significant N losses may occur by volatilisation, denitrification or leaching. In this case which is likely, particularly under field conditions, N immobilisation cannot be simply deduced from the mineral N balance in soil.

The more recent studies have more or less completely taken these criticisms into account. The NH_4^+ and NO_3^- pools are considered in all studies. The problem which then arises is to estimate separately the microbial immobilisation coming from NH_4^+ (rate i_a) or NO_3^- (rate i_n). Some authors calculate the global immobilisation by a N balance assuming that immobilisation represents the whole "consumption" (Davidson *et al*., 1990; Barraclough, 1991) which can be criticised (Schimel, 1996). Others consider that the microbial assimilation of nitrate i_n is negligible relative to i_a (Nishio *et al*., 1985; Myrold and Tiedje, 1986; Smith *et al*., 1994). A few studies do not make this assumption and try to calculate i_n (Bjarnason, 1988; Schimel *et al*., 1989; Ambus *et al*., 1992; Wessel and Tietema, 1992). Fewer studies take N losses into account (Myrold and Tiedje, 1986).

Another main difference between methods is the technique of calculation itself which can be either analytical or numerical. The analytical methods are based essentially on the "isotopic dilution" and "isotopic enrichment" principles (Mary and Recous, 1994; Monaghan and Barraclough, 1995). The isotopic dilution applies to a pool initially labelled which is replenished with unlabelled material exclusively. The isotopic enrichment applies to an "accumulation" pool which has no output fluxes. The isotopic dilution formula derived have been largely used to calculate the mineralisation rate m in experiments where the NH_4^+ pool has been labelled. Revising the various analytical formulations given in the literature, Smith *et al*. (1994) have shown that those of Blackburn (1979), Nishio *et al*. (1985), Barraclough (1991) were all similar and could be described by the same formula

$$m = -\frac{\Delta A}{\Delta t}\frac{\ln(e_{A2}/e_{A1})}{\ln(A_2/A_1)} \qquad (1)$$

or if A is constant

$$m = -\frac{A}{\Delta t}\ln(e_{A2}/e_{A1}) \qquad (1')$$

where A is the ammonium content and e_A its ^{15}N isotopic excess (difference between ^{15}N abundance in sample and natural abundance in soil). These formula are also equivalent to equations (6) and (7) given by Wessel and Tietema (1992).

A similar formulation can be derived for the nitrification rate n in experiments where the NO_3^- pool has been labelled (Davidson *et al*., 1990, 1991; Barraclough, 1991):

$$n = -\frac{\Delta N}{\Delta t}\frac{\ln(e_{N2}/e_{N1})}{\ln(N_2/N_1)}, \qquad (2)$$

where N is the nitrate content and e_N its ^{15}N isotopic excess.

In order to determine both rates simultaneously, the latter authors used "paired" experiments. The situation is not as clear concerning the application of isotopic enrichment principle since various formula have been proposed for example to calculate immobilisation rates (e.g. Shen *et al*., 1984; Guiraud *et al*., 1992). This is due to the fact that they are only approximations (Wessel and Tietema, 1992; Barraclough and Puri, 1995). The alternative to make accurate calculations is to use a numerical technique.

In fact, the "numerical" methods which have been proposed combine a description of the modelled system, a numerical resolution of the differential equations and a non linear optimization procedure for estimating N rates. Their interest compared to the analytical approach has already been shown by Myrold and Tiedje (1986), Bjarnason (1988), Nason and Myrold (1991), Wessel and Tietema (1992), Smith *et al*. (1994). This type of method is essential to solve the differential system when multiple fluxes can simultaneously dilute or enrich the ^{15}N composition of a given pool: this is particularly the case with the rates i_a and i_n, or with rates m and r. However none of the previously mentioned works gave us

complete satisfaction regarding to the modelled system; for example biomass-N was not included. This paper presents the model FLUAZ which was then developed and applied to two laboratory data sets dedicated to test it.

MATERIALS AND METHODS

Model description

The basic compartmental model which is taken into account in the FLUAZ calculation program is shown in Fig. 1(a). Six fluxes are considered: mineralisation, immobilisation of ammonium and nitrate, nitrification, volatilisation and denitrification. These fluxes can be determined by a single $^{15}NH_4^+$ labelling experiment, involving five measurements: ammonium-N and ^{15}N, nitrate-N and ^{15}N, organic-^{15}N or microbial biomass-^{15}N. Two hypotheses must be made since the system is underdetermined: one about gaseous losses (volatilisation or denitrification) and one about relative immobilisation of ammonium and nitrate.

FLUAZ also allows simulation by the more complete model given in Fig. 1(b). Four additional N fluxes can be considered: N mineralisation coming from plant residue decomposition (flux *s*), direct

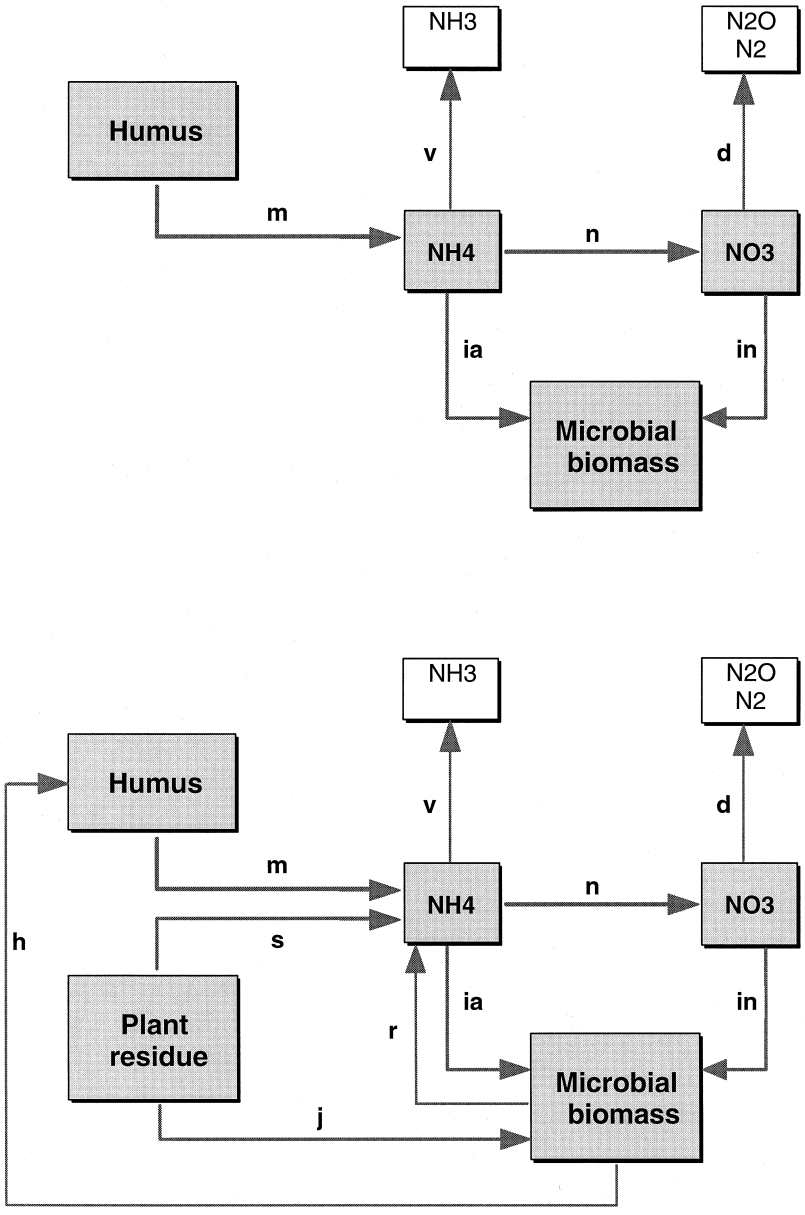

Fig. 1. Compartmental model of N rates considered in FLUAZ. (a) Basic model with 6 processes (b) more detailed model with 10 processes.

assimilation of organic N by microorganisms (j), remineralisation (r) and N humification (h). The microbial biomass which is considered in our model is smaller than the "active" organic pool of some authors, because it represents only the part of the total biomass which is actively growing and incorporating some of the labelled N added. It corresponds to the "zymogenous" biomass in the case of a plant residue addition (Winogradski, 1949).

The N fluxes considered in Fig. 1(b) are assumed constant (zero order rate) during each measurement interval, but may vary from one interval to another. This assumption is acceptable for mineralisation: over short-time steps, the first order kinetics is almost similar to zero-order kinetics. Immobilisation rates are also considered constant since they are not proportional to ammonium or nitrate concentration, but rather depend on C decomposition rates which are not modelled here. In contrast, nitrification, volatilisation and denitrification rates are allowed to follow first order kinetics relative to either NH_4^+ concentration for the first two or to NO_3^- concentration for the last process. NH_4^+ fixation onto clay lattices is supposed to be very rapid and complete at the first measurement date.

The FLUAZ program combines a numerical model (Runge–Kutta algorithm, 4th order, with a variable time-step) which solves the differential system given by the N and ^{15}N mass equations, and a non linear fitting program (based on Marquardt's algorithm) which enables calculation of one or several N rates (or rate constants) described in Fig. 1. Each N rate (or rate constant) can be either imposed or fitted. FLUAZ minimizes the quadratic weighted error (QWE) which can be defined for each time interval j

$$\mathrm{QWE}_j = \sum_{i=1}^{n} \frac{(\bar{Y}_{ij} - \hat{Y}_{ij})^2}{s_{ij}^2}, \tag{3}$$

where n is the number of variables, $\bar{Y}_{ij}$ is the mean of the observed values of variable i, $\hat{Y}_{ij}$ is the predicted value of variable i and s_{ij} is the standard deviation between replicates. Minimizing this value instead of the usual sum of squares has two main advantages (Huet *et al.*, 1992): it accounts for the variance of the measurements (those with the greatest variability have the lowest weight) and it "normalizes" the various variables, which can then be summed up. In our case the five variables are the amounts of ammonium and nitrate-N and the isotopic atom% excess of ammonium, nitrate and organic N in soil.

The mean weighted error (MWE_j) can be calculated over each time interval

$$\mathrm{MWE}_j = \sqrt{\frac{\mathrm{QWE}_j}{n}} \tag{4}$$

This term which is unitless can be compared to 1, since

$$\forall i \in [1, n] \quad |\bar{Y}_{ij} - \hat{Y}_{ij}| \leq s_{ij} \Longrightarrow \mathrm{MWE}_j \leq 1$$

If different time intervals are available, one can calculate the global mean weighted error

$$\mathrm{MWE} = \frac{1}{N} \sum_{j=1}^{N} \mathrm{MWE}_j \tag{5}$$

This equation indicates that the term MWE will be lower than 1 if each measured variable does not differ by more than one standard deviation from the predicted value.

The model can use data from a *single experiment*, i.e. whose initially labelled pool(s) is (are) NH_4^+ or NO_3^- or both. In this case, five independent variables must be measured at least at two dates: Q_a (amount of NH_4^+–N), E_a (isotopic excess of $^{15}NH_4^+$), Q_n (amount of NO_3^-–N), E_n (isotopic excess of $^{15}NO_3^-$) and E_o (isotopic excess of total organic ^{15}N). These measurements enable determination of a maximum of five N rates. Therefore only the simplified model shown in Fig. 1(a) can be considered in this case. Moreover, at least one more hypothesis must be made since the model considers six N processes. The hypothesis can be that one gaseous loss rate is null (volatilisation or denitrification) or that the immobilisation of nitrate-N is null. The first possibility can be checked looking at the ^{15}N balance or by direct NH_3 or ($^{15}N_2 + {}^{15}N_2O$) gas flux measurements.

The model can also consider data from *an experiment involving "paired" treatments*, i.e. the same ^{14}N treatments but labelled either on NH_4^+ or on NO_3^- (called inorganic paired treatments). The great interest of these "mirror images" treatments has already been shown (Barraclough, 1991). In this case, $2 \times 5 = 10$ variables are measured; in fact, 8 of them are completely independent, since the amounts of ammonium and nitrate are supposed to be similar. These measurements enable calculation of up to 6 processes out of those described in Fig. 1(b). They are of particular interest to determine the importance of rates i_n, immobilisation of nitrate-N, and r, remineralisation of recently immobilized-N. With the inorganic paired treatments, the mineralisation of N derived from added organic residues (rate s) cannot be separated from the mineralisation of humified organic matter (rate m): the mineralisation rate calculated by the model is then equal to $m + s$. However, the model can also simulate the "mixed" paired treatments (^{15}N-labelled residue + unlabelled mineral N; unlabelled residue + ^{15}N-labelled mineral N): in this case rate m and s can be both calculated. The rate j represents the direct assimilation of organic N from organic residues by the microbial biomass. Recent work has indicated that this pathway could be significant

(Barak *et al.*, 1990; Hadas *et al.*, 1992). Since the determination of this process requires ^{15}N labelled residues (Mary *et al.*, 1993), the flux j has been fixed in our study. Similarly, since microbial and humified ^{15}N have not been measured, the N humification rate h has been fixed at 0.

The amount of total organic N at time 0 is also required. Biomass-N or ^{15}N measurements would theoretically be as interesting as or more than total organic N and ^{15}N. However, absolute measurements of biomass-N and -^{15}N are still difficult: in the case of the chloroform fumigation technique (CFIM or CFEM), the difficulty lies in the determination of the k_N coefficient (ratio of extracted-N to biomass-N). This leads us to think that it is safer to use total organic ^{15}N and consider that all organic ^{15}N is microbial ^{15}N, at least over short periods of time. This hypothesis will be discussed later. Furthermore, measuring organic ^{15}N is essential to make a ^{15}N balance and then indirectly estimate the gaseous losses which may have occurred. The model calculates the evolution of N and ^{15}N pools and their isotopic excess, using fixed or fitted N transformation rates, during each time interval. When several intervals are available (at least three dates of measurement), the simulated values at the end of a time interval are used as initial values for the next interval. N rates can vary from one interval to another. The minimisation procedure used to find the best parameters may fail by finding "local minima", particularly when 5 or 6 parameters are fitted simultaneously. In order to limit this risk, the program automatically repeats the minimisation search for three different initial values of each of the n fitted parameters (3^n combinations) and selects the lowest minimum. The various minima obtained are displayed in order to identify situations in which convergence is more difficult to reach and where local minima are more frequent. This procedure was good enough to give the best fit in all our simulations.

The program also calculates the correlation coefficient matrix between parameters, the slope $\partial MWE/\partial Pi$ (relative to each fitted parameter Pi) and the confidence intervals of parameters: these criteria rapidly show if the fitted parameters can adequately be determined with a given data set. For example, they indicated that mineralisation rate $m + s$ cannot be precisely determined with a single treatment involving $^{15}NO_3^-$ labelling.

Experiments

Two "paired" incubation experiments were used to test the model. The experiments were realized successively but, in both cases, soil cores were taken from the ploughed layer (0–20 cm) of an arable soil in the same field (Mons-en-Chaussée). The soil was a loamy soil (Typical Hapludalf) containing 16% clay, 76% silt, 7% sand and traces of $CaCO_3$. Its organic C content was 9.5 g kg^{-1} soil and its total N content was 980 mg kg^{-1} soil. In both experiments, the moist soil was slightly air-dried to reach a moisture content of about 160 mg water g^{-1} dry soil and sieved at 3.15 mm. The visible organic residues remaining were then removed by hand picking. The soil was conditioned at 4°C during 2 weeks. Finally it was amended with wheat straw and mineral ^{15}N and incubated at 15°C and 20% moisture content (−60 kPa water potential). Each soil sample (30 g moist soil) was incubated in a 1 l jar containing one beaker of NaOH 0.2 N for CO_2 trapping. All samplings and analysis were made in triplicate. Measurements included the amounts of CO_2 evolved, the amounts of exchangeable NH_4^+, NO_3^- and total organic N, the isotopic excess of $^{15}NH_4^+$, $^{15}NO_3^-$ and total organic ^{15}N. Organic + fixed N was determined by a Dumas combustion method after elimination of inorganic N by successive washings. The procedures and methods of analysis are described in Recous *et al.* (1995). All isotopic excess were calculated relatively to air composition whose isotopic abundance equals 0.3663 atom%, but actual natural soil ^{15}N composition (0.3688 atom%) was accounted for in FLUAZ calculations. The amounts of ^{15}N found in soil at time 0 (in fact 30 min after $^{15}NH_4^+$ addition) were attributed to fixed-$^{15}NH_4^+$ and used to calculate fixed NH_4^+.

Experiment 1 Straw and mineral N were applied in a single pulse at the beginning of incubation ($t = 0$). Wheat straw was applied at the rate of 4.0 g kg^{-1} of dry soil, corresponding to an addition of 1707 mg C kg^{-1} soil and 12.4 mg N kg^{-1} soil. Mineral N was added as $(NH_4)_2SO_4$ and KNO_3 at the rate of 45 mg NH_4–N kg^{-1} and 20 mg NO_3–N kg^{-1} soil. The soil initially contained 0.7 mg NH_4–N kg^{-1} and 5.4 mg NO_3–N kg^{-1} soil. Two real "paired" treatments were realized:

(1a) addition of $(^{15}NH_4)_2SO_4$ (9.62 atom% excess) + $^{14}NO_3K$

(1b) addition of $(^{14}NH_4)_2SO_4$ + $^{15}NO_3K$ (8.05 atom% excess)

The soil was incubated for 28 d and sampled for CO_2, mineral and organic N on 8 dates.

Experiment 2 Straw was added first, at the rate of 2.4 g kg^{-1} of dry soil, corresponding to an addition of 1003 mg C kg^{-1} soil and 8.6 mg N kg^{-1} soil. Labelled mineral N was applied later at two dates, in order to vary the molar ratio of soil N concentrations $NH_4^+/(NH_4^+ + NO_3^-)$. The first N application was taken as time $t = 0$, straw addition date corresponding to $t = -3.1$ d. Each application of mineral N was made at the rate of 25 mg N kg^{-1} soil. The soil initially contained 1.2 mg NH_4–N kg^{-1} and 4.1 mg NO_3–N kg^{-1}. Two treatments were realized

(2a) addition of $(^{15}NH_4)_2SO_4$ (9.36 atom% excess) at time $t = 0$ and $t = 6.8$ d

(2b) addition of $^{15}NO_3K$ (10.02 atom% excess) at time $t = 0$ and $t = 6.8$ d.

The incubation which lasted 17 d (starting at $t = -3.1$ d and ending at $t = +13.7$ d) included 10 sampling dates where CO_2, mineral and organic N were determined. In this experiment, NH_3 volatilisation was measured by trapping in H_2SO_4 0.02 N and back titrating with NaOH. The treatments were not pure "paired" treatments since they were different in their unlabelled mineral forms. However they contained similar amounts of mineral N and straw. In the calculations with FLUAZ, all fluxes were supposed similar in the two treatments except nitrification and the proportion of ammonium and nitrate immobilisation.

RESULTS

Simulation of the paired treatments in experiment 1

The model FLUAZ was first applied to experiment 1 using the hypothesis H0 described in Table 2. This was the more general hypothesis tested, since six N rates were fitted: $m + s$, i_a, i_n, n, v (or d) and r. Nitrification was supposed to be a first order kinetics. The direct N assimilation from straw and the N humification rates were assumed to be negligible ($j = h = 0$), as well as the initial size of the (newly-formed) biomass at the date of straw incorporation ($B_o = 0$). Gaseous losses were considered but only at the dates when the ^{15}N recovery in the soil (NH_4–$^{15}N + NO_3$–^{15}N + organic-^{15}N) decreased significantly; volatilisation and denitrification were supposed to be exclusive to each other.

The values of the five variables simulated in each treatment with this hypothesis are plotted vs time in Fig. 2. A very good agreement was obtained between observed and simulated values. The only noticeable difference relative to the standard error of the measurements was a slight overestimation of the NH_4–^{15}N isotopic excess in the treatment 1a between d 8 and 16. The observed decrease in ^{15}N recovery was simulated by supposing NH_3 volatilisation immediately after NH_4^+ application and denitrification between d 5 and 8.

The good quality of fit was confirmed by the low values of the mean weighted error (MWE) which varied between 0.34 and 1.73 for the seven time intervals during which the calculations were made. The average value for the whole incubation period (0–28 d) was MWE = 1.04. This result validated the model since the system was overdetermined: 5 or 6 parameters were calculated with 8 independent measured variables.

The other cumulative N fluxes are plotted in Fig. 3. The model calculated high amounts of N mineralised and immobilised: 21.3 and 67.4 mg N kg^{-1} after 28 incubation d, respectively. The resulting effect was a net immobilisation, which was due to the active straw decomposition. Ammonium concentration dropped from 46 to 1 mg N kg^{-1} during the first 9 d of incubation. The model indicated that ammonium immobilisation was dominant but not exclusive during this period: significant nitrate immobilisation occurred before full depletion of the ammonium pool. In order to test this result more thoroughly, we operated a variable change:

Table 2. Description of the different hypotheses tested in experiment 1 with paired treatments. Only the changing assumptions (relatively to hypothesis H0) are displayed

	N rate*									N pool†
Hypothesis	$m + s$	i_a	i_n	n	v	d	r	j	h	B_o
H0	F0	F0	F0	F1	F0	F0	F0	I0 ($j = 0$)	I0 ($h = 0$)	I0 ($B_o = 0$)
H1	–	–	I0 ($\beta = 0.05$)							
H2	–	–	I0 ($\beta = 0$)							
H3	–	–	–	F0						
H4	–	–	–	–	I0 ($v = 0$)	I0 ($d = 0$)				
H5	–	–	–	–	–	–	I0 ($r = 0$)			
H6	–	–	–	–	–	–	–	I0 ($j = 0.44$‡)		
H7	–	–	–	–	–	–	–	–	I0 ($h = 1.50$)	
H8	–	–	–	–	–	–	–	–	–	I0 ($B_o = 10$)
H9	I0 (i. dilution)	–	I0 ($\beta = 0$)	I0 (i. dilution)						

F0 is the fitted rate, zero order, I0 the imposed rate, zero order, F1 the fitted rate, first order and I1 the imposed rate, first order.
*N rate in mg N kg^{-1} d^{-1}.
†N pool in mg N kg^{-1} soil.
‡The rate $j = 0.4$ mg N kg^{-1} d^{-1} corresponds to the maximum flux coming from straw if all straw-N would have been directly assimilated in 28 d.

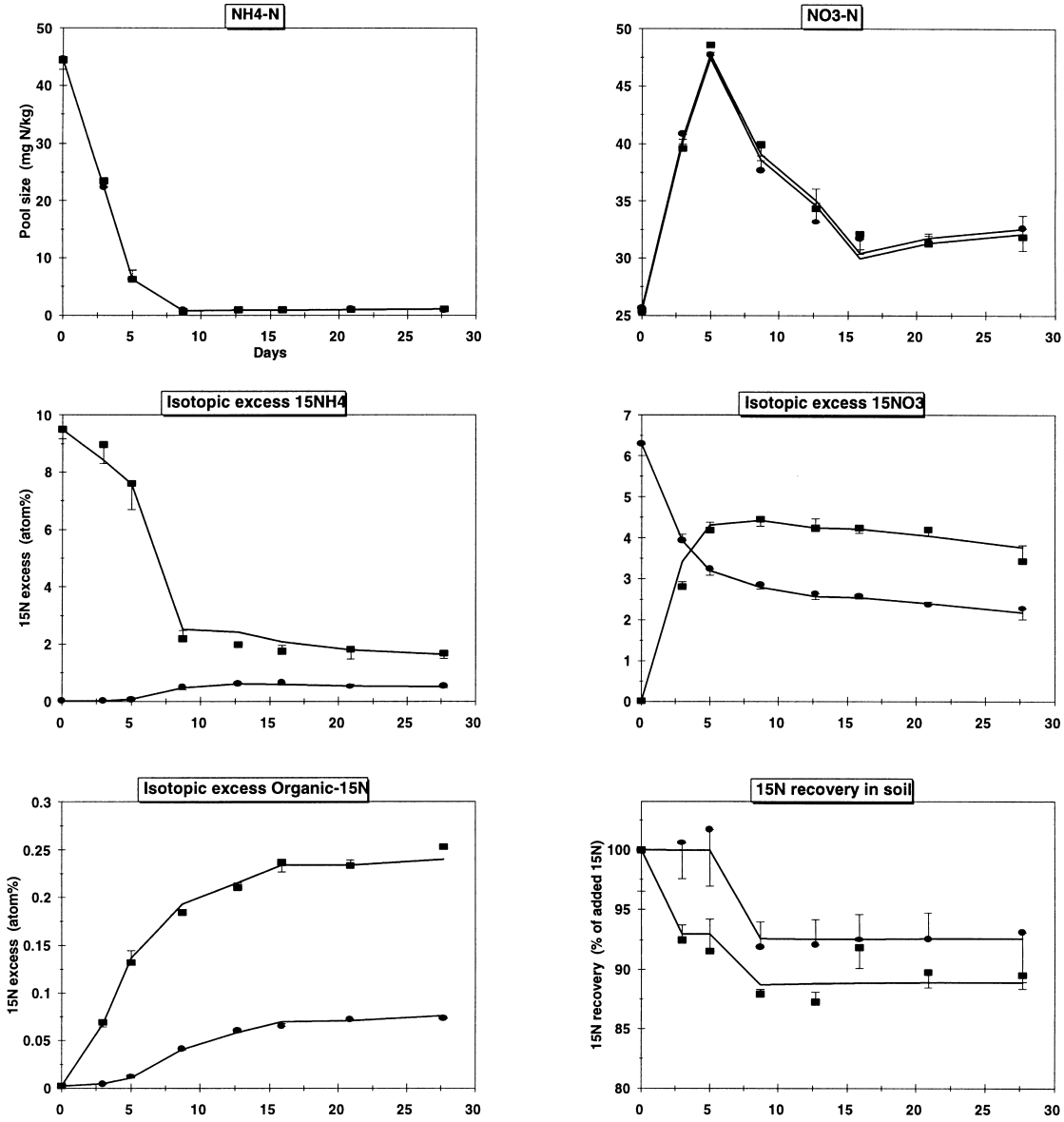

Fig. 2. Amounts of NH_4–N, NO_3–N (mg kg^{-1} soil), ^{15}N isotopic excess of NH_4, NO_3, organic N (atom% excess) and ^{15}N recovery (% of added ^{15}N) vs incubation time in experiment 1. Square symbols: treatment 1a ($^{15}NH_4 + NO_3$). Round symbols: treatment 1b ($NH_4 + {}^{15}NO_3$). Continuous lines: simulated values. Vertical bars represent one standard deviation.

we assumed that the fraction of ammonium immobilised (i_a) relative to the total immobilisation ($i = i_a + i_n$) was a function of the fraction of exchangeable ammonium (Q_a) relative to the mineral pool ($Q = Q_a + Q_n$), and that the function could be represented by a Langmuir-type equation

$$\frac{i_a}{i} = \frac{Q_a/Q}{\beta + (1-\beta)Q_a/Q} \qquad (6)$$

In this equation, the parameter β is related to the importance of nitrate immobilisation. It can vary between 0 and 1; the value $\beta = 0$ corresponds to exclusive NH_4^+ immobilisation whereas the value $\beta = 1$ would represent an absence of preference for NH_4^+ or NO_3^- in microbial N assimilation. In the subsequent work, the parameters (i_a, i_n) were replaced by the parameters (i, β). We first checked that this substitution did not affect the results, neither the MWE nor the values of rates i_a and i_n. The validity of equation (6) was then studied by testing the stability of β coefficients obtained on each time interval.

Simulation of the paired treatments in experiment 2

Although the second data set (experiment 2) did not include real "paired" treatments, the model

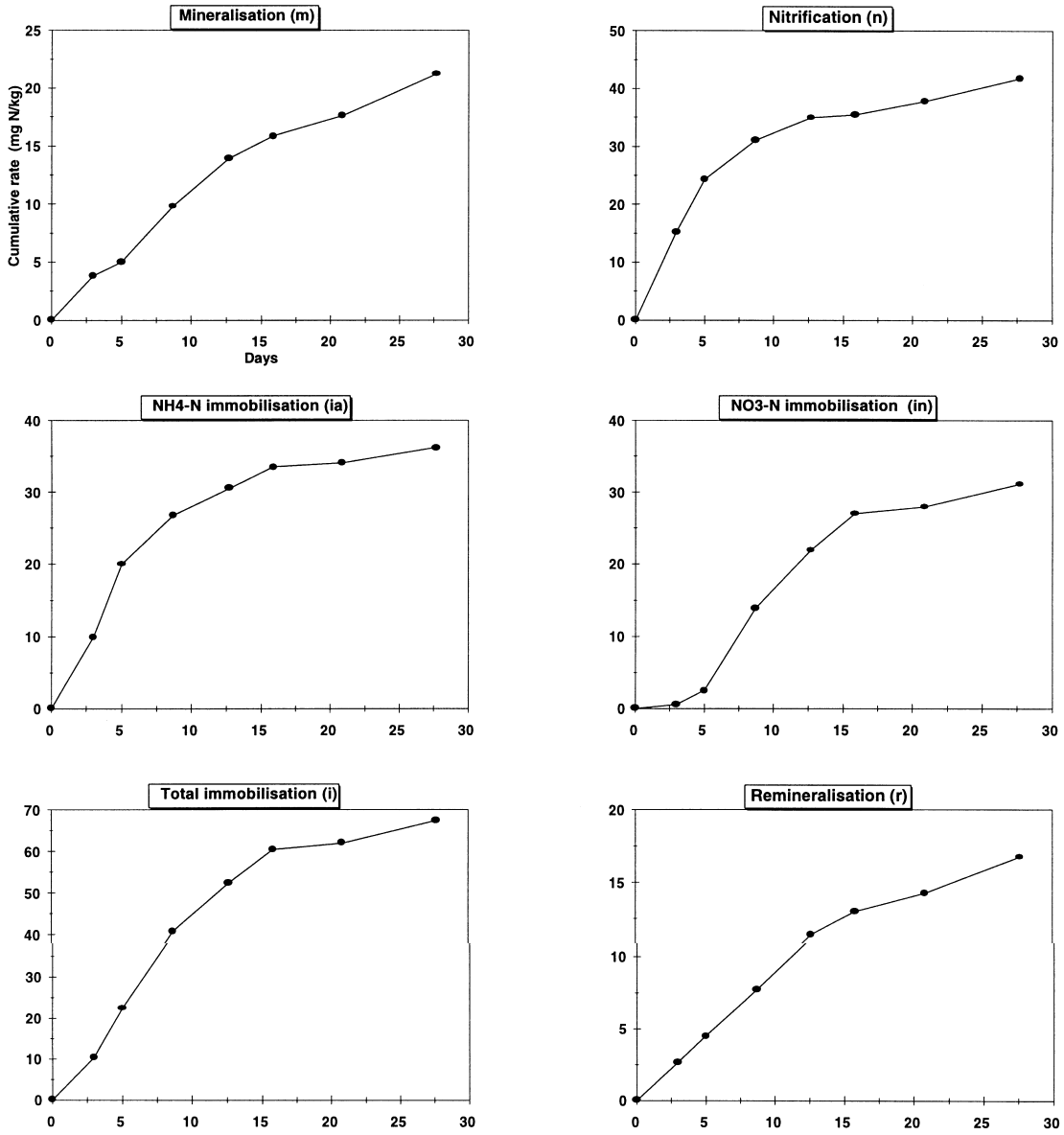

Fig. 3. Cumulative amounts of gross mineralisation ($m + s$), nitrification (n), NH_4–N immobilisation (i_a), NO_3–N immobilisation (i_n), total immobilisation (i) and remineralisation (r) calculated by FLUAZ with hypothesis H1 in experiment 1.

could be applied to both treatments simultaneously considering that:

(i) nitrification rates were different in the two treatments but could be described by first order kinetics, the rate constants being supposed similar;

(ii) immobilisation of ammonium and nitrate also differed in the two treatments but equation (6) was used to calculate each of them assuming that total immobilisation was identical in both treatments and that the parameter β was the same.

The results obtained with this second data set using hypothesis H0 were good too, since the MWE varied between 0.56 and 2.65 for the eight time intervals during which the calculations were made. The average value for the whole period (0–13.7 d) was MWE = 1.62. These figures were slightly higher than for experiment 1, due to two reasons: (i) the test of the model was more severe: 5 or 6 parameters were calculated using 10 independent measured variables; (ii) measurements in experiment 2 had a lower variance than those of experiment 1: the mean coefficient of variation of all variables was 4.1% in the second experiment vs 6.7% in the first experiment.

The evolution of the different measured and simulated variables is shown in Fig. 4. Since NH_3 volatilisation measured in this experiment was found negligible, the small deficit in ^{15}N recovery

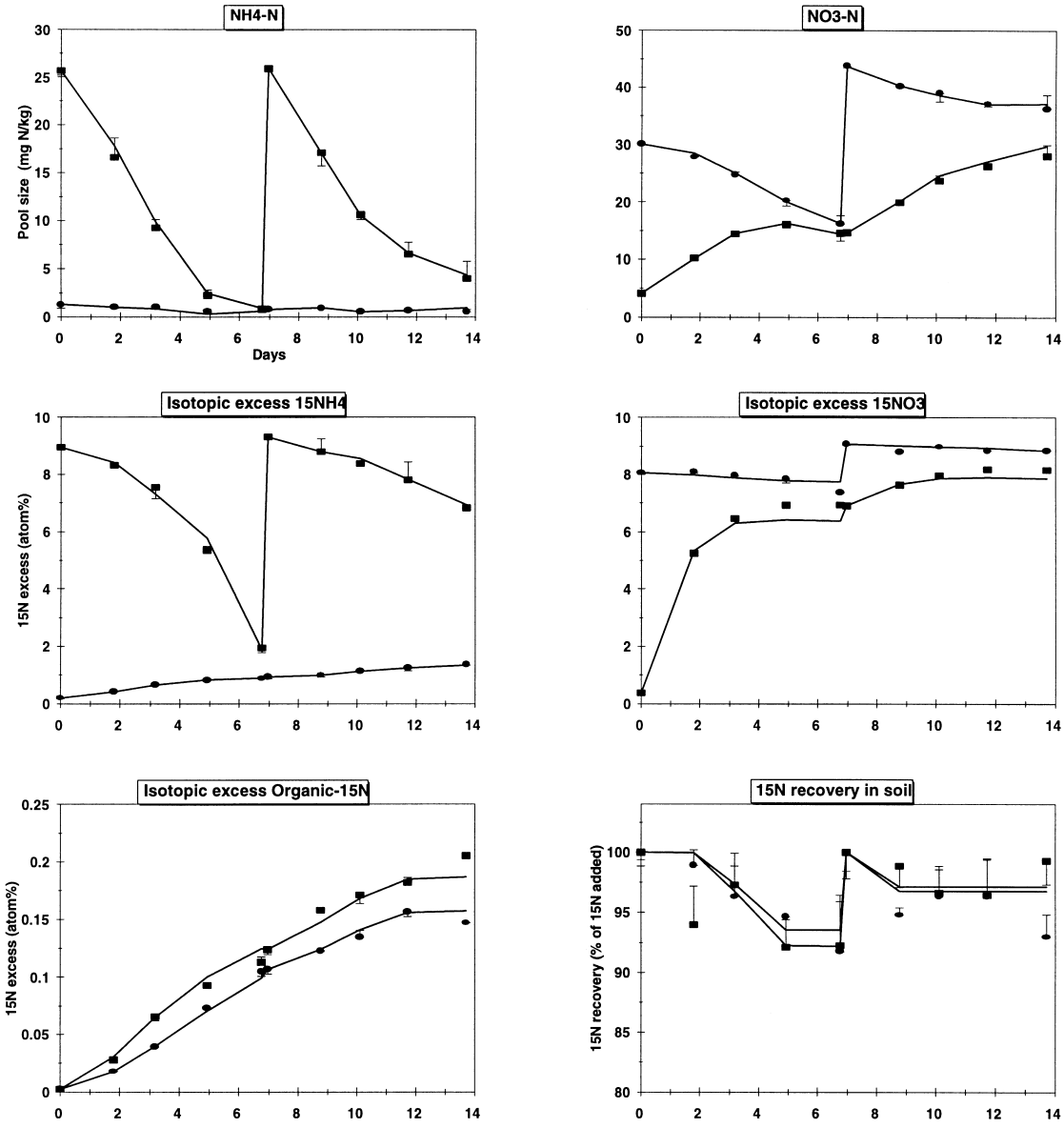

Fig. 4. Amounts of NH_4–N, NO_3–N (mg kg^{-1} soil), ^{15}N isotopic excess of NH_4, NO_3, organic N (atom% excess) and ^{15}N recovery (% of added ^{15}N) vs incubation time in Experiment 2. Square symbols: treatment 2a ($^{15}NH_4$). Round symbols: treatment 2b ($^{15}NO_3$). Continuous lines: simulated values.

was attributed to denitrification. Two moderate but significant discrepancies appeared concerning (i) the isotopic excess of nitrate in treatment 2a: the model predicted lower values than observed at d 5 and 7; (ii) the isotopic excess of organic N at d 14 was not well simulated in both treatments. No satisfactory explanation could be found to these discrepancies.

The cumulative N fluxes calculated are given in Fig. 5. The results fully confirmed those obtained in experiment 1, i.e. *i*) the high rates of mineralisation and immobilisation (8.9 and 28.5 mg N kg^{-1} after 13.7 incubation days, respectively) (ii) the existence of significant immobilisation of nitrate in the $^{15}NH_4^+$ treatment and conversely immobilisation of ammonium in the $^{15}NO_3^-$ treatment (iii) the existence of N remineralisation flux, occurring almost simultaneously with N immobilisation.

Immobilisation of ammonium and nitrate

The results obtained with the two data sets using the more general hypothesis H0 provided various estimates of the parameter β (one estimate per time interval). We excluded time intervals for which the ammonium pool was very low and the confidence interval on β was very large: 4 intervals were excluded out of 15. The remaining 11 values of β and therefore of i_a/i could be plotted vs Q_a/Q according to equation (6). The results are given in

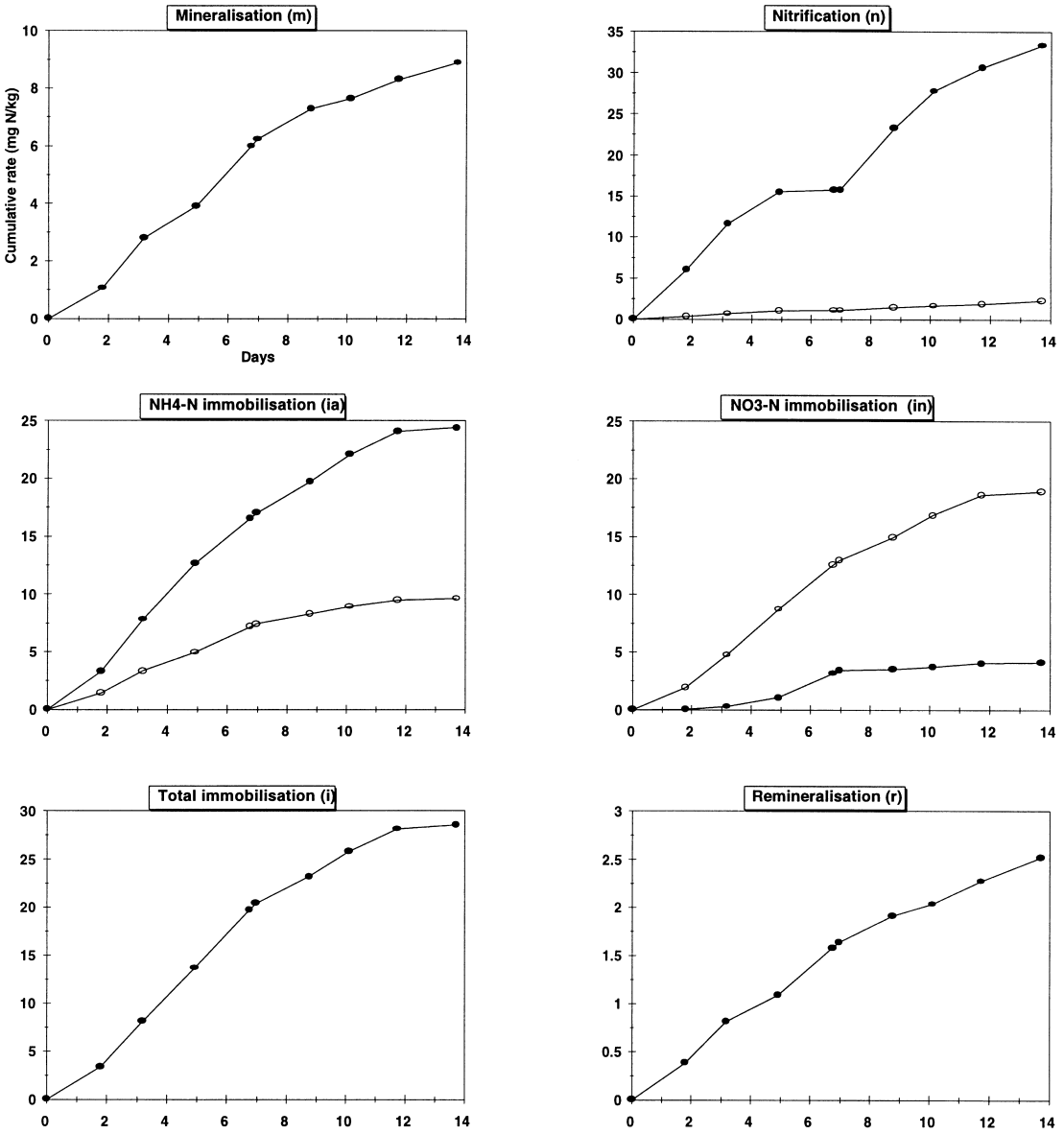

Fig. 5. Cumulative amounts of gross mineralisation ($m + s$), nitrification (n), NH_4–N immobilisation (i_a), NO_3–N immobilisation (i_n), total immobilisation (i) and remineralisation (r) calculated by FLUAZ with hypothesis H1 in Experiment 2. Closed symbols: treatment 2a ($^{15}NH_4$). Open symbols: treatment 2b ($^{15}NO_3$).

Fig. 6. The experimental data (calculated by FLUAZ) appeared to be correctly explained by equation (6) when taking the mean value $\beta = 0.05$. They all fell within the two envelope curves defined by $\beta = 0.03$ and $\beta = 0.08$.

Experiments 1 and 2 were simulated again with the imposed value $\beta = 0.05$. The quality of fit obtained in experiment 1 for the first incubation period was very close (MWE = 0.98) to that obtained with the most general hypothesis H0 (MWE = 0.87). The same result was found in experiment 2: imposing the value $\beta = 0.05$ gave a mean weighted error MWE = 1.95, close to the one found with hypothesis H0 (MWE = 1.62) for the whole incubation period. These results indicate the interest of equation (6) to describe the relative immobilisation of ammonium and nitrate when both are present, particularly when a single ^{15}N treatment is available.

Sensitivity analysis

Table 2 presents the nine different hypotheses (H1 to H9) which have been tested relative to hypotheses H0. The sensitivity to a change in each N

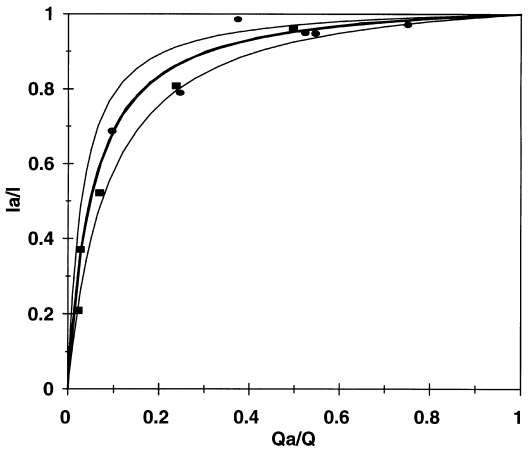

Fig. 6. Relationship between the fraction of N immobilised as NH_4 (i_a/i) and the fraction of mineral N present as NH_4 (Q_a/Q). The experimental points have been calculated by FLUAZ with hypothesis H0. The three curves correspond to equation (6) for $\beta = 0.05$ (thick line) and $\beta = 0.03$ and 0.08 (thin lines).

rate was tested in hypothesis H1–H7. N rates were either fitted (F) or imposed at a given value (I). Each rate was supposed constant over each time interval, except for nitrification which could follow a zero order (0) or a first order (1) kinetics. In hypothesis H8, the initial size of the growing biomass was changed. Hypothesis H9 used the classical dilution equation (1) in the $^{15}NH_4^+$ treatment and equation (2) in the $^{15}NO_3^-$ treatment for calculating m and n, respectively. It also considered that NH_4^+ immobilisation was exclusive, as often assumed. In the FLUAZ model, "exclusive" immobilisation has the following meaning: only NH_4^+ is immobilised when enough NH_4^+ is available to satisfy microbial requirements, but NO_3^- is immobilised when the exchangeable NH_4^+ pool cannot meet all microbial needs.

The results of the sensitivity analysis are summarised in Fig. 7 and Table 3. Figure 7 shows the mean weighted error (MWE) obtained with the various

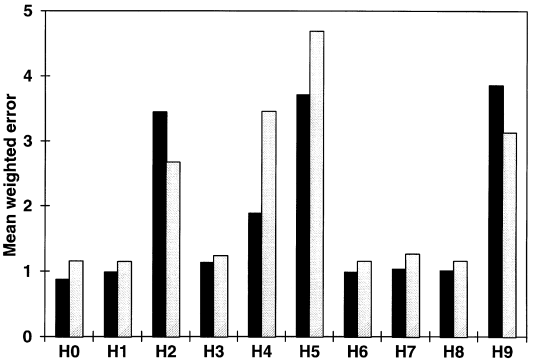

Fig. 7. Mean weighted error (MWE) obtained with the 10 different hypotheses tested using the paired treatments of experiment 1. Full bars: period 0 – 8.7 d; open bars: period 8.7–28 d. Hypotheses are defined in Table 2.

hypotheses for two incubation periods: 0–8.7 d, when the soil contained significant amounts of ammonium; 8.7–28 d, when the ammonium pool was almost completely depleted (< 1.2 mg N kg^{-1}). The accuracy of sensitivity analysis was greater during the first period but the results obtained during the second period confirmed the former ones. We only consider now the results obtained during the 0–9 d period. The best quality of fit, corresponding to the lowest value of MWE, was obviously obtained with the most general hypothesis H0: MWE = 0.87. However the hypotheses H1, H3, H6, H7, H8 provided almost as good results, since MWE ranged between 0.98 and 1.13. In contrast, hypotheses H2, H5, H9 resulted in a much higher MWE: $3.44 \leq \text{MWE} \leq 3.85$. These three hypotheses resulted in lower mineralisation, immobilisation and nitrification rates than the other ones (Table 3). Hypothesis H4 (absence of gaseous losses) gave intermediate results: MWE = 1.89.

H2 supposed that microbial N assimilation, at least for straw decomposers, occurred exclusively as NH_4^+. With this assumption, the model markedly overestimated the isotopic excess of the organic ^{15}N in the $^{15}NH_4$ treatment (1a) and underestimated it in the $^{15}NO_3$ treatment (1b). The hypothesis H5 which assumed no remineralisation of labelled N during the first 9 d could explain neither the increase in the isotopic excess of the $^{15}NH_4^+$ found in treatment 1b, nor its stabilisation observed in treatment 1a (Fig. 2).

Our conclusion is that simultaneous immobilisation of NH_4^+ and NO_3^-, gaseous losses and remineralisation were likely to occur and must be accounted for to obtain precise estimates of N fluxes. First order kinetics for nitrification is preferred to zero-order (H3) since it could simulate better the experimental results. None of the hypotheses H1, H3, H6, H7 and H8 which gave similar MWE could be rejected. The effect of these hypotheses on the calculation of N rates is variable:

(i) the existence of a direct assimilation pathway for microbial assimilation (H6) or a rapid humification affecting the N immobilised in the newly-formed biomass (H7) had very little effect on the calculation of the other N rates (Table 3).

(ii) increasing the initial size of the "zymogenous" biomass from 0 to 10 mg N kg^{-1} (1% of total organic N; H8) led to the calculation of a lower mineralisation rate and higher remineralisation rate. The remineralisation rate would then represent 63% of gross immobilisation during the first 3 d. Such a high value does not seem realistic, particularly during the initial stage of straw decomposition. Much higher values would be obtained if the immobilising pool included the whole microbial biomass or an "active" fraction representing 10–20% of total organic N. Setting the initial size of the zymo-

Table 3. N rates (mg N kg^{-1} d^{-1}) calculated by FLUAZ with the paired treatments of experiment 1 during the first incubation period (0–8.7 d), for the different hypotheses (see Table 2)

Hypothesis	N rate					
	$m + s$	i	n	r	i–r	$m + s - (i - r)$
H0	0.97	4.44	3.57	0.80	3.63	–2.67
H1	1.13	4.68	3.57	0.89	3.79	–2.66
H2	0.81	4.31	3.52	0.80	3.51	–2.70
H3	1.33	5.17	3.56	1.19	3.98	–2.65
H4	0.73	4.90	3.30	0.85	4.05	–3.32
H5	0.60	3.39	3.15	0.00	3.39	–2.79
H6	1.13	4.68	3.57	0.89	3.79	–2.66
H7	1.12	4.56	3.52	0.78	3.78	–2.66
H8	0.52	4.65	3.56	1.47	3.18	–2.66
H9	0.82	3.95	3.36	0.38	3.57	–2.75

genous biomass to 0 or a low value is probably a reasonable assumption in many cases.

Table 3 also shows that the net immobilisation rate ($i - r$) was less variable with the different hypotheses than the gross rate. The net mineralisation rate ($m + s - i + r$) was even more stable.

The sensitivity analysis was also realised onto data of experiment 2 (results not shown here). It confirmed the conclusions drawn from experiment 1.

Comparison between single and paired treatments

The previous results obtained with the paired treatments can be compared to calculations made with a single treatment. In experiments 1 and 2, only the $^{15}NH_4$ treatment (1a or 2a) was selected, since the $^{15}NO_3$ treatment does not allow to determine the mineralisation rate $m + s$. We first conducted a sensitivity analysis with this single treatment.

Table 4 displays the five hypotheses which were compared. H10 was the most general hypothesis since it did not make any assumption on rates $m + s$, i_a, i_n and n. Nitrification was supposed to obey a first order reaction rate. The remineralisation rate r was fixed to 0. Hypothesis H11 was similar to H10 but assumed that the partition between ammonium and nitrate immobilisation was determined by equation (6) with the value $\beta = 0.05$. H12 was similar to H11 but imposed the gross mineralisation rate at the value calculated by the isotopic dilution formula equation (1). H13 is the hypothesis most frequently used: gross mineralisation is calculated by isotopic dilution, nitrification is a zero order rate, ammonium immobilisation is exclusive. Finally H14 is identical to H10, except that N remineralisation rate was imposed at a positive value (equal to the average of the values previously found).

The MWEs obtained for these different hypotheses are shown at Fig. 8, for each of the two experiments. Experiment 1 always gave lower MWEs than experiment 2, as observed previously, due to the higher variance of the measurements in experiment 1. However, the effect of the various hypotheses on MWE was independent on the data set: MWE increased from H10 to H13; H14 gave as a good fit as H10. The latter result confirmed that it is not possible to calculate a remineralisation rate with a single $^{15}NH_4^+$ treatment.

The corresponding N rates calculated by FLUAZ are given at Table 5 (experiment 1) and 6 (experiment 2). Mineralisation and immobilisation rates were only slightly affected by the hypotheses H10–H13. The greatest variation was caused by hypothesis H14: imposing a positive remineralisation rate always resulted in calculating a higher immobilisation rate. However, the "net" immobilisation rate $i - r$ was much less affected. The "net" mineralisation rate $m + s - (i - r)$ was almost independent on the hypothesis chosen.

The consequence of using single or paired treatments to estimate the different N fluxes can be drawn from Tables 5 and 6, by comparing hypotheses H11 and H1 which are analogous. Small differences in N rates were found with data of experiment 2 (Table 6), whereas markedly higher rates of mineralisation, immobilisation and nitrifica-

Table 4. Description of the different hypotheses tested in experiment 1 and 2 with a single treatment ($^{15}NH_4$). Only the changing assumptions (relatively to hypothesis H10) are displayed

Hypothesis	N rate				
	$m + s$	*ia*	*in*	*n*	*r*
H10	F0	F0	F0	F1	I0 ($r = 0$)
H11	–	–	I0 ($\beta = 0.05$)		
H12	I0 (i. dilution)	–	I0 ($\beta = 0.05$)		
H13	I0 (i. dilution)	–	I0 ($\beta = 0$)	F0	
H14	–	–	–	–	I0 ($r > 0$)

F0 is the fitted rate, zero order, I0 the imposed rate, zero order, F1 the fitted rate, first order and I1 the imposed rate, first order.

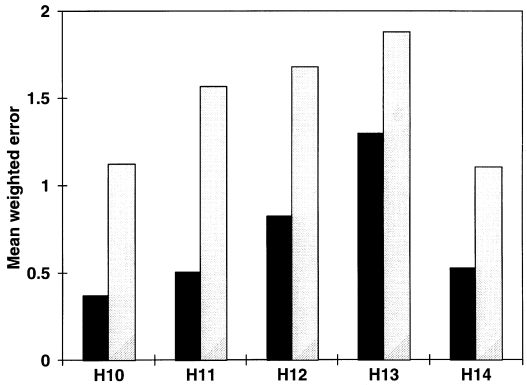

Fig. 8. Mean weighted error (MWE) obtained with the five different hypotheses tested using a single treatment. Full bars: experiment 1 (period 0–8.7 d); open bars: experiment 2 (period 0–13.7 d). Hypotheses are defined in Table 4.

tion were obtained with the "paired" treatments in experiment 1 (Table 5). This result is not attributable to the use of single or paired treatments *per se*, but to the importance of the remineralisation rate: the higher the remineralisation rate (either imposed or fitted), the higher the immobilisation rate. This was clearly shown in experiment 1 for which FLUAZ calculated a much greater remineralisation rate than in experiment 2. Moreover, hypotheses H14 (single treatment) and H1 (paired treatment) which considered similar remineralisation rates resulted in almost identical mineralisation, immobilisation and nitrification fluxes.

Relationship between C and N fluxes

CO_2 measurements made in experiments 1 and 2 indicated that C mineralisation kinetics were absolutely identical in the two treatments studied (Fig. 9). The absence of difference, expected at least in experiment 1, bears out the assumption previously made of similar fluxes in the two treatments. The slight difference between the two experiments is probably due to the use of a different wheat straw.

The N assimilation rates by straw decomposers, i.e. either the immobilisation flux i calculated with hypothesis H1 or the assimilation flux $i + j$ calculated with hypothesis H6, were then compared to C mineralisation rates (m_C) obtained during the different time intervals in the two experiments. A significant but moderate correlation was found between i and m_C: the correlation coefficient was $r = 0.55$ ($n = 15$, $P < 0.05$). The correlation between $i + j$ and m_C was slightly higher: $r = 0.69$ ($P < 0.01$). In fact the relationship between the cumulative i (or $i + j$) vs cumulative m_C was not linear but curvilinear with a slope decreasing regularly. Therefore, the assimilation rate per unit of mineralised C decreased with time.

We then plotted the N assimilation rates calculated on each time interval k vs the C mineralisation rates measured on each time interval $k + 1$. With this shift, a strong correlation was found for the immobilisation rate ($r = 0.965$, $P < 0.001$) as well as for the assimilation rate ($r = 0.975$; Fig. 10). This result can be explained by either a change in microbial metabolism, e.g. a decline in the C assimilation yield, or a delayed C mineralisation relative to N assimilation by microorganisms (the delay being about 2 d for straw at 15°C). Although the latter hypothesis has not been mentioned earlier, it may be worth considering: the results presented by Swift *et al.* (1979) for an amino-acid decomposition (Fig. 5.8, p. 195) showed that N mineralisation proceeded faster than O_2 uptake. Hopkins *et al.* (1995) found much greater rates of N than C mineralisation (20–200× greater) during the first 5 h of decomposition of methionine, MSO and MSX. Both results suggest that N mineralisation (and probably also N assimilation) may precede C mineralisation.

We obtained the following regression equations:

$$i = -0.9 + 0.200 m_C \quad (7)$$

and

$$i + j = 0.174 m_C \quad (8)$$

equation (7) gives a similar slope (0.200) to that found by Hart *et al.* (1994) for immobilisation in unamended forest soils, but has a negative intercept. The linear relationship equation (8), easier to analyse, indicates that the ratio N immobilisation: C mineralisation was lower than 174 mg N g^{-1} C. Schimel (1988) proposed the following formula to calculate the C assimilation yield

$$Y = \frac{R_B \cdot a_N}{R_B \cdot a_N + m_C} \quad (9)$$

Table 5. N rates (mg N kg^{-1} d^{-1}) calculated by FLUAZ with the single treatment 1a ($^{15}NH4 + NO_3$) of experiment 1 during the first incubation period (0–8.7 d), for the different hypotheses (see Table 4)

Hypothesis	N rate					
	$m + s$	i	n	r	$i - r$	$m + s - (i - r)$
H10	0.70	3.21	3.35	0.00	3.21	–2.51
H11	0.75	3.23	3.05	0.00	3.23	–2.48
H12	0.82	3.30	3.04	0.00	3.30	–2.48
H13	0.82	3.18	2.68	0.00	3.18	–2.36
H14	1.04	4.32	4.20	0.80	3.52	–2.49
H1	1.13	4.68	3.57	0.89	3.79	–2.66

Table 6. N rates (mg N kg^{-1} d^{-1}) calculated by FLUAZ with the single treatment 2a ($^{15}NH_4$) of experiment 2 during the whole incubation period (0–13.7 d), for the different hypotheses (see Table 4)

Hypothesis	N rate					
	$m + s$	i	n	r	$i - r$	$m + s - (i - r)$
H10	0.60	1.99	2.89	0.00	1.99	−1.39
H11	0.61	1.96	2.34	0.00	1.96	−1.35
H12	0.67	2.02	2.34	0.00	2.02	−1.36
H13	0.67	1.91	2.20	0.00	1.91	−1.25
H14	0.56	2.15	2.95	0.20	1.95	−1.39
H1	0.63	2.03	2.47	0.18	1.85	−1.22

where R_B is the C–N ratio of the decomposers and a_N the gross N immobilisation rate. In fact a_N represents the total assimilation rate and better corresponds to $i + j$ than to i. Combining equations (8) and (9) then gives

$$Y = \frac{0.174 R_B}{0.174 R_B + 1} \quad (10)$$

In this equation, assimilation yield values of 0.62, 0.60, 0.50 and 0.40 g C g^{-1} C would be obtained for C–N ratios of 9.4, 8.6, 5.75 and 3.85, respectively. The latter C–N values do not seem realistic. Therefore, straw decomposition must have proceeded with a high yield efficiency (about 0.60), close to the theoretical maximum.

DISCUSSION

The consistency of our approach can be tested by comparing the N rates calculated with other published data using ^{15}N tracing. However, the reported experiments widely vary in soil organic N content, amount of plant residues added, temperature and moisture conditions, amount of NH_4–N, all factors which strongly influence the N mineralisation, immobilisation and nitrification rates. The gross mineralisation rates reported vary from around 1 mg N kg^{-1} d^{-1} (Myrold and Tiedje, 1986; Bjarnason, 1988; Watkins and Barraclough, 1996) to 5–20 (Davidson *et al.*, 1990, 1991; Ambus *et al.*, 1992; Wessel and Tietema, 1992; Nishio, 1994; Smith *et al.*, 1994) and up to 50–200 mg N kg^{-1} d^{-1} (Schimel *et al.*, 1989; Tietema and Wessel, 1992; Wessel and Tietema, 1992). In our work, the gross mineralisation rate was estimated at 970 μg N kg^{-1} d^{-1} in Experiment 1 and 770 μg N kg^{-1} d^{-1} in Experiment 2 during the 7 d following straw addition. Watkins and Barraclough (1996) also incubated an agricultural soil with wheat straw and obtained a similar value: 900 μg N kg^{-1} d^{-1}.

Compared to the analytical methods, the method presented here has several points of interest:

(i) it does not make any approximation in the calculations. Indeed there are no exact analytical solutions of the system presented in Fig. 1, if immobilisation of nitrate and ammonium simultaneously occur, or if remineralisation occurs, or if reactions are not zero order (e.g. first order or Michaëlis–Menten kinetics). The numerical method may account for any case without appreciable error as was pointed out by Nason and Myrold (1991).

(ii) it enables testing the consistency of the data set relative to the modelled system since it combines mineral and organic N and ^{15}N measurements. This is particularly interesting in experiments with paired

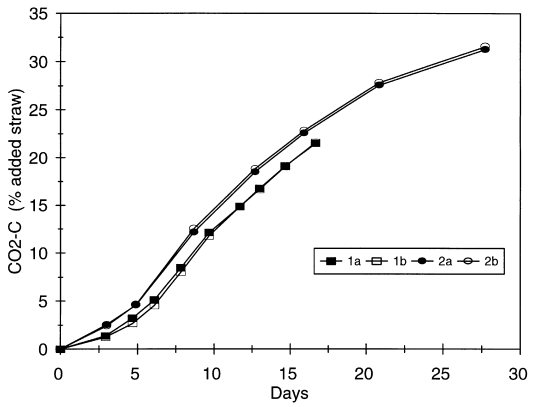

Fig. 9. Kinetics of C mineralisation observed in experiments 1 and 2. Treatment 1a (■), treatment 1b (□), treatment 2a (●) and treatment 2b (○).

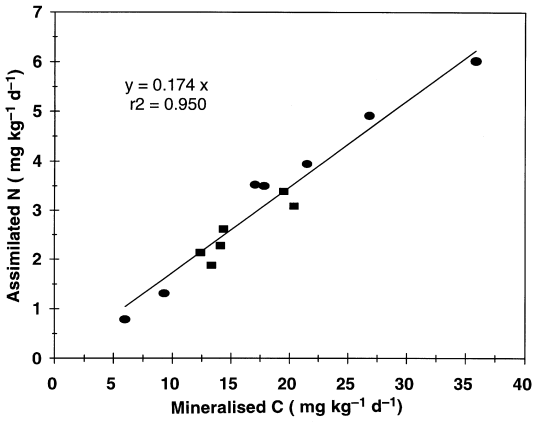

Fig. 10. Relationship between N assimilation rate (rate $i + j$) and C mineralisation rate (rate m_C) calculated during each time interval in experiments 1 and 2. Square symbols: experiment 1. Round symbols: experiment 2.

treatments which produce an overdetermined system (8 measured variables compared to 4 or 5 calculated fluxes).

(iii) the criterion to minimize, MWE, takes the data variability into account. This avoids putting too much weight on variables having a high coefficient of variation, such as NH_4^+ pool when it is depleted and favours the variables more precisely determined. The importance of weighting data has been emphasized by Nason and Myrold (1991). The mean weighted error scales and gathers all variables in one index. The differences between each observed and measured variable can be further tested using the other variance ratio F = LOFIT/SSE proposed by Whitmore (1991) and complementary statistical tests (Smith *et al.*, 1996).

(iv) FLUAZ calculates all rates simultaneously and provides confidence intervals and a correlation matrix between parameters. Analytical methods calculating the rates successively, starting with gross mineralisation rate, may result in transmitting errors and transferring all the variability on the last fluxes calculated, without any indication of the accuracy of the estimated rates.

(v) the program is easy and rapid to run. Non linear fitting procedures and numerical calculations used to be tedious and time-consuming. This drawback no longer holds with present PC computers. This allows running simulations and carrying out sensitivity analyses in order to check the accuracy of the calculations performed.

Sensitivity analysis applied to our data has shown the importance of considering gaseous losses when estimating N rates. Rapid disappearance of the ^{15}N tracer is often observed soon after its application. Davidson *et al.* (1991) found a ^{15}N recovery varying from 63% to 91% within 15 min in the laboratory and even lower values in the field. In the case of $^{15}NH_4^+$ application, the drop can be attributed either to NH_4^+ fixation or to NH_3 volatilisation. Both processes must be studied carefully and measured as far as possible. Incomplete recovery of labelled N after addition of $^{15}NO_3^-$ was also found in well-aerated soils (Myrold and Tiedje, 1986; Davidson *et al.*, 1991; this work) indicating that small but significant amounts of nitrate may be denitrified. Again, direct measurements of ^{15}N–$(N_2O + N_2)$ would be useful to improve accuracy of N rates.

We found that N remineralisation was significant and occurred almost simultaneously with N immobilisation. Wessel and Tietema (1992) also concluded that "recycling was relevant in the majority of $^{15}NH_4$ enrichment experiments and should be reckoned with even in short experiments". Bjarnason (1988) calculated slightly delayed remineralisation and concluded that estimates of gross N transformations could be seriously erroneous if remineralisation is not accounted for. Nishio (1994) did not consider remineralisation in his model but did consider the possible reduction of nitrate into ammonium. The two processes are in fact difficult to distinguish from each other: only ^{15}N-labelled microbial biomass measurements can separate them. However, dissimilatory reduction of nitrate into ammonium occurs only at very low oxido-reduction potentials, lower than denitrification (Fazzolari *et al.*, 1990). This process is therefore unlikely in aerobic incubations, remineralisation being more plausible. Accurate measurement of the remineralisation process is difficult, because it relies on the quality of measurements of atom% excess of low quantities of ammonium (in the $^{15}NO_3^-$ treatment) and it strongly depends on the initial size of the microbial biomass which is considered (cf. hypothesis H8 in Table 3). The existence of rapid remineralisation implies that single $^{15}NH_4$ experiments (in which remineralisation cannot be assessed) provide approximate values of gross fluxes and particularly underestimates N immobilisation. Nevertheless, we have shown that they provide accurate estimates of the "net" immobilisation rates $(i - r)$.

Our results also indicated that immobilisation of NH_4^+ and NO_3^- take place simultaneously in soil, even though there is a preferential uptake of NH_4^+. This observation could mainly result from the lower mobility of NH_4^+ ions in soil solution due to adsorption rather than the physiological response of microbes, which would bear out our formula (6). Our results are consistent with those of Rice and Tiedje (1989) and Recous *et al.* (1990) who found that NO_3^- assimilation was strongly but not completely inhibited by the presence of NH_4^+. We could simulate the results of these authors (Fig. 1 and Fig. 2(b) respectively) using equation (6) and a β value greater than ours (0.10–0.25), indicating that microbial "preference" for NH_4^+ was even smaller than what we found (results not shown). Therefore we think that equation (6) can be usefully applied to single experiments to obtain better estimates of immobilisation than by supposing exclusive immobilisation of ammonium ($\beta = 0$) or by supposing that immobilisation of nitrate is nil ($i_n = 0$) which is even a more severe assumption.

The FLUAZ program (with a small brochure) is available on request to the first author.

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