

# Explicit consideration of microbes in quantification of gross N rates based on N-tracing model

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## Abstract

When studying the nitrogen cycle in soils, the principle of  $^{15}\text{N}$  isotope dilution and enrichment is commonly used to measure gross N transformation rates. The measuring data of both organic and inorganic nitrogen substrates are therefore analysed by *Ntrace* model. Although all transformations between organic and mineral N pools are microbial, the measurement of microbial  $^{15}\text{N}$  has rarely been done due to technical difficulties and uncertainties. To gain a deeper understanding of the effects of microbes in the mineralization-immobilization turnover, a new version of the *Ntrace* model was developed. By considering microbial  $^{15}\text{N}$  in the tracing model, this research investigates whether explicit consideration of microbial biomass is essential during the quantification of gross N rates and the practicability of our new model. In this study we used previously published data to test the new developed model *Nmicr* compared to the frame conceptual  $^{15}\text{N}$  trace model *Nbas*. By adding two additional parameters: release of ammonium from the microbes ( $R_{\text{NH}_4}$ ) and the release of organic N from microorganisms to the organic nitrogen pool ( $I_{\text{Nmic}}$ ), a total of eight N transformation rates were included in the *Nmicr*. To reassure the basic rule of organic nitrogen assimilates before conversion to ammonium in *Nbas*, we also made a third model *Nmit* with a direct route from organic N to  $\text{NH}_4^+$ . In conclusion, The *Nmicr* simulation is generally convincing with well-defined probability density functions (PDFs). Our result showed an inseparable interaction between the organic N pool and the microbial biomass in *Nmicr* simulation: (1) Strong correlation coefficient ( $\approx 0.96$ ) of gross rate within the efflux ( $M_{\text{Norg}}$ ) and influx ( $I_{\text{Nmic}}$ ) of organic nitrogen pool in the permanent grassland soil samples (data GM). (2)  $I_{\text{Nmic}}$  and  $M_{\text{Norg}}$  were both presenting the largest or the second largest gross rate in both datasets. (3) The calculated gross rate of  $I_{\text{Nmic}}$  was bigger than  $R_{\text{NH}_4}$  in both datasets. This study also suggested the importance of including explicit microbial biomass in the simulation: The result of *Nmit* simulation indicated a necessary efflux coming from the organic pool to the microbial pool ( $M_{\text{Norg}}$ ) which could lead to a downward trend in microbial  $^{15}\text{N}$ . Further research could be carried out with two or more organic N pools. It would also be interesting to include the oxidation of organic N pool to nitrate ( $O_{\text{Norg}}$ ) and the dissimilatory reduction of nitrate to ammonium ( $D_{\text{NO}_3}$ ).

*key words: N-tracing model, microbial biomass, gross N quantification, organic N, mineralization, immobilization*

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## 1. Introduction

Nitrogen cycle is a biogeochemical transformation in which nitrogen is converted into organic and mineral forms that circulate among the atmosphere, terrestrial and aquatic ecosystems. Nitrogen is an essential nutrient for all living creatures: it is a constituent of RNA, DNA, and protein. Being the most abundant element in the earth's atmosphere, nitrogen could, however, be utilized by living organisms only when the atmospheric nitrogen ( $N_2$ ) is converted into biologically usable form through fixation in the soils. In general, processes that could release or restrain plant-available N in the soils such as the mineralization-immobilization turnover are considered to be microbial (Gottschalk, 1986; Merrick and Edwards, 1995; Cronan, 2018, p.34). Mineralization-immobilization turnover is showing two microbial processes of organic N intake and  $NH_4^+$  release in mineralization (Waksman, 1927), and the preferably  $NH_4^+$  consumption with a release of organic nitrogen back to the organic N pool during immobilization (Jansson, 1958). This phenomenon occurs to adjust the soil N content. Mineralization, in which the organic N being decomposed to inorganic N, controls soil mineral N availability. Immobilization, on the other hand, which takes up organic N and could contribute to a decrease of N leaching, regulates N concentrations in soil solution (Sawada et al. 2015; Qiu et al. 2007). It is believed that the microbes could cope with resource imbalances by regulating nitrogen use efficiency (NUE), which partitioned the organic N uptake within growth and released inorganic N back to the environment (Mooshammer et al., 2014). Therefore, consideration of microbial biomass is important when it comes to quantifying soil mineralization and immobilization rate (Kreutzer et al, 2009; Tahovská et al, 2013).

$^{15}N$  isotope dilution and enrichment has been regarded as a valuable technique to investigate the gross N fluxes on land. Soil samples were injected with  $^{15}N$  enrichments then set in laboratory incubation from one day to several weeks. The principle of the method is to measure the decrease N abundance of an enriched pool for gross production. To have a better look at the nitrogen cycle system in the soil, conceptual N models have been developed to analyse these data. The model was originally based on two pools, the mineral nitrogen, and the organic nitrogen (Kirkham & Bartholomew, 1954). The inorganic N was then divided into  $NH_4^+$  and  $NO_3^-$  measurement with the same immobilization rate by Nishio et al., (1985) while later studies found a dominant of ammonium in microbial metabolism (Rice and Tiedje, 1989). This has changed the assumption of immobilization rate from regarding nitrate and ammonium as one rate into two separate immobilization rates (Schimel, 1996).

Compared to the mineral pool, the organic N is showing an initialization of the total soil nitrogen. Previous study (Mary et al., 1998) has considered the microbial biomass in FLUAZ tracing model, however, absolute measurements of microbial  $^{15}N$  are difficult due to the determination of the ratio of extracted N to biomass N. It is noted that the microbial biomass has conceptually no difference from the total organic nitrogen pool if the data of microbial biomass has not been applied. In addition, due to the limitation of Levenberg-Marquardt algorithms method, the technique has its uncertainty in searching for global minimum when having more than six parameters during the simulation (Aster et al., 2005). Therefore, preselection of the N transformations is needed to simplify the model. This could however lead to preference bias which affects the gross N calculation since important fluxes might be excluded at the beginning of the experiment (Müller et al., 2005).

In order to solve these problems, a new developed model *Nmicr* which has been developed in this study was based on *Ntrace*. The *Ntrace* model considers different parameters in its simulation. These

processes are fluxes between ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and organic nitrogen pool (Norg). The program is made up of several equations with non-linear optimization routes which could be used as a method to minimize the misfit between modelled and observed data (Müller et al., 2007). Moreover, *Ntrace* has the advantage of including all fluxes into conception via Monte Carlo sampling. Yet the microbial  $^{15}\text{N}$  has not been applied to *Ntrace*, by simply adding influx and efflux from the microbial biomass in the new simulation, we can compare the calculation results between the two models without excluding any other N transformations.

The main question of this research is to understand whether the consideration of microbial biomass is essential by applying existing datasets through the *Ntrace* model (*Nbas*) and comparing its gross rate with that of our new model (*Nmicr*). New fluxes contained in *Nmicr* were (1) processes of ammonium that released from the microbial biomass and (2) the release of organic nitrogen from *Nmicr* to Norg. Since the pioneer work of Kirkham and Bartholomew in 1954, realistic conceptual N models are now able to include more than one organic N pool and additional N losses (Mary et al., 1998) while in this study, the N losses were excluded, and we only considered one organic pool for simplicity of the experiment. Further application could be done once the *Nmicr* model works. Side question of this research is to examine the concept of mineralization passing the microbial biomass before entering the ammonium pool. Various models assumed that all nitrogen mineralized to ammonium before assimilation (Jansson and Persson, 1982), while discrepancy results in later studies (Barak et al, 1990; Barraclough, 1997) showed that during the composition of amino acids, N could go directly to the microbial biomass consistent with a route which describes the uptake of ammonium by the microbial biomass released from the ammonium pool. Hence, we had another *Nmit* model which was adjusted from the *Nmicr* model prepared for the test. In this system, the mineralization process is not going through the microbial biomass, but the organic nitrogen is converted directly into ammonium. As a result, parameter  $R_{\text{NH}_4}$  is precluded as no ammonium is released from the microbes ( $R_{\text{NH}_4}$ ) in model *Nmit*.

## 2. Materials and Methods

### 2.1 Model development

Microbial activities play an important role in the mineralization and immobilization processes, to know whether explicit consideration of microbial biomass is requisite, here we used two conceptual models *Nbas* and *Nmicr* to optimize existing datasets. Both *Ntrace* models had its model setup in Simulink (R2020a Update 6, The MathWorks Inc.) and its algorithm in MATLAB (R2020a Update 6, The MathWorks Inc.). Models are common methods used to describe the interactions within various product pools. The main difference between these two  $^{15}\text{N}$  tracing models is whether the measurement of microbial  $^{15}\text{N}$  is applied or not. The basic *Ntrace* model, which is referred to as *Nbas* here, was used as a reference frame. The algorithm is called the  $^{15}\text{N}$ -tracing model. Six fluxes were reflected within three different organic and inorganic pools, namely nitrification ( $\text{NH}_4^+ \rightarrow \text{NO}_3^-$ ,  $O_{\text{NH}_4}$ ), dissimilatory reduction of nitrate ( $\text{NO}_3^- \rightarrow \text{NH}_4^+$ ,  $D_{\text{NO}_3}$ ), mineralization ( $\text{N}_{\text{org}} \rightarrow \text{NH}_4^+$ ,  $M_{\text{Norg}}$ ), ammonium immobilization ( $\text{NH}_4^+ \rightarrow \text{N}_{\text{org}}$ ,  $I_{\text{NH}_4}$ ), nitrate immobilization ( $\text{NO}_3^- \rightarrow \text{N}_{\text{org}}$ ,  $I_{\text{NO}_3}$ ) and the heterotrophic nitrification ( $\text{N}_{\text{org}} \rightarrow \text{NO}_3^-$ ,  $O_{\text{Norg}}$ ) (Fig. 1).

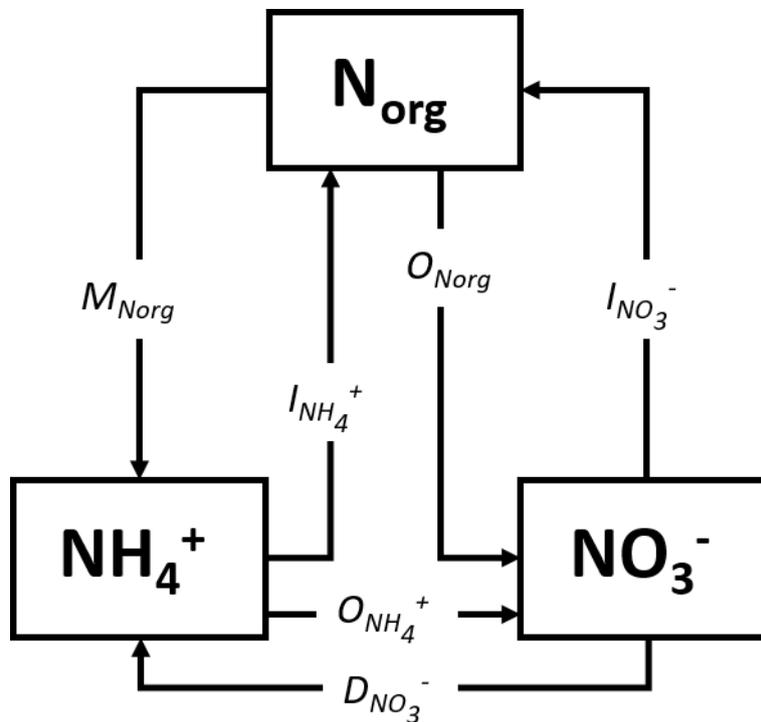


Fig. 1. Conceptual  $^{15}\text{N}$  tracing model *Nbas* was used as a reference frame. Six fluxes were reflected within three different organic and inorganic pools, namely nitrification ( $\text{NH}_4^+ \rightarrow \text{NO}_3^-$ ,  $O_{\text{NH}_4}$ ), dissimilatory reduction of nitrate ( $\text{NO}_3^- \rightarrow \text{NH}_4^+$ ,  $D_{\text{NO}_3}$ ), mineralization ( $\text{N}_{\text{org}} \rightarrow \text{NH}_4^+$ ,  $M_{\text{Norg}}$ ), ammonium immobilization ( $\text{NH}_4^+ \rightarrow \text{N}_{\text{org}}$ ,  $I_{\text{NH}_4}$ ), nitrate immobilization ( $\text{NO}_3^- \rightarrow \text{N}_{\text{org}}$ ,  $I_{\text{NO}_3}$ ) and the heterotrophic nitrification ( $\text{N}_{\text{org}} \rightarrow \text{NO}_3^-$ ,  $O_{\text{Norg}}$ ).

*Nmicr* is a more complicated model compared to *Nbas* (Fig. 2). An overall of eight processes were taken in its simulation after considering the  $^{15}\text{N}$  of the microbial biomass and its N content. Two new fluxes were included in the system: the transformation in which microorganisms consume organic

nitrogen and release ammonium ( $R_{NH_4}$ ) and the efflux of organic nitrogen from the microbial biomass to the organic N pool ( $I_{Nmic}$ ). The  $M_{Norg}$  written here indicates the output of an organic nitrogen pool in which organic N is consumed by the microbial biomass ( $N_{org} \rightarrow N_{mic}$ ), but not the mineralization process of  $M_{Norg}$  ( $N_{org} \rightarrow NH_4^+$ ) in *Nbas*.

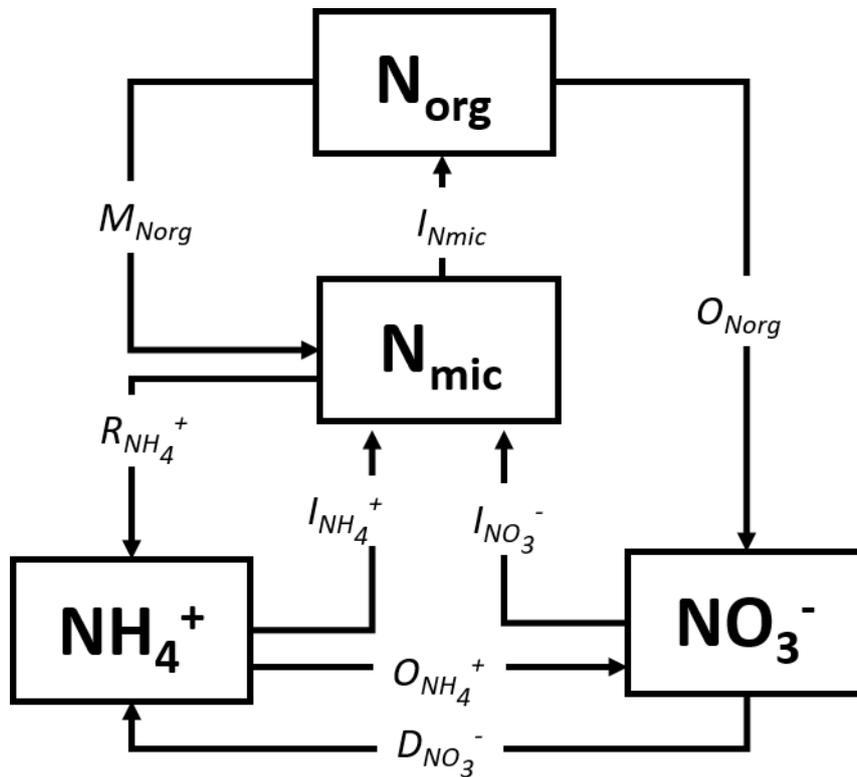


Fig. 2. *Nmicr* includes eight fluxes in the simulation. Two new fluxes have been applied in the system: the flux where microorganisms consume organic nitrogen and release ammonium ( $R_{NH_4}$ ) and the immobilization which refers to the mineral nitrogen (ammonium and nitrate) taken up by the microbes ( $I_{Nmic}$ ).

To understand whether the organic nitrogen turns into ammonium before microbial assimilation, the two datasets were tested in another *Nmit* model as well. The *Nmit* model is formed on *Nmicr*, while one contrast is that the organic nitrogen could turn directly into ammonium without passing through the microbial biomass (Fig. 3). Seven fluxes were included in *Nmit*, but one process was eliminated: a release of ammonium from the microbial biomass ( $R_{NH_4}$ ).

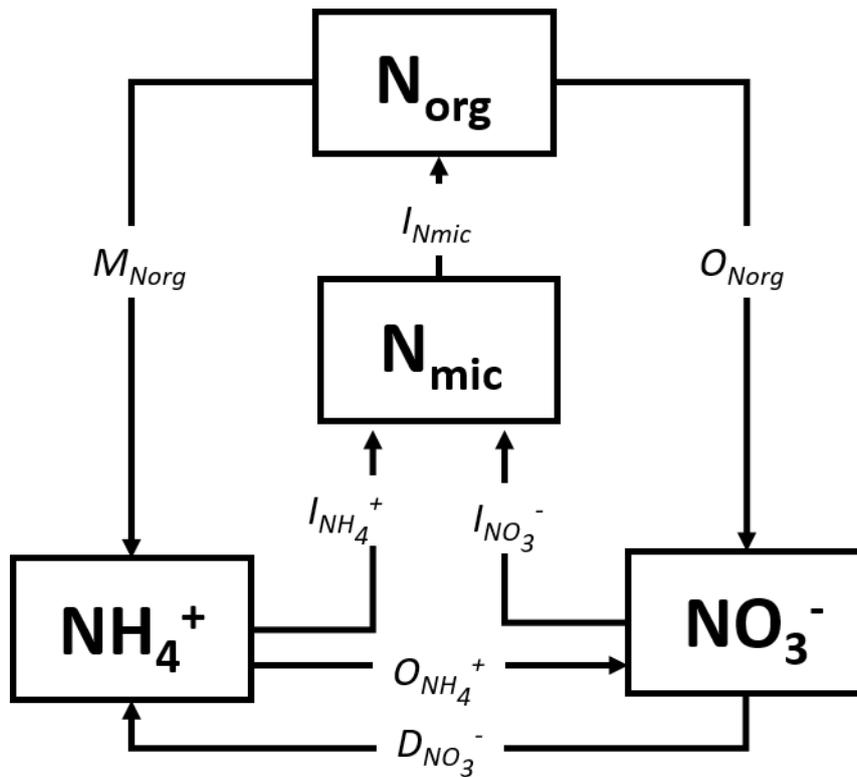


Fig. 3. The third model *Nmit* tests if the organic N could convert directly into ammonium before being consumed by microbial biomass. Compared to *Nmicr*, no ammonium is released from the microbes ( $R_{NH_4}$ ).

When doing the simulation, whether the flux can be simulated is determined from the original tracing experiment. In this study, the initial values of the two nitrogen processes: the oxidation of  $N_{org}$  to  $NO_3^-$  ( $O_{Norg}$ ) and the dissimilatory reduction of  $NO_3^-$  to  $NH_4^+$  ( $D_{NO_3}$ ) were excluded in our simulation since  $^{15}NO_3^-$  did not carry out as a labelling treatment in the original research.

## 2.2 Preselection of datasets

$^{15}N$  isotope enrichment and dilution are key principles when calculating gross N transformation rates, measurements on N concentration and  $^{15}N$  are available based on different labels in the tracing experiments. To quantify gross rates, gross N production and gross N consumption rates could be calculated from the changing size of the total nitrogen pool and from the decline in  $^{15}N$  enrichment above natural abundance over time (Barraclough, 1991; Hart et al., 1994; Kirkham and Bartholomew, 1954; Murphy et al., 2003).

In each optimization run, parameter values could be represented as its own probability density plots, therefore, a proper data set should be selected before starting the first run. The objective is to find datasets that present a reasonable decline in  $^{15}NH_4^+$  due to mineralization after the ammonium pool has been labelled. Meanwhile, it should be expected that  $^{15}NO_3^-$  is increasing because of nitrification. Ideal plot should be shown as follows (Fig. 4).

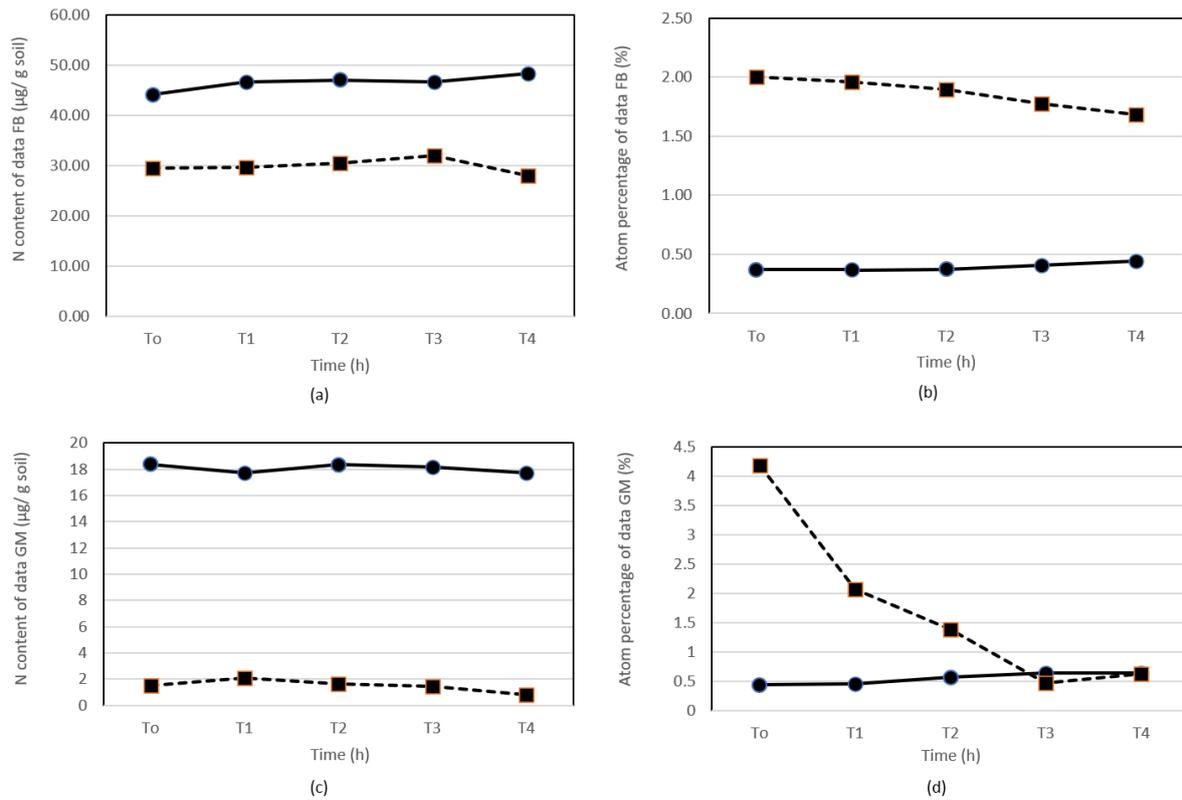


Fig. 4. Plots of N content ( $\mu\text{g/g soil}$ ) and  $^{15}\text{N}$  variation of the selected datasets (Braun et al., 2018). Two datasets were collected from a beech forest (FB) and from a permanent grassland (GM). To have reasonable simulation result, data sets that present a reasonable decline in  $^{15}\text{NH}_4^+$  (dotted line with square mark) due to mineralization with  $^{15}\text{NO}_3^-$  (solid line with circular mark) increment because of nitrification is needed. T0-T4 indicates five-time intervals: 0h, 0.25h, 3.5h, 24h and 48h after applying  $^{15}\text{NH}_4^+$  tracer.

### 2.3 Dataset description

The datasets used in this study were based on live (non-sterilized) soil samples taken from two sites in Austria, October of 2014 (Braun et al., 2018). Five consecutive measurements of concentrations and isotope composition of the ammonium, nitrate and microbial biomass pools were conducted in different incubation time intervals: 0h, 0.25h, 3.5h, 24h and 48h within 48 hours after adding the  $^{15}\text{NH}_4^+$  tracer. Two of these datasets will be referred to as data FB and data GM separately later in this study. Data FB was taken from the upper 10 cm of the mineral soil of a beech (*Fagus sylvatica* L.) forest and data GM indicated permanent grassland. Due to different vegetation composition and soil pH, the total N of data FB was  $0.2 \pm 0.0 \%$  and that of  $0.3 \pm 0.1 \%$  in data GM. The soil ammonium contents were determined as  $29.2 \pm 0.7 \mu\text{Ng.d.w.}$  in soil FB and  $1.3 \pm 0.1 \mu\text{Ng.d.w.}$  in soil GM. The soil description of two datasets is presented in Table 1.

Table 1. Two datasets (Braun et al., 2018) which were collected in beech forest (FB) and permanent grassland (GM) were used in our simulation. Due to different vegetation composition and soil pH, the total N of data FB was  $0.2 \pm 0.0 \%$  and that of  $0.3 \pm 0.1 \%$  in data GM. The soil ammonium contents were determined as  $29.2 \pm 0.7 \mu\text{Ng}$  and  $1.3 \pm 0.1 \mu\text{Ng}$ .

Soil parameter	Data FB	Data GM
Vegetation type	Beech forest	Permanent grassland
Soil pH	4.0 ± 0.0	6.0 ± 0.0
Total N (%)	0.2 ± 0.0	0.3 ± 0.1
NH <sub>4</sub> <sup>+</sup> concentration (µg/ g soil)	29.2 ± 0.7	1.3 ± 0.1
Incubation time	0, 0.25, 3.5, 24 and 48	0, 0.25, 3.5, 24 and 48

## 2.4 Calculation of average N content and standard deviation

The original research was performed in triplicates, therefore the average values of N concentration, isotope composition and standard deviations of ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) and the microbial biomass (Nmic) should be calculated in both samples before the simulation run. The average values of data FB and GM are shown in Table 2.

Table 2. Average values and standard deviations of N content (NH<sub>4</sub><sup>+</sup>, Nmic and NO<sub>3</sub><sup>-</sup>) and the <sup>15</sup>N enrichment (<sup>15</sup>N-NH<sub>4</sub><sup>+</sup>, <sup>15</sup>N-Nmic and <sup>15</sup>N-NO<sub>3</sub><sup>-</sup>) of the three replicates of data FB and data GM were calculated.

Datasets	Time	NO <sub>3</sub> <sup>-</sup>		<sup>15</sup> N-NO <sub>3</sub> <sup>-</sup>		Nmic		<sup>15</sup> N-Nmic		NH <sub>4</sub> <sup>+</sup>		<sup>15</sup> N-NH <sub>4</sub> <sup>+</sup>	
		avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
FB	T0	44.17	0.76	0.37	0.00	17.37	1.89	0.41	0.01	29.45	0.79	2.01	0.01
	T1	46.70	1.40	0.37	0.00	14.57	1.18	0.35	0.02	29.62	0.78	1.96	0.02
	T2	47.10	2.01	0.37	0.00	14.18	0.47	0.40	0.02	30.55	0.51	1.89	0.02
	T3	46.76	1.23	0.41	0.00	15.35	1.55	0.30	0.14	32.08	0.18	1.78	0.00
	T4	48.42	1.77	0.44	0.01	13.46	2.63	0.30	0.06	28.08	0.37	1.69	0.01
GM	T0	18.39	0.22	0.43	0.00	9.46	1.14	0.47	0.01	1.48	0.04	4.20	0.18
	T1	17.70	0.48	0.45	0.00	9.47	0.27	0.43	0.02	2.08	0.36	2.07	0.18
	T2	18.35	0.07	0.56	0.01	9.74	1.95	0.43	0.01	1.62	0.27	1.38	0.10
	T3	18.17	0.66	0.64	0.01	9.70	0.43	0.42	0.01	1.44	0.15	0.47	0.09
	T4	17.70	0.86	0.63	0.01	11.59	1.54	0.42	0.02	0.82	0.47	0.63	0.26

## 2.5 Model simulations

In this study, a reliable optimization run included 3 sequences with 10000 to 50000 iterations. Most of the simulations iterated 10000 times while optimization might be put into 50000 iterations if the results showed a better distribution performance with higher iterations. All fluxes considered in the algorithm were set to either first- or zero-order kinetics (Kirkham and Bartholomew, 1955). The natural abundance of the soil <sup>15</sup>N in the *Ntrace* model was 0.3663 atom% (Mariotti, 1983). Since the soil samples were carried as a single <sup>15</sup>NH<sub>4</sub><sup>+</sup> labelling experiment (Braun et al., 2018), two parameters: the oxidation of Norg to NO<sub>3</sub><sup>-</sup> (O<sub>Norg</sub>) and the dissimilatory reduction of NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> (D<sub>NO3</sub>) was not considered here in the simulation run. The initial values of the two parameters were therefore set to zero in our experiment. The values of measured concentration, nitrogen <sup>15</sup>N and the standard deviation of the ammonium and nitrate were recorded in separate sheets as <sup>15</sup>N label1 in excel file named “data” while performing the *Nbas* model simulation, whereas adding measurements of the microbial biomass in the *Nmicr* simulation. During the run, Akaike information criterion (AIC) and cost function were two important values which provide the quality of our model performance. A lower AIC indicated a higher quality of the given model as less information was lost. Cost function calculated the difference between both model and actual outputs; therefore, the lower cost function showed a better simulation result.

The simulations were firstly tested in conceptual *Nbas* model with data FB and data GM. Gross transformation and its uncertainties could be obtained after the mean parameters were calculated in the run. We then performed the optimization again but in *Nmicr*, which had the measurements of microbial biomass as an additional pool called *Nmic*. With new gross transformation rates, we compared the differences in the result. The determination of the microbial N content (*Nmic*) and its isotope ratio ( $^{15}\text{N}_{\text{mic}}$ ) was carried out in the original experiment (Braun et al., 2018): *Nmic* was calculated from the differences of total dissolved nitrogen (TDN) with and without liquid chloroform and the isotopic mass balance equation was used to obtain  $^{15}\text{N}_{\text{mic}}$ .

## 2.6 Calculation of gross transformation rate and the uncertainties

After model simulation of each dataset, the mean parameter density function values (paramean) and standard deviation (stdev) of the six parameters ( $M_{\text{Norg}}$ ,  $I_{\text{NH}_4}$ ,  $O_{\text{NH}_4}$ ,  $I_{\text{NO}_3}$ ,  $O_{\text{Norg}}$  and  $D_{\text{NO}_3}$ ) could be obtained for further calculation from the MATLAB's workspace. Paramean were six values of the nitrogen processes we considered in the *Nbas* and *Nmicr* models. The corresponding kinetics were either zero-order or first-order. If the nitrogen transformation process was regarded as zero-order kinetic, the gross flux was equal to the kinetic factor constant value "k", which had already been showing as paramean that has been optimized by the tracing model:

$$F = k \text{ [ } \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1} \text{ ]} \quad (1)$$

When it was first-order kinetic, we computed the average value of either ammonium or nitrate substrate before multiplying the paramean. The data could be acquired from *Model\_values* stated in the workspace, in which the concentration and  $^{15}\text{N}$  values of different nitrogen pools were stored. The formula could be written as below:

$$F = N * k \text{ [ } \mu\text{mol} \cdot \text{g}^{-1} \text{ ] [ h}^{-1} \text{ ]} \quad (2)$$

For the uncertainties, we used the standard deviation values to divide paramean and multiply gross rate under first-order kinetic. The zero-order kinetic, could be the same value as standard deviation values simulated from the model.

### 3. Results

To compare nitrogen transformation rates of each dataset between *Nbas* and *Nmicr*, simulations were done in the two models separately. Optimizations were carried out in three sequences, 10000 to 50000 iterations with six parameters in *Nbas* while eight parameters in *Nmicr*. Parameters could be omitted once their probability density function (PDFs) plots showed a truncated distribution during the optimization. The correlation coefficient (CR) between the parameters of data FB in the *Nbas* and *Nmicr* model were listed in Table 3 and 4, data GM in Table 5 and 6. The mean values of the parameters and standard deviation which were obtained from the run were tabulated in Table 7 and 8. Net production of the four different nitrogen pools were calculated in Table 9 and 10.

#### 3.1 Simulation of the data set FB

Data FB was first run in the *Nbas* model with 10000 iterations. In this optimization run (Fig. 5) the measured average concentration nitrate content at T0 (44.17) was replaced by the average of five interval values (46.63) to reduce the misfit between measured points and the simulation. Overall, the PDFs of three fourths parameters  $M_{\text{NoTg}}$ ,  $I_{\text{NH}_4}$  and  $O_{\text{NH}_4}$  were well distributed (Fig. 6). The PDF of parameter  $I_{\text{NO}_3}$  showed a truncated distribution in various testing results, so this particular flux was omitted in the simulation. The AIC of this simulation was 300.5 and the cost function was 349.8.

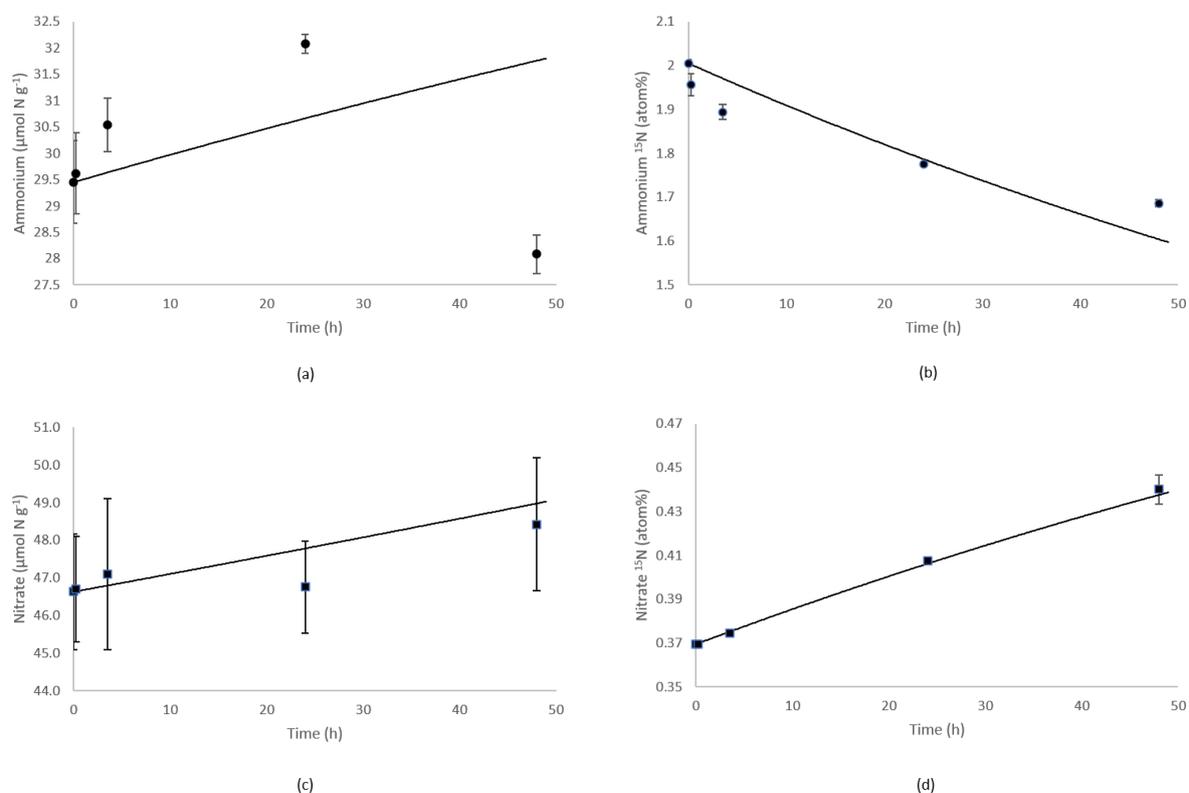


Fig. 5. Measured and simulated concentrations and  $^{15}\text{N}$  enrichments of ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) of data FB via *Nbas* model from T0 to T4 (0h, 0.25h, 3.5h, 24h and 48h). The solid line illustrated the simulation result from *Nbas*, and the measured data points were marked with different patterns (circular indicated  $\text{NH}_4^+$  and square indicated  $\text{NO}_3^-$ ) and error bars (uncertainties).

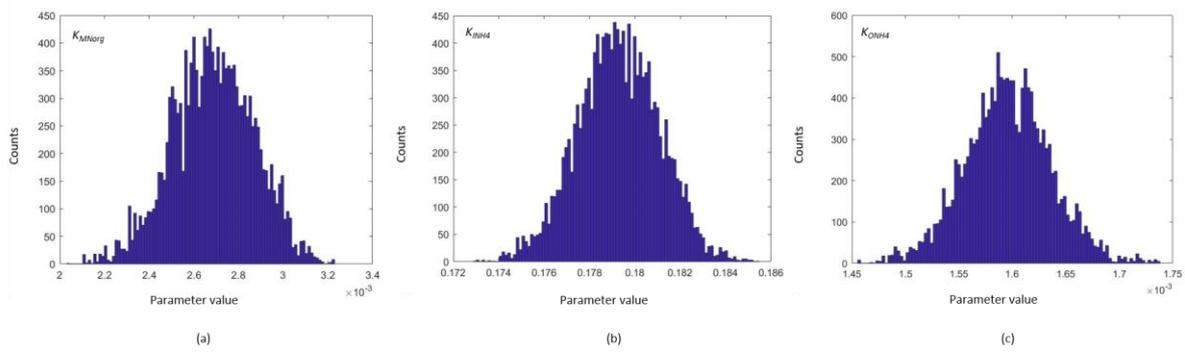


Fig. 6. Probability density plots of the four parameters ( $k_{MNorg}$ ,  $k_{INH4}$ ,  $k_{ONH4}$  and  $k_{INO3}$ ) in data FB via *Nbas*. Note that parameter  $I_{NO3}$  ( $k_{INO3}$ ) was omitted in the simulation as it showed a truncated distribution in our optimization. ( $k_{MNorg}$  = mineralization from Norg to  $NH_4^+$ ;  $k_{INH4}$  = immobilization of  $NH_4^+$  to Norg;  $k_{ONH4}$  = oxidation of  $NH_4^+$  to  $NO_3^-$  and  $k_{INO3}$  = immobilization of  $NO_3^-$  to Norg)

In our newly developed *Nmicr* model, the simulation included six parameters but had an exclusion of  $I_{NH4}$  and  $I_{NO3}$  due to a truncated distribution. The simulated results of the concentrations and  $^{15}N$  enrichments of ammonium and nitrate were generally promising (Fig. 7). The cost function of this run was 573.6 and the AIC was 498.6. Two PDFs of parameter  $R_{NH4}$  and  $I_{Nmic}$  had the distribution skewed to the left (Fig. 8). As for the microbial biomass, we saw a progressively decrease in both  $Nmic$  content simulation and the  $^{15}N$ . However, a gap between the microbial  $^{15}N$  simulated value and the measuring point in 48 h (T4) was observed. The general microbial  $^{15}N$  trend started with a slightly higher consumption rate followed by a steady decline through time with lowest point at 0.37%, yet the measured value 0.30%. This could have a high possibility due to the measuring data of microbial  $^{15}N$  had a lower atom percentage than the natural abundance (0.3663%) we had set in the *Ntrace* model. To test our thoughts, we first ran the original dataset in which the microbial biomass  $^{15}N$  in 24 h (T3) and 48 h (T4) were both measured as 0.30% and the lowest simulated point dropped by 0.38%. Later, we replaced the original values into the average of microbial  $^{15}N$  from T0 to T5 (0.35) then ran the simulation again. The simulated value lowered but not fully improved. The result showed that the lowest point of the microbial  $^{15}N$  decrement in the simulation stopped by 0.37% and could not go further down.

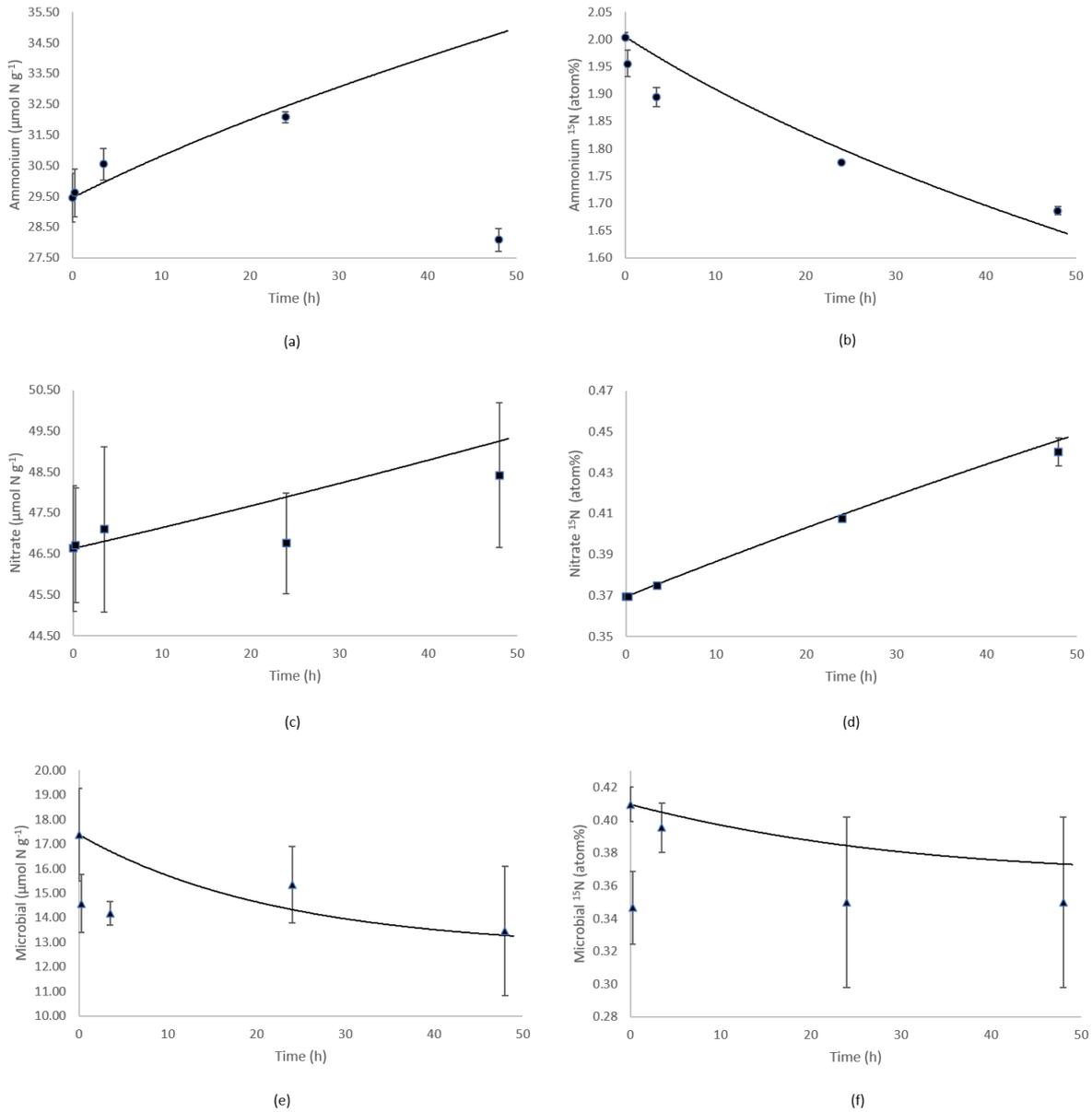


Fig. 7. Measured and simulated concentrations and  $^{15}\text{N}$  enrichments of ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and microbial biomass ( $\text{N}_{\text{mic}}$ ) of data FB via *Nmicr* model from T0 to T4 (0h, 0.25h, 3.5h, 24h and 48h). The solid line illustrated the simulation result from *Nmicr*, and the measured data points were marked with different patterns (circular indicated  $\text{NH}_4^+$ , square indicated  $\text{NO}_3^-$  and triangle indicated  $\text{N}_{\text{mic}}$ ) and error bars (uncertainties).

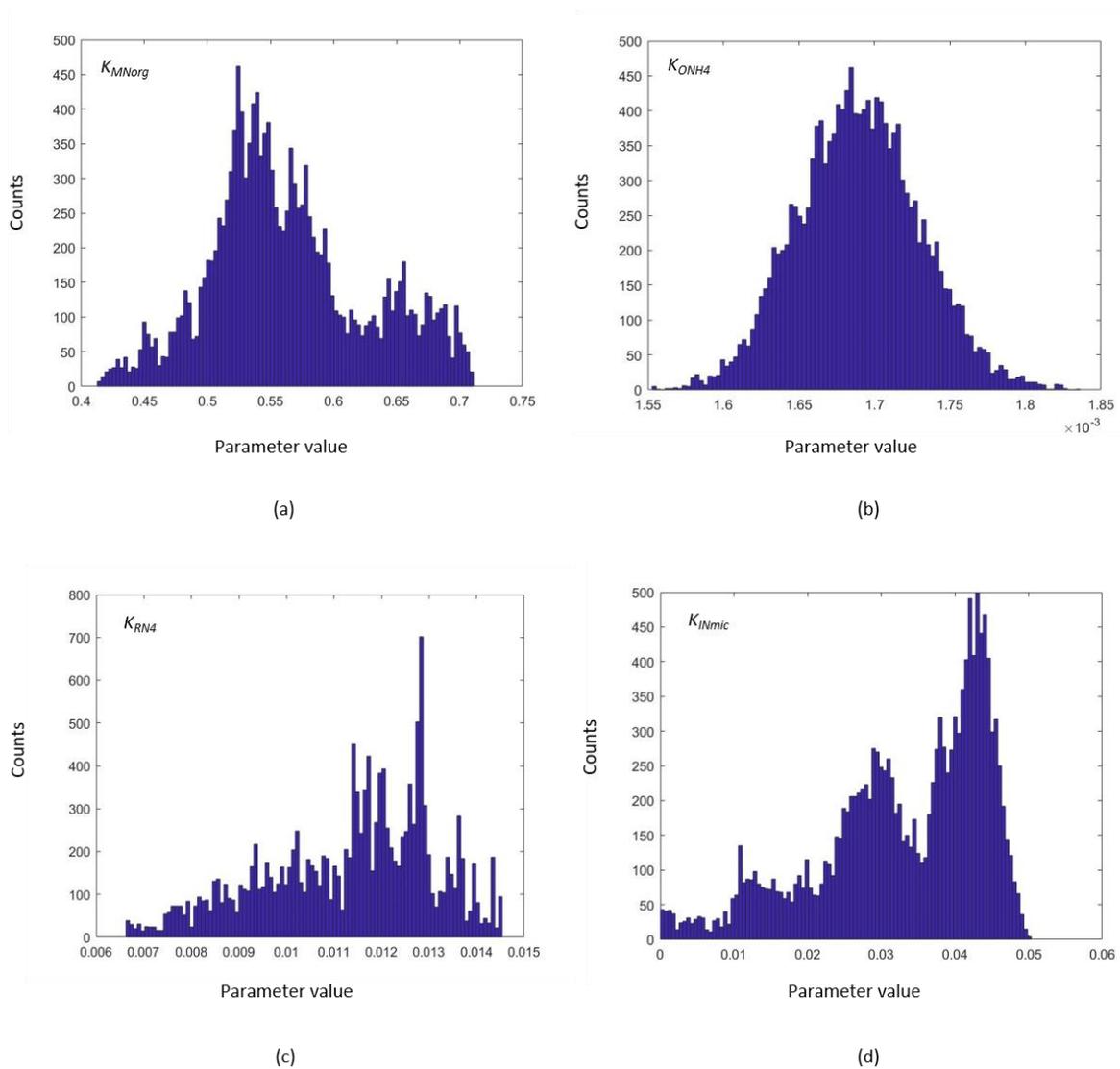


Fig. 8. Probability density plots of the four parameters ( $k_{MNorg}$ ,  $k_{ONH_4}$ ,  $k_{RNH_4}$  and  $k_{INmic}$ ) in data FB via *Nmicr*. Two parameters  $R_{NH_4}$  ( $k_{RNH_4}$ ) and  $I_{Nmic}$  ( $k_{INmic}$ ) were left skewed ( $k_{MNorg}$  = organic N released from Norg to Nmic;  $k_{ONH_4}$  = oxidation of  $NH_4^+$  to  $NO_3^-$ ;  $k_{RNH_4}$  = mineralization from Nmic to  $NH_4^+$  and  $k_{INmic}$  = organic N released from Nmic to Norg)

When looking at the correlation coefficient (CR) between the parameters of data FB in the *Nbas* and *Nmicr* model (Table 3 and 4), only two parameters  $R_{NH_4}$  and  $I_{Nmic}$  were observed to have correlation values higher than 0.8 in *Nmicr* (Table 4), meaning they were more easily to be constrained together (Quinn and Keough, 2002). A negative CR within  $O_{NH_4}$  and  $I_{NH_4}$  was marked.

The *Nmit* model removed the process of  $R_{NH_4}$  because the mineralization was not considered as a microbial transformation in this system. In this simulation, the organic nitrogen turned into ammonium before microbial assimilation. With a lack of  $NH_4^+$  released from the microbial biomass, meaning that no organic N was passing from Norg to Nmic, we saw no decrease but an increase of microbial  $^{15}N$  in the simulation (Fig. 9). Parameter  $I_{NO_3}$  was added into the optimization in different runs but did not adjust the microbial  $^{15}N$  simulation result (Fig. 10).

Table 3. Correlation matrix between parameters of data FB in the *Nbas* model. Three parameters  $I_{NO_3}$ ,  $O_{Norg}$  and  $D_{NO_3}$  were not included in the optimization and were therefore not shown. None of the parameters above were observed to have a strong tendency to be constrained together.

	$K_{MNorg}$	$K_{INH4}$	$K_{ONH4}$
$K_{MNorg}$	1	0.0675	0.0052
$K_{INH4}$	0.0675	1	-0.1773
$K_{ONH4}$	0.0052	-0.1773	1

Table 4. Correlation matrix between parameters of data FB in the *Nmicr* model. Three parameters  $I_{NO_3}$ ,  $O_{Norg}$  and  $D_{NO_3}$  were not included in the optimization and were therefore not shown. A strong correlation between  $R_{NH4}$  and  $I_{Nmic}$  was observed.

	$K_{MNorg}$	$K_{ONH4}$	$K_{RNH4}$	$K_{INmic}$
$K_{MNorg}$	1	0.0044	-0.2853	0.0448
$K_{ONH4}$	0.0044	1	0.0606	0.0637
$K_{RNH4}$	-0.2853	0.0606	1	0.9400
$K_{INmic}$	0.0448	0.0637	0.9400	1

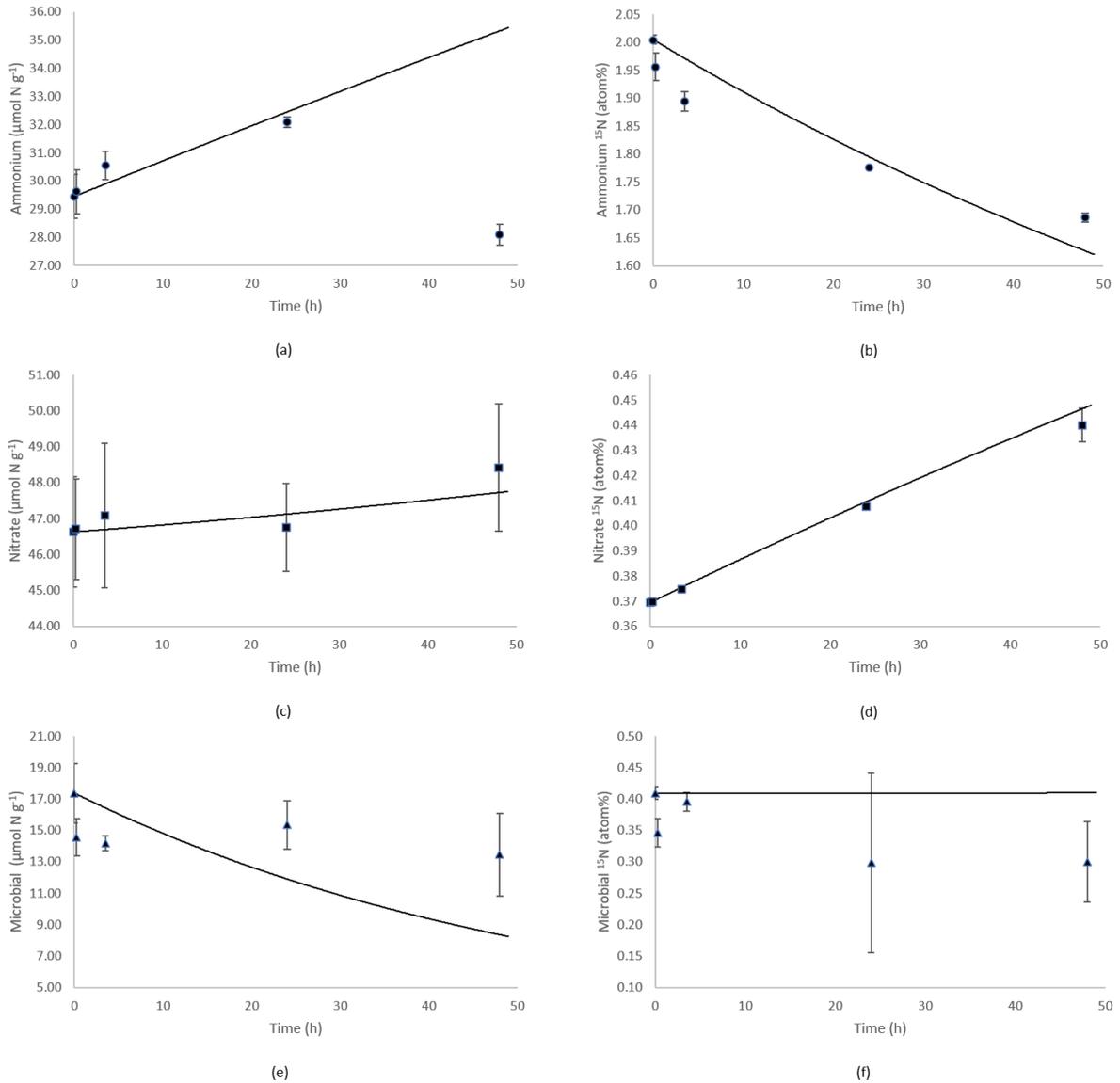


Fig. 9. Measured and simulated concentrations and  $^{15}\text{N}$  enrichments of ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and microbial biomass ( $\text{N}_{\text{mic}}$ ) of data FB via *Nmit* model from T0 to T4 (0h, 0.25h, 3.5h, 24h and 48h). The solid line illustrated the simulation result from *Nmicr*, and the measured data points were marked with different patterns (circular indicated  $\text{NH}_4^+$ , square indicated  $\text{NO}_3^-$  and triangle indicated  $\text{N}_{\text{mic}}$ ) and error bars (uncertainties). Unlike the measured data points, the optimized atom percentage of the microbial  $^{15}\text{N}$  was not decreasing.

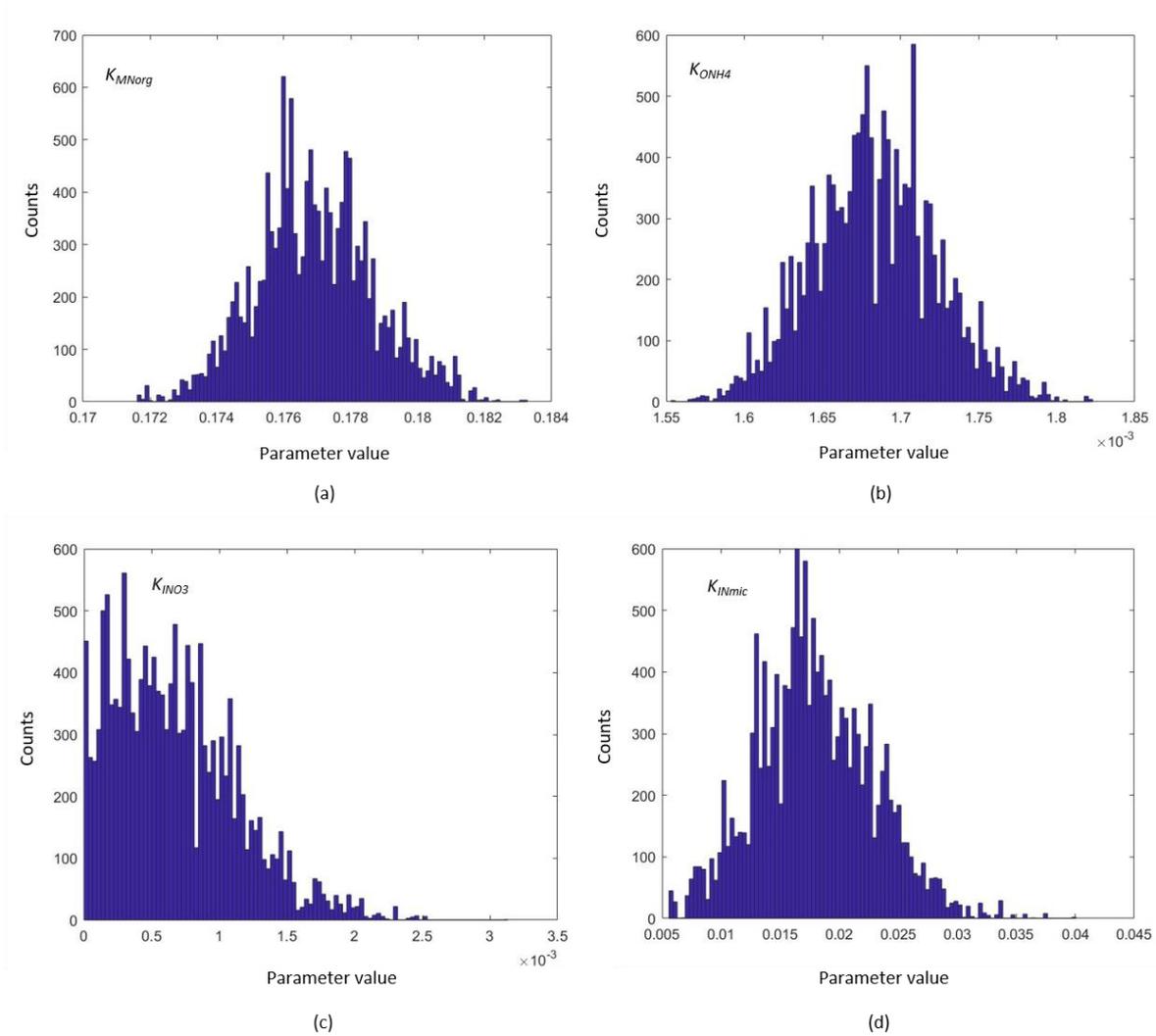


Fig. 10. Probability density plots of the four parameters ( $k_{MNorg}$ ,  $k_{ONH4}$ ,  $k_{INO3}$  and  $k_{INmic}$ ) in data FB via *Nmit*. Three parameters  $I_{NH4}$  ( $k_{INNH4}$ ) and  $R_{NH4}$  ( $k_{RNH4}$ ) were omitted in the simulation. Note that parameter  $I_{NO3}$  ( $k_{INO3}$ ) did not impact the simulation result. ( $k_{MNorg}$  = mineralization from Norg to  $NH_4^+$ ;  $k_{ONH4}$  = oxidation of  $NH_4^+$  to  $NO_3^-$ ;  $k_{INO3}$  = immobilization from  $NO_3^-$  to Nmic and  $k_{INmic}$  = organic N released from Nmic to Norg)

### 3.2 Simulation of the data set GM

Data GM was run in the *Nbas* model with 50000 iterations. In this optimization run (Fig. 11), we set the  $I_{NH4}$  to zero because this parameter was presented as a skewed distribution. A total of three parameters were participated. Generally, PDFs of the parameters  $M_{Norg}$ ,  $O_{NH4}$  and  $I_{NO3}$  in the *Nbas* model were well defined (Fig. 12). The AIC of this simulation was 295.9 and the cost function was 349.8.

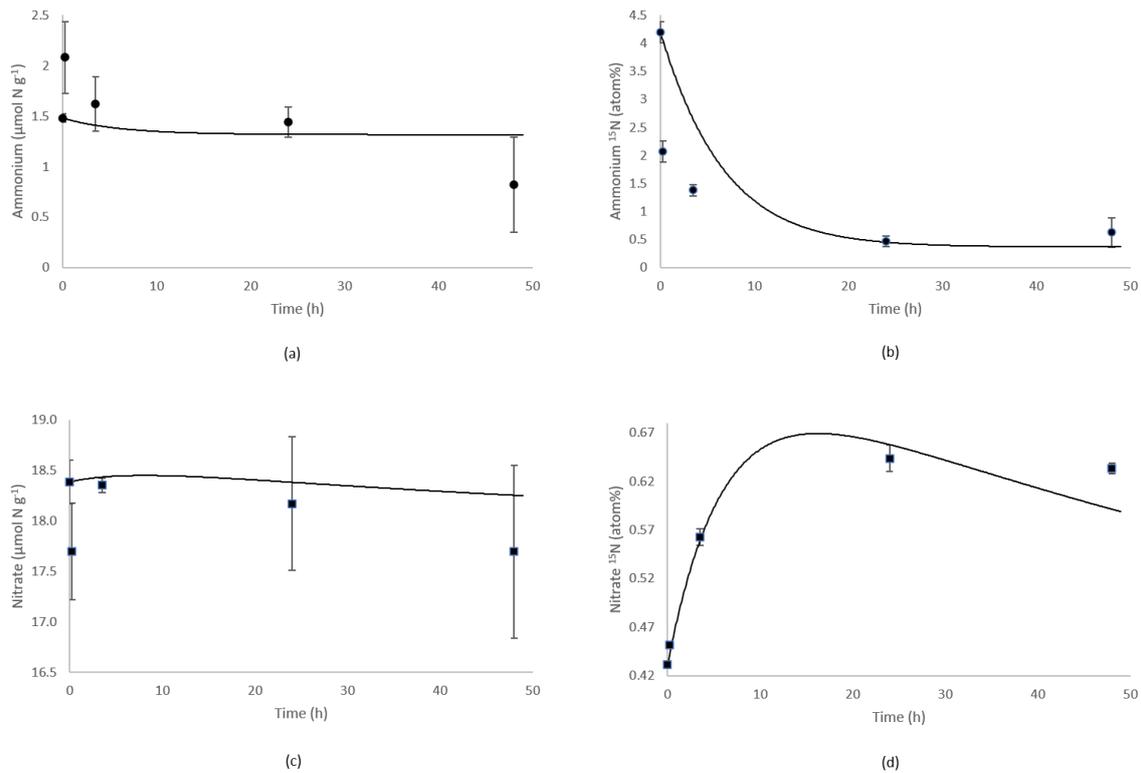


Fig. 11. Measured and simulated concentrations and <sup>15</sup>N enrichments of ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) of data GM via *Nbas* model from T0 to T4 (0h, 0.25h, 3.5h, 24h and 48h). The solid line illustrated the simulation result from *Nbas*, and the measured data points were marked with different patterns (circular indicated NH<sub>4</sub><sup>+</sup> and square indicated NO<sub>3</sub><sup>-</sup>) and error bars (uncertainties).

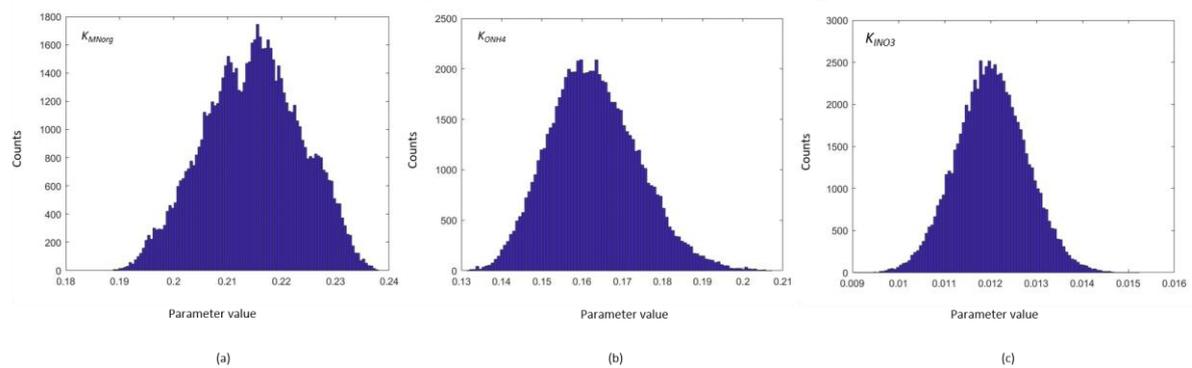


Fig. 12. Probability density plots of the three parameters ( $k_{MNorg}$ ,  $k_{ONH4}$  and  $k_{INO3}$ ) in data GM via *Nbas*. Parameter  $I_{NH4}$  ( $k_{INH4}$ ) was omitted in the simulation. ( $k_{MNorg}$  = mineralization from Norg to NH<sub>4</sub><sup>+</sup>;  $k_{ONH4}$  = oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> and  $k_{INO3}$  = immobilization of NO<sub>3</sub><sup>-</sup> to Norg)

Showing a truncated distribution,  $I_{NH4}$  was omitted in the simulation when running data GM in the *Nmicr* model. The optimization results were shown in Fig. 13. There was in general a rapid consumption rate of ammonium <sup>15</sup>N before T4, 15 hours precisely, coming with a steady decrease at T5. A quick production rate of the nitrate <sup>15</sup>N was illustrated from 0h to 15h. The microbial <sup>15</sup>N was having its highest consumption rate which continuously going downwards and reaching its lowest point at 48h. Had a closer look at the N content of ammonium and microbial biomass, here we saw a rapid consumption and production rate in the three pools between T0 to T3. The two nitrogen pools, NH<sub>4</sub><sup>+</sup> and Nmic, were sharing a similar trend but in a reverse form: the concentration of the

ammonium pool was slightly decreasing compared to a marginal rise in microbial N content. Nitrate content reached its highest concentration around T3, then gradually decreased. The cost function of this run was 338.9 and the AIC was 265.9. All PDFs were showing a unimodal distribution except parameter  $R_{NH4}$  ( $k_{RNH4}$ ) (Fig. 14).

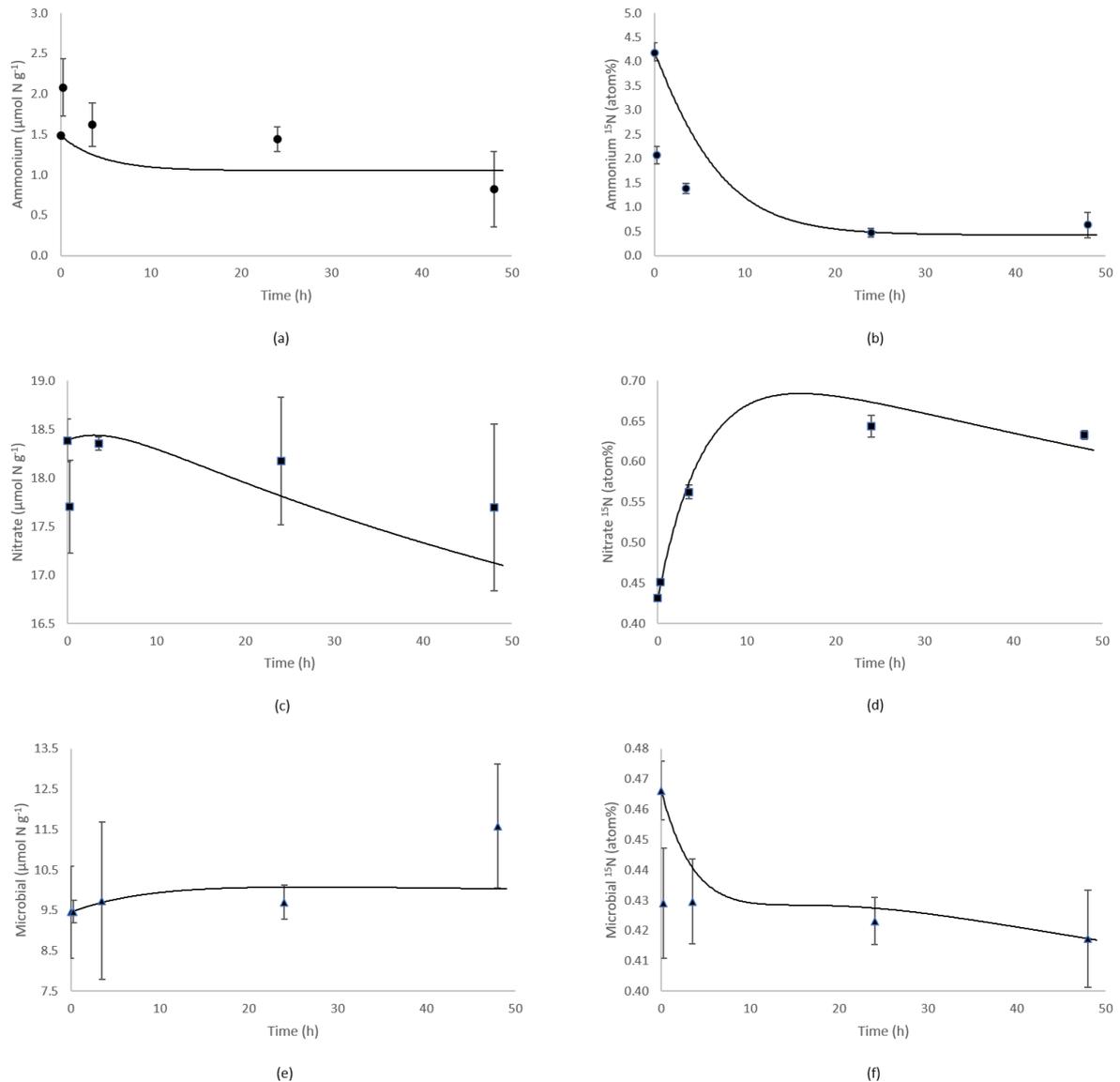


Fig. 13. Measured and simulated concentrations and  $^{15}\text{N}$  enrichments of ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and microbial biomass ( $\text{N}_{\text{mic}}$ ) of data GM via *Nmicr* model from T0 to T4 (0h, 0.25h, 3.5h, 24h and 48h). The solid line illustrated the simulation result from *Nbas*, and the measured data points were marked with different patterns (circular indicated  $\text{NH}_4^+$ , square indicated  $\text{NO}_3^-$  and triangle indicated  $\text{N}_{\text{mic}}$ ) and error bars (uncertainties).

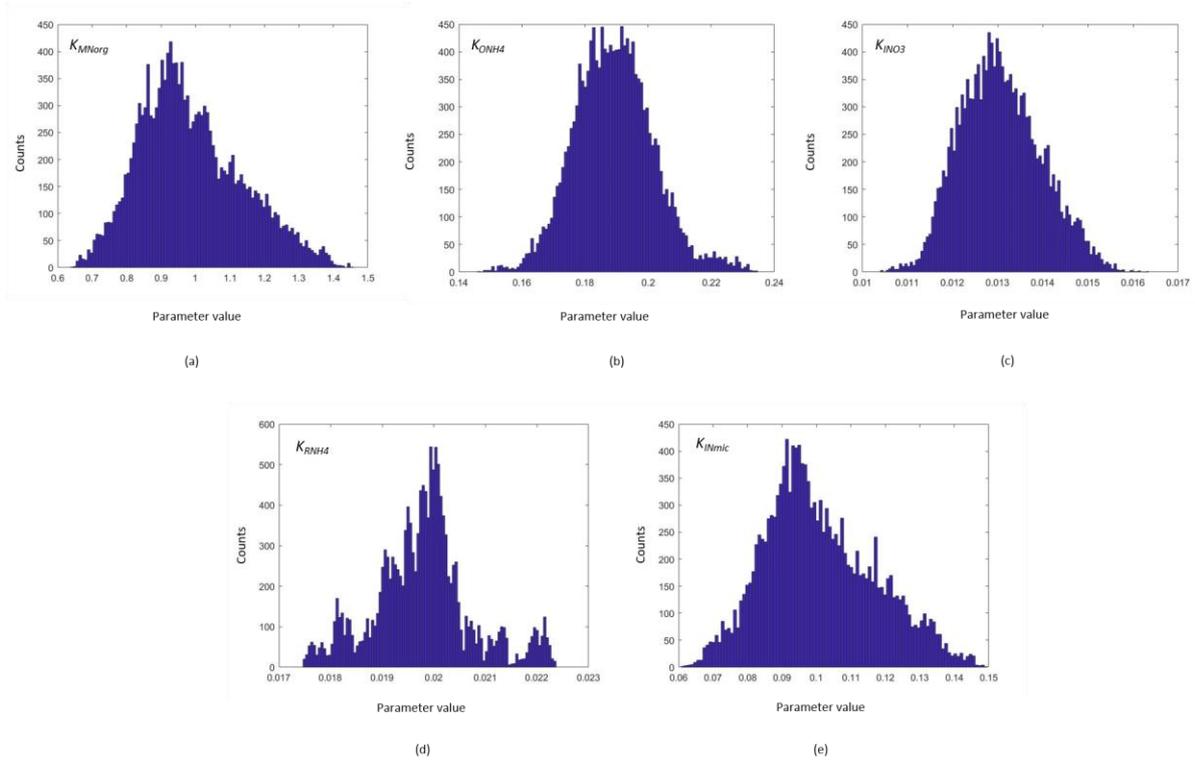


Fig. 14. Probability density plots of the five parameters ( $k_{MNorg}$ ,  $k_{ONH4}$ ,  $k_{INO3}$ ,  $k_{RNH4}$  and  $k_{INmic}$ ) in data GM via *Nmicr*. Parameter  $I_{NH4}$  ( $k_{INH4}$ ) was omitted in the simulation. All parameters were well defined except  $k_{RNH4}$ , drawing a multimodal distribution in the run. ( $k_{MNorg}$  = organic N released from Norg to Nmic;  $k_{ONH4}$  = oxidation of  $NH_4^+$  to  $NO_3^-$ ;  $k_{INO3}$  = immobilization from  $NO_3^-$  to Nmic;  $k_{RNH4}$  = mineralization from Nmic to  $NH_4^+$  and  $k_{INmic}$  = organic N released from Nmic to Norg)

When applying data GM in *Nbas* and *Nmicr* simulations, the correlation matrix of these parameters was shown in Table 5 and 6. In the *Nbas* model, parameters  $I_{NH4}$  was not included in the optimization since it was truncated (Table 5). We saw no strong correlation ( $<0.3$ ) and a negative value between  $M_{Norg}$  and  $O_{NH4}$  could be observed in *Nbas*. In the *Nmicr* model, parameter  $I_{NH4}$  was omitted in the simulation run. The highest CR value, which was larger than 0.8, could be found between  $M_{Norg}$  and  $I_{Nmic}$  ( $\approx 0.96$ ). Negative CR was shown in parameters  $M_{Norg}$ ,  $R_{NH4}$ ,  $I_{Nmic}$  and  $R_{NH4}$  (Table 6). Same optimization problem could be obtained when applying the data GM in *Nmit* that the microbial  $^{15}N$  was not decreasing but growing significantly violated the measuring data points (Fig. 15), therefore, the correlation matrix of *Nmit* was not further discussed as it could not describe the observed N dynamics in both datasets. Higher discrepancy could be shown between the simulation and the measuring data points in data GM.

Table 5. Correlation of coefficient of parameters of data GM in the *Nbas* model. Three parameters  $I_{NH4}$ ,  $O_{Norg}$  and  $D_{NO3}$  were not included in the optimization and were therefore not shown. ( $k_{MNorg}$  = organic N released from Norg to Nmic;  $k_{ONH4}$  = oxidation of  $NH_4^+$  to  $NO_3^-$ ;  $k_{INO3}$  = immobilization from  $NO_3^-$  to Nmic)

	$K_{MNorg}$	$K_{ONH4}$	$K_{INO3}$
$K_{MNorg}$	1	-0.1087	0.1504
$K_{ONH4}$	-0.1087	1	0.3079
$K_{INO3}$	0.1504	0.3079	1

Table 6. Parameter  $I_{NH4}$  was omitted in this simulation of data GM. The highest CR value, which was larger than 0.8, could be found between  $M_{Norg}$  and  $I_{Nmic}$ . Negative CR was shown in parameters  $M_{Norg}$ ,  $R_{NH4}$ ,  $I_{Nmic}$  and  $R_{NH4}$ . ( $k_{MNorg}$  = organic N released from Norg to Nmic;  $k_{ONH4}$  = oxidation of  $NH_4^+$  to  $NO_3^-$ ;  $k_{INO3}$  = immobilization from  $NO_3^-$  to Nmic;  $k_{RNH4}$  = mineralization from Nmic to  $NH_4^+$  and  $k_{INmic}$  = organic N released from Nmic to Norg)

	$K_{MNorg}$	$K_{ONH4}$	$K_{INO3}$	$K_{RNH4}$	$K_{INmic}$
$K_{MNorg}$	1	0.0130	0.2745	-0.1166	0.9597
$K_{ONH4}$	0.0130	1	0.3207	-0.1219	0.0239
$K_{INO3}$	0.2745	0.3207	1	0.2869	0.3096
$K_{RNH4}$	-0.1166	-0.1219	0.2869	1	-0.0494
$K_{INmic}$	0.9597	0.0239	0.3096	-0.0494	1

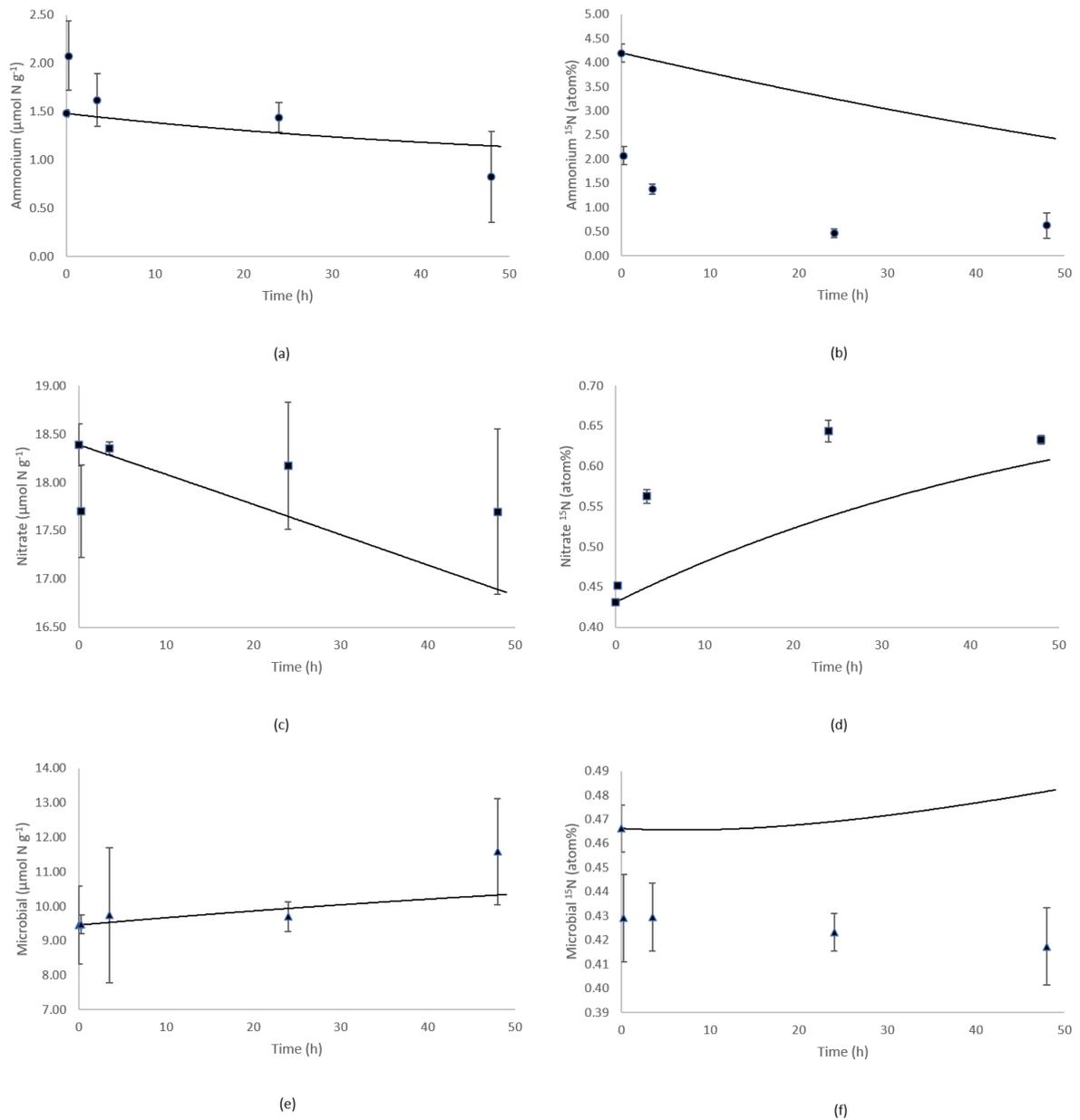


Fig. 15. Measured and simulated concentrations and  $^{15}\text{N}$  enrichments of ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and microbial biomass ( $\text{N}_{\text{mic}}$ ) of data GM via *Nmit* model. The solid line illustrated the simulation result from *Nbas*, and the measured data points were marked with different patterns (circular indicated  $\text{NH}_4^+$ , square indicated  $\text{NO}_3^-$  and triangle indicated  $\text{N}_{\text{mic}}$ ) and error bars (uncertainties). The optimized atom percentage of the microbial  $^{15}\text{N}$  was not decreasing but going upwards.

### 3.3 Gross mineralization and immobilization rate

The mean of parameters and standard deviations of the two datasets were presented in Table 7 and Table 8. Absent data was shown in two parameters  $R_{\text{NH}_4}$  and  $I_{\text{N}_{\text{mic}}}$  as they were not considered when running *Nbas*. Immobilization of nitrate to the organic nitrogen pool ( $I_{\text{NO}_3}$ ) in *Nbas* and *Nmicr* was omitted in the simulation of data FB (Table 7).  $I_{\text{NH}_4}$  was precluded in both *Nbas* and *Nmicr* optimization when running data GM but when having data FB,  $I_{\text{NH}_4}$  was only neglected in *Nmicr*.

In data FB, the mineralization rate which indicated the production of inorganic N from organic N in *Nbas* ( $M_{\text{Norg}}$ ) was larger than *Nmicr* ( $R_{\text{NH}_4}$ ). Additionally, higher  $\text{NH}_4^+$  immobilization rate (outflow of  $\text{NH}_4^+$  pool) could also be observed in the *Nbas* model (Table 7). A similar paramean value of parameter  $O_{\text{NH}_4}$  could be found in both models, but a rather higher gross rate was observed in the second model. In data GM, the mineralization rate in the second model was smaller when running our second model (Table 8). Since the immobilization of the ammonium pool was omitted in this simulation, the immobilization rate of nitrate in both models were quite similar. We saw in parameter  $O_{\text{NH}_4}$ , the values between two different models were quite the same but the gross rate was slightly lower in *Nmicr*. Parameter  $I_{\text{Nmic}}$  and  $M_{\text{Norg}}$  were either the biggest or second largest gross rate when applying microbial biomass into the simulation system (*Nmicr*) in both datasets. In other words, a strong interaction between the microbial biomass and the organic N pool could be observed in the second model system.

Table 7. Transformation rates of data FB in *Nbas* and *Nmicr*. Absent data in the column was shown as dash due to deficiency of microbial biomass measurement. Zero indicated no participation of specific parameters in the simulation.

Parameter	Description	Kinetics	Paramean		Gross rate		Stdev		Uncertainties	
			Nbas	Nmicr	Nbas	Nmicr	Nbas	Nmicr	Nbas	Nmicr
MNorg	Mineralization of Norg to $\text{NH}_4^+$ (Nbas)	0 ( $\mu\text{mol Ng}^{-1}\text{h}^{-1}$ )	0.1793	0.5639	0.1793	0.5639	0.0018	0.0625	0.0018	0.0625
	Assimilation of Norg to microbes (Nmicr)									
INH <sub>4</sub>	Immobilization of $\text{NH}_4^+$ to Norg (Nbas)	1 ( $\text{h}^{-1}$ )	0.0027	0.0000	0.0824	0.0000	0.0002	0.0000	0.0056	0.0000
	Immobilization of $\text{NH}_4^+$ to microbes (Nmicr)									
ONH <sub>4</sub>	Oxidation of $\text{NH}_4^+$ to $\text{NO}_3^-$	1 ( $\text{h}^{-1}$ )	0.0016	0.0017	0.0489	0.0548	0.0000	0.0000	0.0012	0.0013
INO <sub>3</sub>	Immobilization of $\text{NO}_3^-$ to Norg (Nbas)	1 ( $\text{h}^{-1}$ )	0	0	0	0	0	0	0	0
	Immobilization of $\text{NO}_3^-$ to microbes (Nmicr)									
RNH <sub>4</sub>	Release of $\text{NH}_4^+$ from Nmic (mineralization)	1 ( $\text{h}^{-1}$ )	-	0.0113	-	0.0720	-	0.0018	-	0.0112
INmic	Organic N efflux from Nmic to Norg	1 ( $\text{h}^{-1}$ )	-	0.0330	-	0.2099	-	0.0112	-	0.0714

Table 8. Transformation rates of data GM in *Nbas* and *Nmicr*. Absent data in the column was shown as dash due to deficiency of microbial biomass measurement. Zero indicated no participation of specific parameters in the simulation.

Parameter	Description	Kinetics	Paramean		Gross rate		Stdev		Uncertainties	
			Nbas	Nmicr	Nbas	Nmicr	Nbas	Nmicr	Nbas	Nmicr
MNorg	Mineralization of Norg to $\text{NH}_4^+$ (Nbas)	0 ( $\mu\text{mol Ng}^{-1}\text{h}^{-1}$ )	0.2147	0.9853	0.2147	0.9853	0.0089	0.1478	0.0089	0.1478
	Assimilation of Norg to microbes (Nmicr)									
INH <sub>4</sub>	Immobilization of $\text{NH}_4^+$ to Norg (Nbas)	1 ( $\text{h}^{-1}$ )	0	0	0	0	0	0	0	0
	Immobilization of $\text{NH}_4^+$ to microbes (Nmicr)									
ONH <sub>4</sub>	Oxidation of $\text{NH}_4^+$ to $\text{NO}_3^-$	1 ( $\text{h}^{-1}$ )	0.1630	0.1892	0.2180	0.2065	0.0111	0.0126	0.0148	0.0137
INO <sub>3</sub>	Immobilization of $\text{NO}_3^-$ to Norg (Nbas)	1 ( $\text{h}^{-1}$ )	0.0120	0.0131	0.2208	0.2327	0.0008	0.0009	0.0140	0.0159
	Immobilization of $\text{NO}_3^-$ to microbes (Nmicr)									
RNH <sub>4</sub>	Release of $\text{NH}_4^+$ from Nmic (mineralization)	1 ( $\text{h}^{-1}$ )	-	0.0198	-	0.1977	-	0.0010	-	0.0095
INmic	Organic N efflux from Nmic to Norg	1 ( $\text{h}^{-1}$ )	-	0.1010	-	1.0086	-	0.0161	-	0.1612

### 3.4 Net production of the microbial biomass

The net production of ammonium, nitrate, organic nitrogen, and the microbial biomass pool in *Nbas* (Table 9) and *Nmicr* (Table 10), the net production of ammonium was higher when excluding the microorganisms in the simulation. It is worth to note that under the *Nmicr* simulation, the Norg was the smallest net production in data FB but the largest in data GM. The microbial biomass, on the other hand, had the largest net production in data FB and the second largest net production in data GM.

Table 9. Net production of four nitrogen pools (ammonium, nitrate, microbial biomass, organic pool) of data FB in the *Nmicr* model. ( $M_{Norg}$  = organic N released from Norg to Nmic;  $I_{NO_3}$  = immobilization from  $NO_3^-$  to Nmic;  $I_{NH_4}$  = immobilization from  $NH_4^+$  to Nmic;  $O_{NH_4}$  = oxidation from  $NH_4^+$  to  $NO_3^-$ ;  $R_{NH_4}$  = mineralization from Nmic to  $NH_4^+$  and  $I_{Nmic}$  = organic N released from Nmic to Norg)

Nitrogen pool	Description		Net production (Influx-Efflux)	
	Nbas	Nmicr	Nbas	Nmicr
Ammonium ( $NH_4^+$ )	$M_{Norg} + I_{NO_3} - I_{NH_4} - O_{NH_4}$	$R_{NH_4} - O_{NH_4}$	0.175	0.0096
Nitrate ( $NO_3^-$ )	$O_{NH_4} - I_{NO_3}$	$O_{NH_4} - I_{NO_3}$	0.0016	0.0017
Microbial biomass (Nmic)	-	$M_{Norg} + I_{NH_4} + I_{NO_3} - I_{Nmic} - R_{NH_4}$	-	0.5196
Organic pool (Norg)	$I_{NO_3} + I_{NH_4} - M_{Norg}$	$I_{Nmic} - M_{Norg}$	-0.1766	-0.5309

Table 10. Net production of four nitrogen pools (ammonium, nitrate, microbial biomass, organic pool) of data GM in the *Nmicr* model. ( $M_{Norg}$  = organic N released from Norg to Nmic;  $I_{NO_3}$  = immobilization from  $NO_3^-$  to Nmic;  $I_{NH_4}$  = immobilization from  $NH_4^+$  to Nmic;  $O_{NH_4}$  = oxidation from  $NH_4^+$  to  $NO_3^-$ ;  $R_{NH_4}$  = mineralization from Nmic to  $NH_4^+$  and  $I_{Nmic}$  = organic N released from Nmic to Norg)

Nitrogen pool	Description		Net production (Influx-Efflux)	
	Nbas	Nmicr	Nbas	Nmicr
Ammonium ( $NH_4^+$ )	$M_{Norg} + I_{NO_3} - I_{NH_4} - O_{NH_4}$	$R_{NH_4} - O_{NH_4}$	-0.0033	-0.0088
Nitrate ( $NO_3^-$ )	$O_{NH_4} - I_{NO_3}$	$O_{NH_4} - I_{NO_3}$	-0.0028	-0.0262
Microbial biomass (Nmic)	-	$M_{Norg} + I_{NH_4} + I_{NO_3} - I_{Nmic} - R_{NH_4}$	-	0.0117
Organic pool (Norg)	$I_{NO_3} + I_{NH_4} - M_{Norg}$	$I_{Nmic} - M_{Norg}$	0.0061	0.0233

## 4. Discussion

### 4.1 Simulation of *Nmicr*: misfit of microbial $^{15}\text{N}$ in data FB

Main reason for a low microbial  $^{15}\text{N}$  was based on the high uncertainties using indirect measurement to obtain microbial N content and particularly  $^{15}\text{N}$ . Unlike the direct measurement of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , microbial N content could only be calculated by the differences of total dissolved nitrogen (TDN) with and without fumigated, and the microbial  $^{15}\text{N}$  used isotopic mass balance equation which required the data of the ammonium, nitrate, TDN concentrations and their respectively  $^{15}\text{N}$  to make a new calculation of microbial  $^{15}\text{N}$ . The six measures have their own uncertainty and when considering the  $^{15}\text{N}$  mixing model, error propagation could lead to larger uncertainties. Take TDN for example, which is composed of dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON), could be determined by the oxidation of dissolved organic nitrogen (DON) and  $\text{NH}_4^+$  to  $\text{NO}_3^-$ . However, there is no direct measurement of DON. According to Braun (2018), DON concentration was calculated from the difference between total dissolved N (TDN) and inorganic N (i.e.,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , DIN) ( $\text{DON} = \text{TDN} - \text{DIN}$ ), and the N isotope ratio of DON was calculated using an isotopic mass balance equation (Fry, 2006). This explains the reason why some of the microbial  $^{15}\text{N}$  values were below natural abundance. Furthermore, the decreasing of the microbial  $^{15}\text{N}$  indicated *Nmicr* received N from the Norg. Since organic N was the lowest substrate (0.3663%) compared to nitrate (0.37%) and ammonium (1.69%), the microbial  $^{15}\text{N}$  was constrained by the organic N and could not go below 0.37% (Table 2).

### 4.2 Correlation coefficient within organic N pool and the microbial biomass in *Nmicr* simulation

When applying to datasets in the *Nbas* and *Nmicr* model, we saw a strong correlation matrix only in the new developed model (*Nmicr*). The organic N released from the microbial biomass to Norg ( $I_{\text{Nmic}}$ ) was more likely to be constrained with ammonium influx ( $R_{\text{NH}_4}$ ) in data FB but with the output of organic nitrogen pool consumed by microorganisms ( $M_{\text{Norg}}$ ) in data GM. This might be due to the difference of  $^{15}\text{N}$  in two soil samples. Data FB had its greatest net production in *Nmic* (Table 9), which indicated a larger influx was entering the microbial biomass compared to efflux. In our simulation run, the gross rates of the mineral pools were both zero (Table 7), while the  $I_{\text{Nmic}}$  showed to be the second largest flow. Despite the microbial assimilation having the largest rate ( $M_{\text{Norg}} = 0.5639$ ) in this system, the mineralization rate ( $R_{\text{NH}_4} = 0.072$ ) was only about one tenth. This indicated that the organic N which Norg received was mainly coming from *Nmic* after the microbial assimilation instead of mineralization into ammonium. As the lowest enrichment, *Nmic* could restrained other N pools. Hence, being less competitive, the efflux of ammonium released from the microbial biomass ( $R_{\text{NH}_4}$ ) could be restricted by the output of organic N ( $I_{\text{Nmic}}$ ) (Fig. 16).

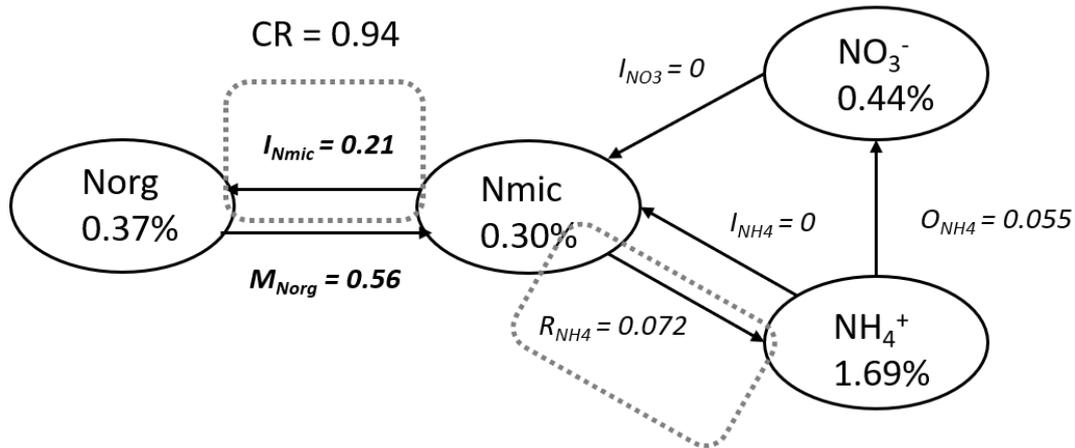


Fig. 16. Strong correlation coefficient was shown between parameters  $I_{Nmic}$  and  $R_{NH4}$  in *Nmicr* when applying data FB. Being the lowest substrate, the output of the *Nmic* was restrained by its production rate. ( $M_{Norg}$  = organic N released from *Norg* to *Nmic*;  $I_{NO3}$  = immobilization from  $NO_3^-$  to *Nmic*;  $I_{NH4}$  = immobilization from  $NH_4^+$  to *Nmic*;  $O_{NH4}$  = oxidation from  $NH_4^+$  to  $NO_3^-$ ;  $R_{NH4}$  = mineralization from *Nmic* to  $NH_4^+$  and  $I_{Nmic}$  = organic N released from *Nmic* to *Norg*)

In data GM (Fig. 17), the ammonium pool was the largest substrate at 0 h (T0), driving the immobilization of two mineral pools ( $I_{NH4}+I_{NO3}$ ) and causing an  $I_{Nmic}$  efflux entering *Norg*. As *Norg* was the lowest enrichment, it constrained the microbial assimilation ( $M_{Norg}$ ) to *Nmic*, leading to strong CR between the two parameters  $M_{Norg}$  and  $I_{Nmic}$ . This could also be supported by the gross rates we gained from the simulation: the  $I_{Nmic}$  had the biggest rate (1.009) followed by  $M_{Norg}$  (0.99). We could also find the largest net production in *Norg* (0.0233) and the second one in *Nmic* (0.0117) (Table 10).

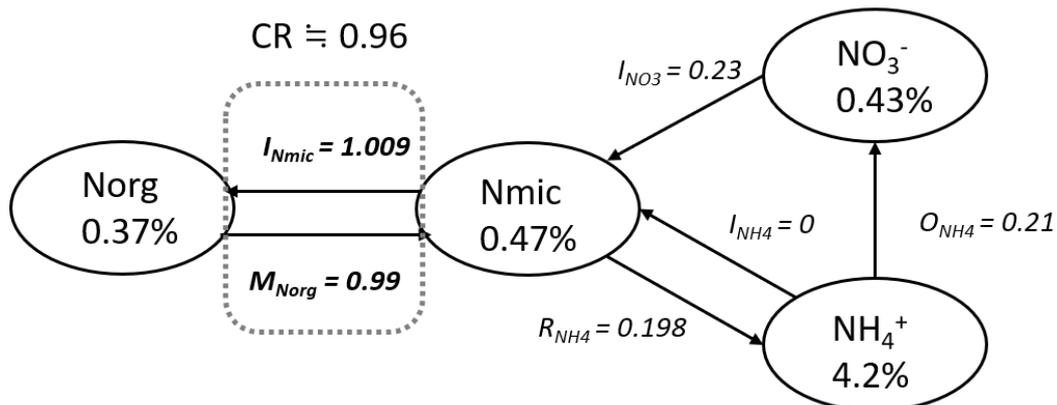


Fig. 17. Strong correlation coefficient was shown between parameters  $I_{Nmic}$  and  $M_{Norg}$  in *Nmicr* when applying data GM. Being the lowest substrate, *Norg* constrained the microbial assimilation after receiving organic nitrogen from the *Nmic*. ( $M_{Norg}$  = organic N released from *Norg* to *Nmic*;  $I_{NO3}$  = immobilization from  $NO_3^-$  to *Nmic*;  $I_{NH4}$  = immobilization from  $NH_4^+$  to *Nmic*;  $O_{NH4}$  = oxidation from  $NH_4^+$  to  $NO_3^-$ ;  $R_{NH4}$  = mineralization from *Nmic* to  $NH_4^+$  and  $I_{Nmic}$  = organic N released from *Nmic* to *Norg*)

#### 4.3 The impact of microbial biomass: smaller mineralization rate in *Nmicr* simulation

Simulation showed that the mineralization rate in *Nmic* ( $R_{NH4}$ ) was one to two times smaller than *Nbas* ( $M_{Norg}$ ) and a greater outflow of organic N pool in *Nmicr* compared to *Nbas*. Both could be revealed by a high microbial nitrogen use efficiency (NUE) in *Nmicr* model due to the process of microbial assimilation before ammonification (Barraclough, 1997). During mineralization, microbes decompose organic matter by depolymerizing proteins into oligopeptides and amino acids via their

extracellular proteases. These simple compounds could then be directly and rapidly utilized by microbes as both an energy and nutrient sources (Jones et al., 2004; Jones, D. L. et al., 2009; Mooshammer et al., 2014). High microbial NUE expresses an efficient microbial N intake process and a concomitant release of a small fraction of ammonium back to the environment. Thus, a smaller efflux (mineralization) could be released from the microbial biomass ( $N_{micr}$ ) than the organic N pool ( $N_{bas}$ ).

Our result also highlighted the importance of considering microbial biomass efflux ( $I_{Nmic}$  and  $R_{NH4}$ ) in the N transformation system, especially  $I_{Nmic}$  since it was a larger output compared to  $R_{NH4}$  in both datasets (Fig. 18 and 19). Even when no mineral influx ( $I_{NO3}$  and  $I_{NH4}$ ) was entering the microbial biomass in data FB (Fig. 18), the output of ammonium ( $R_{NH4}$ ) from the microorganisms was always lower than the efflux from the microbes to the Norg ( $I_{Nmic}$ ). The phenomenon which microbes returned organic N back to Norg rather than mineralized into  $NH_4^+$  possibly revealed a strong interaction between the Norg and the Nmic.

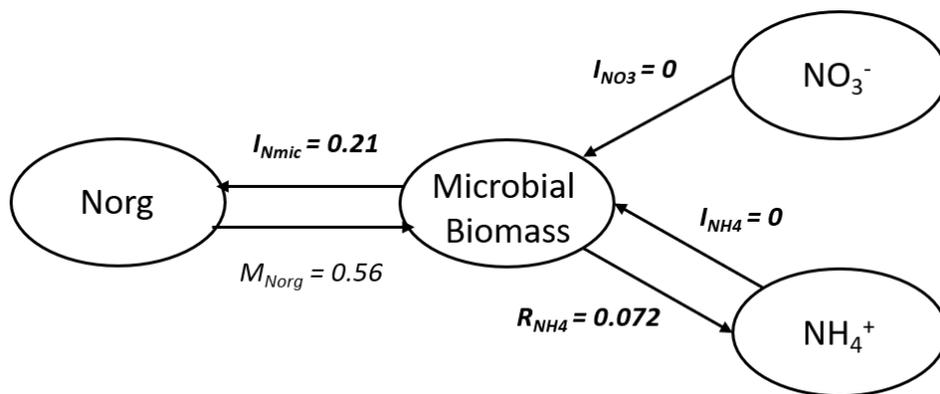


Fig. 18. The intake and output of microbial biomass in  $N_{micr}$  of data FB. Our result highlighted the importance of considering microbial biomass efflux in the N transformation system since that even when no influx ( $I_{NO3}$  and  $I_{NH4}$ ) was entering the microbial biomass in data FB, the output of ammonium ( $R_{NH4}$ ) from the microorganisms was always lower than the efflux from the microbes to the Norg ( $I_{Nmic}$ ). ( $M_{Norg}$  = organic N released from Norg to Nmic;  $I_{NO3}$  = immobilization from  $NO_3^-$  to Nmic;  $I_{NH4}$  = immobilization from  $NH_4^+$  to Nmic;  $R_{NH4}$  = mineralization from Nmic to  $NH_4^+$  and  $I_{Nmic}$  = organic N released from Nmic to Norg)

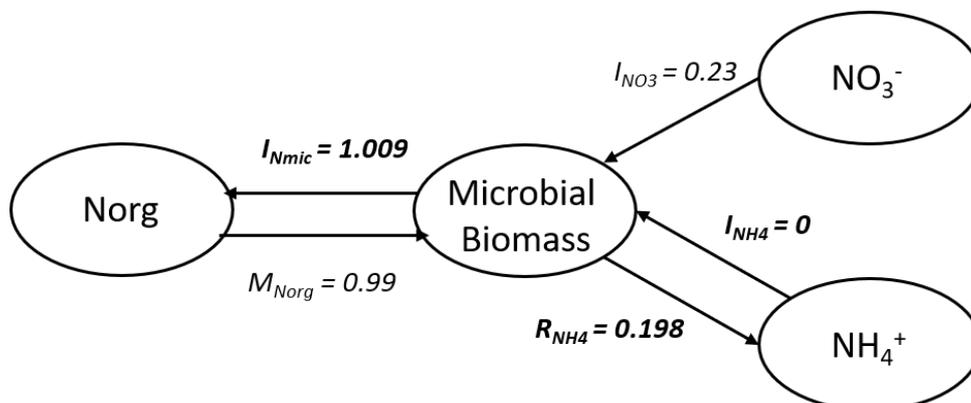


Fig. 19. The intake and output of microbial biomass in  $N_{micr}$  of data GM. Larger efflux from the microbial biomass to the organic nitrogen pool ( $I_{Nmic}$ ) was observed compared to the microbial output of ammonium ( $R_{NH4}$ ). In data GM, it was expected to have a bigger  $I_{Nmic}$  efflux than  $M_{Norg}$  regarding an immobilization input ( $I_{NO3}$ ) coming from the nitrate pool. ( $M_{Norg}$  = organic N released from Norg to Nmic;  $I_{NO3}$  =

immobilization from  $\text{NO}_3^-$  to  $\text{Nmic}$ ;  $I_{\text{NH}_4}$  = immobilization from  $\text{NH}_4^+$  to  $\text{Nmic}$ ;  $R_{\text{NH}_4}$  = mineralization from  $\text{Nmic}$  to  $\text{NH}_4^+$  and  $I_{\text{Nmic}}$  = organic N released from  $\text{Nmic}$  to  $\text{Norg}$ )

#### 4.4 Major obstacle of *Nmit* simulation: no flux coming directly from the organic nitrogen

The main problem of microbial  $^{15}\text{N}$  not decreasing in the *Nmit* simulation was due to a lack of lower  $^{15}\text{N}$  production from a source. Conversely, whether adding  $I_{\text{NO}_3}$  or not did not change the simulated result: the trend remained going slightly upwards (Fig. 9). In the original database (Table 2), the microbial biomass had the lowest  $^{15}\text{N}$  at T5 compared to ammonium and nitrate, thus an additional flow coming from the organic nitrogen to the microbial biomass is needed for leading a downward trend of  $^{15}\text{N}$  in the microbial pool. This however contradicts the way *Nmit* works here as we considered the mineralization is not passing through the microbial biomass but turning into ammonium. Consequently, the *Nmit* simulation in this study showed no agreement to the assumption of all nitrogen mineralized to ammonium before assimilation (Jansson and Persson, 1982) but agreed with the conception of parallel route (Barraclough, 1997) that the ammonium is mineralized via the microbial biomass into  $\text{NH}_4^+$  pool and the ammonium is immobilized by the microbes before turning back to organic nitrogen.

#### 4.5 Limitations and future research

The main goal of this work was to examine the practicability of our new developed Ntrace model *Nmicr*, and whether it is necessary to consider the microbial biomass in gross N rates quantification. We did not discuss the microbial diversity in different soil characteristics and therefore neglected the N competition between different types of mycorrhiza fungi and free-living microbes (Orwin et al., 2011; Averill et al., 2014). Soil physiochemistry affects N transformation steps, differs in forests. According to Tatsumi et al (2020), the participation of mycorrhiza fungi during mineralization controls the decomposition of soil organic matter (SOM). Despite the two sampling sites showing similar soil properties, the composition of mycorrhiza fungi is not the same. In the forest, EMC fungi tend to limit N available for microbes and slow down SOM decomposition; in grassland, which the arbuscular mycorrhizal (AM) fungi are unable to decompose soil organic matter (SOM), must wait for the assimilation process by free-living microbes. To investigate how mycorrhiza fungi impact the N transformation rate, we suggested to adjust the *Nmicr* model with additional fungi measurement for a more realistic system.

The datasets were not specifically designed for the study but were the best one available. Hence, specifically designed datasets are needed to further dig into this topic. In our case, the general simulation result of the new developed model *Nmicr* was convincing. However, two parameters  $O_{\text{Norg}}$  and  $D_{\text{NO}_3}$  were removed as precaution because no  $^{15}\text{NO}_3^-$  tracer was added in the original research. It would be worth having  $^{15}\text{NO}_3^-$  labelled as well in the samples so that the interaction between organic N to nitrate could be observed. Furthermore, since we only considered one organic N pool in this study for simplicity reasons, two or more organic N could be applied. Finally, parameters could be evaluated from setting each parameter with different step-sizes but not sharing the same value to increase model accuracy. It is worthy to mention that the study was based on a lab incubation approach thus could not fully represent the microbial activities under natural conditions.

## 5. Conclusions

The consideration of microorganisms is important when studying the N transformation between mineral and organic nitrogen pools. However, measurement of microbial  $^{15}\text{N}$  has hardly been done or being applied due to technical obstacles. The aim of this research is to know whether the consideration of microbial biomass is essential and the practicability of our new developed model *Nmicr*. Our result indicated the importance of considering microbial biomass when studying the N cycle. It is worth discussing that the mineralization rate which indicated the release of ammonium from the organic N or microbial biomass was smaller in the second model *Nmicr*. Results also showed that the gross rate of influx and efflux of the organic nitrogen pool were the greatest in *Nmicr*. The phenomenon could not be observed if we exclude the microbial pool in *Nbas* since the mineralization is expected to pass through the microbial biomass before entering to the ammonium pool (Jansson, 1958; Barak et al, 1990; Barraclough, 1997). Due to the uptake, less ammonium will be released to the  $\text{NH}_4^+$  pool as organic N is partly utilized as an energy source of microbes. Moreover, four fluxes that took part in the *Nbas* simulation showed no correlation relationship but once we ran the *Nmicr*, the organic N efflux of microbes ( $I_{\text{Nmic}}$ ) was observed to be strongly constrained with  $R_{\text{NH}_4}$  in data FB and  $M_{\text{Norg}}$  in data GM. Our research also recognized a strong interaction within the organic N pool and the microbial biomass as the microbial efflux ( $I_{\text{Nmic}}$ ) was always larger than the output of ammonium in both datasets ( $R_{\text{NH}_4}$ ). The necessity of  $R_{\text{NH}_4}$  and  $I_{\text{Nmic}}$  could also be supported by our *Nmit* simulation: when having the direct route from Norg to  $\text{NH}_4^+$ , a lack of organic N flux from Norg could lead to an upward trend of microbial  $^{15}\text{N}$  in the run. Therefore, the simulation result of *Nmit* did not fit the actual measuring data points we had in the original soil samples. The general result of *Nmicr* simulation was convincing: The PDFs of all parameters were well-defined. As the research only applied one organic pool for simplicity, further work could be done by running *Nmicr* in a more complicated system.  $^{15}\text{NO}_3^-$  is also interesting to be added as a tracer so that the flux coming from Norg and the dissimilatory reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  could be observed as well.

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