^{15}N tracing models with a Monte Carlo optimization procedure provide new insights on gross N transformations in soils

Tobias Rütting[a], Christoph Müller[a,b,*]

[a]*Department of Plant Ecology, University of Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany*
[b]*School of Biology and Environmental Science, UC Dublin, Belfield, Dublin 4, Ireland*

Abstract

^{15}N tracing studies in combination with analyses via process-based models are the current "state-of-the-art" technique to quantify gross nitrogen (N) transformation rates in soils. A crucial component of this technique is the optimization algorithm which primarily decides how many model parameters can simultaneously be estimated. Recently, we published a Markov chain Monte Carlo (MCMC) method which has the potential to simultaneously estimate large number of parameters in ^{15}N tracing models [Müller et al., 2007. Estimation of parameters in complex ^{15}N tracing models by Monte Carlo sampling. Soil Biology & Biochemistry 39, 715–726].

Here, we present the results of a reanalysis of datasets by Kirkham and Bartholomew [1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Proceedings 18, 33–34], Myrold and Tiedje [1986. Simultaneous estimation of several nitrogen cycle rates using ^{15}N: theory and application. Soil Biology & Biochemistry 18, 559–568] and Watson et al. [2000. Overestimation of gross N transformation rates in grassland soils due to non-uniform exploitation of applied and native pools. Soil Biology & Biochemistry 32, 2019–2030] using the MCMC technique. Analytical solutions such as the ones derived by Kirkham and Bartholomew [1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Proceedings 18, 33–34] result in gross rates without uncertainties. We show that the analysis of the same data sets with the MCMC method provides standard deviations for gross N transformations. The standard deviations are further reduced if realistic data uncertainties are considered. Reanalyzing data by Myrold and Tiedje [1986. Simultaneous estimation of several nitrogen cycle rates using ^{15}N: theory and application. Soil Biology & Biochemistry 18, 559–568] (Capac soil) resulted in a model fit similar to the one of the original analysis but with more precise estimates of gross N transformations. In addition, our analysis showed that small N transformations such as heterotrophic nitrification, which was neglected in the original analysis, could be quantified for this soil. Watson et al. [2000. Overestimation of gross N transformation rates in grassland soils due to non-uniform exploitation of applied and native pools. Soil Biology & Biochemistry 32, 2019–2030] provided evidence of a non-uniform exploitation of applied and native N that led to an overestimation of gross N transformations. Reanalyzing the data (CENIT soil, low N application) with the Müller et al. [2007. Estimation of parameters in complex ^{15}N tracing models by Monte Carlo sampling. Soil Biology & Biochemistry 39, 715–726] model where NH_4^+ oxidation was set to Michaelis–Menten kinetics resulted in a satisfactory fit between modeled and observed data, indicating that the observed artifact by Watson et al. [2000. Overestimation of gross N transformation rates in grassland soils due to non-uniform exploitation of applied and native pools. Soil Biology & Biochemistry 32, 2019–2030] was mainly due to inappropriate kinetic settings. Our study shows that the combination of a MCMC method with ^{15}N tracing models is able to consider more complex and possibly more realistic models and kinetic settings to estimate gross N transformation rates and thus overcomes restriction of previous ^{15}N tracing techniques.

*Corresponding author. Department of Plant Ecology, University of Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany.
Tel.: +49 641 9935315; fax: +49 641 9935309.
  E-mail addresses: christoph.mueller@bot2.bio.uni-giessen.de, Christoph.Mueller@ucd.ie (C. Müller).

1. Introduction

The internal nitrogen (N) cycle in soil involves all processes which transform N from one chemical form to another and the transport of N between different pools (Hart et al., 1994). A pool is defined as "a fraction of nitrogen distinguishable by chemical analysis" (Barraclough, 1991). Gross N transformation rates provide important information on the mechanisms and dynamics of the internal N cycle. Therefore, the quantification of gross N transformations is of major interest for soil scientists, plant nutritionists and ecologists. In the 1950s Kirkham and Bartholomew (1954) developed the theoretical basis for quantifying gross N transformations based on the labeling and subsequent dilution of ^{15}N in soil N pools. The traditional ^{15}N dilution technique is based on the labeling of a product pool and its subsequent monitoring over time for isotopic excess and N content. A dilution of this pool indicates a flow of N into this pool at natural abundance or low ^{15}N enrichment, while the outflow of this pool uses the isotopes at the given proportion and therefore does not change the isotopic composition (Hart et al., 1994; Stark, 2000; Booth et al., 2005). The dilution and pool size changes are used to estimate the production and consumption of the labeled N pool using at least two measuring points and an analytical model (Kirkham and Bartholomew, 1954). In contrast ^{15}N tracing techniques rely on the labeling of a substrate pool and the subsequent determination of the ^{15}N label in various product pools (Stark, 2000). This technique was originally developed to determine the fate of ^{15}N after a period of exposure (Schimel et al., 1989). Measuring the isotopic composition and the size of different N pools over a period of time allows the quantification of various gross N transformation rates. Combining both, the dilution and tracing technique, provides the means for a more detailed analysis of the N cycle in soils. For instance, applying the principles of isotopic dilution and enrichment, Barraclough and Puri (1995) were able to separate gross NO_3^- production into a heterotrophic and autotrophic pathway.

Kirkham and Bartholomew (1954) presented for the first time an analytical solution to quantify gross N transformation rates based on the ^{15}N dilution theory. Their model considered one organic ("unavailable nitrogen in soil organic matter") and one mineral ("nitrate nitrogen") N pool with one in- and outflow, i.e. mineralization and immobilization. In the following year the same authors presented a more complex solution (Kirkham and Bartholomew, 1955), which considered the same two N pools as above but they were now connected via two transformations. Since this pioneering work other analytical equations for mineralization and immobilization have been developed. Smith et al. (1994) and Takahashi (2001) reviewed the analytical solutions and came to the conclusion that they are most suitable for short measurement intervals where remineralization can be neglected. Furthermore, Nishio et al. (1985) presented an analytical equation to quantify also gross nitrification.

Building more complex and therefore arguably more realistic models of the N cycle requires numerical solutions (Nason and Myrold, 1991). Moreover analytical solutions can only estimate combined transformations. So in reality they do not quantify specific processes (e.g. mineralization and immobilization) but only the combined gross production and consumption of the labeled pool (Barraclough and Puri, 1995; Schimel, 1996), which can lead to inconsistencies. For instance ignoring remineralization of labeled N (as in most analytical solutions) can lead to an underestimation of the gross N rates (Tietema and van Dam, 1996). Another drawback of analytical solutions is that they do not provide uncertainties for the estimated parameters, i.e. according to Nason and Myrold (1991) "these solutions are attractive because they are exact and require only initial and final conditions to obtain parameter estimates. However, no statistical information on the reliability of the rate estimates is given by this method".

The limitations of the analytical solutions resulted in the development of several dynamic models which are solved numerically (Myrold and Tiedje, 1986; Bjarnason, 1988; Müller et al., 2004). The purpose of these so-called ^{15}N tracing models is to calculate gross N rates for particular data sets (Mary et al., 1998). Therefore they are fundamentally different from simulation models which should be valid for diverse data sets and therefore provide a general process description. Tracing models represent always the most simple description of the N cycle which is sufficient to analyze the data set in question and may vary for different soils (Myrold and Tiedje, 1986). Hence, tracing models allow the simultaneous estimation of several N transformations in particular when multiple fluxes dilute or enrich a N pool with ^{15}N (Myrold and Tiedje, 1986; Mary et al., 1998). They consist at least of three N pools (i.e. NH_4^+, NO_3^- and organic N) but more complex models are also possible (e.g. Tietema and van Dam, 1996; Mary et al., 1998; Müller et al., 2004; Stange and Döhling, 2005). In contrast to analytical solutions, numerical ^{15}N tracing models take into account possible interactions between transformations. All numerical ^{15}N tracing models have in common that model parameters are estimated via non-linear optimization routines. Myrold and Tiedje (1986) and Müller (2004) used the Simplex algorithm and the Levenberg–Marquardt algorithm, respectively, to estimate a unique parameter set for the entire duration of an experiment. In contrast, the FLUAZ model (Mary et al., 1998) estimates transformation rates only between two successive time points via the Haus–Marquardt algorithm and calculates afterwards average N transformation rates over the entire experimental duration. In all of the above mentioned analysis models the number of fitted parameters should be ideally much lower than the number of observed variables to avoid problems due to over-parameterization. The optimization algorithms used in these models are prone to finding parameters which are representative for local but not inevitably for global minima (Müller et al., 2007). This provides a severe restriction for the development

of more complex ^{15}N tracing models (e.g. consideration of Michaelis–Menten kinetics and further N species such as nitrite and gaseous N) (Müller et al., 2007).

To overcome these restrictions we recently presented a new approach for parameter estimation in ^{15}N tracing models based on a Markov chain Monte Carlo (MCMC) method in combination with the Metropolis algorithm (MA) (Müller et al., 2007). We showed that this technique is able to unambiguously estimate a large number of parameters in ^{15}N tracing models and allows therefore complex process descriptions of the N cycle in soils (Müller et al., 2007). The key feature of this technique is a random walk algorithm, which avoids trapping in local minima of the misfit function.

The aim of the present study was to show the applicability of the MCMC technique to estimate gross N transformation rates of previously published data sets by Kirkham and Bartholomew (1954), Myrold and Tiedje (1986) and Watson et al. (2000). In particular the Watson et al. (2000) results have been controversially discussed (Stark and Schimel, 2001; Cliff et al., 2002; Watson et al., 2002; Luxhøi et al., 2003) and already reanalyzed with different models (Watson et al., 2002; Herrmann et al., 2005).

All abbreviations used in the text are explained in Box 1.

2. Methods

2.1. Monte Carlo sampling

The method used to quantify gross N transformation rates is described in detail by Müller et al. (2007). This ^{15}N analysis tool combines an analysis model, i.e. the different N pools and transformations, with an algorithm for parameter estimation. Both parts are independent and can therefore easily be replaced by different versions. Different analysis models were used for each data set which are described in detail in Section 2.2. For the parameter estimation we used the MA (Metropolis et al., 1953), the most efficient MCMC method (Tarantola, 2005).

Harmon and Challenor (1997) have shown that the estimated parameter values obtained with the MA in combination with Monte Carlo sampling provide the true parameter ranges. In each iteration a new parameter set is tested and compared with the previous one. The change of each parameter is governed by a random step width and direction. After creating the new parameter set the algorithm calls the analysis model.

After each model run the misfit between observed data and model output is calculated with a misfit-function $f(\mathbf{m})$ and compared to the misfit of the previous iteration. If the misfit is reduced (i.e. a better fit between the model and observed data) the new parameter set is accepted. Otherwise, if the misfit is increased, a likelihood-function

$$L(\mathbf{m}) = \exp(-\tfrac{1}{2} f(\mathbf{m})) \qquad (1)$$

Box 1
Abbreviations used in the text

Abbreviation	Description
N	Nitrogen
NH_4^+	Ammonium
NO_3^-	Nitrate
N_{org}	Organic Nitrogen
N_{lab}	Labile organic Nitrogen
N_{rec}	Recalcitrant organic Nitrogen
$NH_{4\ ads}^+$	Adsorbed Ammonium
$NO_{3\ sto}^-$	Stored Nitrate
M_{Norg}	Mineralization of N_{org}
M_{Nrec}	Mineralization of N_{rec}
M_{Nlab}	Mineralization of N_{lab}
I_{NH_4}	Immobilization of NH_4^+
I_{NO_3}	Immobilization of NO_3^-
O_{NH_4}	Oxidation of NH_4^+ to NO_3^-
O_{Norg}	Oxidation of N_{org} to NO_3^-
O_{Nrec}	Oxidation of N_{rec} to NO_3^-
$R_{NH_{4a}}$	Release of adsorbed NH_4^+
$R_{NO_{3s}}$	Release of stored NO_3^-
D_{NO_3}	Dissimilatory NO_3^- reduction to NH_4^+
R_{NO_3}	Denitrification
MCMC	Markov chain Monte Carlo
MA	Metropolis algorithm
PDF	Probability density function
OLS	Ordinary least square
QWE	Quadratic weighted error
avg.	Average
S_D	Standard deviation
S_e	Standard error

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is compared with a uniformly distributed random number (X_{rand}). The new parameter set is only rejected if $L(\mathbf{m}) < X_{rand}$. This step of the MA allows the acceptance of an increased misfit and avoids the algorithm to be trapped in local minima (Müller et al., 2007). The acceptance rate is calculated by dividing the number of accepted steps with the total number of iterations. The acceptance rate should be between 0.23 and 0.44 (Gelman et al., 2003).

After a sufficient number of iterations (in most cases 10,000–50,000) the algorithm stops the optimization procedure. This stop point is controlled by a user defined minimal number of iterations and a reduction factor $\hat{R}$ (Gelman et al., 2003; Müller et al., 2007). This factor compares the within and between variance of three simultaneously occurring optimization sequences. The algorithm stops if the minimal number of iterations is reached and $\hat{R}$ is near one for each parameter (in most cases < 1.1). The optimization procedure results in a

probability density function (PDF) for each model parameter, from which parameter averages, standard deviations and a correlation coefficient matrix are calculated. Parameter averages for left-truncated PDFs were calculated for the distribution between zero and the median. Tests showed that this parameter range is normally distributed and can therefore be used for statistical calculations (note: this method was only applied to the PDF of the parameter O_{Norg} for the Myrold and Tiedje (1986) data set).

The MA is programmed in the software MatLab (Version 7.2, The MathWorks Inc.), which calls models that are separately set up in Simulink (Version 6.4, The MathWorks Inc.).

2.2. Data sets

2.2.1. Kirkham and Bartholomew (1954)

Kirkham and Bartholomew (1954) derived analytical solutions for their 2-pool model (organic and mineral N, Fig. 1) to calculate gross N mineralization and immobilization rates of seven data sets which differed in soil type and amendments (see Kirkham and Bartholomew, 1954, Table 1). We rebuilt their model in Simulink to estimate the two N transformation rates with the MCMC method for all seven data sets. Apart from the analytical solutions developed by Kirkham and Bartholomew (1954) we also
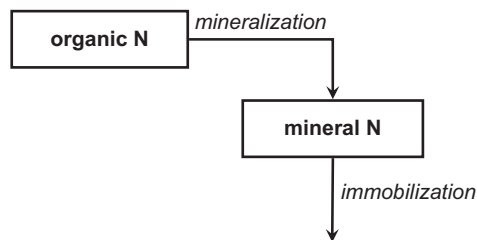

Fig. 1. Conceptual ^{15}N tracing model to analyze gross N transformations (Kirkham and Bartholomew, 1954).

compared our results with the three additional analytical solutions presented by Takahashi (2001). Since Kirkham and Bartholomew (1954) did not provide any uncertainties of their data we evaluated $f(\mathbf{m})$ using an ordinary least square (OLS). To estimate the effect of uncertainties in experimental data on parameter uncertainties we used additionally $f(\mathbf{m})$ in form of a quadratic weighted error (QWE) assuming a 1% standard deviation of experimental data. The deviation is very narrow but is ideal to illustrate the difference between $f(\mathbf{m})$ using OLS and QWE. Because of the pioneering character of the work by Kirkham and Bartholomew (1954) and the fact that the values of the originally reported gross N transformations are well known in the scientific community, we decided to use the original units [lb acre^{-1} d^{-1}] instead of the SI units (note one lb acre^{-1} d^{-1} corresponds to 0.1121 g m^{-2} d^{-1}).

2.2.2. Myrold and Tiedje (1986)

Around 30 years after Kirkham and Bartholomew (1954) the first numerical ^{15}N tracing model to quantify gross N transformations was presented by Myrold and Tiedje (1986). They analyzed several data sets with their model but in only one data set both NH_4^+ and NO_3^- were concurrently labeled with ^{15}N (data from Capac soil). Such a "mirror image" approach is required for the simultaneous estimation of process specific gross N transformation rates (e.g. division of gross NO_3^- production into NH_4^+ oxidation and organic N oxidation, see Barraclough and Puri, 1995). Since experimental data were no longer available (Myrold, D. D.; pers. comm.), we extracted the data from Fig. 3 in Myrold and Tiedje (1986) using the software SigmaScan 3.02 (Jandel Scientific). For the analysis of the data we used the model presented by Myrold and Tiedje (1986) (Fig. 2), but allowed heterotrophic oxidation (O_{Norg}) to proceed. In accordance with Myrold and Tiedje (1986) the kinetics of all transformations were set to first-order. From time point 288 h onwards we used for both ^{15}N treatments the $^{15}NO_3^-$ excess reported for the NH_4^+ treatment, because no data

Table 1
Estimated values for mineralization and immobilization [lb acre^{-1} d^{-1}] with the MCMC method (OLS function) and different analytical solutions for data sets reported by Kirkham and Bartholomew (1954)

Soil	Amendment	Time (days)	Mineralization						Immobilization			
			MCMC		Analytical solutions[a]				MCMC		Analyt.sol.[a]	
			Avg.	S_D	K&B	S	T	Y	Avg.	S_D	K&B	S
Marshall	Corn stalks	0–10	1.64	0.26	1.62	1.60	1.62	1.61	3.24	0.23	3.22	3.20
Clarion	Corn stalks	0–10	1.55	0.24	1.54	1.53	1.53	1.54	1.75	0.21	1.74	1.73
		10–73	0.89	0.07	0.87	0.82	0.85	0.86	1.20	0.07	1.17	1.12
Marshall	None	0–10	3.39	0.23	3.40	3.41	3.39	3.41	0.59	0.20	0.60	0.61
		10–73	0.88	0.06	0.87	0.86	0.86	0.87	0.69	0.05	0.68	0.67
Clarion	Alfalfa	0–10	1.48	0.27	1.46	1.46	1.46	1.46	1.38	0.25	1.36	1.36
		10–73	1.24	0.06	1.22	1.22	1.19	1.22	0.62	0.06	0.61	0.61

[a]K&B: Kirkham and Bartholomew (1954); S: Shen et al. (1984); T: Tiedje et al. (1981); Y: Yamamuro, 1988 [formulas taken from Smith et al. (1994) and Takahashi (2001)].
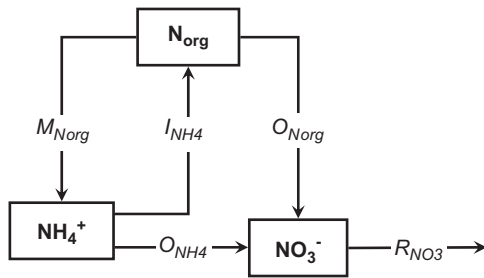
Fig. 2. Conceptual ^{15}N tracing model to analyze gross N transformations (Myrold and Tiedje, 1986).
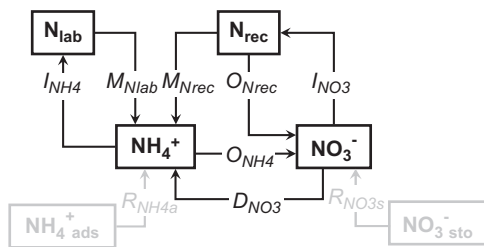

Fig. 3. Conceptual ^{15}N tracing model to analyze gross N transformations (Müller et al., 2007). N-pools and -transformations in grey are not considered in the analysis. (Note, the model presented here represents a basic model structure which has to be adapted to the particular situation, see text for further explanations.)

were reported for the second treatment. We assumed that this procedure is valid because of the similarity of the previous ^{15}N abundance in both treatments (see Myrold and Tiedje, 1986, Fig. 3). For the misfit function $f(\mathbf{m})$ we used a QWE function. We assumed a standard deviation for the observed data of 10%. This was needed due to difficulties to extract single data from Fig. 3 in Myrold and Tiedje (1986).

2.2.3. *Watson et al. (2000)*

Recently, Watson et al. (2000) found evidence for an overestimation of gross N transformation rates caused by an unequal exploitation of native and applied N in particular after small N additions with high ^{15}N enrichments. This hypothesis lead to a controversial discussion (Stark and Schimel, 2001; Cliff et al., 2002; Watson et al., 2002; Luxhøi et al., 2003) and subsequent reanalyzes of the data sets with different models (Watson et al., 2002; Herrmann et al., 2005). Because of the immense importance of whether such an artifact exists or not, we decided to reanalyze the Watson et al. (2000) data using the ^{15}N tracing model presented by Müller et al. (2007; Fig. 3). The underlying assumption in this model is that applied and native N pools are uniformly exploited. We selected the CENIT data because this was also the data set used in the "response to the editor" by Watson et al. (2002) and is therefore ideal for model comparisons. Nitrogen ($2\,\mu g\,N\,g^{-1}$ soil) was applied to the soil so that either NH_4^+ or NO_3^- was labeled with ^{15}N at 99.8 atom% excess.

To compare estimates for gross N transformations with published rates for the same experiment (Watson et al., 2000, 2002; Herrmann et al., 2005) we calculated total mineralization $(M_{Nlab} + M_{Nrec})$, NH_4^+ consumption $(I_{NH_4} + O_{NH_4})$, total nitrification $(O_{NH_4} + O_{Nrec})$ and NO_3^- consumption $(I_{NO_3} + D_{NO_3})$ (Fig. 3). For N transformations following first-order kinetics, average rates were calculated by integrating the gross N rates over the experimental period divided by the total time. This was also applied to gross N transformations reported by Herrmann et al. (2005). Following Müller et al. (2007) we analyzed the data either with NH_4^+ oxidation set to first-order kinetics (run1) or set to Michaelis–Menten kinetics (run2). All other kinetic settings were identical to Müller et al. (2007).

3. Results

3.1. *Kirkham and Bartholomew (1954)*

Kirkham and Bartholomew (1954) analyzed seven data sets which differed in soil type and amendments. While the analytical calculations resulted in gross N transformation rates without uncertainties the MCMC technique produces a PDF from which parameter averages and uncertainties can be calculated. For all seven data sets the two gross N transformations were characterized by well-defined PDFs. Fig. 4A shows exemplary the PDFs for data set 1 (Marshall-soil amended with corn stalks). The estimated average gross N transformations were for all seven data sets in good agreement with the analytical solutions (Table 1). The analytical solutions resulted in slightly different rates but were all within the estimated standard deviations and even the interquartile range (i.e. between the 25th and 75th percentile) (see boxplot Fig. 4). Due to the high number of iterations ($i = 10{,}000$) a statistical test for the comparison of results is inappropriate (Yoccoz, 1991). Assuming a 1% standard deviation of observed data resulted in PDFs with similar average values but considerably reduced parameter uncertainties. This is illustrated in Fig. 4 for the Marshall soil amended with corn stalks. Kirkham and Bartholomew (1954) calculated mineralization and immobilization rates of 1.62 and 3.22 [lb acre^{-1} d^{-1}], respectively, while the MCMC method resulted in values of 1.64 ± 0.26 and 3.24 ± 0.23 [lb acre^{-1} d^{-1}] (OLS) or 1.63 ± 0.14 and 3.22 ± 0.09 [lb acre^{-1} d^{-1}] (QWE).

3.2. *Myrold and Tiedje (1986)*

The MCMC method resulted in a fit similar to the one presented by Myrold and Tiedje (1986) (Fig. 5). While Myrold and Tiedje (1986) considered four gross N transformations we also included O_{Norg} (heterotrophic nitrification). This rate was very small and characterized by a left-truncated PDF (inset Fig. 5). However, our analysis indicated that a slightly positive O_{Norg} rate contributed to a reduction of $f(\mathbf{m})$. At a first glance it looks like that the estimated parameters of both methods
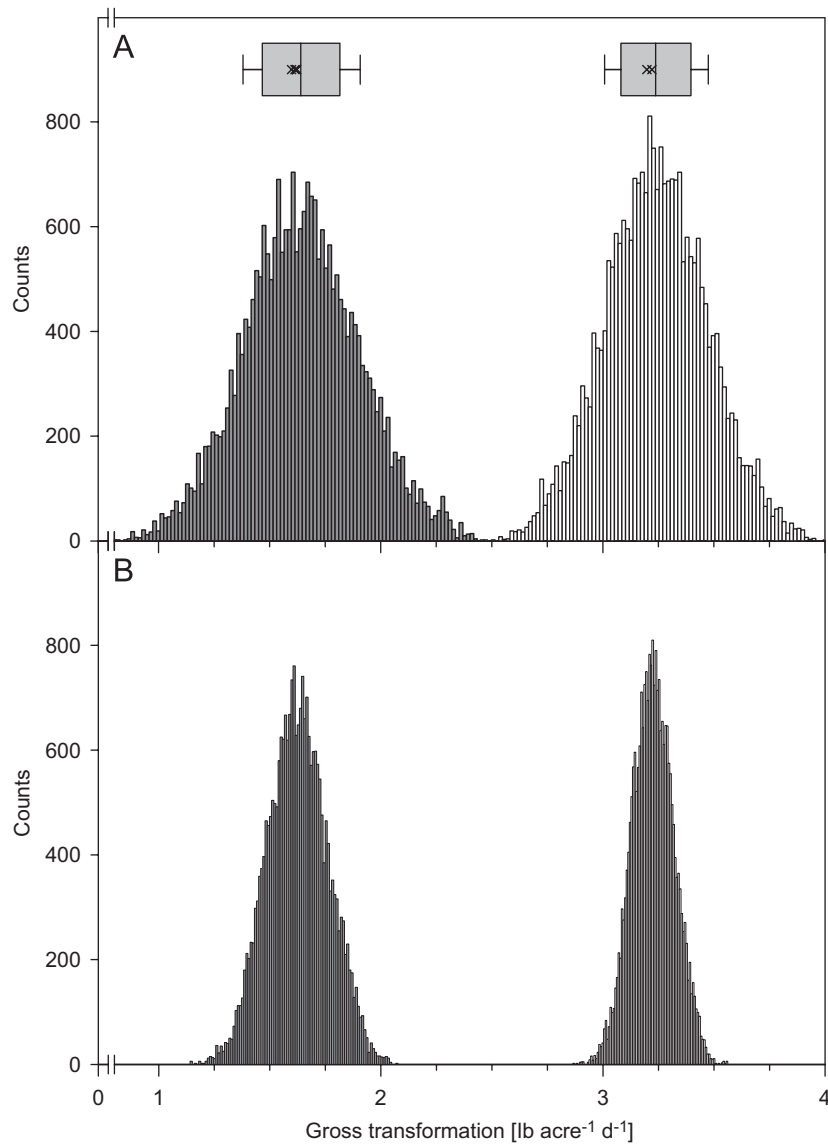
Fig. 4. Probability density plots for results of OLS (A) and QWE (B) optimization and boxplots (median, interquartile range and S_D) of mineralization (left) and immobilization (right) estimated by the MCMC algorithm and analytical solutions (X) (Marshall-soil, amendment of corn stalks; Kirkham and Bartholomew, 1954).

(Table 2) were markedly different. However, considering the reported parameter uncertainties a different picture emerges. While Myrold and Tiedje (1986) reported uncertainties in standard errors (S_e) we report our uncertainties in standard deviations (S_D). It is unclear how Myrold and Tiedje (1986) derived the S_e, in particular how many replications were considered for calculation. Assuming a number of $n = 5$ (from Fig. 3 in Myrold and Tiedje, 1986) it can easily be seen that the averages $\pm S_D$ reported by Myrold and Tiedje (1986) include the values estimated by the MCMC method or at least overlap (R_{NO_3}). Therefore, the MCMC results in much lower uncertainty ranges and therefore provides a method to estimate gross N rates with increased precision.

3.3. Watson et al. (2000)

The two optimization runs for the data from CENIT ($2 \mu g \, N \, g^{-1}$ soil) differed in the kinetics used for NH_4^+ oxidation (O_{NH_4}). In run1 where O_{NH_4} was described by first-order kinetics a distinct discrepancy between measured and modeled ^{15}N excess values of the NO_3^- pool was observed (Fig. 6 A2, B2). This discrepancy diminished in run2 where O_{NH_4} was described by Michaelis–Menten kinetics (Fig. 6). Introducing Michaelis–Menten kinetics influenced the values of the other parameters and the combined N transformations (Tables 3 and 4). Except for I_{NH_4} all gross N transformations were different between both optimization runs. Our estimated gross NH_4^+ immobilization I_{NH_4} (Table 3) was in both runs
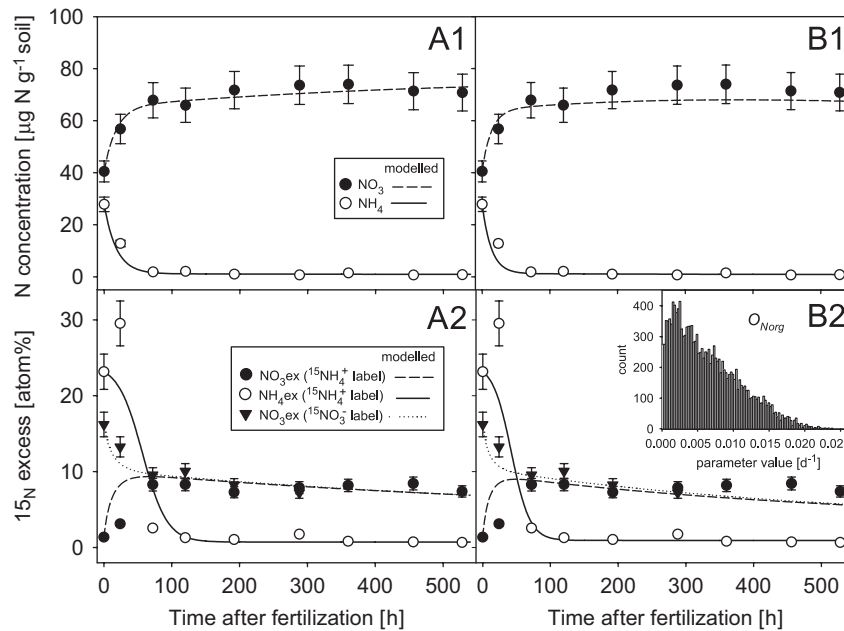
Fig. 5. Measured and modeled N concentrations and ^{15}N enrichments of ammonium (NH_4^+) and nitrate (NO_3^-) of the Capac soil for the Myrold and Tiedje (1986) analysis (A) and the MCMC method (B) and the probability density plot for heterotrophic nitrification (O_{Norg}).

Table 2
Estimated first-order parameters of N transformation rates [day^{-1}] for the Capac soil with the MCMC method and values reported by Myrold and Tiedje (1986)

Nrate	MCMC		Myrold and Tiedje (1986)	
	Avg.	S_D[a]	Avg.	S_e[a]
M_{Norg}	0.0142	0.0020	0.0104	0.0210
I_{NH_4}	0.2312	0.0517	0.1080	0.0810
O_{NH_4}	1.5418	0.1139	1.2300	0.1400
R_{NO_3}	0.0261	0.0092	0.0132	0.0052
O_{Norg}	0.0026	0.0015	ND	

[a]Note: while Myrold and Tiedje (1986) report uncertainties in standard errors (S_e) we report our uncertainties in standard deviations (S_D).

similar to the rate of $0.7525\,\mu g\,N\,g^{-1}\,soil\,h^{-1}$ reported by Watson et al. (2000). The total mineralization ($1.03 \pm 0.05\,\mu g\,N\,g^{-1}\,soil\,h^{-1}$) is the only combined N transformation which was equal among the MCMC method (run1) and the previous studies, while NH_4^+ consumption and total nitrification were smaller using the MCMC method and NO_3^- consumption was higher than reported values (Table 4). Using Michaelis–Menten kinetics approximately halved the total mineralization and NO_3^- consumption compared to the first run, while NH_4^+ consumption and total nitrification were not influenced by the change in the kinetics for O_{NH_4} (Table 4). NO_3^- consumption was still greater in run2 than the reported values by Watson et al. (2000, 2002) and Herrmann et al. (2005) (Table 4).

4. Discussion

4.1. Kirkham and Bartholomew (1954)

The main difference between the data analysis via analytical solutions and the MCMC method is that the numerical technique is able to calculate true parameter uncertainties. Irrespective of observed data uncertainties the MCMC method determines the PDF for each gross N transformation, i.e. the statistical probability that the gross N transformations are within a certain range (Fig. 4). The various analytical solutions were different but all within the average $\pm S_D$ ranges of the MCMC calculated values. This example illustrates the analysis power of the MCMC technique not only to provide a value for the gross N transformation but also to determine uncertainty ranges. The MCMC method provides more realistic parameter uncertainties if misfit calculations are weighted by observed data uncertainties (Müller et al., 2007) which can clearly be seen comparing the analyses when $f(\mathbf{m})$ was calculated as OLS or QWE (Fig. 4). So far only one other method exists to estimate standard errors of gross N transformations calculated with analytical solutions which requires information on data uncertainties (Luxhøi and Brockhoff, 2004). In contrast the calculation of gross N transformations via analytical solutions is usually based on observed average values without consideration of data uncertainties. Thus, our analysis highlights the importance of a confidence interval for gross N transformations rather than just the average rate. This is in line with theoretical considerations and ecosystem studies emphasizing the
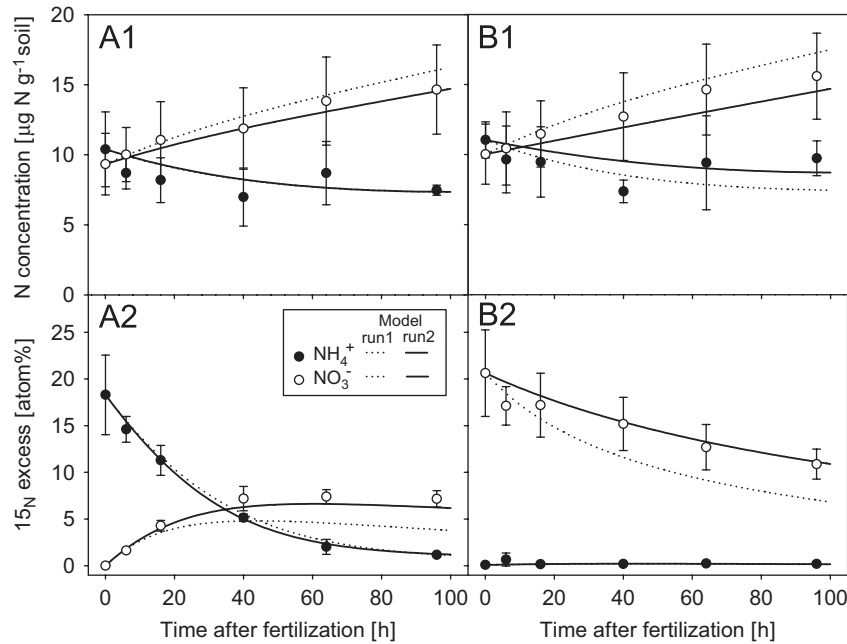
Fig. 6. Measured and modeled N concentrations and ^{15}N enrichments of ammonium (NH_4^+) and nitrate (NO_3^-) of the CENIT soil (Watson et al., 2000) for the two optimization runs (according to Table 3). $A = {}^{15}NH_4$ label; $B = {}^{15}NO_3$ label.

Table 3
Cumulated gross N transformation rates [μg N g^{-1} soil h^{-1}] for the CENIT soil (Watson et al., 2000) estimated with the MCMC method

Nrate	Kinetic	run1		run2	
		Avg.	S_D	Avg.	S_D
I_{NH_4}	Zero	0.708	0.088	0.738	0.098
I_{NO_3}	Zero	0.297	0.078	0.117	0.068
M_{Nrec}	Zero	0.985	0.045	0.494	0.093
M_{Nlab}	First	0.041	0.017	0.090	0.026
O_{NH_4}	First/MM[a]	0.152	0.011	0.062	0.026
O_{Nrec}	Zero	0.010	0.009	0.093	0.031
D_{NO_3}	Zero	0.019	0.002	0.041	0.006

[a]run1: first-order; run2: Michaelis–Menten kinetics.

importance of parameter confidence intervals (Yoccoz, 1991; Quinn and Keough, 2002; Knorr and Kattge, 2005).

The model by Kirkham and Bartholomew (1954) (Fig. 1) is able to quantify gross rates for total mineral N production and consumption (Schimel, 1996). These two rates are the sum of various process specific transformations (see e.g. Barraclough and Puri, 1995). However, to quantify process specific gross N transformation rates more detailed process-based ^{15}N tracing models in combination with more detailed ^{15}N tracing experiments are required.

4.2. Myrold and Tiedje (1986)

Myrold and Tiedje (1986) presented the first numerical model to quantify process specific gross N transformations. Our reanalysis with the MCMC method (Table 2) of their most detailed data set (Capac soil, Myrold and Tiedje,

1986, Fig. 3) resulted in a fit between experimental data and model output which was similar to the original analysis (Fig. 5).

One of the main differences of our analysis to the original one is that we were also able to identify oxidation of organic N to NO_3^- (heterotrophic nitrification, O_{Norg}). This process was included in the model by Myrold and Tiedje (1986) but was set to zero, probably because the value of this process was indistinguishable from zero. Based on the well-defined PDF for O_{Norg} (see inset Fig. 5) our analysis provides for this process a value close to zero but with an average $\pm S_D$ clearly above zero (Table 2). In general, the parameter uncertainties reported by Myrold and Tiedje (1986) are much higher than the MCMC values. In case of the mineralization rate, the uncertainty by Myrold and Tiedje (1986) is even higher than the average value (Table 2), thus covering a wide range of possible values. The parameter values (avg. $\pm S_D$) determined with the MCMC technique are in most cases within the range reported by Myrold and Tiedje (1986) (Table 2). Therefore, our method reduces the uncertainty ranges and provides more precise estimates of gross N transformations.

The Myrold and Tiedje (1986) model (Fig. 2) did not include processes such as immobilization of NO_3^- (I_{NO_3}) and dissimilatory NO_3^- reduction to NH_4^+ (D_{NO_3}) which are sometimes observed in soils (Cookson et al., 2006). Parkin et al. (1985) who also used the Capac soil found an increase of ^{15}N abundance in the NH_4^+ pool in the treatment where the NO_3^- pool had been labeled with ^{15}N, which could have been caused by D_{NO_3}. Another explanation could be a fast immobilization in combination with re-mineralization. Both processes were tested in a previous study (Müller et al., 2004) where it was shown that D_{NO_3} was the most

Table 4
Combined N transformations [$\mu g \, N \, g^{-1} \, soil \, h^{-1}$] for the CENIT soil (Watson et al., 2000) using the MCMC method and previously published rates (Watson et al., 2000, 2002; Herrmann et al., 2005)

Nrate	MCMC run1		MCMC run2		Watson et al.[a]			Herrmann (2005)	
	Avg.	S_D	Avg.	S_D	Avg1	S_D	Avg2	Avg.	S_D
Total mineralization	1.03	0.05	0.58	0.10	0.99	0.04	0.99	1.10	0.08
NH_4^+ consumption	0.86	0.09	0.80	0.10	1.12	0.07	1.12	1.21	0.05
Total nitrification	0.16	0.01	0.15	0.04	0.33	0.07	0.33	0.28	0.03
NO_3^- consumption	0.32	0.08	0.16	0.07	0.10	0.01	0.08	0.03	0.05

[a]Avg1: Watson et al. (2000); Avg2: Watson et al. (2002) (no uncertainties reported).

likely process to explain the increase in ^{15}N abundance in the NH_4^+ pool when only NO_3^- was labelled. In addition, Parkin et al. (1985) concluded that their calculated denitrification rate could have been overestimated if I_{NO_3} would occur. We included both rates (D_{NO_3} and I_{NO_3}) in the model by Myrold and Tiedje (1986) to test the likelihood of these processes in the Capac soil. However, the misfit of this model run was not improved (data not shown). Our results showed that the model without D_{NO_3} and I_{NO_3} but considering O_{Norg} is the best model to use. Such a decision is in line with Cox et al. (2006) who emphasized that "models should be as simple as possible, but no simpler".

One of the reasons for the differences of the uncertainty ranges between our and the Myrold and Tiedje (1986) analysis is related to the kind of misfit function used for the optimization. While Myrold and Tiedje (1986) used the OLS function we implemented a QWE function. As discussed above, the parameter uncertainty range is dependent on the type of misfit function used. The uncertainties of the data are needed to calculate realistic parameter uncertainties. This was already demonstrated on the Kirkham and Bartholomew (1954) data comparing the analysis when $f(\mathbf{m})$ was set to OLS or QWE (1% data uncertainty) (Fig. 4). Using OLS in a situation where observed data are characterized by totally different value ranges (e.g. see Fig. 5 A1, B1) would bias the optimization procedure towards the highest values within the data set and therefore influence the optimization results (Raupach et al., 2005).

4.3. Watson et al. (2000)

Watson et al. (2000) quantified gross N transformations in four different soils after labeling with ^{15}N at two concentrations. At low N application rates, Watson et al. (2000) found, one day after fertilizer application, that 55% of the added ^{15}N–NH_4^+ had entered the NO_3^- pool (CENIT soil). Since this was observed "without a concurrent increase in the size of the unlabeled NO_3^- pool" (Watson et al., 2000), they concluded that a preferential consumption of applied NH_4^+ took place. The authors confirmed this preferential use of applied N in their second analysis and provided evidence that a "preferential nitrification of applied $^{15}NH_4^+$ occurred" (Watson et al., 2002). Herrmann et al. (2005) reanalyzed the data and introduced separate nitrification rates for added and native N. They estimated a four-fold higher rate for added N and therefore confirmed the preferential use of added N. They presumed that this preferential use of added N resulted in an overestimation of gross mineralization and nitrification. Moreover, Luxhøi et al. (2003), who carried out a study very similar to the one by Watson et al. (2000), concluded that the findings by Watson et al. could be explained by a non-homogenous distribution of applied N.

In our reanalysis we used the model presented by Müller et al. (2007) which presents a suitable model to quantify gross N transformations in temperate grassland soils. In the first optimization run NH_4^+ oxidation was described by first-order kinetics. This first run resulted in average parameter values that were similar to previous analyses (Table 4). We observed a discrepancy between modeled and observed $^{15}NO_3^-$ contents (Fig. 6 A2, B2), which was similar to the one observed by Watson et al. (2002). Based on the results of run1 we confirmed that a preferential uptake of applied NH_4^+ could have occurred. However, after changing the kinetics of NH_4^+ oxidation to Michaelis–Menten (Müller et al., 2007) no preferential usage of applied NH_4^+ could be detected anymore (Fig. 6). This emphasizes how crucial it is to carefully select the most realistic kinetic settings. In general, Michaelis–Menten is conceptually the best kinetics to use for microbial transformations (Myrold and Tiedje, 1986), in particular at low NH_4^+ concentrations where the change from zero- to first-order kinetics occurs (Taylor and Bottomley, 2006; Müller et al., 2007). Our second optimization run resulted in a gross mineralization and gross nitrification which were considerably lower (42% and 55%, respectively) than the transformations estimated by Watson et al. (2000, 2002) (Table 4). Herrmann et al. (2005) found a 1.5–2.5-fold overestimation of gross mineralization for the same data set. It should be noted that our estimate for gross N mineralization with run2 is 1.9-fold lower than the rate estimated by Herrmann et al. (2005). Moreover, Watson et al. (2000) observed a higher mineralization rate under low N application compared to the high N application. If the real mineralization rate was lower, as suggested by Watson et al. (2000), then it is not surprising that an improved model fit to the data resulted in reduced

mineralization rates as observed in run2 when O_{NH_4} was described by Michaelis–Menten kinetics. This example shows that a switch of kinetics of one N transformation can severely change the gross rates of other N transformations and highlights that a single gross N transformation rate can only realistically be interpreted in concert with all other simultaneously occurring N transformations. Using the equation developed by Kirkham and Bartholomew (1954), transformations such as I_{NO_3} could not be simultaneously estimated by Watson et al. (2000). Our reanalysis showed that I_{NO_3} is characterized by a well-defined PDF and thus supported the argumentation by Stark and Schimel (2001). However, we also showed that D_{NO_3}, another NO_3^- consumption process, is responsible for ∼25% of the total NO_3^- consumption (Tables 3 and 4) and therefore is an important process in the CENIT soil. Furthermore, remineralization of immobilized mineral N is an important transformation which was ignored in the original analysis (Watson et al., 2000). Our reanalysis highlights the complexity of N transformations in soil and shows that simple models and inadequate kinetic settings can lead to erroneous results. Great care should be exercised to select both, a suitable model and appropriate kinetics for each N transformation. An approach similar to the one presented by Cox et al. (2006) who selected the most appropriate model after a series of models had been tested seems most promising.

5. Conclusions

The reanalysis of three ^{15}N enrichment data sets published over the last 50 years with a new analysis method (MCMC) to estimate gross N transformations highlighted the following points.

(1) For all gross N transformations not only average values but also standard deviations can be estimated even for data sets where no data uncertainties are available.
(2) The application of the MCMC method resulted in much narrower confidence intervals for the model parameters (i.e. avg. $\pm S_D$) and therefore provides more precise estimates of gross N transformation rates.
(3) The change of kinetic settings for one N transformation can considerably influence the gross rates of other N transformations. NH_4^+ oxidation is most realistically described by Michaelis–Menten kinetics, whereas inappropriate kinetic settings for this transformation may lead to wrong data interpretations.

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