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Estimation of parameters in complex ^{15}N tracing models by Monte Carlo sampling

Christoph Müller[a,*], T. Rütting[a], J. Kattge[b], R.J. Laughlin[c], R.J. Stevens[c]

[a]*Department of Plant Ecology, University Giessen, Heinrich-Buff-Ring 26-32, Giessen 35392, Germany*
[b]*Max-Planck Institute for Biogeochemistry, Hans-Knöll-Str. 10, 07745 Jena, Germany*
[c]*Agriculture, Food and Environmental Science Division, Agri-Food and Biosciences Institute, Newforge Lane, Belfast BT9 5PX, Northern Ireland*

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Abstract

The most widely used method to quantify gross N transformation rates in soils is based on ^{15}N dilution and enrichment principles. To identify rate parameters, ^{15}N-tracing experiments are analysed by models that are linked to algorithms that try to minimize the misfit between modelled and observed data. In currently available ^{15}N-tracing models optimization algorithms are based on the Levenberg–Marquardt method that is suitable for the determination of small number of parameters. Therefore, these models are restricted to a few processes. Methods based on Monte Carlo sampling have the potential to overcome restrictions on parameter numbers but have not been tested for application in ^{15}N-tracing models. Here, for the first time, we use a Markov chain Monte Carlo (MCMC) method with a tracing model to simultaneously determine the probability density functions (PDFs) of the whole set of parameters for a previously published data set [Müller, C., Stevens, R.J., Laughlin, R.J., 2004. A ^{15}N tracing model to analyse N transformations in old grassland soil. Soil Biology & Biochemistry 36, 619–632]. We show that the MCMC method can simultaneously determine PDFs of more than 8 parameters and demonstrate for the first time that it is possible to optimize models where transformations are described by Michaelis–Menten kinetics. Setting the NH_4^+ oxidation rate to Michaelis–Menten kinetics reduced the misfit by 19%. Together with monitoring diagnostics for parameter convergence, the MCMC method is a very efficient and robust technique to determine PDFs for parameters in ^{15}N-tracing models that contain large number of N transformations and complex process descriptions.

Keywords: ^{15}N tracing; Model; N transformations; Metropolis algorithm; Monte Carlo sampling; Markov chain; Michaelis–Menten kinetics

1. Introduction

Techniques based on the principle of ^{15}N isotope enrichment and dilution are widely used to quantify nitrogen (N) transformation rates in terrestrial and aquatic ecosystems (Blackburn, 1979; Stark, 2000). In tracing experiments one or more soil N pools are enriched with ^{15}N and the N concentrations and enrichments are determined in the labelled but also in various product pools over a period of time. The calculation of gross rates is based on models that describe their interactions with individual soil N pools. In their pioneering work, Kirkham and Bartholomew (1954, 1955) considered an organic N pool and one mineral N pool and developed analytical solutions for the mineralization and immobilization process in soil. With the progress in analytical methods since the 1950s, we have now techniques available that can differentiate the pools of organic N and mineral N into compound-specific N species. This led to the development of more detailed ^{15}N tracing models (Myrold and Tiedje, 1986; Mary et al., 1998; Müller et al., 2004). Current ^{15}N-tracing models are based on a set of differential equations that are solved by numerical techniques (e.g. Runge–Kutta algorithm) (Mary et al., 1998). Non-linear optimization routines are used to find sets of kinetic parameters that minimize the misfit between modelled data and a set of

*Corresponding author. Tel.: +49 641 9935315; fax: +49 641 9935309.
E-mail address: christoph.mueller@bot2.bio.uni-giessen.de (C. Müller).

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observed data. The estimation of parameters in those models is usually carried out with algorithms based on the Levenberg–Marquardt method. However, this technique is only suitable for small number of parameters (usually not more than 6 parameters) (Aster et al., 2005) and therefore restricts the development of more complex models. In current ^{15}N-tracing models this problem is solved in two ways: (1) the optimizations are only carried out between two adjacent observations points in time (Mary et al., 1998) so that N transformations with small rates can be ignored or (2) optimizations are carried out over the entire experimental period but in more than one step (Müller et al., 2004). Both approaches have their draw backs because they may ignore transformation rates that are only apparent in longer time sequences (Müller et al., 2005) or may misinterpret ^{15}N-tracing data (Luxhøi et al., 2005). Another disadvantage of current ^{15}N-tracing models that follows from the restriction on parameter numbers is that N transformations are usually described by either zero- or first-order kinetics but not Michaelis–Menten kinetics, which would be conceptually the best kinetics to use but doubles the number of parameters (Myrold and Tiedje, 1986).

Complex ^{15}N-tracing models have recently been published that include nitrite species (Müller et al., 2006) and gaseous N species (Stange and Döhling, 2005) but the currently applied optimization techniques are unable to estimate all parameters in these models simultaneously. Therefore, further progress in ^{15}N-tracing techniques can only be expected once we are able to estimate large number of parameters via robust optimization techniques. The most promising methods that can simultaneously estimate large number of parameters are based on Bayesian data analysis (Gelman et al., 2003). In particular, methods that perform a guided random walk in the model parameter space via Monte Carlo (MC) sampling are considered to be the most efficient techniques to collate parameter sets and their probability density function (PDF) of large-scale problems (Tarantola, 2005). MC sampling techniques have been developed for applications in nuclear physics (Metropolis and Ulam, 1949; Metropolis et al., 1953) and are recently applied successfully to estimate large number of parameters in ecosystem models (Braswell et al., 2005; Knorr and Kattge, 2005; Xu et al., 2006). In this paper, we show for the first time that a Markov chain Monte Carlo (MCMC) method that uses the Metropolis algorithm (MA; Metropolis et al., 1953) is suitable to simultaneously collate parameter sets representing the PDF of all parameters of complex ^{15}N-tracing models. These sets of PDFs are then used to derive optimized parameter values, their error covariances and parameter deviations from Gaussian distribution. Furthermore, we demonstrate that this technique is able to optimize parameters of N transformations that are based on Michaelis–Menten kinetics. We will first describe the algorithm and then use the new method with experimental data and the model (ModelB) described by Müller et al. (2004).

2. Materials and methods

2.1. MC sampling

The aim of an optimization algorithm is to find the so-called global minimum. Fig. 1 illustrates a misfit function, $f(\mathbf{m})$, for a theoretical 2-parameter model. There are two combinations in the parameter space where the misfit function has a minimum: one local and one global minimum. Optimization algorithms should be able to avoid or move in and out of the local minimum in search

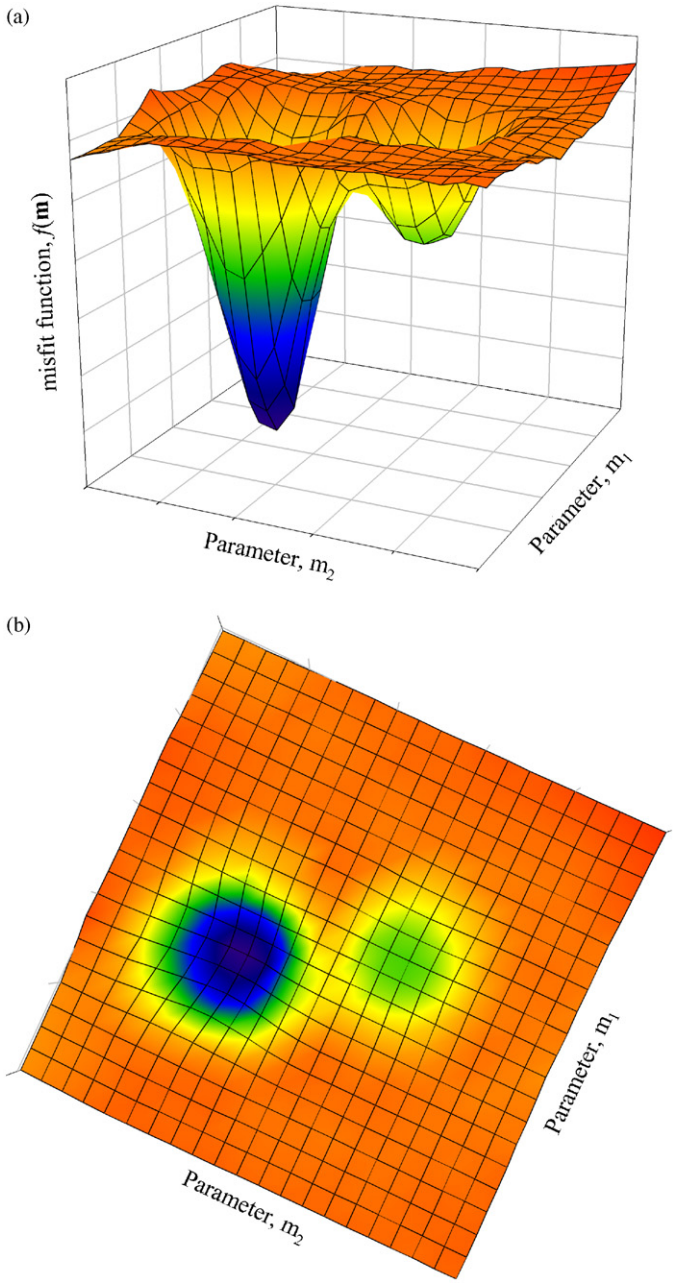

Fig. 1. Model space of a misfit function, $f(\mathbf{m})$, of a theoretical two-parameter model, (a) and (b) represent the view from the side and from above respectively.

for the global minimum. This task is easily achieved with models of low dimension but becomes increasingly more difficult in models with many parameters (Aster et al., 2005).

Optimization routines in ^{15}N-tracing models are often based on the Levenberg–Marquardt (LM) method, which is a very efficient and fast optimization algorithm that approaches the minimum often within a few iterations. However, there exists an uncertainty in using suitable starting conditions that make it difficult to judge if the global minimum was reached (Aster et al., 2005). One of the solutions to that problem is to repeat the optimization with various starting points and monitor if they all converge to the same solution (Mary et al., 1998). However, with the increase in model dimensions the LM method is prone to finding a local minimum closest to the starting point rather than the desired global minimum and it is, therefore, the method of choice only for optimizations of low number of parameters (Aster et al., 2005).

To identify the global minimum, an exhaustive global search method that visits the entire model space would be ideal. However, such a search is only practical for systems with small number of parameters (Mosegaard and Tarantola, 1995). To restrict the number of misfit calculations, random walk techniques based on MC search methods are available. They avoid entrapment in local minima and are therefore ideally suited for highly non-linear optimization problems (Mosegaard and Tarantola, 1995). Each optimization step is only dependent on the previous step and they are, therefore, a mathematical illustration of a Markov chain (Metropolis and Ulam, 1949). The most efficient MC method to sample the model space is the MA (Metropolis et al., 1953; Tarantola, 2005) (Fig. 2). The MA performs a random walk (comparable to a Brownian motion) in the model space. The walk is guided by probabilistic rules that decide if a move is accepted or rejected: moves that improve the fit are always accepted, while moves that degrade the fit are accepted with a certain probability. This allows the algorithm to move in and out of local minima. Generally, the acceptance rule leads towards a minimum in $f(\mathbf{m})$ and enables the algorithm to exactly sample the PDF of parameters in the vicinity of the global minimum (Tarantola, 2005).

A likelihood function, $L(\mathbf{m})$ is calculated and evaluated for each optimization step:

$$L(\mathbf{m}) = k \exp\left(-\frac{1}{2} f(\mathbf{m})\right), \tag{1}$$

with $f(\mathbf{m})$ in our case:

$$f(\mathbf{m}) = [g(\mathbf{m}) - \mathbf{d}]^{\mathbf{T}} \mathbf{C}^{-1} [g(\mathbf{m}) - \mathbf{d}], \tag{2}$$

where k is a normalisation constant, $\mathbf{C}^{-1}$ the error covariance matrix of the observations (i.e. the experimental uncertainties) and $\mathbf{T}$ denotes the transposed vector. In case of independent Gaussian uncertainties, $f(\mathbf{m})$ takes on the form of a weighted quadratic error function:

$$f(\mathbf{m}) = \sum_{i=1}^{N} \frac{(g(\mathbf{m})_i - \mathbf{d}_i)^2}{\mathbf{s}_i^2}, \tag{3}$$

where $g(\mathbf{m})$, the modelled data; $\mathbf{m}$, the vector of model parameters; $\mathbf{d}$, the vector of observed data; $\mathbf{s}^2$, the variance of the individual observations and N the number of variables (note, $\mathbf{s}$ are the measured individual standard deviations). Thus, the variation in the experimental data is taken into account for parameter quantification.

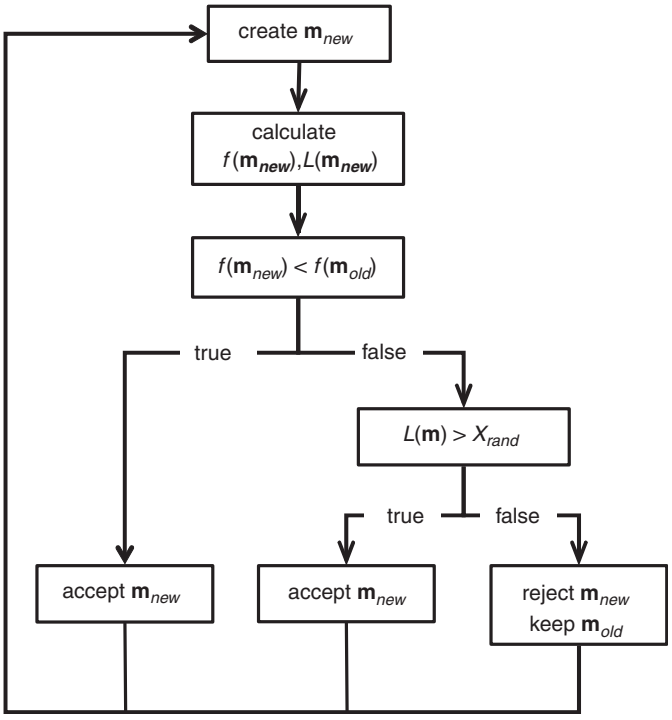

Fig. 2. Flow diagram of the Markov chain Monte Carlo method using the Metropolis algorithm (see text for explanation of variables).

2.2. Metropolis decision

The acceptance probability, P_{accept}, of an optimization step (i.e. the Metropolis decision) is given by

$$P_{\text{accept}} = \begin{cases} f(\mathbf{m}_{new}) \leqslant f(\mathbf{m}_{old}) & \Rightarrow \quad 1 \\ f(\mathbf{m}_{new}) > f(\mathbf{m}_{old}) & \Rightarrow \quad \exp\left(-\frac{1}{2}\Delta f\right), \end{cases} \tag{4}$$

with $\Delta f = f(\mathbf{m}_{new}) - f(\mathbf{m}_{old})$ (the subscripts new and old refer to the current and the previous iteration).

In case of $\Delta f > 0$ (i.e. the new optimization step degrades the fit) the step is accepted with a probability given by $\exp\left(-\frac{1}{2}\Delta f\right)$. In practice the step is accepted when

$$\exp\left(-\frac{1}{2}\Delta f\right) > X_{\text{rand}},$$

where X_{rand} is a uniformly distributed random number between 0 and 1 (Fig. 2).

To start the random walk the algorithm is supplied with an initial set of parameters which is always accepted. A new

set of parameters ($\mathbf{m}_{new}$) is generated by altering the old parameters by a parameter-specific step that is in our case multiplied with a uniformly distributed random number between −1 and 1. The new parameter vector is evaluated as outlined above (Fig. 2). If a parameter value of the new vector is outside a prior defined range the new parameter vector is rejected. The prior defined range of all parameters was between 0 and 1 except for run 6 that evaluated Michaelis–Menten parameters (K_{mHN4} ranged between 0 and 10). Depending on the size of the parameter vector ($\mathbf{m}$) the acceptance rate of $\mathbf{m}_{new}$ should be between 0.44 and 0.23 (Gelman et al., 2003).

2.3. Monitoring parameter convergence

The global minimum of the misfit function $f(\mathbf{m})$ is approached after a 'burn-in time'. From this point onwards the algorithm samples the model space in the vicinity of the global minimum. Sampling should continue till the PDF is well characterised for all aspects of interest, in our case parameters. The accuracy of the sampling can be monitored by a reduction factor ($\hat{R}$) according to Gelman et al. (2003). This requires the simultaneous evaluation of a series of sequences. In this study we tested 3–5 sequences, each starting with a different parameter vector. This was achieved by taking starting parameters from Müller et al. (2004) (Table 2) and multiply each parameter with a random number between 0.5 and 2. The between- and within-sequence variances (B, W) are defined by

$$B(\mathbf{m}) = \frac{n}{J-1}\sum_{j=1}^{J}(\overline{\mathbf{m}}_j - \overline{\mathbf{m}})^2, \tag{5}$$

$$W(\mathbf{m}) = \frac{1}{J}\sum_{j=1}^{J}\mathbf{s}_j^2, \tag{6}$$

where J is the number of sequences, n the number of iterations, $\overline{\mathbf{m}}_j$ the vector of the within-sequence parameter mean, $\overline{\mathbf{m}}$ the vector of the parameter mean over all sequences and $\mathbf{s}_j^2$ the vector of the within-sequence parameter variance.

The reduction factor ($\hat{R}$) can now be computed which should decline to 1 if iterations continue for $n \to \infty$ (Gelman et al., 2003):

$$\hat{R}(\mathbf{m}) = \sqrt{\frac{((n-1)/n)W(\mathbf{m}) + (1/n)B(\mathbf{m})}{W(\mathbf{m})}}. \tag{7}$$

In practice, a value of $\hat{R} < 1.2$ is acceptable for most cases and can serve as a stop criteria for the optimization run. In our study we stopped the sampling once the reduction factors of all parameters had reached values below 1.2 (i.e. $\hat{R}$ was much lower than 1.2 for most parameters). The initial half of the final number of iterations within each sequence is considered as 'burn-in time' and these parameter sets are discarded (Gelman et al., 2003).

2.4. Parameter values, their uncertainties and statistical considerations

The sampling from the end of the 'burn-in time' (b) till the last optimization run (n) from all sequences is used to calculate the mean, median and standard deviation of all values of the parameter vector $\mathbf{m}$.

The mean value is calculated by

$$\overline{\mathbf{m}} = \frac{1}{J(n-b)}\sum_{j=1}^{J}\sum_{i=b}^{n}\mathbf{m}_{ij}, \tag{8}$$

and the standard deviation is calculated by

$$\mathbf{m}_\sigma = \sqrt{\frac{1}{[J(n-b)]-1}\sum_{j=1}^{J}\sum_{i=b}^{n}(\mathbf{m}_{ij} - \overline{\mathbf{m}})^2} \tag{9}$$

(alternatively, $\mathbf{m}_\sigma$ can be computed as the square root of the diagonal elements of the covariance matrix). The median is the 50th percentile of the sorted parameter vector.

The final value of the misfit function, $f(\mathbf{m})$, is calculated with the mean parameter values ($\overline{\mathbf{m}}$).

The probability distribution characterised by MC sampling between the end of the 'burn-in time' and the end of the run is visualized by bar-charts (histograms) and analysed for normality via a probit-analyis. The range of values obtained for each parameter in vector $\mathbf{m}$ is divided into 100 equally spaced 'bins'. The counts per 'bin' are plotted as histograms. The probability ($\mathbf{p}$) for each bin is calculated

$$\mathbf{p}_i = \frac{\mathbf{c}_i}{\sum_{i=1}^{100}\mathbf{c}_i}$$

and expressed as cumulative probability ($\mathbf{p}_I = \sum_{i=1}^{I}\mathbf{p}_i$, where $\mathbf{c}$ is the count per 'bin' and i refers to the 'bin' number).

Probits ($\mathbf{p}_{D_i}$) are calculated for each 'bin' by

$$\mathbf{p}_{D_i} = \Phi^{-1}(\mathbf{p}_I) + 5, \tag{10}$$

where Φ^{-1} is the inverse of the cumulative normal distribution with a mean of zero and a standard deviation of one (5 is added, so that all probits obtain positive values).

Normally distributed parameters are indicated by a close fit of the probit plot to the linear probit regression line. The probit reference points ($\mathbf{p}_{D_{i\_\mathrm{ref}}}$) for the regression line are calculated for each 'bin' by

$$\mathbf{p}_{D_{i\_\mathrm{ref}}} = \frac{\mathbf{m}_i - \overline{\mathbf{m}}}{\mathbf{m}_\sigma} + 5. \tag{11}$$

The covariance and correlation matrix is calculated from the parameter vector of iterations after the 'burn-in time'. The correlation matrix provides information on error correlation (Tarantola, 2005). In particular, it identifies groups of parameters that tend to be constrained together (Knorr and Kattge, 2005).

2.5. Performing model simulations

The MCMC routine is programmed in the software MatLab (Version 7.1, The MathWorks Inc.). The algorithm calls the ^{15}N-tracing model, which is separately set up in Simulink (Version 6.3, The MathWorks Inc.), a companion software to MatLab. A set up in such a way has the advantage that various ^{15}N-tracing models can easily be tested and the optimization routine can also be used for other optimization problems. In this paper, we only use ModelB described by Müller et al. (2004). MatLab has in-built solvers for ordinary differential equations which are available in Simulink. We used the solver *ode5* that is based on the Runge–Kutta formula. Initial values of the six N pools in the model (Fig. 3) were taken from Müller et al. (2004) (Table 1).

In the first optimization run we simultaneously optimized all nine rate parameters including those that were either kept constant (k_{DNO3}) or set to zero (k_{RNO3}) in the original analysis (Müller et al., 2004). Other tests are described in Section 3. A typical optimization run with 50,000 iterations, 3 sequences and 9 parameters would take approximately 3 h on a Pentium M780 with 2.26 GHz clock speed and 1 Gb ram running on Windows XP Professional.

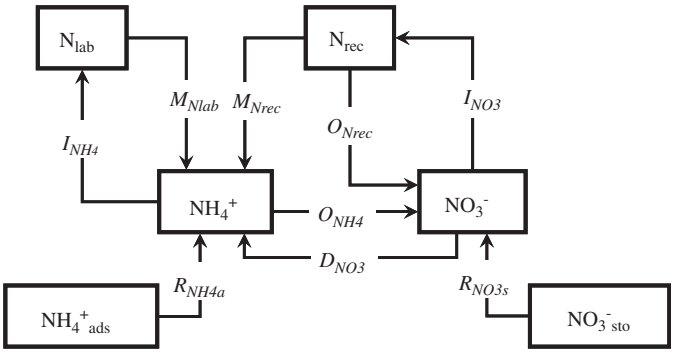

Fig. 3. Conceptual ^{15}N tracing model to analyse N transformations in an old grassland soil (Müller et al., 2004) (N_{lab} = labile soil organic N, N_{rec} = recalcitrant soil organic N, NH_4^+ = ammonium, NO_3^- = nitrate, $NH_{4\ ads}^+$ = adsorbed NH_4^+, $NO_{3\ sto}^-$ = stored NO_3^-; for explanation of N transformations and parameters see Table 2).

Table 1
Value of the misfit function of six optimization runs (run 2 corresponds to the set up of the original model described by Müller et al., 2004)

Optimization run	Parameter settings	$f(\mathbf{m})$ value
1	All parameters	882.23
2	$k_{RNO3s} = 0$	858.90
3	$k_{INO3} = 0$	859.92
4	$k_{RNO3s} = k_{INO3} = 0$	858.17
5	Same as 2 but k_{INO3} set to first-order kinetics	865.05
6	Same as 2 but O_{NH4} set to Michaelis–Menten kinetics	693.78

2.6. Michaelis–Menten kinetics

A disadvantage of ^{15}N-tracing techniques that use LM-type optimization algorithms is the limitation on parameter numbers, precluding the use of Michaelis–Menten kinetics which would increase the number of parameters for optimization (Myrold and Tiedje, 1986; Müller et al., 2005). However, transformations such as NH_4^+ oxidation are likely to follow Michaelis–Menten kinetics rather than zero- or first-order kinetics (Tate, 1995). The activity of nitrifying microorganisms is crucially dependent on the availability of NH_4^+, which undergoes a rapid change during the experiment from non-NH_4^+ limiting conditions (zero-order kinetics) to NH_4^+-limiting conditions (first-order kinetics). Michaelis–Menten kinetics describes such a smooth transition from zero- to first-order kinetics (Müller, 2000). The Michaelis–Menten notation used to describe NH_4^+ oxidation is

$$O_{\mathrm{NH_4}} = \frac{V_{mNH4}\ NH4^+}{K_{mNH4} + NH4^+}, \tag{12}$$

with parameters V_{mNH4} and K_{mNH4} that have to be optimized.

2.7. Description of the data set and the model

The data set and the model used in this study are described in detail by Müller et al. (2004) and only a summary is given here. A laboratory study was carried out with soil from an old grassland soil near Giessen/Germany. Nitrogen in form of NH_4NO_3 was applied at a rate of 100 μg N g^{-1} and either NH_4^+, NO_3^- or both moieties were labelled with ^{15}N at 60 at% excess. Soil extractions were carried out 3, 23, 46, 118, 190 and 286 h after fertilizer application and extracts were analysed for concentrations of NH_4^+ and NO_3^- and their respective ^{15}N contents by methods based on their conversion to N_2O (Stevens and Laughlin, 1994; Laughlin et al., 1997).

In the original analysis by Müller et al. (2004) first a basic conceptual model was used to analyse the data. However, to obtain a more realistic representation of the data the model had to be modified. The final model contains 6 N pools and 9 N transformation rates either at zero- or at first-order kinetics (Fig. 3). At this point we want to stress that we do not see ModelB as the ultimate model that can be used to quantify gross N transformations in all soils (Müller et al., 2004). In other soils, N transformations, which were not included in ModelB, such as adsorption of NH_4^+ on exchange sites, immobilization of NH_4^+ into N_{rec} or immobilization of NO_3^- into N_{lab}, may have to be considered. The same applies for the choice of the kinetics of the various N transformations. It is one of the main points of this paper to show that the value of the misfit function, $f(\mathbf{m})$, will ultimately indicate whether a certain model structure or kinetic choice is adequate to describe the data set. Optimizations by Müller et al. (2004) were performed with the LM-method in two steps by

considering first the transformations influencing the NH_4^+ and then those affecting the NO_3^- pool. To prevent the algorithm entering a local minimum, great care was taken to define an appropriate set of initial parameters by a series of sensitivity analyses.

3. Results

3.1. MC sampling

To test the MC sampling technique a series of optimization runs was carried out (Table 1). Initially, all model parameters were included in the optimization (run 1). In this run all parameters except k_{RNO3s} and k_{INO3} showed a satisfactory sampling (see Table 2 for explanation and kinetics of parameters). For parameters k_{RNO3s} and k_{INO3} the sampling indicated that values were close to zero. Therefore, in optimization runs 2–4 either k_{RNO3s} or k_{INO3} or both parameters were set to zero and the optimization was repeated with all remaining parameters. The values of $f(\mathbf{m})$ of optimization runs 2–4 were more or less identical but considerably lower than in optimization run 1 (Table 1). Setting the kinetics of k_{INO3} to first-order kinetics was tested in optimization run 5 but did not improve the misfit between modelled and observed data (Table 1). Therefore, it was decided to present optimization results from optimization run 2. This run also corresponded exactly to the set up of the model analysis by Müller et al. (2004). As a example, results of the MC sampling are presented for the parameter k_{MNrec} for a run with 5 sequences (Fig. 4a) and 3 sequences (Fig. 4b). In both runs the sampling continued for 50,000 iterations. Irrespective of the number of sequences, k_{MNrec} approached the same value in both runs. The reduction factor of k_{MNrec} had reached the threshold value of 1.2 after approximately 2500 iterations and declined with further iterations to values close to 1. After 2500 iterations k_{MNrec} did not noticeably change (Fig. 4) confirming that a threshold value of $\hat{R}<1.2$ is a good indicator for sufficient sampling. Furthermore, the analysis showed that in our case a run with 3 sequences was enough to safely characterise the PDF.

The sampling of the 8 parameters optimized in run 2 (Table 1) is presented in Fig. 5 for one sequence. All parameters converged to a steady level after approximately 5000 iterations (Fig. 5). The corresponding counts per 'bin' and the probit analysis of the accepted samplings after the 'burn-in-time' shows that all parameters except k_{INO3} are characterised by PDFs with a well-defined normal distribution (Fig. 6). For k_{INO3} only half of a Gaussian distribution just above zero is shown that indicates that the parameter value is close to zero. Setting k_{INO3} to zero in run 4 (Table 1) resulted in an equally good minimum of misfit, indicating that immobilization of NO_3^- had only a negligible contribution to N transformations in this experiment.

3.2. Parameter values and comparison with results from Müller et al. (2004)

Based on the sampling, average parameter values ± standard deviations were computed. Table 2 presents results from the optimization with the MCMC method and the LM algorithm (Müller et al., 2004). All values obtained with the LM algorithm are outside the 95% confidence interval of the parameter values obtained with the MCMC method. However, in general the average values obtained with the two methods are in the same range except k_{INO3} with clearly different values (Table 2). The advantage of using the MCMC method is obvious because it does not require a step-wise analysis or an extensive preliminary sensitivity analysis. The satisfactory fit of the model is indicated by the close agreement with the experimental data (Fig. 7).

Table 2
Description of parameters and values optimized (avg ± sd) with the Markov chain Monte Carlo method (MCMC) for optimization run 2 (see Table 1) and the Levenberg–Marquardt method (LM) (see Müller et al., 2004)

Parameter	Description	Kinetics [units][a]	MCMC[b]	LM[c]
k_{MNrec}	Mineralization of N_{rec}	0 ($\mu mol\,N g^{-1} h^{-1}$)	0.00937 (0.00016)	0.01073
k_{MNlab}	Mineralization of N_{lab}	1 (h^{-1})	0.00314 (0.00011)	0.0022
k_{IND4}	Immobilization of NH_4^+	0 ($\mu mol\,N g^{-1} h^{-1}$)	0.02370 (0.00057)	0.02001
k_{INO3}	Immobilization of NO_3^-	0 ($\mu mol\,N g^{-1} h^{-1}$)	0.00010 (0.00007)	0.00133
k_{ONH4}	Oxidation of NH_4^+ to NO_3^-	1 (h^{-1})	0.03723 (0.00033)	0.04156
k_{ONrec}	Oxidation of N_{rec} to NO_3^-	0 ($\mu mol\,N g^{-1} h^{-1}$)	0.00308 (0.00007)	0.00428
$k_{RNH4\alpha}$	Release of adsorbed NH_4^+	1 (h^{-1})	0.00899 (0.00020)	0.00770
k_{RNO3s}	Release of stored NO_3^-	1 (h^{-1})	0	0
k_{DNO3}	Dissimilatory reduction of NO_3^- to NH_4^+	0 ($\mu mol\,N g^{-1} h^{-1}$)	0.00027 (0.00001)	0.00021

[a] 0 = zero-order kinetics, 1 = first-order kinetics.
[b] Results of the optimization with the Markov chain Monte Carlo method (MCMC) presented in this paper, average ± standard deviation (avg + sd) in brackets.
[c] Results of the optimization with the Levenberg-Marquardt method (LM) (Müller et al., 2004), average values. Standard deviations were in principle estimated by the LM-method but were not presented and therefore also omitted in the current paper. In general the errors calculated with the LM-method were much higher than the errors calculated by the MCMC-method.

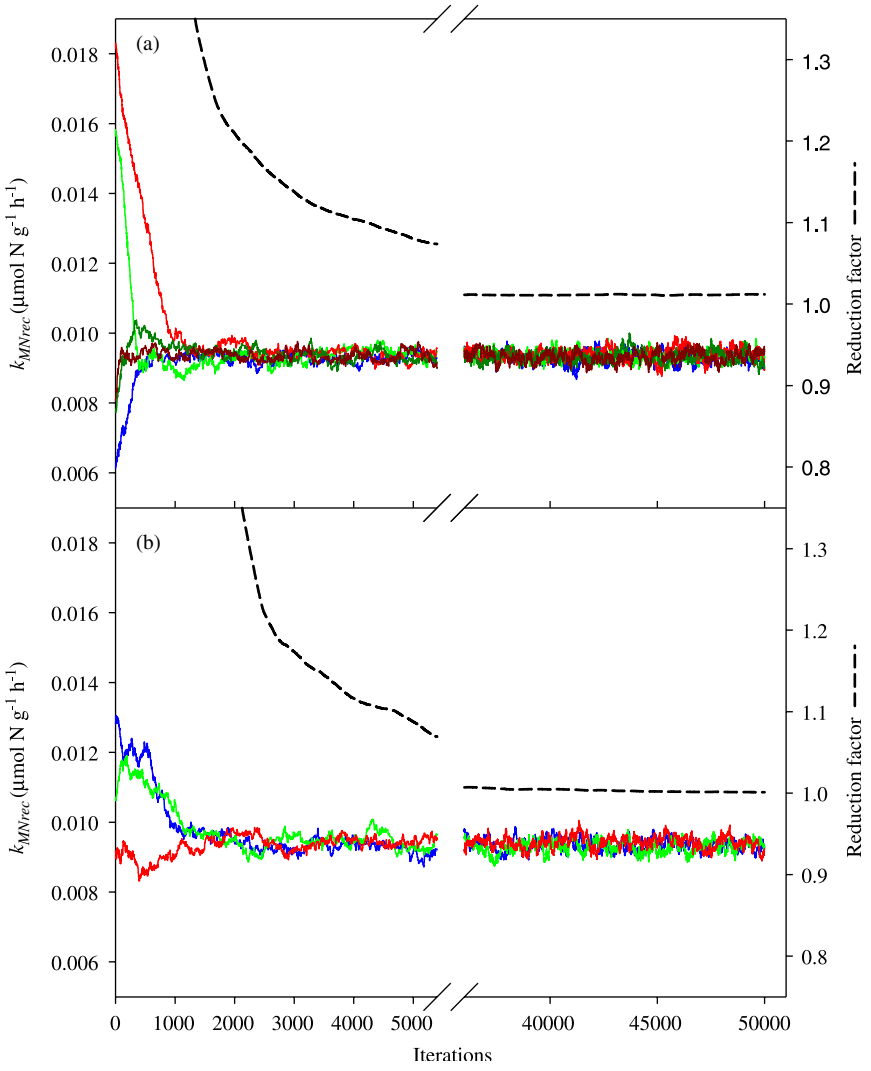

Fig. 4. Example of an optimization run for parameter k_{MNrec} with five sequences (a) and three sequences (b) together with the reduction factor.

The correlation matrix for all parameters optimized in run 2 is presented in Table 3. Correlation values (r) above 0.8 indicate pairs or groups of parameters that tend to be strongly constrained together (Quinn and Keough, 2002). In the analysis presented here only parameters related to the turnover of NH_4^+, namely k_{MNlab}, k_{INH4} and $k_{RNH\alpha}$ showed such a tendency. A weak negative correlation was observed between k_{MNrec} and k_{ONrec} (Table 3).

3.3. Michaelis–Menten kinetics

In optimization run 6 parameter settings were equal to run 2 except that O_{NH4} was set to Michaelis–Menten kinetics (Table 1). The PDFs of Michaelis–Menten parameters K_{mNH4} and V_{mNH4} were both ormally distributed with values of 2.284 ($\pm$0.173) and 0.101($\pm$0.006), respectively and a correlation coefficient of $r > 0.9$ (Fig. 8). Compared to optimization run 2 the $f(\mathbf{m})$ value in run 6 was reduced by 19% (Table 1), thus, indicating a much closer fit of the model to the data when O_{NH4} was described by Michaelis–Menten kinetics.

4. Discussion

4.1. MC sampling

Models to analyse data obtained from ^{15}N-tracing experiments to quantify gross N transformation rates have increased in complexity over the last 50 years with increasing number of pools and transformation rates. Estimation of rate parameters in current models cannot be obtained by analytical solutions anymore but rely totally on optimization procedures. Standard optimization algorithms have difficulties to simultaneously identify the optimum parameter sets in models with high number of transformations (Aster et al., 2005; Tarantola, 2005). Robust optimization techniques that can unambiguously determine parameters of models with large number of parameters are often based on MCMC methods but have

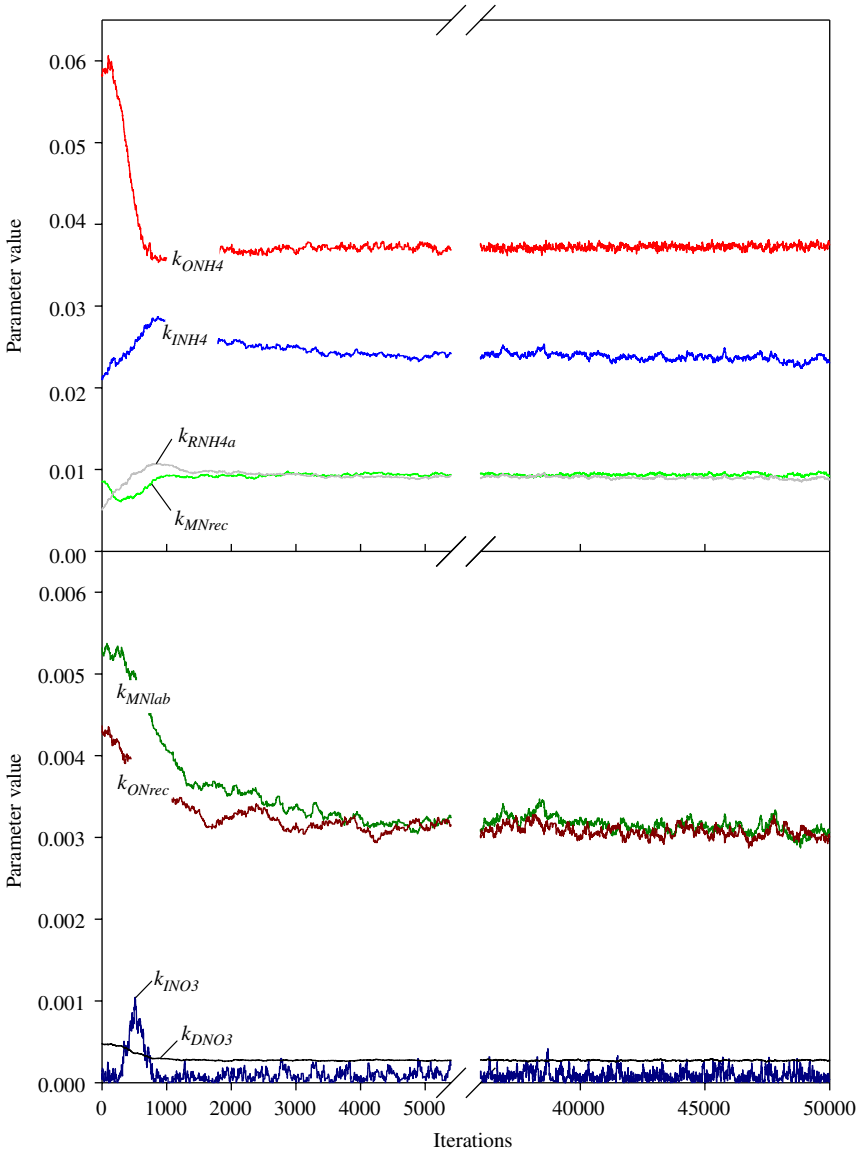

Fig. 5. Values of all model parameters during 50,000 optimization steps of one sequence during optimization run 2 (k_{RNO3s} was set to zero).

never been used for estimation of parameters in ^{15}N-tracing models.

Here we demonstrate for the first time that a MCMC method is able to simultaneously define the PDFs of more than 8 parameters in a tracing model within a single optimization run (Figs. 4 and 5). MC methods base their determination of PDFs on large number of iterations. Monitoring the parameter convergence according to Gelman et al. (2003) helps to restrict the number of iterations and provides us with additional assurance that the MCMC algorithm was reporting PDFs of parameters in the vicinity of the global minimum with respect to the misfit. Tests showed that in our case runs with 3 sequences, where each starts with a randomly different starting point, were sufficient to monitor parameter convergence (Figs. 4 and 5).

The MCMC method has the potential to simultaneously handle up to 50 parameters (Gelman et al., 2003). Therefore, the restrictions on number of parameters that can be observed when using LM-type methods do not apply here. The advantage of the MCMC method is that it is able to simultaneously sample all parameters that are assumed to be variable, therefore, preventing an arbitrary selection of parameters. Parameters with little impact on model results will only be poorly constrained by the optimization. Therefore, the MCMC method can detect processes that are unimportant with respect to the data. In the analysis presented here, this was the case for parameters k_{RNO3s} and k_{INO3} that could have been set to zero and excluded from the optimization without deterioration of $f(\mathbf{m})$ (Table 1, Fig. 5). On the other hand PDFs with well-constrained uncertainties indicate that those N transformations are needed to reduce the misfit between model and data. In case of parameter k_{INO3} another important issue can be highlighted. The PDF of this parameter in run 2 is not normally distributed because a

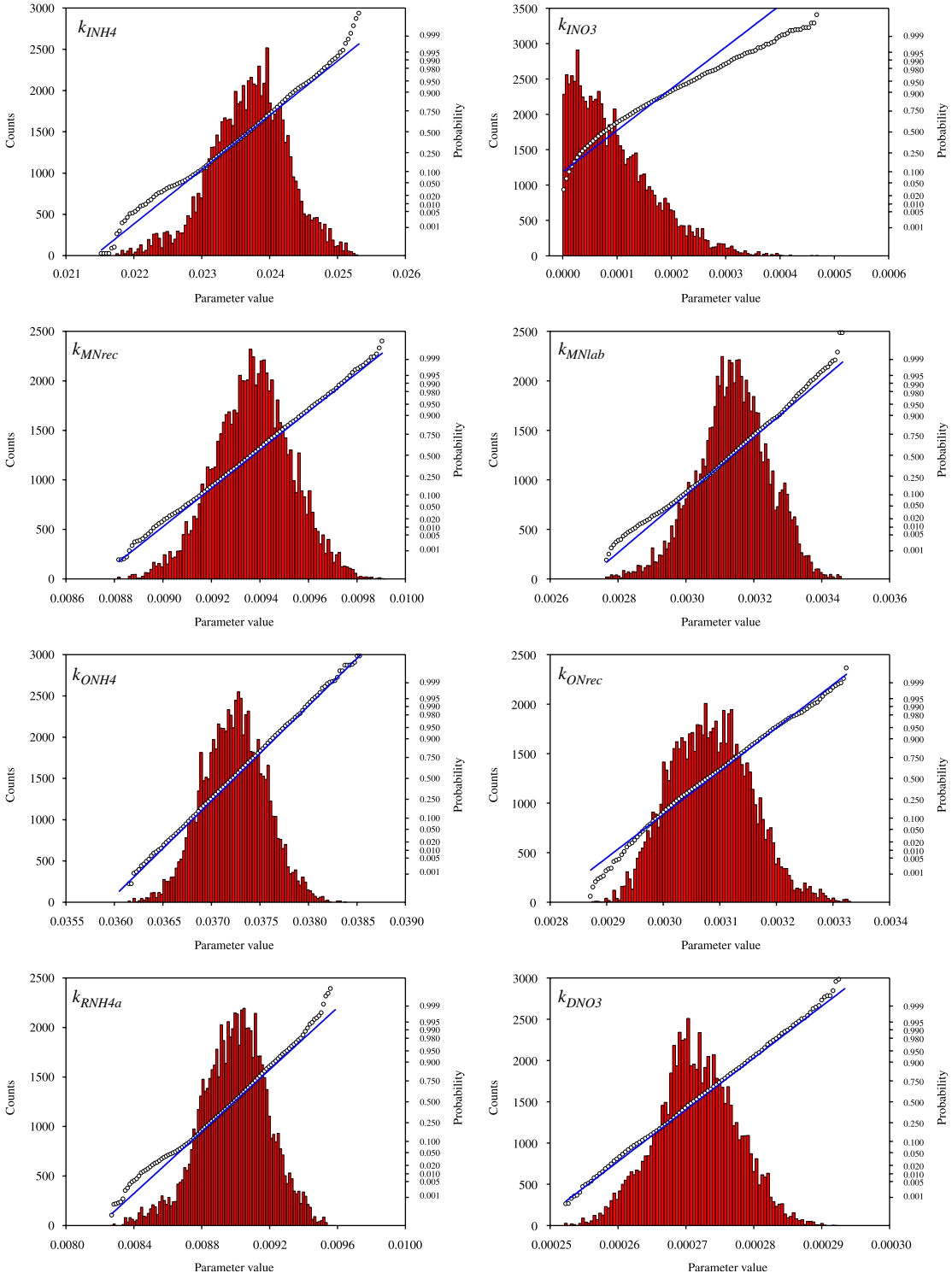

Fig. 6. Probability density plots of all parameters shown in Fig. 5. The probit plots and probit regression lines are superimposed (see text for details).

part of the distribution is truncated on the left side (Fig. 6). Assuming that k_{INO3} is characterised by a full normal distribution we would expect a mean value for k_{INO3} that is lower than 0.0001 (Table 2). We confirmed this by applying the method of Cicchinelli (1965). The calculation resulted in a mean value (standard deviation) of −0.00002 (±0.00012). Since a value below zero is unrealistic, the best estimate for the parameter value for k_{INO3} is considered to be zero.

A recent critique on our original model analysis questioned the importance of parameters related to NH_4^+ release from exchange sites (k_{RNH4a}), oxidation of organic N to NO_3^- (k_{ONrec}) and DNRA (k_{DNO3}) (Luxhøi et al., 2005). Here we show that those parameters have well

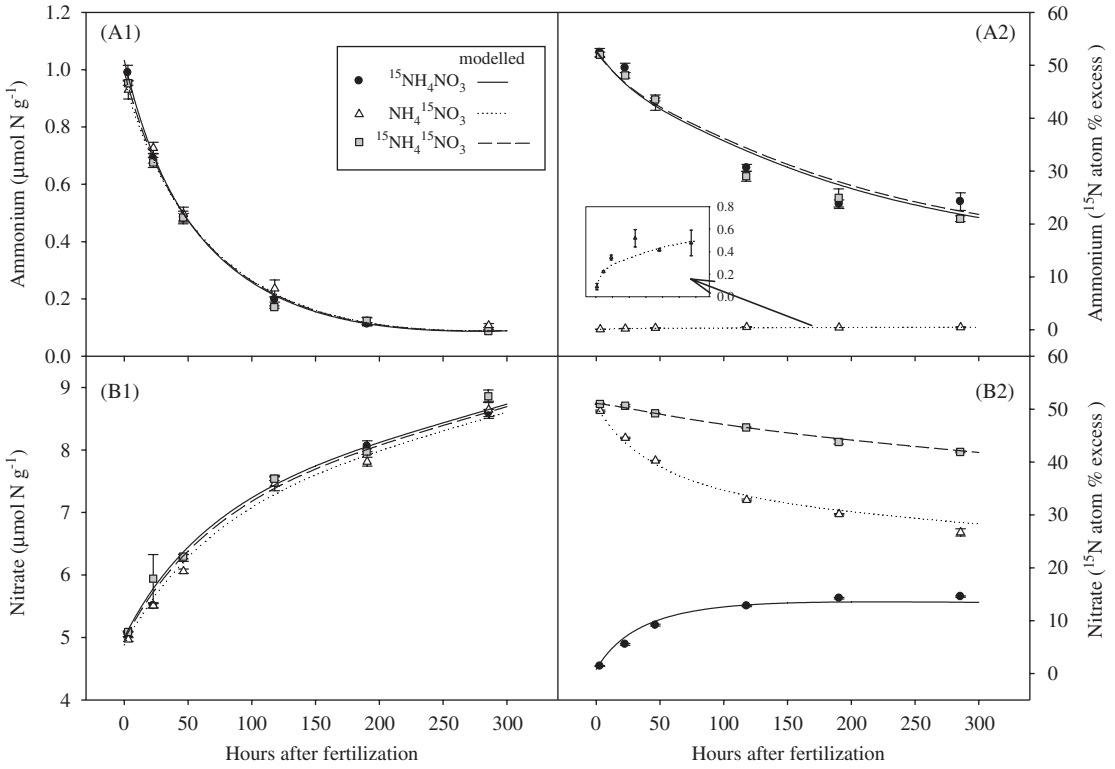

Fig. 7. Measured and modelled concentrations and ^{15}N enrichments of ammonium (NH_4^+) and nitrate (NO_3^-) of an old grassland soil after application of 100 µg N g^{-1} as NH_4NO_3 at 60 at% ^{15}N excess (optimization run 2, Table 2).

Table 3
Correlation matrix for all parameter of optimization run 2 (see Table 1, note, k_{NO3s} was not included in the optimization and is therefore not shown)

	k_{MNlab}	k_{INH4}	k_{INO3}	k_{ONH4}	k_{ONrec}	k_{RNH4a}	k_{DNO3}
k_{MNrec}	−0.030	0.462	−0.129	−0.018	−0.433	0.416	0.370
k_{MNlab}		0.862	−0.048	−0.325	0.382	0.855	0.173
k_{INH4}			−0.132	−0.377	0.166	0.957	0.351
k_{INO3}				0.103	0.179	−0.255	−0.219
k_{ONH4}					−0.346	−0.361	−0.117
k_{ONrec}						−0.012	−0.092
k_{RNH4a}							0.345

defined PDFs (Fig. 6). Parameters that are non-zero with well-defined PDFs contribute to the improvement of the model fit to the data and cannot be neglected. The two optimization methods (LM, MCMC) that are used to analyse the data set came up with similar parameter values except for k_{INO3} (Table 2), but only the MCMC method unambiguously determined the parameters by simultaneous optimization in one run which indicates the superiority of this method for parameter optimization.

MCMC methods have been successfully used for determination of large number of parameters in ecosystem models (Braswell et al., 2005; Knorr and Kattge, 2005; Sacks et al., 2006; Xu et al., 2006). Therefore the MCMC method can be of enormous advantage for the optimization of parameters in complex N-tracing models that include N species (Stange and Döhling, 2005; Müller et al., 2006) that are not considered here. Subsequently to the analysis presented here the method was successfully used for the estimation of parameters using the model by Müller et al. (2006) that considers the dynamics of nitrite (data not shown).

4.2. Michaelis–Menten kinetics

The most important progress for ^{15}N-tracing models is related to the ability of MCMC to determine PDFs in high-dimensional parameter space. This allows the implementation of Michaelis–Menten kinetics for calculating N transformation rates. Already 20 years ago Myrold and Tiedje (1986) pointed out, that Michaelis–Menten kinetics would conceptually be the best kinetics to use. In particular, N transformations where the product pool undergoes a rapid change during the experimental period may be best described by Michaelis–Menten kinetics. In our study, we tested the feasibility of using Michaelis–Menten kinetics for the oxidation of NH_4^+ (O_{NH4}) and for the first time we were able to simultaneously determine the PDFs of all parameters by MCMC sampling (Fig. 8). This resulted in a considerably improved misfit (Table 1), most likely due to the more realistic representation of NH_4^+ oxidation dynamics in this soil. While first-order kinetics is linearly related to the NH_4^+ concentration, Michaelis–Menten kinetics described a non-linear response in the relevant NH_4^+ concentration range (Fig. 9). From studies

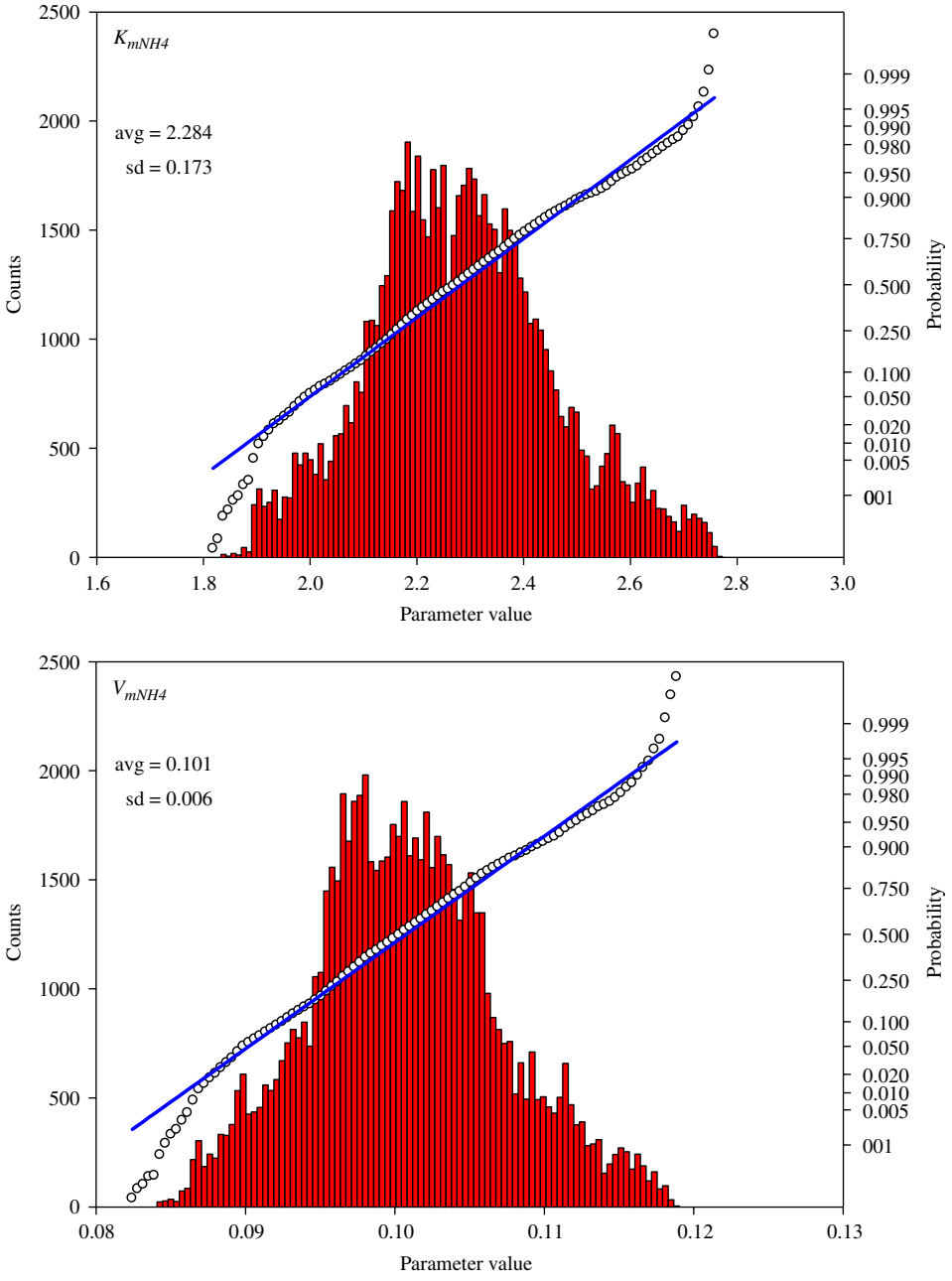

Fig. 8. Probability density plot for the Michaelis–Menten parameters related to NH_4^+ oxidation. The number in the graph refer to the average (avg) and standard deviation (sd).

with pure cultures of autotrophic organisms (e.g. genus *Nitrosomonas* or *Nitrospira*) it is known that O_{NH4} follows Michaelis–Menten kinetics (Taylor and Bottomley, 2006). Here we present strong evidence that Michaelis–Menten kinetics is also the best kinetics to use for describing NH_4^+ oxidation in ^{15}N-tracing studies. Michaelis–Menten parameters obtained with the MCMC technique should be referred to as apparent Michaelis–Menten parameter because they are likely a conglomeration of kinetic parameters from various autotrophic and heterotrophic NH_4^+ oxidizing microorganisms (Focht and Verstraete, 1977).

5. Conclusions

To obtain a realistic understanding of the internal N cycle in soil, methods based on ^{15}N tracing in combination with a model analysis are most promising. However, the development of realistic models that contain known N pools and N transformations is severely restricted by current optimization techniques that cannot cope with large number of parameters. Here we present a robust optimization method based on MC sampling that is able to simultaneously determine high number of parameters in a ^{15}N-tracing model. The MCMC method allows us to focus

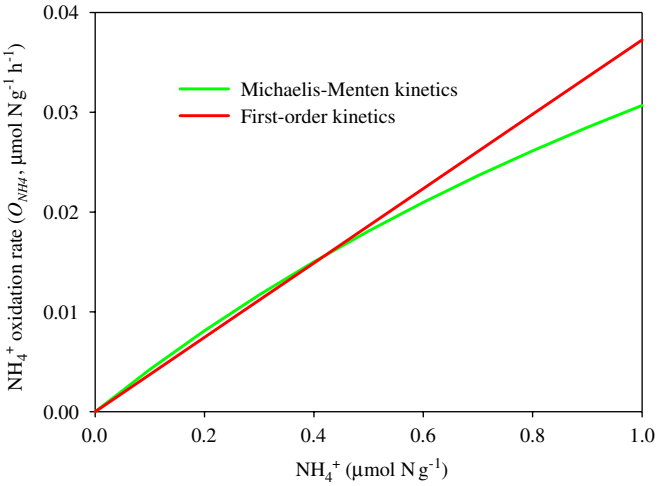

Fig. 9. NH_4^+ oxidation calculated with ModelB for the data presented in Müller et al. (2004) by using first-order kinetics or Michaelis–Menten kinetics.

on realistic model development rather than be restricted by the requirements of the optimization algorithm. As shown in this study, this method has a high potential for the development and evaluation of ^{15}N-tracing models with high-dimensional parameter space (e.g. consideration of Michaelis–Menten kinetics and further N species such as nitrite and gaseous N).

People interested to use the ^{15}N-tracing algorithm described in this paper should contact the corresponding author.

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