UNIVERSITÀ DEGLI STUDI DI MILANO

SCUOLA DI DOTTORATO
TERRA, AMBIENTE E BIODIVERSITÀ

Ph.D. in Agricultural Ecology

XXIII Cycle

Carbon and nitrogen dynamics in soil after repeated manure applications: measurements and simulations

Ph.D. Thesis

Daniele Cavalli
N° R07597

Supervisor	**Academic Year**	*Coordinator*
Prof. Marco Acutis	**2009-2010**	**Prof. Graziano Zocchi**

Daniele Cavalli

Carbon and nitrogen dynamics in soil after repeated manure applications: measurements and simulations

Ph. D. Thesis
Department of Plant Production
University of Milan
Via Celoria 2, 20133 Milan – Italy
daniele.cavalli@unimi.it

Titolo in Italiano: "Dinamiche di mineralizzazione del carbonio e dell'azoto in seguito a ripetute applicazioni di liquame: misure sperimentali e simulazioni modellistiche."

Tesi di Dottorato in Ecologia Agraria

XXIII Ciclo, Anno Accademico 2009-2010

UNIVERSITÀ DEGLI STUDI DI MILANO

Ph.D. in Agricultural Ecology - XXIII Cycle

Daniele Cavalli

Cavalli, D., 2010. Carbon and nitrogen dynamics in soil after repeated manure applications: measurements and simulations

The first part of this Ph. D. Thesis (Chapters 1 and 2) is relative to the calibration of a soil organic matter simulation model (the CN-SIM model) by applying it to an experimental data-set, using two different methodologies, in order to verify its conceptual structure and obtaining parameters to describe the partitioning of C and N within the manures. Based on the optimization results, it emerged that the low model performance could be attributed to the lack of some processes involving mineral nitrogen transformations into the soil. In order to better understand the nitrogen dynamics following manure application to the soil, an incubation experiment was build up. In the experiment, two different manures were applied to two soils of different texture, and, in order to estimate the contribution of manure recalcitrant fractions to the mineralization dynamics of C and N in the soil (residual effect of added manure carbon and nitrogen), each manure was

applied four times on the same soil (Chapter 3). In the incubation experiment were included measurements of non-exchangeable ammonium, with the aim of better understand if the lower recoveries of added manure ammonium, often found on the clay soils, were due to microbial biomass immobilization or ammonium clay-fixation. Measurements obtained in the incubation experiment were the used to perform a preliminary modification of the CN-SIM model to take into account the ammonium fixation and release processes (Charter 4).

Credits evaluation

Courses:

- Elements of statistics.
- Instrumental analysis.
- Biogeochemical cycles.

Other courses:

- Programming course: “Introduzione alla programmazione procedurale in C++”, Cilea, Segrate, Milano, Italy. 10-12 November 2008.
- Programming course: “Introduzione alla programmazione orientata agli oggetti in C++”, Cilea, Segrate, Milano, Italy. 10-12 November 2008.

Summer school:

- Fifth summer school on Sensitivity Analysis, University of Venezia, San Servolo, Venezia, Italy. 9-12 September 2008.

Attendance at international/national congress:

- Anaerobic digestion: opportunities for agriculture and environment, 24-25 January 2008, Sesto San Giovanni, Milano, Italy.
- X Congress of the European Society for Agronomy, Bologna, Italy, 15-19 September 2008.
- 16th Nitrogen Workshop: Connecting different scales of nitrogen use in agriculture. June 28th – July 1st 2009, Turin, Italy.
- 38th Italian Society of Agronomy Congress, 21-23 September 2009 Firenze, Italy.
- 14th Ramiran Conference, Lisboa, Portugal, 13-15 September 2010.

Attendance at seminars:

- Seminar: "Basic and advanced geostatistics" (8,5 hours) hold by Prof. Alfred Stein at University of Milano – Faculty of Agricultural Science. 12-13-15 May 2008.

Poster presentation at international/national congress:

- Bechini, L., Cavalli, D., Marino, P., 2008. Can a unique model simulate C and N dynamics of different liquid dairy manures? In: Rossi Pisa, P. (Ed.), Multifunctional agriculture. Agriculture as a Resource for Energy and Environmental Preservation, 10th Congress of the European Society for Agronomy, Bologna, Italy, 15–19 September 2008. Italian Journal of Agronomy/Rivista di Agronomia, Vol. 3, No. 3 Supplemento pp. 849–850.
- Cavalli, D., Marino, P., Bechini, L., 2009. Sensitivity analysis and multi-objective calibration of a manure mineralization model. Pages 511-512 in: Proceedings of the 16th Nitrogen Workshop - Connecting Different scales of Nitrogen use in Agriculture. Facoltà di Agraria, Università degli Studi di Torino, Turin (Italy).

- Cavalli, D., Invernizzi, A., Ponzini, R., Bechini, L., 2009. Modelli di simulazione della sostanza organica nel suolo: una libreria C++ orientata agli oggetti implementata in ambiente High Performance Computing. In Atti del XXXVIII Convegno della Società Italiana di Agronomia (M. Bindi Ed.). Società Italiana di Agronomia - Università degli Studi di Firenze, Firenze (Italia), pp. 311-312.
- Cavalli, D., Marino, P., Bechini, L., 2009. Analisi della sensibilità e calibrazione multi-obiettivo di un modello per la mineralizzazione di reflui zootecnici. In Atti del XXXVIII Convegno della Società Italiana di Agronomia (M. Bindi Ed.). Società Italiana di Agronomia - Università degli Studi di Firenze, Firenze (Italia), pp. 309-310.
- Cavalli, D., Marino, P., Occhi S., Bechini, L., 2010. Carbon and nitrogen dynamics in soil under repeated manure manure applications: preliminary results. Proceedings of the 14th Ramiran Conference, Lisboa, Portugal, from the 13-15 September 2010.
- Manfredini A., Negri M., Cavalli, D., Bechini, L., Marino, P. 2010. Carbon and nitrogen mineralization of raw and separated, digested animal manures. Proceedings of the 14th Ramiran Conference, Lisboa, Portugal, from the 13-15 September 2010.

Internships:

- Internship at the Institute for Plant Nutrition (Justus Liebig University, Giessen, Germany) under the guide of Prof. D.Steffens. From April 12th to June 3th 2010.

UNIVERSITÀ DEGLI STUDI DI MILANO

Supervisor:
Prof. Marco ACUTIS

Co-Supervisors:
Dr. Luca BECHINI
Dr. Pietro MARINO

Coordinator:
Prof. Graziano ZOCCHI

Acknowledgements

I wish to thank my supervisior Prof. Marco Acutis, Dr. Luca Bechini and Dr. Pietro Marino for valuable professional assistance during all the development of the Ph.D. Thesis.

I' am deeply grateful to the following people who helped me:

Dr. Marco Negri, Dr. Stefano Occhi, Gaia Brenna, Daniele Baronchelli, Dr. Roberto Fuccella, Dr. Andrea Manfredini, Dr. Francesco Savi, Dr. Nicola Castoldi, Dr. Mattia Fumagalli, Dr. Roberto Gonfalonieri, Prof. Matteo Provetto, Prof. Tommaso Maggiore, Dr. Liliana Krotz, Dr. Guido Giazzi, Dr. Raffaele Ponzini, Dr. Alice Invernizzi. Prof. S. Shubert, Dr. D. Steffens and Mr. R. Pfanshilling, Dr. Maino Matteo, Prof. Massimo Setti, Dr. Rizzi, Ditto Domenico.

I'm also deeply grateful to Ida, my parents and to my sister.

CONTENTS

GENERAL INTRODUCTION

The region Lombardia (northern Italy) has, particularly in the plain, an intensive agricultural production. Livestock breeding is characterised by raising large quantities of animals using a relatively small area of land to produce the feed. This makes manure management complicated and potentially dangerous for the environment, due to a disproportion between the nutrients available in the manure and those taken up by crops. To maintain water quality, to reduce CO_2 emissions and the use of mineral fertilisers, animal manure has to be managed properly (Grignani et al., 2003).

To estimate the nutrient fertiliser value of animal manure, one could use the concentration of ammonium as a first estimate. However, the dynamics of the organic fraction of the manure is extremely important as well. Many authors have shown that part of manure ammonium, together with that produced by rapid mineralisation of easily degradable organic materials with low C/N (e.g. aminoacids and urea), is immobilised by soil microbial biomass immediately after manure application to soil (about in the first two-three weeks). This happens mostly due to the rapid mineralisation of compounds poor in N (e.g. volatile fatty acids, that can represent up to 30% of total C of stored slurries; Sørensen, 1998a; Sørensen, 1998b). Microbial N immobilisation continues during degradation of more recalcitrant compounds contained in faeces (undigested feed). Moreover, microbial immobilisation of N is also due to the decomposition of native organic materials, as the residues of microbial cell walls that, if mineral N is available in the medium, are rapidly decomposed (Sørensen and Amato, 2002). The decrease of soil mineral N concentration that happens during the incubation of soil + manure mixtures can also be due to the fixation of ammonium in the interlayers of clay minerals (e.g. Sørensen, 1998b) and to denitrification, which occurs also in soils far from saturation (e.g. Calderón et al., 2004).

Following initial immobilisation, N is remineralised due to the death of microbial biomass and degradation of cellular residues of bacteria and fungi. The turnover time of microbial biomass and its residues depends on the type and concentration of clay minerals in the soil, on the size and distribution of soil pores, and on soil water content and temperature. Higher clay content, together with a granular soil structure (characterised by the presence of macro and micropores), determines a higher protection of living soil microbial biomass from the attack of predators (protozoa and nematodes). Moreover, clay reduces the accessibility of microbial residues to living microorganisms. This generates a lower decomposition rate and therefore a lower release of CO_2. The decomposition rate of residues is also influenced by their chemical and physical complexity, and therefore varies according to the type of microorganism originating them (Six et al., 2006).

When ^{15}N is used to label manure N and to follow its fate after soil incorporation (Sørensen, 2004; Powell et al., 2005; Muñoz et al., 2003), half of applied N with the manure remains in soil (mostly in organic form) 2.5 years after application. Therefore it is possible to make the hypothesis that the forms of immobilised N are rather stable (at least part of them).

The amount of C lost as CO_2 during organic matter decomposition depends on the substrate use efficiency (Microbial Growth Efficiency, MGE) by microbial biomass. MGE represents the substrate C assimilated by microorganisms, expressed as a fraction of total decomposed C. The values of MGE reported in the literature depend on the substrate, climatic conditions, type of microorganism, method used to measure it, and on the availability of other nutrients. They vary between 1 and 85% (Six et al., 2006; Thiet et al., 2006).

Therefore, the complexity of the interactions among manure components (organic and inorganic) and soil components (microbial biomass, native organic matter and mineral fraction) makes it difficult to estimate the nutrient fertilising value of animal manure and the fate of manure N, both in the short and the long term. This issue is further complicated in common farming practice by the fact that manures are repeatedly applied to soil, year after year. To properly manage manure N, therefore, it is important to understand and quantify the residual effect. The residual effect consists in higher crop N availability in soils that were manured in previous years, compared to historically unmanured soils. Soils that receive manure regularly accumulate organic N (due to microbial immobilization and to recalcitrance of added N: Sørensen and Amato, 2002). Subsequent mineralisation of accumulated N contributes to the residual effect. The residual effect is traditionally estimated using tabbed values of apparent N recovery, which depend on frequency, date and method of application and on manure type. However, the apparent N recovery is an empirical representation of many complex processes: storage of N in (and release of N from) microbial biomass, recalcitrant organic fractions and clay minerals. To our knowledge, the effects of repeated manure applications on soil N pools are seldom reported in the literature (Monaco et al., 2008). A mechanistic understanding of these dynamics would be extremely useful for a better formulation of nutrient management plans and for the interpretation of field experiments. Residual effect is traditionally determined based on crop N uptake measured under field conditions (Schröder et al., 2005). Laboratory incubations of a mixture of soil and manure, without a crop and under constant conditions of soil temperature and water content, are frequently adopted to isolate the effect of the added N and to estimate net N immobilization.

Simulation models are increasingly used to describe the dynamics of soil organic matter (Causarano et al., 2007; Manzoni and Porporato, 2009). The evaluation of their performance allows understanding if the representation of the real system that they provide is adequate or not. Therefore, they represent a scientifically sound tool to integrate and improve scientific knowledge. CN-SIM (Petersen et al., 2005a and 2005b) is a SOM simulation model developed to properly simulate both short- and long-term C and N dynamics. It was successfully applied at many different conditions, and demonstrated to be adequate for the simulation of organic matter dynamics in a wide range of field experiments (Petersen et al., 2005a) and incubation experiments (Petersen et al., 2005b). However, liquid dairy manures sometimes were not simulated properly. In particular, there was a frequent overestimation of soil mineral N (SMN) concentration (Petersen et al., 2005b, experiment F). Moreover, the liquid dairy manures tested by Petersen et al. (2005b) consisted only of one fresh and one stored manure, incubated with or without straw on two different soils.

This PhD work draws upon an incubation experiment ("DINA.MI.RE.") carried out at the Department of Plant Production of the University of Milano. The incubation (Bechini and Marino, 2009) included eighteen treatments, obtained as the combination of five liquid dairy manures (plus one unfertilised control) and three soils of increasing clay content. The five manures used in the experiment were selected from a set of 101 liquid dairy manures that had been collected from livestock farms located in Lombardia. The incubation was conducted by maintaining the experimental units (soil + manure mixture) at a soil matrix potential of -0.05 MPa in the dark at 25°C and at an air humidity near saturation. On Days 0, 2, 4, 8, 12, 16, 21, 29, 41, 72, 121, and 181 after the addition of manure, measurements of respired CO_2–C and soil nitrate and ammonium concentration were performed.

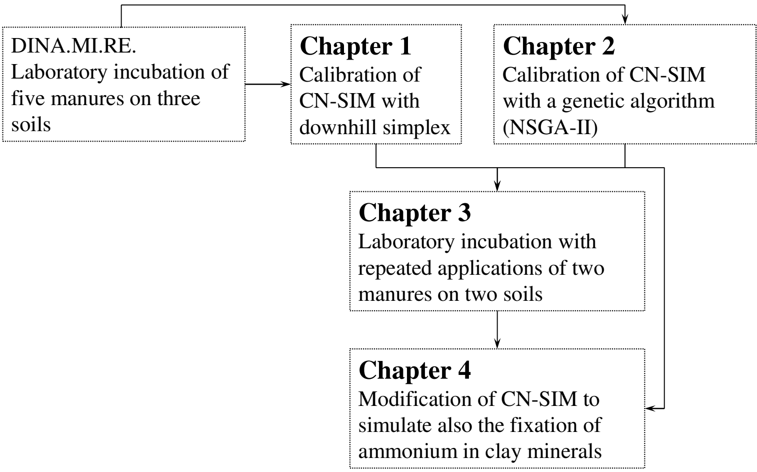
Objectives of the thesis and flow-chart

The objectives of this PhD work were therefore:

- To calibrate the CN-SIM model by applying it at the "DINA.MI.RE." experimental data set, by verifying its conceptual structure and obtaining parameters to describe the partitioning of C and N within the manures (Chapters 1 and 2).
- To carry out experimental measurements aimed at improving the understanding of manure N dynamics after manure addition to soil, in particular after repeated manure applications (Chapter 3).
- To improve the CN-SIM model to take into account the new experimental data (Chapter 4).

The flow-chart in Figure 1 describes the organisation of the thesis and the logical connection among the chapters.

Figure 1. Flow-chart of the work carried out during the PhD and organisation of the chapters of this thesis.



Chapter 1 describes the first calibration of the CN-SIM model using the experimental data set "DINA.MI.RE.". This calibration was treated as a single-objective optimisation problem, and the downhill simplex algorithm (Nelder and Mead, 1965) was used. In **Chapter 2** the calibration of CN-SIM with the same data set was carried out using NSGA-II, a multi-objective optimisation algorithm (Deb et al., 2002). Both model calibrations have shown that it is impossible to achieve a satisfactory simulation performance using the CN-SIM model, due to exaggerated simulated microbial immobilisation. We then made the hypothesis that adding the simulation of clay fixation to the

model could improve its performance. We therefore set up an appropriate incubation experiment to measure C respiration and N dynamics (including clay fixation of ammonium), under repeated manure applications, which is described in **Chapter 3**. Finally, **Chapter 4** reports the preliminary results of the application of a modified version of CN-SIM (which simulates clay fixation of ammonium) to the incubation experiment of Chapter 3.

CHAPTER 1 - Sensitivity analysis and calibration of CN-SIM to simulate the mineralisation of liquid dairy manures.

(Submitted to Soil Biology and Biochemistry on September, 2010. Accepted with minor revision on December 2010).

Introduction

The intensification of animal production systems has led to high livestock densities. This means that high amounts of nutrients are available per unit area of cultivated land. More than 6 million cows are raised in Italy (National Statistical Institute, 2008), and 67% of them are concentrated in the northern regions, which cover only 37% of the Italian agricultural area. Livestock operations have concentrated in fewer farms during the past decades, with 17 bovine heads per farm in 1982, 36 in 2000 (National Statistical Institute, 2000) and 42 in 2007 (National Statistical Institute, 2008). The careful management of manure N requires consideration of pedo-climatic conditions, cropping systems management and N fate in the soil and agroecosystem.

Animal manures are complex materials, made of widely different compounds. Manure organic matter comprises volatile fatty acids (VFA), cellulose, hemicellulose and lignin (Van Kessel et al., 2000). These compounds have different decomposabilities and C/N ratios (Van Kessel et al., 2000). Complex interactions develop among these materials when they decompose after manure incorporation in the soil. Some organic compounds are quickly decomposed, while others are attacked much more slowly. If easily degraded compounds have low N/C ratio (or zero in the case of VFA), inorganic N is immobilised from the soil solution (Probert et al., 2005; Sørensen, 1998a, 1998b and 2004;

Thomsen and Olesen, 2000) to allow microbial growth. Later, immobilised nitrogen is remineralised or remains stored in the soil (Sørensen, 2004).

Soil organic matter (SOM) models describe the carbon and nitrogen turnover of old organic matter and recently added organic materials to soil (Probert et al., 2005; Causarano et al., 2007; Manzoni and Porporato, 2009). Simulation models allow studying experimental results by comparing predictions with measurements, and therefore checking the validity of the theory embedded in the models, which describe system behaviour.

CN-SIM (Petersen et al., 2005a and 2005b) is a SOM simulation model developed to properly simulate both short- and long-term C and N dynamics. It was successfully applied at many different conditions, and demonstrated to be adequate for the simulation of organic matter dynamics in a wide range of field experiments (Petersen et al., 2005a) and incubation experiments (Petersen et al., 2005b). However, liquid dairy manures sometimes were not simulated properly. In particular, there was a frequent overestimation of soil mineral N (SMN) concentration (Petersen et al., 2005b, experiment F). Moreover, the liquid dairy manures tested by Petersen et al. (2005b) consisted only of one fresh and one stored manure, incubated with or without straw on two different soils.

Model optimisation is an essential step for model testing and development; its purpose is to achieve a good fit between simulated model outputs and corresponding experimental observations. For this purpose, optimisation algorithms are used to automatically explore different combinations of parameters, looking for the solutions that provide the best agreement between measured and simulated variables. Prior to model optimisation, it is important to explore the effects of model parameters on model outputs through sensitivity analysis (SA) (Saltelli et al., 2004). During SA, several parameter combinations are generated and, for each of them, a simulation is performed. Thereafter variations in model outputs are related to variation in model inputs, highlighting the contribution of each parameter and of parameters interactions on model output variation. Therefore SA helps in selecting the most important parameters that need to be varied during parameter optimisation. Examples of SA are not frequent in SOM models. Most published studies of SA use simplified local methods, which change one parameter at a time in a specific point of the input space, while keeping the others constant (e.g. Heinen, 2006). Petersen et al. (2005a and 2005b) have evaluated the effects on optimisation results by separately changing CN-SIM parameters of ± 10% and then re-running optimisation. However, they only tested the sensitivity of soil and microbial parameters, and did not include manure parameters. Moreover, this procedure did not rank parameters according to their sensitivity, did not explore their full range of variation and did not provide information on parameters interaction. These limitations are overcome by global SA methods (Chan et al., 1997; Saltelli, 2002; Saltelli et al., 2004). Global methods of SA were applied in agricultural models by Causarano et al. (2007), Confalonieri (2010) and Richter et al. (2010). To further explore parameter optimisation for the simulation of liquid dairy manures with CN-SIM, we have carried out global SA and model optimisation using a published data set from an incubation experiment (Bechini and Marino, 2009), with measurements of C and N dynamics of five liquid dairy manures (with more variable characteristics compared to those used by Petersen et al., 2005b) incorporated in three soils of increasing clay content.

The objectives of this paper are to report our results of the test of CN-SIM, and to identify the improvements of CN-SIM structure that can be proposed, based on the model performance with our data set.

Material and methods

Experimental data

The experimental data set consisted of measurements of respired CO_2–C and concentrations of NH_4–N and NO_3–N from three manured soils (see Bechini and Marino, 2009, for the details). The three soils (Table 1.1) differed in clay content, while they were similar for the other properties (pH, C content and C/N ratio). The manures had been selected from a larger data set to ensure variability in their chemical properties (Table 1.2). All soil × manure treatments plus three unamended treatments (one for each soil) were incubated in three replicates at constant temperature (25°C) and non-limiting soil water content (corresponding to a soil water potential of -0.05 MPa). Rates of CO_2–C respiration and soil concentrations of NH_4–N and NO_3–N were destructively measured 0.25, 1.50, 3.50, 7.50, 11.50, 20.50, 28.50, 41.50, 72.50, 121.50 and 181.50 d after manure addition to soil.

Table 1.1. Soils used in the incubation experiment.

Soil	pH (H_2O)	Organic C ($g\ C\ kg^{-1}$)	Total N ($g\ N\ kg^{-1}$)	C/N	Clay ($g\ kg^{-1}$)
1	6.54	13.96	1.47	9.50	102
2	6.73	14.87	1.66	8.98	209
3	6.61	18.03	1.84	9.80	337

Table 1.2. Manures used in the incubation experiment.

Variable	Unit	Man. 1	Man. 2	Man. 3	Man. 4	Man. 5
Dry matter	g kg^{-1}	80	102	117	49	67
Ash	g kg^{-1} dry weight	205	218	157	251	206
Organic C	g kg^{-1} dry weight	386	393	412	367	411
Water soluble C	g kg^{-1} dry weight	107	46	82	174	93
Total N	g kg^{-1} dry weight	48.5	26.1	38.8	75.2	38.8
Organic N	g kg^{-1} dry weight	21.9	17.4	25.5	37.9	22.6
NH_4–N	g kg^{-1} dry weight	26.6	8.7	13.3	37.3	16.2
Water soluble N	g kg^{-1} dry weight	23.8	8.0	14.3	35.5	14.8
Storage time	d	23	72	10	40	25

The simulation model

The CN-SIM model (Petersen et al., 2005a and 2005b) describes carbon and nitrogen turnover during organic matter degradation in the soil. It uses seven pools to represent soil organic matter (Fig. 1.1). Two pools represent the added organic materials (AOM_1 and AOM_2), two represent the microbial biomass (‘*autochthonous*’, SMB_1 and ‘*zymogenous*’, SMB_2), and three represent the more stable fractions, i.e. SMR (soil microbial residues), NOM (native organic matter) and IOM (inert organic matter). Mineral nitrogen is represented with two pools, ammonium and nitrate. All organic pools, except IOM that is supposed to be inert, decay with first-order kinetics, whose rates are modified through environmental adjustments that depend on soil water content and temperature. Microbial biomass rates are also affected by the clay content of the soil.

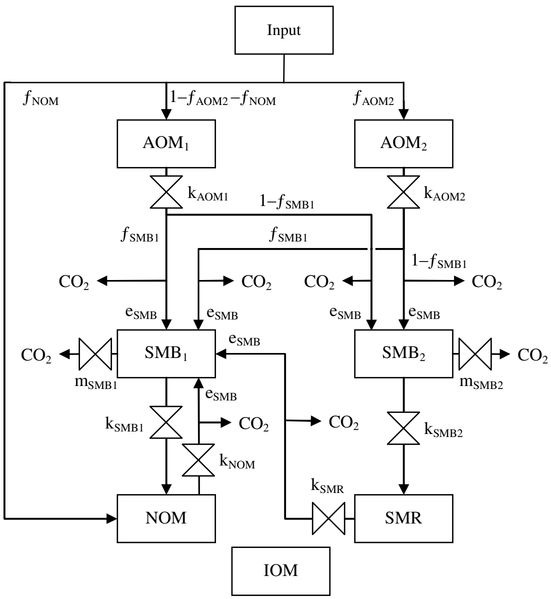
The heterogeneous composition of added organic materials is simulated by splitting their C and N content (C_i and N_i) in two new AOM_1 and AOM_2 pools, and, only for animal manure, in the humified fraction added to the NOM pool. Part of the added N can also be input to the NH_4–N pool. Model parameters are listed in Table 1.3. We refer the reader to Petersen et al. (2005a and 2005b) for more details about CN-SIM.

Model implementation and application

We implemented the model in a computer program written in C++ that produces identical results as the original. Common to the original implementation is also a function that allows each simulation to start with steady-state conditions for organic pools. This procedure was needed because if the user would provide initial sizes of organic pools that are not in equilibrium, this would generate trends of C and N dynamics that would affect model parameterisation.

Temperature and soil water content were fixed at 25°C and -0.05 MPa, as in the incubation experiment, and adjustment factors were calculated by the model. This was needed because the parameter values by Petersen et al. (2005a and 2005b) are given with reference to a different temperature (10 °C) and soil water potential (between -0.0310 and -0.0031 MPa). In the analysis described in this paper, we have always used a time step of 0.25 d.

Figure 1.1. Relational diagram of the CN-SIM model (redrawn from Petersen et al., 2005a). Rectangles are state variables (organic pools), valves are rate variables, continuous lines are flows of C. AOM1 = Added Organic Matter 1 (resistant); AOM2 = Added Organic Matter 2 (labile), SMB1 = '*Autochthonous*' Soil Microbial Biomass; SMB2 = '*Zymogenous*' Soil Microbial Biomass; SMR = Soil Microbial Residues; NOM = Native Organic Matter; IOM = Inert Organic Matter.



Our program implements also the downhill simplex method (Nelder and Mead, 1965) to perform the automatic optimisation of parameter values. We coded in C++ the simplex version of Acutis and Confalonieri (2006), which implements the original Nelder and Mead (1965) algorithm and adds the multiple start procedure. Compared to the Levenberg-Marquardt optimisation algorithm used by Petersen et al. (2005a and 200b), the downhill simplex has often similar performances in finding local or global optimal solutions (e.g. Maes et al., 1999) and, due to the multiple start procedure, better guarantees that the global optimal solution is found. Our program performs one or more simulations, each with its own parameters; it also performs automatic parameter optimisation using data of one or more experimental treatments.

Petersen et al. (2005a and 2005b) parameterised CN-SIM iteratively, by alternating long- and short-term optimisations. Long-term optimisation involved the use of measurements from long-term field experiments and the calibration of f_{IOM}, k_{NOM}, f_{NOM} and t_{SMB1}. Laboratory incubations were used for short-term optimisation, where k_{AOM1}, k_{AOM2}, f_{AOM2}, f_{SMB1}, r_{SMB2}, t_{SMB2}, k_{SMR}, CN_{SMB2}, CN_{SMR} and f_{AOM2N} were calibrated. In our case this procedure was not needed, because we were only interested in short-term dynamics after manure application to soil during an incubation experiment. In this study we tested the influence of all parameters through SA and calibrated only those that have a marked influence on model outputs in the period 0-180 d.

We introduced another innovation in our implementation to guarantee the proper execution of SA. Some combinations of parameters generated during SA resulted in inconsistent values for added organic pools (i.e. negative values for C or N in the AOM pools) because the fractions in which the organic input is split are determined independently one from the other during SA. This problem did not arise during parameter optimisation because when inconsistencies with respect to the theoretical model formulation occurred, an extremely high value of the objective function was assigned, thus forcing the optimisation algorithm to explore different areas of the input parameters space.

To avoid unreliable values of model parameters during SA, we wrote a shell around the original model. The shell modifies the way to specify how manure C and N are partitioned among pools. In the original formulation, the amount of manure C allocated to AOM_1 (C_{AOM1}) is calculated as the difference between added manure C and the amount of C directed to AOM_2 (C_{AOM2}) and NOM (C_{NOM}). Similarly, the amount of manure N allocated to the AOM_1 pool (N_{AOM1}) is calculated as the difference between added organic manure N and the amount of N directed to NH_4, AOM_2 and NOM. Because parameters are changed independently during SA, negative values of C_{AOM1} and N_{AOM1} may occur if the sum of the fractions of C and N allocated to AOM_2 and NOM exceeds one. In order to ensure positive values for the three fractions in which manure C and N are split, we changed the rules for C and

N partitioning. For this purpose, we first calculated f_{NOM} and f_{AOM1} as:

$$f_{NOM} = (1 - f_{AOM2}) \times f_{NOM*} \quad (1)$$

$$f_{AOM1} = (1 - f_{AOM2}) \times (1 - f_{NOM*}) \quad (2)$$

where f_{NOM*} is a unitless partitioning factor (introduced with the shell) used to ensure that f_{AOM1} is positive. Next we calculated the nitrogen fractions. To do this, we made a first guess of f_{MIN}, which was set at its lowest possible threshold for liquid dairy manures (0.23; Marino et al., 2008); we then calculated the other N fractions:

$$f_{AOM2N} = f_{AOM2} \times CN_{org} / CN_{AOM2} \quad (3)$$

$$f_{NOMN} = f_{NOM} \times CN_{org} / 10 \quad (4)$$

$$f_{AOM1N} = 1 - f_{AOM2N} - f_{NOMN} \quad (5)$$

where CN_{org} is the C/N ratio of the organic manure pool = CN_i / (1 – f_{MIN}), and CN_{AOM2} is the C/N ratio of the AOM_2 pool (a parameter introduced with the shell); CN_i is the C/N ratio of the added manure. We then verified that CN_{AOM1} was higher than 4.2, the average C/N ratio of the organic compounds (amino acids) that are richest in N. In other words, we did not want AOM_1 to be richer in N than amino acids, otherwise AOM_1 would not represent a realistic pool. If $CN_{AOM1} < 4.2$, then CN_{AOM1} was set at 4.2 and the excess N was considered to be mineral (i.e. it was added to the ammonium pool by increasing f_{MIN}). Finally, the nitrogen fractions were recalculated. The shell did not introduce conceptual differences in the simulation model, because input parameters for model execution remained f_{AOM2}, f_{AOM2N} and f_{NOM}, as originally defined by Petersen et al. (2005a). Compared to the original model, the difference was that f_{AOM2N} and f_{NOM} were not input by the user (which was the SA algorithm in this case), but were calculated by the shell based on f_{NOM*} and CN_{AOM2}.

Sensitivity analysis

As a support to parameter optimisation, SA was carried out to assess the contribution of each model parameter in determining model output variation (Saltelli et al., 2004). The output variables for which sensitivity was evaluated were net simulated CO_2–C and net SMN, on the same dates when measurements were carried out. 'Net' means that we calculated the difference between the simulated amended and the simulated control treatment, for both variables. Sensitivity analysis was performed in two steps: a first analysis (Factors Fixing experiment) was performed using the method of Morris with the aim of fixing the value of those parameters that do not significantly influence model outputs; a second analysis (Factors Prioritisation experiment) was carried out with the purpose of ranking parameters in order of importance on impacting model output variance, to diminish the number of parameters subjected to optimisation. We used the software SIMLAB version 2.2 (SIMLAB, 2004). SIMLAB version 2.2 is a free computer program to carry out global uncertainty and sensitivity analysis, developed by the Joint Research Centre of the European Commission. The general steps for both analyses can be summarized as follows: (1) a matrix containing different combinations of parameter values was created by SIMLAB, sampling each parameter value from a probability distribution function specified by the user; (2) each combination of parameter values was then used to run a simulation with our CN-SIM implementation; (3) model outputs were analysed by SIMLAB together with their corresponding inputs, thus providing the sensitivity indices for each parameter with respect to each output variable. The details about the generation of combinations of parameter values are available below and in the referenced literature.

First sensitivity analysis (method of Morris)

The method of Morris (Campolongo et al., 2007; Morris, 1991; Saltelli et al., 2004) provides two sensitivity indices: μ and σ, which represent the overall effect of the parameter on model output and its higher order effects (non-linear effects and interactions with other parameters), respectively. The two indices are computed based on the calculation of Elementary Effects (EEs). An EE is the variation in model output (ΔY) due to a variation in one model input (ΔX_i). The average of all EEs of a parameter X_i, calculated in different regions of the input parameters space, is represented by μ, while σ is their standard deviation. The mean value provided by SIMLAB is calculated based on the absolute value of all EEs (μ^*); this avoids that positive and negative effects counterbalance (Campolongo et al., 2007). In our analysis for each parameter 10 EEs (r = 10) were calculated (see Campolongo et al., 2007 for further details). This choice provided an input sample of 230 ($r \times (k + 1)$) sets of parameters, where $k = 22$ is the number of parameters. Using the values of μ^* and σ we identified non-influent parameters according to the following steps: (i) we calculated at each date the average value of each output variable (accumulated respired CO_2–C and SMN); (ii) parameters having μ^* and σ lower than 10% of the average value for both model outputs at all dates were considered to have a negligible effect on model outputs and therefore their value during the rest of this study was fixed at the optimised value reported in Petersen et al. (2005a and 2005b). The other parameters (i.e. those having μ^* or σ higher than 10% for at least one date and one output variable) were subjected to the second SA. To evaluate the uncertainty due to random generation of the input parameter space, the first SA was performed three times by using different input samples obtained through randomisation.

Second sensitivity analysis (method of Sobol')

The second SA was carried out using the method of Sobol' (Saltelli et al., 2004; Sobol', 1993; Sobol' and Kucherenko, 2005). This method is based on the decomposition of the output variance ($V(Y)$) into different sources of variation, i.e. the partial variances due to single parameters (V_i), and all possible interactions of order k between model parameters (V_{ij}, V_{ijm}, etc.):

$$V(Y) = \sum_{i} V_i + \sum_{i<j} V_{ij} + \sum_{i<j<m} V_{ijm} + \cdots + V_{12\ldots k}$$

where i, j, m, ..., and k represent model parameters. The sensitivity indices, given by the ratios between the conditional variances (V_i, V_{ij} and so on) and the total unconditional variance ($V(Y)$), represent the contribution to the total output variance of model parameters alone or in combination with other parameters. In particular, for each model parameter, two indices are provided by the method: (1) the first-order index (S_i) of the i^{th} parameter, calculated as the ratio $V_i/V(Y)$, where $V_i = V(E(Y|X_i))$ represents the expected amount of output variance that would be removed from the unconditional (total) variance having fixed the value of X_i at its true value; (2) the total-order index (ST_i) of the i^{th} factor, calculated as the ratio $E(V(Y|X_{\sim i}))/V(Y)$, where $E(V(Y|X_{\sim i}))$ represents the expected amount of output variance that would remain unexplained (residual variance) if X_i was left free to vary over its uncertainty range, having fixed the value of all other factors ($X_{\sim i}$) (Homma and Saltelli, 1996). The first index represents the contribution of the i^{th} factor individually on diminishing the total variance, while the second index estimates the global contribution of the i^{th} parameter on output variance, including its interactions with the other parameters. The total number of combinations explored in the experiment is $c = n \times (2 \times k + 2)$, where n is a sample size and k is the number of parameters (equal to 15 in our case). We have chosen a high value for n (2048), resulting in a sample of $c = 65536$ parameters combinations. In our application, the clay content of the soil and the C/N ratio of the manure are two parameters defined by the user that were not allowed to vary during parameter optimisation. Therefore, it was not important to test the sensitivity of the model to these parameters. However, it was important to know if the sensitivity of the model to the other 15 parameters was different under various combinations of clay content and C/N ratio of the manure. Therefore, in order to explore the effects of parameters in conditions similar to the experimental incubation, the analysis with the method of Sobol' was carried out four times, by combining two C/N ratios of the manure and two clay contents; the generated input space was the same for the four combinations. According to Saltelli et. al (2004), the most important parameters have to be selected based on their first-order sensitivity indices (S_i); we chose to calibrate the parameters having a value of S_i higher than 0.1 (i.e. those that are responsible for a minimum of

10% of model output variation) for at least one output variable (simulated CO_2 or SMN).

Statistical distributions of the parameters during sensitivity analysis

Lacking information about the distribution of the 22 parameters tested in the first SA (Table 1.3), uniform distributions were defined. For the decomposition constants, the C to N ratios, and the microbial biomass turnovers, the lower limit was assumed to be 50% lower than the minimum published value by Petersen et al. (2005a, 2005b); similarly, for these parameters the upper limit was 50% higher than the maximum published value by Petersen et al. (2005a, 2005b). The boundaries for f_{SMB1}, e_{SMB} and r_{SMB2} were set as large as possible, under the constraint to have positive values of r_{SMB1} (see Petersen et al., 2005a). The parameter CN_{AOM2} was given boundaries from 15 to 100; these limits were chosen in order to allow the creation of N–rich or N–poor AOM_2 pools. The lower limit was set at 15 in order to provide positive values for all C and N fractions, reliable values for CN_{AOM1}, and to ensure values of f_{MIN} in the range 0.23 – 0.64. These are the 1st and 99th percentiles of the statistical distribution of f_{MIN} for 93 liquid dairy manures described in Marino et al. (2008). Table 1.3 describes how each parameter was handled during the first and the second SA (either fixed or allowed to vary within a range) and during optimisation. For parameters that were not subjected to SA or that were not calibrated, the fixed value used is indicated.

Parameter optimisation

We integrated CN-SIM with a single-objective optimisation algorithm, the downhill simplex (Nelder and Mead, 1965). This optimisation method is based on the geometrical figure called "simplex", having a number of vertexes (m) equal to the number of parameters subjected to optimisation (n) plus one. Each vertex corresponds to a complete set of model parameters under calibration. The values of these parameters are the coordinates of the vertex in the n-dimensional space. The vertexes are randomly initialised at the start of the procedure and then evaluated. A vertex is evaluated by calculating an objective function (OF) that depends on model outputs obtained with the set of parameters corresponding to that vertex. During the optimisation, the search is guided outside the simplex area (reflection and expansion) or inside it (contraction and shrinking). While reflection is always performed, expansion and contraction are mutually exclusive. The search process stops either when the user-defined maximum number of iterations is reached, or when the simplex collapses, that is when the objective function of each vertex of the simplex is extremely close to the others. In this study, in order to explore most of the space of parameters and to prevent fast collapse of simplexes into local minima, the method was repeatedly applied by generating many simplexes (300) that started from different randomly defined initial vertexes (Acutis and Confalonieri, 2006). At the end of optimisation, the vertex with the lowest OF was selected as the best set of parameters to be retained. The range of parameters used during optimisation (Table 1.3) was the same used during SA. The shell was not used during parameter optimisation; the ranges of parameters f_{NOM} and f_{AOM2N} were calculated based on the ranges of parameters f_{NOM*} and CN_{AOM2}, using Eqs. (1) and (3). To control the variability of optimisation results due to random vertexes generation, optimisation of model parameters was performed three times. Results of the three runs were compared and checked for stability and the vertex with the lowest OF was chosen. The objective function used in this study was the average of the relative root mean squared errors (RRMSEs) between simulated and measured rates of net CO_2–C respiration (RRMSE–CO_2) and net SMN (RRMSE–SMN). The agreement of measured and simulated rates was described by also calculating the coefficient of determination (CD), the coefficient of residual mass (CRM), the modelling efficiency (EF), and the parameters of the linear regression (Slope, Intercept and R^2) (Bellocchi et al., 2009; Loague and Green, 1991).

Regressions among model parameters and manure properties

After model calibration we have evaluated the linear regression between measured manure properties (ash, C, total N, organic-N, NH_4–N, storage time, C/N, C/organic-N, water soluble-C, water soluble-N, soluble-C/soluble-N and NH_4–N/C) and optimised model parameters (or quantities derived from model parameters). The regression was separately evaluated for the three soils (n = 5); we comment only the regressions with $R^2 > 0.90$ and the slope significantly different than zero ($P < 0.05$) for the three soils. The regressions were calculated using the REGRESSION procedure of the statistical package SPSS ver. 18.0.0.

Table 1.3. Model parameters. Parameters were either varied within a range (during sensitivity analysis and eventually during parameter optimisation) or fixed at a constant value throughout the simulation.

Symbol	Description	Unit	Studied with sensitivity analysis?			Calibrated during parameter optimisation?		
			Yes		No	Yes		No
			Min	Max	Fixed value	Min	Max	Fixed value
Original CN-SIM parameters								
Clay	Soil clay content	mg clay g soil^{-1}	102 [a]	337 [a]	102 or 307 [b]	–	–	Measured soil clay content
CN_{AOMs}	C to N ratio of $AOM_{1,2}$ initially present in the soil	mg C mg N^{-1}	35 [a]	105 [a]	70 [b]	–	–	70
CN_i	C to N ratio of the organic input	mg C mg N^{-1}	5 [a]	15 [a]	5 or 15 [b]	–	–	Measured input C/N
CN_{IOM}	C to N ratio of IOM	mg C mg N^{-1}	–	–	Calculated	–	–	Calculated
CN_{NOM}	C to N ratio of NOM	mg C mg N^{-1}	–	–	Calculated	–	–	Calculated
CN_{SMB1}	C to N ratio of SMB_1	mg C mg N^{-1}	3	10	–	3	10	–
CN_{SMB2}	C to N ratio of SMB_2	mg C mg N^{-1}	5	14	–	–	–	9.33
CN_{SMR}	C to N ratio of SMR	mg C mg N^{-1}	3	9	–	3	9	–
e_{SMB}	Substrate utilization efficiency of $SMB_{1,2}$	mg C mg C^{-1} or mg N mg N^{-1}	0.50	0.90	–	0.50	0.90	–
f_{AOM2}	Fraction of input C allocated to AOM_2	mg C mg C^{-1}	0.01	0.66	–	0.01	0.66	–
f_{IOM}	Fraction of inert soil	mg C mg C^{-1} or	0.147 [a]	0.617 [a]	0.402 [b]	–	–	0.402

Symbol	Description	Unit	Studied with sensitivity analysis?			Calibrated during parameter optimisation?		
			Yes		No	Yes		No
			Min	Max	Fixed value	Min	Max	Fixed value
	organic C and N	mg N mg N^{-1}						
f_{MIN}	Fraction of input N in the form of NH_4	mg N mg N^{-1}	–	–	–	–	–	Measured f_{MIN}
f_{SMB1}	Fraction of outflows of C and N from $AOM_{1,2}$ directed to SMB_1	mg C mg C^{-1} or mg N mg N^{-1}	0.27	0.70	–	–	–	0.312
k_{NIT}	Nitrification constant of the NH_4 pool	d^{-1}	0.05 [a]	0.15 [a]	0.1 [b]	–	–	0.1
k_{AOM1i}	Decomposition constant of the AOM_1 pool of organic input (resistant)	d^{-1}	0.0000425	0.0225	–	0.0000425	0.0225	–
k_{AOM2i}	Decomposition constant of the AOM_2 pool of organic input (labile)	d^{-1}	0.0065	0.42	–	0.0065	0.42	–
k_{AOM1s}	Decomposition constant of the AOM_1 pool initially present in the soil (resistant)	d^{-1}	0.002 [a]	0.006 [a]	0.004 [b]	–	–	0.004
k_{AOM2s}	Decomposition constant of the AOM_2 pool initially present in the soil (labile)	d^{-1}	0.02 [a]	0.06 [a]	0.04 [b]	–	–	0.04
k_{NOM}	Decomposition constant of NOM	d^{-1}	0.0000565	0.000219	–	–	–	0.000146

Symbol	Description	Unit	Studied with sensitivity analysis?			Calibrated during parameter optimisation?		
			Yes		No	Yes		No
			Min	Max	Fixed value	Min	Max	Fixed value
k_{SMR}	Decomposition constant of SMR	d^{-1}	0.001630	0.008805	–	0.001630	0.008805	–
r_{SMB2}	Turnover ratio of SMB_2	–	0.42	0.66	–	–	–	0.528
t_{SMB1}	Total turnover of SMB_1	d^{-1}	0.0055	0.0180	–	0.0055	0.0180	–
t_{SMB2}	Total turnover of SMB_2	d^{-1}	0.0615	0.2500	–	–	–	0.128
Additional parameters introduced to carry out SA								
CN_{AOM2i}	C to N ratio of AOM_2 pool of organic input	mg C mg N^{-1}	15	100	–	–	–	–
f_{NOM*}	Partitioning fraction of "recalcitrant" input C between AOM_1 and NOM	mg C mg C^{-1}	0.01	0.35	–	–	–	–
Original CN-SIM parameters recalculated from additional parameters [c]								
f_{AOM2N}	Fraction of input organic N allocated to AOM_2	mg N mg N^{-1}	–	–	–	0.01	0.66	–
f_{NOM}	Fraction of input C allocated to NOM	mg C mg C^{-1}	–	–	–	0.0034	0.3465	–

a For these parameters, the ranges indicated are for the first sensitivity analysis, using the Morris method.
b For these parameters, the fixed values were used in the second sensitivity analysis, using the method of Sobol'.
c Only for SA, these parameters were calculated from the additional parameters introduced to carry out SA, using Eqs. (9) and (11).

Results

Sensitivity analysis

The results of the sensitivity analysis (method of Morris) showed that few parameters did not have substantial influence on model outputs because their μ^* and σ were lower than 10% of the simulated value for both variables at all dates (data not shown in figures or tables). These parameters were CN_{AOMs}, f_{IOM}, k_{AOM1s}, k_{AOM2s} and k_{NIT}. For the parameter k_{NOM} μ^* and σ for SMN were in most dates lower (and in the other dates – from day 12 to day 72 – only slightly higher) than the 10% threshold, and always lower than the 10% threshold for CO_2 respiration. Most of the other parameters had a substantial influence on one or both simulated variables, but only at some dates, depending on the parameter and on the response variable considered (CO_2 respiration or SMN). In particular, k_{AOM2i} and CN_{AOM2} were substantially influent only at the beginning of the cycle, while f_{NOM*} was effective only at the end; the effect of CN_{AOM2} lasted longer on SMN (until day 72) compared to CO_2 (until day 4 – 21). CN_{SMB2} had a marked influence only until day 72 for both variables. The 10% threshold was overcomefor CN_{SMR} and k_{SMR} frequently for SMN and seldom for CO_2 respiration. Few parameters were important at all dates for both variables. These were CN_i, e_{SMB}, f_{AOM2} and k_{AOM1i}. After this analysis we fixed the value of CN_{AOMs}, k_{AOM1s}, k_{AOM2s}, k_{NOM} and k_{NIT} (Table 1.3).

The results of the second sensitivity analysis (method of Sobol') showed that the most important parameters determining variation of net accumulated CO_2 respiration (Fig. 1.2a) were microbial efficiency (e_{SMB}) and four parameters describing the composition of manure both in terms of C allocation to AOM_1, AOM_2 and NOM (f_{AOM2} and f_{NOM*}) and in terms of decomposition rates of AOM_1 and AOM_2 (k_{AOM1i} and k_{AOM2i}). The decomposition rate of the labile pool (k_{AOM2i}) had an important effect only in the first period (~ Day 8), while the decomposition rate of the resistant pool (k_{AOM1i}) had an increasing effect until Day 41; after this date its effect remained constant. The effect of the partitioning parameter f_{AOM2} was most important until Day 28, but did not nullify later. The other partitioning factor f_{NOM*} had an increasing effect over time. The sensitivity of net accumulated respired CO_2 to manure-related parameters (f_{AOM2}, k_{AOM1i}, k_{AOM2i}) was identical for manures with different C/N ratios, while the effect of clay was evident only for k_{AOM1i}, with larger sensitivities for the soil with low clay content. The opposite effect of clay content was observed for microbial efficiency (e_{SMB}). First-order Sobol' sensitivity indexes for SMN (Fig. 1.2b) confirmed that microbial efficiency and manure parameters were important in the initial period if related to the labile pool (f_{AOM2}, k_{AOM2i}) and later if related to the resistant pools (f_{NOM*}, k_{AOM1i}). The parameters

CN_{SMR}, CN_{SMB1}, k_{SMR} and t_{SMB1} had an effect on SMN but not on CO_2 respiration. The manure parameter CN_{AOM2} did not have effects on SMN. The effect of k_{AOM1i} was extremely important for the manure with a low C/N ratio, while it had a much smaller effect for the manure with a high C/N ratio. Microbial parameters (e_{SMB}, CN_{SMB1}, t_{SMB1}, CN_{SMR} and k_{SMR}) were more important for the manure with a high C/N ratio. The effect of clay was evident for CN_{SMB1} (higher effect for the soil rich in clay) and k_{AOM1i} (higher effect for the soil low in clay).

Figure 1.2a. Sobol' first-order sensitivity indices of CN-SIM model parameters for the net accumulated CO2 respiration of added manure. Four sensitivity analyses are reported, obtained as the combination of two soil clay content and two C/N ratio of the added manure.

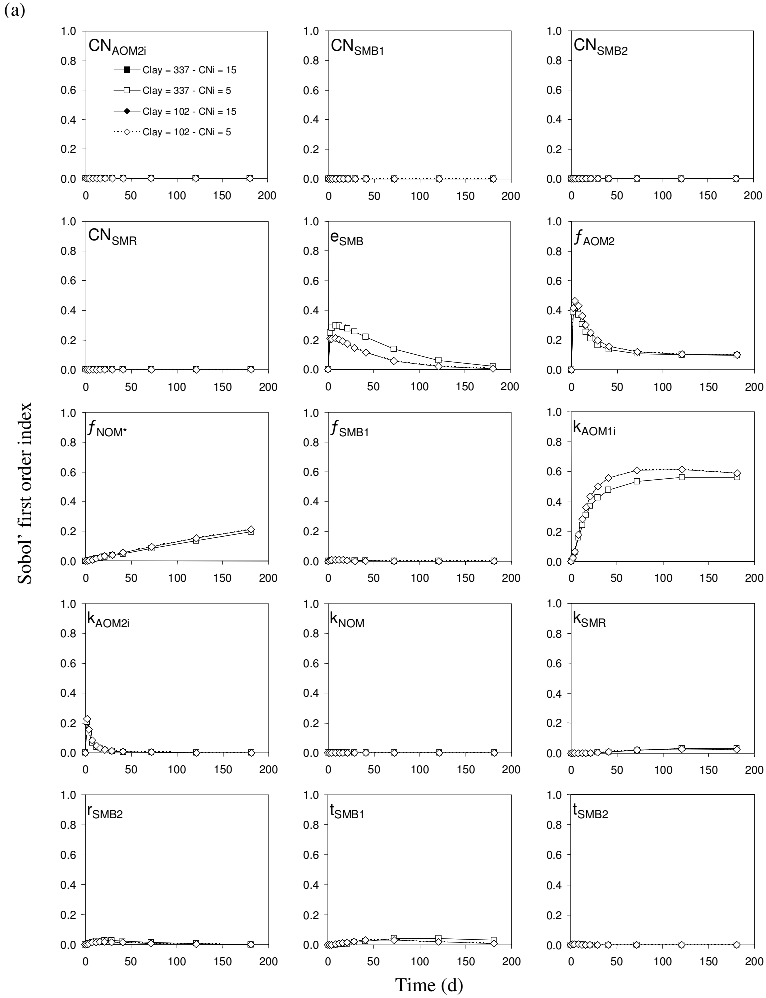
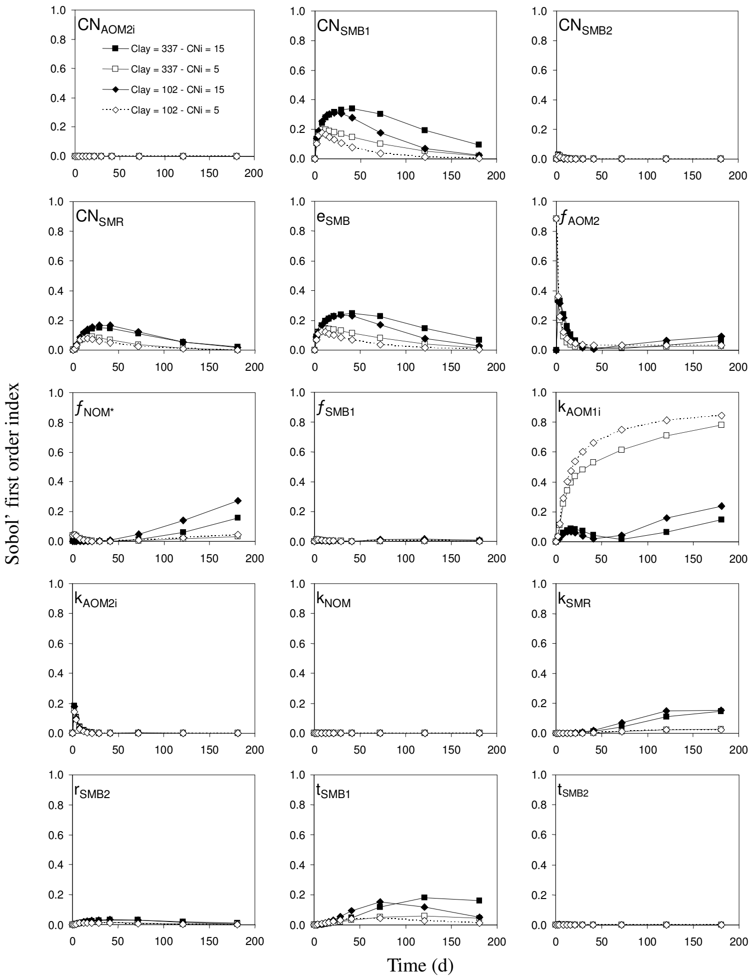


Figure 1.2b. Sobol' first-order sensitivity indices of CN-SIM model parameters for net soil mineral nitrogen (SMN). Four sensitivity analyses are reported, obtained as the combination of two soil clay content and two C/N ratio of the added manure

(b)



Parameter optimisation

We first have carried out parameter optimisation by sharing manure parameters for the three simulations of each manure on three soils simultaneously. This joint optimisation gave unsatisfactory model performance (Table 1.4), in particular for manure 2 and 4. We made the hypothesis that imposing the same manure parameters simultaneously on the three soils was limiting the ability of the model to reach a satisfactory agreement between measured and simulated variables. We therefore optimised the rates of CO_2 respiration and SMN for 15 treatments separately, i.e. by allowing the set of manure parameters to be soil- and manure- dependent. The optimised parameter values were highly variable among manures and even within each manure (Table 1.5). In 13 out of 15 cases, the more recalcitrant AOM_1 pool (i.e. with lower decomposition constant, k_{AOM1i}) was richer in N compared to the more labile AOM_2. In most situations AOM_2 contained 19 – 34% of added C, while the humified fraction (i.e. allocated to NOM) represented 15% of added C on average, with a maximum of 30%. The remaining C was allocated to AOM_1 for 18 – 69%, with most of the manures above 50%. After parameter optimisation the agreement between measured and simulated respired CO_2 was very good for most soil × manure combinations (Fig. 1.3 and Table 1.6a), with the absolute value of RRMSE always smaller than 17%. Model performance was very poor for

SMN (Fig. 1.3 and Table 1.6b), with absolute RRMSE larger than 17%. The modelling efficiency was always positive for both variables, indicating that the average of measured rates would not be a better predictor than the model. The linear regression between measured and simulated rates had a slope and an R^2 close to 1, in particular for CO_2. The indices of model performance calculated only for the period 0 – 41 d (not shown in tables) indicated a much better agreement between measured and simulated rates in this initial period for both variables compared to the 71 – 181 d period (Fig. 1.3). For SMN the CRM indicated a slight underestimation for Day 0 – 41 and a strong overestimation for Day 71 – 181 when a similar deterioration of model performance is described by all the other indices. The predictive performance of the model was highly variable among manures (Fig. 1.3, Table 1.6a and 1.6b). One example of good model fitting was that of manure 3 on soil 2: the pattern of C mineralisation was reproduced correctly, with initial high rates and slower rates thereafter; while SMN was poorly simulated after Day 41. One example of poor fitting for both variables was for manure 2 on soil 3, where the initial phase of respiration was underestimated before Day 41 and strongly overestimated after that date.

Table 1.4a. Indices of agreement between measured and simulated rates of CO2 respiration for optimisation carried out on three soils simultaneously. RRMSE = Relative Root Mean Squared Error, EF = Modelling Efficiency; CRM = Coefficient of Residual Mass; CD = Coefficient of Determination; Slope, Intercept and R2 = parameters of the linear regression.

(a)

Manure	RRMSE (%)	EF (-)	CRM (-)	CD (-)	Slope (-)	Intercept (mg kg^{-1} d^{-1})	R^2 (-)
1	18	0.99	0.03	1.01	0.99	0.77	0.99
2	51	0.67	0.20	1.26	0.80	5.59	0.77
3	22	0.97	0.07	1.03	0.97	2.02	0.97
4	42	0.95	0.04	0.95	1.00	0.40	0.95
5	13	0.99	-0.02	0.98	1.01	-0.45	0.99

Table 1.4b. Indices of agreement between measured and simulated rates of SMN for optimisation carried out on three soils simultaneously. RRMSE = Relative Root Mean Squared Error, EF = Modelling Efficiency; CRM = Coefficient of Residual Mass; CD = Coefficient of Determination; Slope, Intercept and R2 = parameters of the linear regression. The RRMSE is always negative for SMN because the average of SMN rates of change is always negative

(b)

Manure	RRMSE (%)	EF (-)	CRM (-)	CD (-)	Slope (-)	Intercept (mg kg^{-1} d^{-1})	R^2 (-)
1	-44	0.98	0.09	0.97	1.00	-0.17	0.98
2	-54	0.98	-0.05	0.95	1.01	0.08	0.98
3	-42	0.98	0.13	0.97	1.01	-0.15	0.98
4	-124	0.93	0.31	0.66	1.22	-0.20	0.96
5	-58	0.97	0.01	0.96	1.00	-0.01	0.97

Table 1.5. CN-SIM parameter values obtained after optimisation with the downhill simplex for five liquid dairy manures on three soils of increasing clay content.

	Manure														
	1			2			3			4			5		
	Soil														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Model parameters															
CN_{SMB1}	7.19	3.01	4.54	9.92	9.39	8.54	10.00	4.82	9.61	10.00	3.43	3.01	7.98	4.49	4.14
t_{SMB1}	0.013	0.010	0.006	0.016	0.018	0.018	0.018	0.006	0.010	0.017	0.018	0.006	0.016	0.0091	0.0119
e_{SMB1}	0.86	0.70	0.67	0.55	0.90	0.90	0.52	0.69	0.64	0.69	0.70	0.64	0.56	0.74	0.60
k_{SMR}	0.002	0.002	0.005	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.009	0.008	0.002	0.002	0.002
CN_{SMR}	9.00	5.42	9.00	9.00	9.00	8.82	3.60	6.26	5.29	3.01	7.66	8.90	4.58	9.00	8.99
f_{AOM2}	0.60	0.34	0.31	0.07	0.25	0.30	0.20	0.25	0.21	0.63	0.66	0.53	0.19	0.32	0.19
f_{AOM2N}	0.62	0.02	0.04	0.01	0.14	0.43	0.08	0.01	0.09	0.38	0.01	0.23	0.01	0.01	0.01
f_{NOM}	0.003	0.156	0.173	0.304	0.136	0.010	0.115	0.066	0.161	0.020	0.164	0.205	0.281	0.173	0.267
k_{AOM1i}	0.020	0.023	0.019	0.011	0.011	0.007	0.016	0.023	0.025	0.024	0.001	0.013	0.023	0.0192	0.0209
k_{AOM2i}	0.420	0.420	0.420	0.271	0.420	0.420	0.225	0.419	0.326	0.378	0.420	0.420	0.333	0.420	0.420

	Manure														
	1			2			3			4			5		
	Soil														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Partitioning of added organic C and N, calculated from model parameters															
CN_{AOM1}	18.6	12.5	14.0	47.3	24.8	28.4	15.2	12.6	15.6	5.6	2.1	4.5	20.2	13.6	19.6
CN_{AOM2}	17.1	358.5	121.3	148.9	41.9	15.9	39.2	297.5	38.0	16.1	507.0	22.2	336.2	582.9	321.7
C_{AOM1} [a]	39.8	50.3	52.0	63.0	61.0	69.1	68.5	68.8	62.7	35.1	17.6	26.7	53.3	50.5	54.4
C_{AOM2} [a]	59.8	34.1	30.7	6.6	25.4	29.9	20.0	24.7	21.2	62.9	66.0	52.7	18.6	32.2	18.9
C_{NOM} [a]	0.3	15.6	17.3	30.4	13.6	1.0	11.5	6.6	16.1	2.0	16.4	20.5	28.1	17.3	26.7
N_{AOM1} [b]	37.8	70.9	65.1	30.1	55.5	55.1	73.1	88.0	64.9	60.3	82.8	57.1	47.9	67.6	50.4
N_{AOM2} [b]	61.6	1.7	4.5	1.0	13.7	42.7	8.3	1.3	9.0	37.7	1.3	23.0	1.0	1.0	1.1
N_{NOM} [b]	0.6	27.4	30.4	68.9	30.8	2.2	18.7	10.7	26.1	2.0	15.9	19.9	51.1	31.4	48.5

a Expressed as a percentage of the added C.
bExpressed as a percentage of the added organic N.

Table 1.6a. Indices of agreement between measured and simulated rates of CO_2 respiration. RRMSE = Relative Root Mean Squared Error, EF = Modelling Efficiency; CRM = Coefficient of Residual Mass; CD = Coefficient of Determination; Slope, Intercept and R2 = parameters of the linear regression.

(a)

Soil	Manure	RRMSE (%)	EF (-)	CRM (-)	CD (-)	Slope (-)	Intercept (mg kg^{-1} d^{-1})	R^2 (-) [a]
1	1	11	0.99	0.01	1.00	1.00	0.20	0.99
1	2	12	0.98	-0.04	0.94	1.02	-0.84	0.98
1	3	4	1.00	0.00	1.00	1.00	0.07	1.00
1	4	4	1.00	-0.01	1.00	1.00	-0.08	1.00
1	5	4	1.00	0.00	1.00	1.00	-0.10	1.00
2	1	8	1.00	-0.01	0.98	1.01	-0.31	1.00
2	2	17	0.97	-0.06	0.89	1.05	-1.88	0.97
2	3	3	1.00	-0.02	0.98	1.01	-0.48	1.00
2	4	11	1.00	0.03	1.00	1.00	0.30	1.00
2	5	7	1.00	-0.01	0.99	1.00	-0.20	1.00
3	1	9	1.00	0.00	0.99	1.00	-0.12	1.00
3	2	17	0.96	0.02	1.05	0.96	1.02	0.96
3	3	5	1.00	0.01	1.01	1.00	0.23	1.00
3	4	12	1.00	0.00	1.00	1.00	0.07	1.00
3	5	7	1.00	0.00	0.99	1.00	-0.09	1.00

Table 1.6ab. Indices of agreement between measured and simulated rates of SMN. RRMSE = Relative Root Mean Squared Error, EF = Modelling Efficiency; CRM = Coefficient of Residual Mass; CD = Coefficient of Determination; Slope, Intercept and R2 = parameters of the linear regression.

(b)

Soil	Manure	RRMSE (%)	EF (-)	CRM (-)	CD (-)	Slope (-)	Intercept (mg kg^{-1} d^{-1})	R^2 (-) [a]
1	1	-75	0.96	0.29	1.00	0.99	-0.35	0.97
1	2	-98	0.82	-0.06	0.82	1.01	0.04	0.83
1	3	-110	0.79	0.04	0.81	0.99	-0.03	0.79
1	4	-173	0.83	0.16	0.80	1.02	-0.05	0.83
1	5	-96	0.87	0.01	0.87	1.00	-0.01	0.87
2	1	-40	0.98	0.02	0.98	1.00	-0.06	0.98
2	2	-54	0.97	-0.03	0.97	1.00	0.07	0.97
2	3	-20	1.00	0.04	0.99	1.00	-0.08	1.00
2	4	-37	0.99	0.05	0.99	1.00	-0.11	0.99
2	5	-39	0.98	0.07	1.00	0.99	-0.21	0.99
3	1	-17	1.00	0.04	1.00	1.00	-0.06	1.00
3	2	-41	0.98	-0.06	0.98	1.00	0.08	0.98
3	3	-29	0.98	0.04	1.00	0.99	-0.05	0.98
3	4	-35	0.99	0.08	1.00	1.00	-0.07	0.99
3	5	-26	0.99	0.04	1.00	1.00	-0.07	0.99

a R2 was always significant at the $P < 0.001$ level, except for SMN Soil 1 Manure 2 ($P = 0.004$) and SMN Soil 1 Manure 4 ($P = 0.043$).

Figure 1.3a. Measured and simulated net CO_2 respiration for five liquid dairy manures incorporated in three soils of increasing clay content.

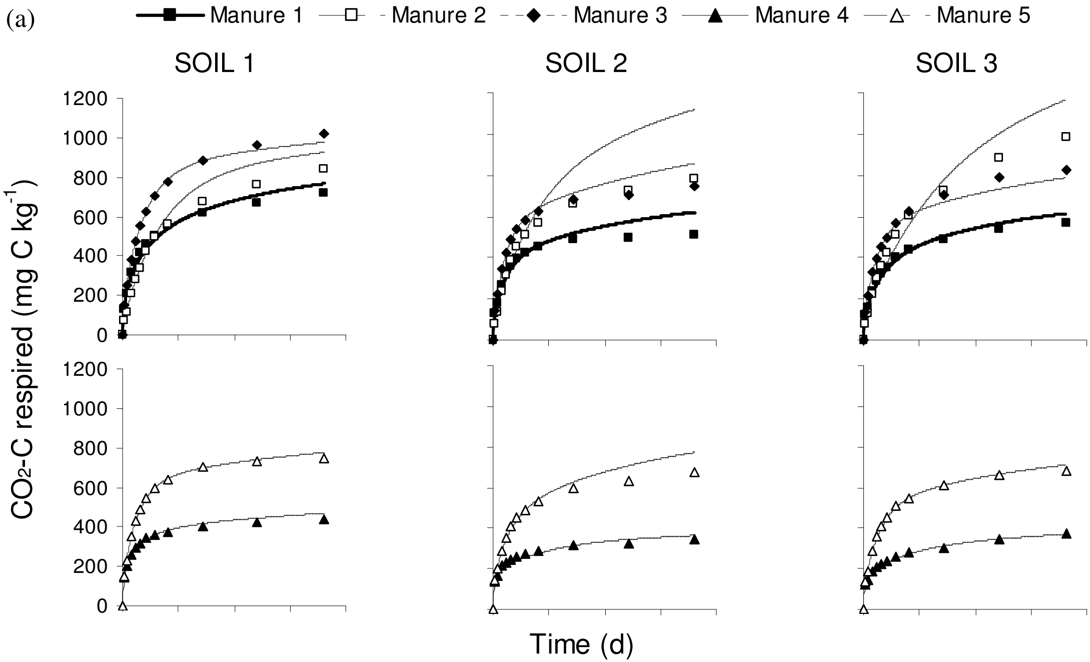
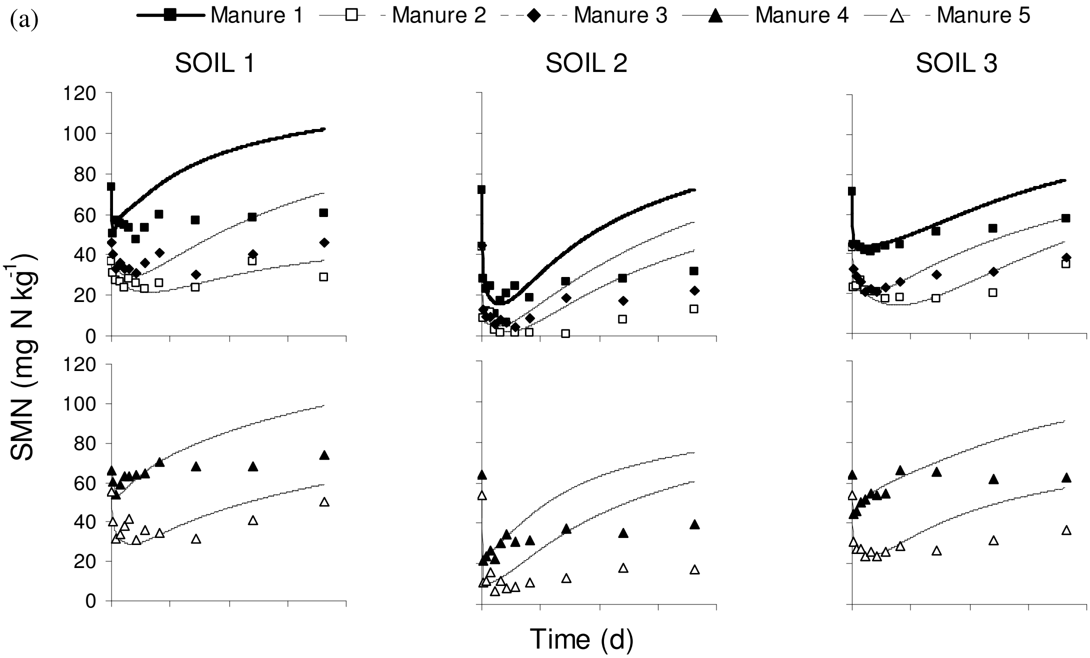


Figure 1.3b. Measured and simulated net soil mineral nitrogen (SMN) for five liquid dairy manures incorporated in three soils of increasing clay content.



Regressions among model parameters and manure properties

We have found good correlations between measured manure properties and two model parameters (C_{AOM1} and CN_{AOM1}). Carbon content of the AOM_1 pool (C_{AOM1}) was significantly ($P < 0.05$) correlated with the ratio NH_4/C ($R^2 = 0.92$, 0.91 and 0.96 for soil 1, 2 and 3, respectively). CN_{AOM1} was significantly ($P < 0.05$) correlated with the ratio manure organic C / manure organic N ($R^2 = 0.94$, 0.98 and 0.97 for soil 1, 2 and 3, respectively). The other manure parameters (C_{AOM2}, N_{AOM2}, C_{NOM}, k_{AOM1i}, k_{AOM2i}) were not correlated with any manure property.

Discussion

Sensitivity analysis

Compared to the local approaches for SA based on changing one parameter at a time while keeping the others constant (Cariboni et al., 2007), the advantage of the methods used here (Morris and Sobol') is that sensitivity is explored under different combinations of parameters, thus showing the contribution of each parameter, alone and in interaction with the others. Nonetheless, it should be remembered that the results of each SA are specific for the well-defined temporal scale and range of parameters of the system explored, and for the output variables studied. Therefore, they cannot be extended to other situations. The results of SA depend on the random generation of the input space. In the case of the Morris method, the reduced number of EEs (r = 10) could limit the exploration of the input space. Previous comparisons between the Morris method and the more computer-intensive method of Sobol' (Campolongo et al., 2007) confirmed the agreement between the two methods in the identification of non-important model parameters. Nonetheless, to increase the exploration of the input space with the Morris method, we repeated this analysis three times, starting from different seeds for random number generation. The results obtained in the three series did not show substantial differences (data not shown). Sensitivity analysis was carried out on the net effects, i.e. on the state variables simulated for the manured treatments minus the simulated variable for the untreated control treatment. As a result the most sensitive parameters were those describing the manure and, for the soil, some of those that are related to pools with higher turnover rates, i.e. microbial biomass and their residues. The analysis with the method of Sobol' (Fig. 1.2a) has shown that some parameters (f_{AOM2}, k_{AOM2i}, e_{SMB}) had more important effects on CO_2 respiration in the initial period than later. This was due to their effect on the allocation of the added manure-C to the labile pool, and its subsequent mineralisation. Microbial efficiency was more important in the initial period due to the larger presence of mineralisable C. The effect of f_{AOM2} did not nullify after Day 41, because it impacts the sizes of both pools (labile and resistant). Two parameters (k_{AOM1i} and f_{NOM*}) had an increasing effect over time, due to the relatively higher importance of the slower pools as simulation continued. The parameters CN_{SMR}, CN_{SMB1}, k_{SMR} and t_{SMB1} had an effect on SMN (Fig. 1.2b) but not on CO_2 respiration (Fig. 1.2a), due to the fact that SA was carried out assuming ample (i.e. non-limiting) SMN availability. Therefore, parameters related to N dynamics had no effect on C dynamics because immobilisation requirements by microbial biomass were always satisfied, and therefore N did not limit manure decomposition. Model parameters did have an impact on simulated SMN concentrations, which explains the sensitivity of SMN to CN_{SMR}, CN_{SMB1}, k_{SMR} and t_{SMB1}. To explore the sensitivity in a range of conditions similar to those of the experiment, we carried out four SA per each model parameter, resulting from two manure C/N ratios and two soil clay contents (Fig. 1.2a and 1.2b). Each set of manure parameters must satisfy the condition that the sum of C allocated to manure pools must equal the amount of manure C applied ($C_{AOM1} + C_{AOM2} + C_{NOM}$ = manure C). The same must be true also for organic N ($N_{AOM1} + N_{AOM2} + N_{NOM}$ = manure organic N). Our shell was built to guarantee that these conditions were satisfied. The value of CN_{AOM2} was allowed to range between 15 and 100; therefore excess N was available when the manure had a C/N < 15; this excess N was then allocated to the AOM_1 pool, which therefore had a low and relatively stable C/N ratio (4.2 – 6.3; data not shown in tables). Therefore, if high values of f_{AOM2} and high values of CN_{AOM2} occur, the shell provides a low CN_{AOM1}. As a consequence, a high sensitivity for the output variable SMN was found to f_{AOM2}. Moreover, a low CN_{AOM1} had the consequence of increasing the mineralisable SMN (and thus the sensitivity to k_{AOM1i}) when manure C/N was low. This might be considered a bias of our analysis, but appeared as a necessary step to avoid unrealistic parameter combinations to be submitted to SA. The higher sensitivity of simulated SMN found at high manure C/N ratios for e_{SMB}, CN_{SMB1}, CN_{SMR} and k_{SMR} can be explained by recalling that: i) these parameters have a strong effect on N request by the receiving pools (SMB1, SMR) and thus they impact N immobilisation/mineralisation; ii) this effect is enhanced when N concentration of added manure is lower (i.e. the C/N ratio is higher). The lower sensitivity of SMN at low clay content for CN_{SMB1}, e_{SMB} and t_{SMB1} can be explained by recalling that clay reduces microbial mortality (Petersen et al., 2005a): thus, the SMB1 pool decays more slowly in a clay soil, and the parameters affecting its N dynamics are therefore less important at low clay. A similar effect was also found for the sensitivity of CO_2 respiration to e_{SMB}.

Parameter optimisation

We initially tried to obtain for each manure a unique set of parameters that could be applied on the three soils. This approach was justified by the fact that CN-SIM describes each manure with manure-specific pools and parameters, while the interactions of the manure with the soil are developed during the simulation. With this kind of optimisation we expected to achieve a unique manure parameterisation, independent of soil, describing C and N partitioning in manure pools and their decomposition constants, and to demonstrate that the interactions of the manure with the soil can be completely explained through the model. As mentioned above, this attempt was unsuccessful (Table 1.4). We therefore reduced our objective to test at least the capability of the model to reproduce single manure × soil combinations, thus loosing generality because manure parameters became soil-specific. Moreover, the calibrated values of manure parameters incorporated information about the interaction of the manure with the soil. Therefore we do not recommend using these calibrated parameters on, for example, a fourth soil.

One important issue of our parameter optimisation is that in all the simulations the optimal solution included values for one or more parameters that were very close or equal to the imposed minimum or maximum boundary, as it can be verified by comparing the optimisation results in Table 1.5 with boundaries in Table 1.3. This indicates that the optimisation algorithm has tried to find the optimal solution outside the allowed parameter space, thus underlying a structural problem of adapting the model to this data set. The parameters that were most frequently subjected to this problem were k_{AOM2i}, CN_{SMR}, CN_{SMB1}, k_{SMR}, and f_{AOM2}. The reasons for this effect will be clarified below.

The good agreement (Fig. 1.3; Table 1.6a) between the simulated and observed dynamics of CO_2 respiration (characterised by an initial rapid phase of C mineralisation, and a successive slower phase) was obtained by the optimisation algorithm by partitioning the added C input into three pools (AOM_1, AOM_2 and NOM) with different decomposition constants (Table 1.5). The values of these parameters were consistent with the observed dynamics: the fraction of manure-C allocated to the faster AOM_2 pool (f_{AOM2})

varied between 0.07 (manure 2 on soil 1, characterised by a low respiration rate) to 0.66 (manure 4 on soil 2, high respiration rate); the decomposition constant k_{AOM2i} varied between 0.225 d^{-1} and 0.420 d^{-1} (the upper boundary); k_{AOM1i} varied between 0.0008 d^{-1} (manure 4 on soil 2) to 0.0253 d^{-1} (manure 3 on soil 3). The optimisation algorithm has forced the model to simulate the microbial immobilisation of mineral N, because it was the only possibility to match the reduction of SMN observed in the experiment in the initial period (0 – 28 d in most cases). The experimental data show that this immobilisation started immediately after the addition of manure (Fig. 1.3). In the model the immobilisation was obtained by assigning a high C/N ratio to the AOM_2 pool; further immobilisation was eventually obtained with a high C/N ratio of AOM_1. After the first 30 – 40 d, simulated remineralisation occurred, due to the decomposition of the pools with k of about 10^{-2} – 10^{-3} d^{-1} (AOM_1, SMR, and SMB1). Remineralisation affected the simulated SMN, which always increased much above the measured values, thus affecting the overall model performance for SMN (Fig. 1.3; Table 1.6b). Despite its negative impact on the adequacy of SMN simulation, this parameterisation was necessary to guarantee the correct simulation of CO_2 respiration in the period 40 – 181 d. To keep the simulated N mineralisation as close as possible to measurements, the optimisation algorithm increased the storage of organic N in the soil (by decreasing k_{SMR} and increasing f_{NOM})

and increased the request of N by SMB_1 and SMR. The set of optimised model parameters is therefore a compromise between the simulation of the observed C and N fluxes, which are strictly interdependent in the model. The model and the parameter values shown in Table 1.5 are thus more appropriate to simulate the short-term dynamics of C and N mineralisation (0 – 41 d), and less appropriate to simulate the processes occurring after Day 41. Similar model parameters and simulation performance are found in the results presented by Petersen et al. (2005b, experiment F): the parameters k_{AOM1i}, k_{AOM2i}, f_{AOM2} and f_{AOM2N} for fresh and stored cattle slurry have the same order of magnitude as ours; moreover, there was a general overestimation of CO_2 respiration (in particular after 35 d) and, similarly to our case, of SMN. The poor model fit for SMN suggests that additional sinks of soil mineral N should be considered in the model, namely denitrified N and clay-fixed ammonium. These fluxes can be relevant in manure-amended soils, with up to 30% of total applied N being denitrified (Calderón et al., 2004) and up to 46% of applied NH_4–N being clay fixed in a clay soil (Chantigny et al., 2004). With the present model version, a decrease in SMN can only be obtained via immobilisation of mineral N in organic pools (SMB_1, SMB_2, SMR and NOM). If denitrification and clay fixation would be implemented in the model, the observed decrease of SMN in the first few weeks of incubation would not be matched only by forcing microbial immobilisation, but also by simulating denitrification and clay fixation. This, in turn, would avoid the excessive SMN concentration (due to remineralisation of immobilised N) after Day 41. Moreover, the fixation and release of ammonium in clay minerals is independent of CO_2 respiration. Therefore, the net simulated C and N fluxes, being less interdependent, might be simulated better. It is worth mentioning that CN-SIM was incorporated in the FASSET model (Chatskikh et al., 2005), where denitrification is simulated. On the contrary, to our knowledge no SOM mechanistic models exist to simulate clay fixation and release of ammonium.

One last comment should be made about how the chosen level of model complexity may influence its ability to represent reality. Models developed for practical applications, like CN-SIM and other SOM models, are relatively simplified, because the soil is inherently complex and difficult to fully reproduce with mathematical equations. For example, not many SOM models represent the soil as a three-dimensional structure. Attempts made in this direction (e.g. Masse et al., 2007) show that the detailed description of soil porosity and interactions between organic and inorganic fractions increase the number of parameters, the overall model complexity and the experimental data needed to support its development and calibration. Moreover, most SOM models (as CN-SIM) use the same pool structure to represent added organic materials that widely differ in composition and decomposition pattern (e.g. crop residues vs. liquid animal manure). This simplifies model structure, but is likely to generate less accurate results.

Conclusions

Sensitivity analysis indicated that all manure parameters (f_{AOM2}, f_{AOM2N}, f_{NOM}, k_{AOM1i} and k_{AOM2i}) and some soil parameters (CN_{SMR}, CN_{SMB1}, k_{SMR}, t_{SMB1} and e_{SMB}) have a substantial effect on simulated net CO_2 respiration and N mineralisation. These parameters have a clear biophysical meaning, and are effectively used by the model to reproduce most of the patterns observed in the decomposition experiment, both initially (rapid CO_2 respiration associated with a decrease of SMN) and later (lower mineralisation rates). However, there was a marked overestimation of SMN after Day 41, because it was impossible to correctly simulate C and N dynamics. Even if the experimental dataset used in this study provides only measurements of CO_2 respiration and SMN, we can make the hypothesis that the processes currently implemented in the model are not sufficient for a complete interpretation of incubation experiments with liquid dairy manures. It is possible that after integrating in CN-SIM the simulation of denitrification and clay fixation the overestimation of SMN might be corrected, at least in part.

CHAPTER 2 - Multi-objective optimization of the simulation of manure mineralisation in soil: insights into model structure.

Introduction

The general aim of optimization is to find the best feasible solution (or solutions) for a problem given a set of limitations (or constraints) (Coello, 2006); for this purpose, optimisation algorithms are used to automatically find such solution corresponding to the extreme (minimum or maximum) value of an objective function or to trade-off values of two or more objective functions in the case of two or more conflicting objectives (Vrugt and Robinson, 2007).

The successful application of simulation models (Duan et al., 1992; Acutis and Confalonieri, 2006) depends on how well the model has been calibrated. Therefore, in many optimization problems of ecological model parameterization, the task of optimization is to obtain a set (or sets) of model parameters able to reproduce the measured dynamics.

When the model output of interest is one, the optimization problem is called single-objective optimization problem, because only one objective function has to be minimized (Deb, 2004). In this type of problems the goal of optimization is to find the best feasible solution (the globally optimal solution) or at least a good approximation of it (Coello, 2006).

When the outputs of interest are more than one (as it happens for most model parameterizations), the optimization problem is called multi-objective optimization problem (Deb, 2004). Often it happens that the different objectives are conflicting one with the other (Vrugt and Robinson, 2007). Therefore, a good fit of one model output may determine a poor fit of the others and vice versa.

A first approach to multi-objective optimization problems, called preference-based multi-objective optimization (Deb, 2004), consists in aggregating, using various techniques, the different objectives of interest into a single objective function, reducing the problem again to a single-objective optimization problem, and to perform optimization using algorithms designed to solve single-objective optimization problems. In this case additional information (higher-level information) is used to aggregate the different objectives into the objective function before optimization is performed.

A second approach consists in obtaining at the end of each optimization run a set of solutions, thus producing a trade-off among the different objectives (Coello, 2006; Vrugt et al., 2003; Wöhling et al., 2008). Thereafter, the user can choose one of these solutions (his/her best solution) using additional information to guide the choice (Deb, 2004). As pointed out by Deb (2004), the second approach is desirable because it is less subjective, using additional information only to select one of the solutions and not to guide the search. The opposite happens in the preference-based strategy, resulting in a high sensitivity to the aggregation of objective functions. Moreover, with the second approach the trade-offs among the different objectives enable the researcher to better understand if the current model structure represents satisfactorily the system for which it was designed, or if it needs improvements (Vrugt et al., 2003; Wöhling et al., 2008).

The conclusions reported in Chapter 1 highlighted that the parameterisation of the CN-SIM model (Petersen et al, 2005a) to simulate manure C and N mineralization in a period of 180 days under laboratory conditions lead to a marked overestimation of soil mineral nitrogen (SMN) concentration after the first six weeks of incubation, due to the remineralization of initially immobilized N. As model parameters had been calibrated for the simultaneous simulation of C and N mineralization, SMN overestimation arose for two main reasons: 1) microbial immobilisation (nitrogen organication) was the only possibility for the model to match the experimental decrease of SMN; 2) to maintain a simulated C respiration consistent with measurements, nitrogen in the organic pools was consequentially mineralized. For this reason it had been hypothesised that the processes currently implemented in the model were not sufficient for a complete interpretation of incubation experiments with liquid dairy manures, and that other processes should be taken into account for a better synchronous simulation of C and N dynamics. Based on this conclusion, the objective of the work described here was to parameterize the CN-SIM model using a multi-objective optimization algorithm (NSGA-II, Deb et al., 2002) in order to: 1) identify trade-offs between errors in the simulation of CO_2–C and SMN, in order to evaluate the degree of compromise for the good simulation of C and N mineralization; 2) understand if it is necessary to include other processes in the model, as previously hypothesized using a single-objective approach for model optimization.

Materials and Methods

Multi-objective optimization

Multi-objective optimization (or multi-criteria optimization or vector optimization) can be defined as the problem of finding "*a vector of decision variables which satisfies constraints and optimizes a vector function whose elements represent the objective functions. These functions form a mathematical description of performance criteria which are usually in conflict with each other. Hence, the term "optimize" means finding such a solution which would give the values of all the objective functions acceptable to the designer.*" (Coello, 2006).

The problem includes a set of n decision variables (called "parameters" when applying the technique to a simulation model), a set of k objective functions, and a set of m constraints. Objective functions and constraints are functions of the decision variables.

The following definitions can be found in Zitzler (2004) and Deb (2004). The task of optimization is to:

minimize (or maximize) $y = f(x) = f(f_1(x), f_2(x), \ldots, f_k(x))$

subject to:

$$e(x) = (e_1(x), e_2(x), \ldots, e_m(x)) \le 0$$
$$x = (x_1, x_2, \ldots, x_n) \in X$$
$$y = (y_1, y_2, \ldots, y_k) \in Y$$

where x is the decision vector, y is the objective vector, X is the n-dimensional decision space and Y is the k-dimensional objective space. The equality/inequality constraints $e(x) \leq 0$ (among which there are parameter boundaries) determinate the set of feasible solutions X_f (i.e. the set of solutions that satisfy the constraints) and the feasible region in the objective space Y_f, the image of X_f in Y. When X_f is equal to X, or Y_f is equal to Y, the problem is said to be unconstrained in the decision space or in the objective space, respectively. In single-objective optimization, the aim of optimization is to find one solution (or more solutions in the case of multi-modal objective functions, where more global optima can be identified) in the feasible space X_f giving the minimum value of the objective function f. Solutions are compared each other according to the following rule: a solution x_1 is better than a solution x_2 if $f\ (x_1) < f\ (x_2)$. This criterion used to compare solutions cannot be fully applied when the objectives are more than one; for example a solution x_1 can be better than a solution x_2 with respect to one objective, but can be worst with respect to another. This happens because when objectives are more than one the feasible objective space (Y_f) is usually partially ordered (Zitlzer, 1999), due to conflicting objectives; in this case three relationships can occur between two objective vectors u and v:

$$u = v \ \ if \ \ \forall\, i \in \{1,2,...k\} : u_i = v_i$$

$$u \leq v \ \ if \ \ \forall\, i \in \{1,2,...k\} : u_i \leq v_i$$

$$u < v \ \ if \ \ u \leq v \ \wedge \ u \neq v$$

The three previous relations lead to the definition of the Pareto Dominance (also called Edgeworth-Pareto Dominance, Coello 2006). Between two decision vectors *a* and *b*:

$$a \prec b \ \ (a \text{ dominates } b) \ \ if \ \ f(a) < f(b)$$

$$a \preceq b \ \ (a \text{ weakly dominates } b) \ \ if \ \ f(a) \leq f(b)$$

$$a \approx b \ \ (a \text{ is indifferent to } b) \ \ if \ \ f(a) \text{ is not} \leq f(b)$$
$$\text{and } f(b) \text{ is not} \leq f(a)$$

A decision vector $x \in X_f$ is said to be non-dominated regarding a set $A \subseteq X_f$ if it does not exist any solution (decision vector) $a \in A$ such that $a \prec x$. The decision vector *x* is said to be Pareto-optimal if *x* is non-dominated regarding X_f. This means that *x* is Pareto-optimal if there exists no feasible vector of decision variables which would improve one objective without causing a simultaneous degeneration in at least one of the others (Coello, 2006).

The entirety of all Pareto-optimal solutions is called the Pareto-optimal set (X_p); the corresponding objective vectors form the Pareto-optimal front (Y_p). Also in multi-objective optimization, as it occurs in single-objective optimization problems, there may be local optima which constitute a non-dominated set of solution in a certain neighborhood (Zitzler, 2004, Deb, 2004); it is therefore desirable that an optimization algorithm designed to solve multi-objective optimization problems is able to find the globally Pareto-optimal front or a good approximation of it throughout a good exploration of the decision variables and the objective spaces. As users of multi-objective optimization algorithms are interested in the evaluation of the trade-offs among objectives and in the choice of one or more compromise solutions from the obtained Pareto set, it is also desirable that the solutions are well distributed along the set. These two last aspects are the main goals of a multi-objective optimization (Deb, 2004, Konak et al, 2006).

For this reason specific optimization algorithms have been designed to handle multi-objectives and to provide, in a single optimization run, a good and uniform approximation of the Pareto-optimal front, using the concept of Pareto dominance to select trade-off solutions (Fig. 2.1).

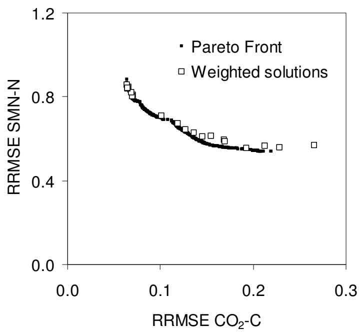


Figure 2.1. Example of trade-off solutions (white empty quadrates) obtained with different weighting vectors using a single-objective optimization algorithm (the Downhill simplex; Nelder and Mead, 1965) and the estimated Pareto front obtained with a multi-objective genetic

algorithm (NSGA-II; Deb, 2002). The two objectives are the Relative Root Mean Squared Error between simulated and measured CO_2–C and SMN; simulations were performed using the CN-SIM model (Petersen et al, 2005a).

NSGA-II algorithm

Among the optimization algorithms specifically designed to solve multi-objective optimization problems, evolutionary algorithms (EAs) have proved to be very efficient and powerful when multiple conflicting objectives exist (Vrugt and Robinson, 2007). EAs are a class of stochastic optimization methods based on the emulation of natural evolution of species (Zitzler, 2004): from a population of N individuals (solutions) some are selected for reproduction to generate (through crossing over) offspring solutions; mutation is then applied to the new individuals. The application of genetic operators (selection, crossover and mutation) guarantees that the evolving population is progressively improved across generations. As EAs work with a population of individuals, in multi-objective optimization they are specifically designed to preserve trade-off solutions instead of converging to a single best one. In order to uniformly explore the Pareto front (or Pareto set), other operators are applied to individuals during evolution.

The NSGA-II algorithm (Nondominated Sorting Genetic Algorithm II) is an evolutionary algorithm designed to solve multi-objective optimization problems developed by Deb in 2002; the choice of this algorithm was supported by the fact that its ability to locate solutions that are well distributed along Pareto sets of known optimization test problems was demonstrated in several studies (for example Deb, 2004).

NSGA-II is an improved version of a previously implemented genetic algorithm (NSGA, Srinivas and Deb, 1994). Compared to NSGA, in the new version the following features were added to improve the location of the globally Pareto-optimal set and to provide a better distribution of the solutions along the Pareto set, avoiding convergence into restricted regions: 1) an elitism-preserving mechanism is introduced, ensuring that the best solutions found are always preserved across generations; 2) a fast non dominated sorting algorithm is used to rank solutions according to non-domination (thus reducing the complexity of the algorithm from $O(MN^3)$ to $O(MN^2)$, where M is the number of objectives and N is the population size); 3) it does not require additional user-defined parameters (σ_{share} used in the NSGA algorithm) to obtain a good distribution of the solutions along the Pareto front; 4) the new version of the algorithm can handle both binary and real parameters representations.

The different steps that form an optimization run with NSGA-II are the following (Deb et. al, 2002):

1. A parent population P of N individuals is created and each individual is randomly initialized. Each individual consists of

a vector of decision variables (model parameters) and a vector of objective values (calculated with the model based on the decision vector).

2. The initial population is sorted according to non-domination and each solution is assigned a fitness (a rank index) equal to its non-domination level (solutions are compared using the sum of constraints violated and the previously described operator "$\prec$"); the set of solutions having the same non-domination level (rank) belongs to a different Pareto front (F_1, F_2, …, F_l).

During each generation the following operations are performed step by step, until a user pre-defined maximum number of generations is reached:

3. A binary tournament selection is recursively applied to the *P* population in order to select *N* pairs of parental solutions; each selected couple of parental solutions is crossed to generate a couple of children solutions; thereafter, the *N* children solutions are mutated and evaluated. At the end of this step a child population *Q* of size *N* is created.
4. A new population *R* of size 2*N* is formed by the union of *P* and *Q*, allowing parent solutions to be compared with the children solutions; in this way elitism is guaranteed (this means that the best solution found is always preserved across generations).

5. The recombined population R is sorted according to non-domination and again different Pareto fronts (F_1, F_2, …, F_l) are found. The new parent population P (at the following generation) is formed by adding solutions from the non-dominated fronts until the number of added individuals exceeds N. Individuals of each front are used to calculate the crowding distance (the distance between one solution an its neighbors solutions). The solutions of the last accepted front (F_i) are not all included into the P population (as the size exceeds N) but are sorted according to a crowded comparison criterion and only a certain number of solutions belonging to F_i are copied into P until the parental population is completely filled. Since the diversity among the solutions is important, with the crowded comparison criterion solutions are selected according to their crowding distance and the solutions far from the others are preferred (that is with a high value of the crowding distance); this helps the algorithm to spread solutions along the full extent of the Pareto front.

The original C++ code of the NSGA-II algorithm is available for download from the web site www.iitk.ac.in/kangal/codes.shtml.

Application of NSGA-II to parameter optimization for the simulation of manure mineralisation

We used the CN-SIM model in the same way and with the same experimental data as already described in Chapter 1. Briefly, each simulation (i.e. each model run during the optimization procedure) started with steady-state conditions for all soil organic pools (AOM_1, AOM_2, SMB_1, SMB_2, SMR and NOM). Soil temperature and soil water content were fixed at 25°C and -0.05 MPa, as in the incubation experiment.

The list of parameters subjected to optimization were the same that was optimized with the downhill simplex method (i.e. CN_{SMB1}, t_{SMB1}, e_{SMB}, k_{SMR}, CN_{SMR}, f_{AOM2}, f_{AOM2N}, f_{NOM}, k_{AOM1i} *and* k_{AOM2i}; see Table 1.3); parameter boundaries and the value of the other model parameters (parameters not subjected to optimization) were those reported in Table 1.3.

The multi-objective optimization problem was constructed by defining a vector of two objective functions: 1) the Relative Root Mean Squared Error between simulated and measured CO_2–C (RRMSE CO_2–C) and 2) the RRMSE between simulated and measured SMN (RRMSE SMN). Both RRMSEs were calculated on rates using net results (i.e.by subtracting the CO_2–C or SMN of the unamended soil from the manured soil). Inequality constraint functions were only applied to model parameters (corresponding to parameters boundaries); when parameter values fell outside boundaries or when infeasible solutions arose, due to inconsistent model parameter values, the values of both objective functions of the individual were set to 10^{10}, ensuring that the solution (individual) did not reproduce across generations.

A first parameter optimization was carried out for each manure on the three soils simultaneously, using the same set of manure parameters (f_{AOM2}, f_{AOM2N}, f_{NOM}, k_{AOM1i} *and* k_{AOM2i}) on the different soils. A second optimization was performed for each for the 15 soil × manure treatments separately, thus allowing the set of optimized parameters to be soil- and manure- dependent. For each optimized treatment, together with the approximated Pareto-optimal front and set, the objective functions and parameters value of the following solutions we also reported: 1) the solution having the lowest RRMSE CO_2–C (SOL1); 2) the solution having the lowest RRMSE SMN (SOL2); 3) the solution having the lowest average RRMSE between RRMSE CO_2–C and RRMSE SMN (SOL3). This last solution (SOL3) was compared to that previously obtained using the downhill simplex (Chapter 1).

Results

The results of the multi-objective optimization with NSGA-II are reported in Fig. 2.2–2.11. For each of the 15 soil × manure treatments the Pareto front approximated by the algorithm at the last generation is reported in Fig. 2.2a-2.11a and the value of model parameters for the solutions in the Pareto-set are reported in Fig. 2.2b-2.11b.

The Pareto fronts found by the algorithm clearly showed that the two selected objectives (RRMSE CO_2–C and RRMSE SMN) are in conflict each other. This was demonstrated by the shape of the fronts, with the two extreme solutions SOL1 (having the lowest RRMSE CO_2–C) and SOL2 (with the lowest RRMSE SMN) located in two different regions of the objective space, and the other trade-off solutions distributed between them (Fig. 2.2a-2.11a).

In a small number of cases the Pareto fronts are located in a relatively restricted area of the space, probably due to a progressive convergence of the different non-dominated solutions across generations; however this was not the common case, indicating that the algorithm was effectively able to satisfactorily explore the objective space by preferring solutions far from the others.

Most of the non-dominated fronts (e.g. manure 2 on soil 1) were shorter along the abscissa axis (RRMSE SMN) and more extended, with a quasi-null slope, along the ordinates axis (RRMSE CO_2–C). This means that: i) many equivalent parameter combinations (solutions) exist, giving similar values of RRMSE SMN at different values of RRMSE CO_2–C; ii) a slight improvement in the simulation of SMN lead to a strong deterioration in the simulation of CO_2–C and iii) no significant improvements can be achieved in the simulation of SMN under a threshold of RRMSE SMN found quite far from SOL2 (the one having the lowest RRMSE SMN). According to Pareto dominance, all of these solutions are non-dominated each other but, in many cases, the improvement achieved in the SMN simulation can be considered inconsistent.

In few treatments (e.g. manure 1 on soils 2 and 3), the two objectives presented a clear rectangular trade-off pattern; in these cases, one "best" compromise solution is represented by the solution located in the bottom-left corner of the front, having both objective functions values close to the minimum.

The best RRMSEs for SMN (SOL2) ranged from 35 to 97% (on average 73%) for the treatments optimized on the three soils simultaneously (Fig. 2.2a-2.6a), and from 20 to 85% (on average 41%) when each manure × soil combination was optimized separately (Fig. 2.7a-2.11a). The RRMSE CO_2–C (SOL1) were in the range 5 – 11% (average of 8%) and 2 – 11 (average of 6%) when manure treatments were optimized on the three soils or separately on each soil, respectively. Also the RRMSEs of the chosen compromise solution SOL3 (best average between the two objective functions), were higher when manures were optimized on the three soils simultaneously (from 30 − 88, on average of 50%) compared to those obtained with separate manure × soil optimizations (from 12 – 87%, on average 34%).

These results showed that the simultaneous calibration of model parameters for the same manure on the three soils lead to lower model performances with respect to both objectives; in particular for SMN, better fits can be achieved when model parameters are manure- and soil-dependent.

Fig. 2.2b-2.11b report the Pareto sets corresponding to the non-dominated solutions belonging to the Pareto fronts found by the NSGA-II algorithm. Different trends in model parameters estimations can be identified. In most of the optimized treatments (e.g. manure 1 on soil 3), the Pareto sets were characterized by solutions having very different combinations of parameter values, indicating that the algorithm explored many areas of the parameters space and that equivalent (non-dominated) solutions in the objective space can be achieved with widely different parameter combinations. This is in agreement with the rather uniformity of the solutions along the Pareto fronts. In most of the Pareto sets, extreme solutions (SOL1 and SOL2) showed a clearly different pattern of calibrated parameter values, often in contrast each other; this is clearly evident when bifurcations occurr in Fig. 2.2b-2.6b and 2.7b-2.11b, indicating that the best simulation of one objective requires a very different solution compared to another objectve. Despite a general uniform distribution of the solutions in the Pareto sets, in some treatments (e.g.manure 2 on soil 2) the solutions converged, for some parameters, around the same values. When manure parameters were optimized for the simultaneous simulation of CO_2–C and SMN on the three soils, this occurred mostly for parameters CN_{SMB1} of soils 1 and 2, CN_{SMR} of soil 2 and k_{AOM2i} (Fig. 2.2b-2.6b). When each manure × soil combination was optimized separately, solutions mostly converged in the values of k_{SMR}, CN_{SMR}, f_{AOM2N} and k_{AOM2i} (Fig. 2.7b-2.11b). An extreme example is represented by the treatment manure 4 × soil 1 (Fig. 2.10b), where all solutions converged in the few areas of the parameters space and the observed variability was mainly due to variations in the value of f_{NOM}. Looking to the obtained Pareto sets, it is also evident that in many cases the values of the parameters were close to the imposed boundaries, indicating that even lower values of the objective functions could be likely achieved if parameters were free to vary in a larger range.

The optimized parameter values obtained for SOL3 (the solution with the best average RRMSE between CO_2–C and SMN), only when each manure was optimized separately on the three soils, were compared with those obtained with a single-objective model parameterization performed using the downhill simplex. The objective of this comparison was not to decide which optimization algorithm performed better, but to understand if selecting a prior or a posterior compromise solution gives similar results in term of objective functions and model parameter values, and, as a consequence, similar model performances. Fig. 2.12 compares the average objective function (RRMSE) and calibrated model parameter values obtained with the two methodologies. The two procedures gave rather similar average RRMSE, with lower values when optimization was performed with the NSGA-II algorithm. For e_{SMB} and f_{AOM2} there was a clear agreement between the values estimated using the two procedures. As previously pointed out, some Pareto fronts showed a typical rectangular shape; in these cases (manure 1 on soil 3, manure 3 on soil 3, and manure 5 on soil 3), the estimated parameter values obtained with the NSGA-II and downhill simplex are closer each other. This is due to the location of the compromise solution SOL3 in the proximity of the bottom-left corner of the Pareto front, corresponding to the best average solution with respect to the two objectives.

Figure 2.2. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #1 on three soils simultaneously. Each solution is represented by a line connecting parameters, indicating, for each model parameter, its optimized value (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

(a) Estimated Pareto front

(b) Estimated Pareto set

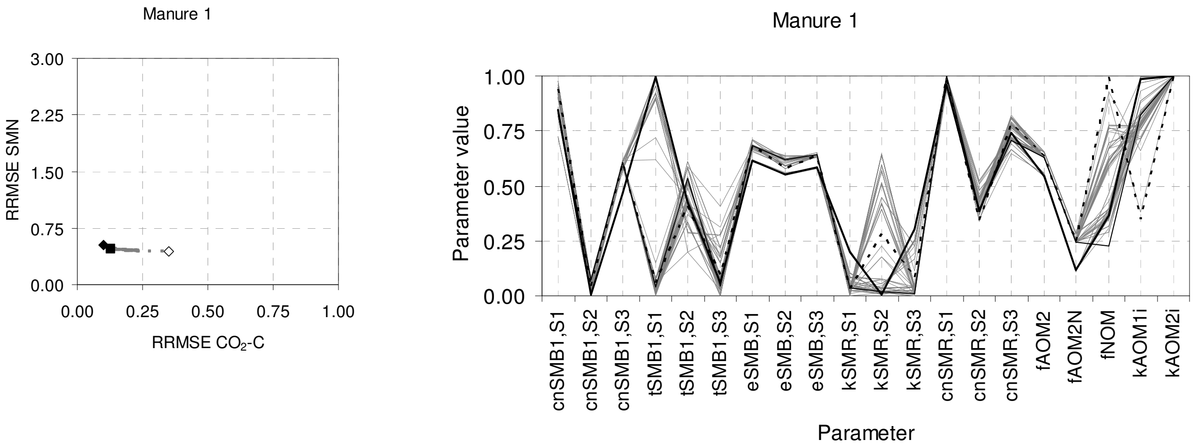
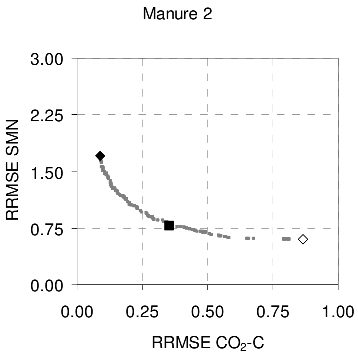


Figure 2.3. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #2 on three soils simultaneously. Each solution is represented by a line connecting parameters, indicating, for each model parameter, its optimized value (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

(a) Estimated Pareto front



(b) Estimated Pareto set

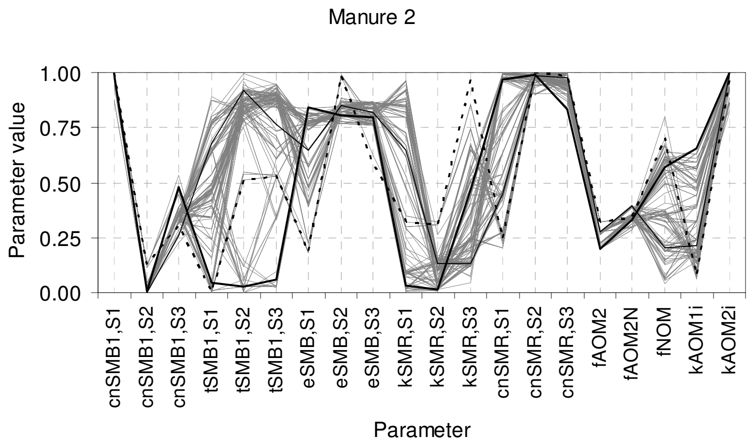
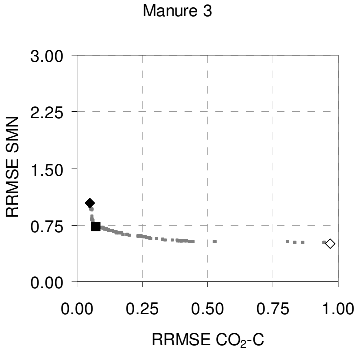


Figure 2.4. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #3 on three soils simultaneously. Each solution is represented by a line connecting parameters, indicating, for each model parameter, its optimized value (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

(a) Estimated Pareto front



(b) Estimated Pareto set

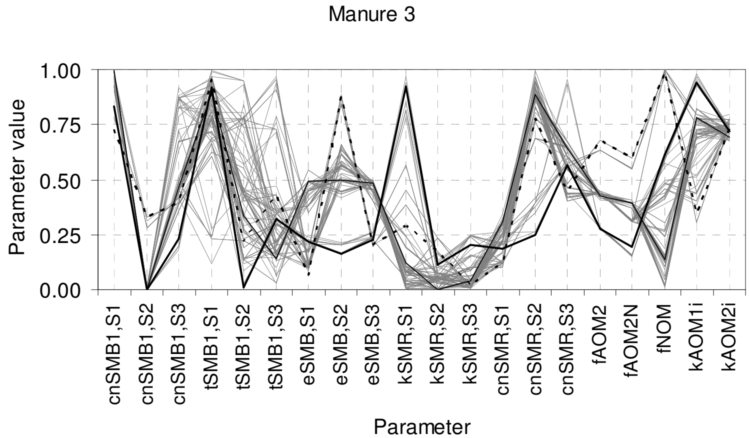


Figure 2.5. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #4 on three soils simultaneously. Each solution is represented by a line connecting parameters, indicating, for each model parameter, its optimized value (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

(a) Estimated Pareto front (b) Estimated Pareto set

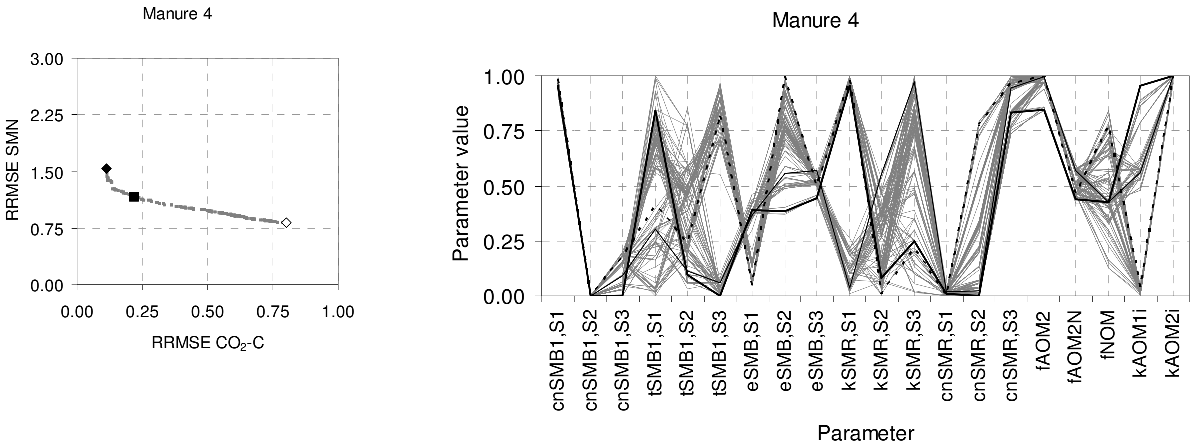


Figure 2.6. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #5 on three soils simultaneously. Each solution is represented by a line connecting parameters, indicating, for each model parameter, its optimized value (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

(a) Estimated Pareto front

(b) Estimated Pareto set

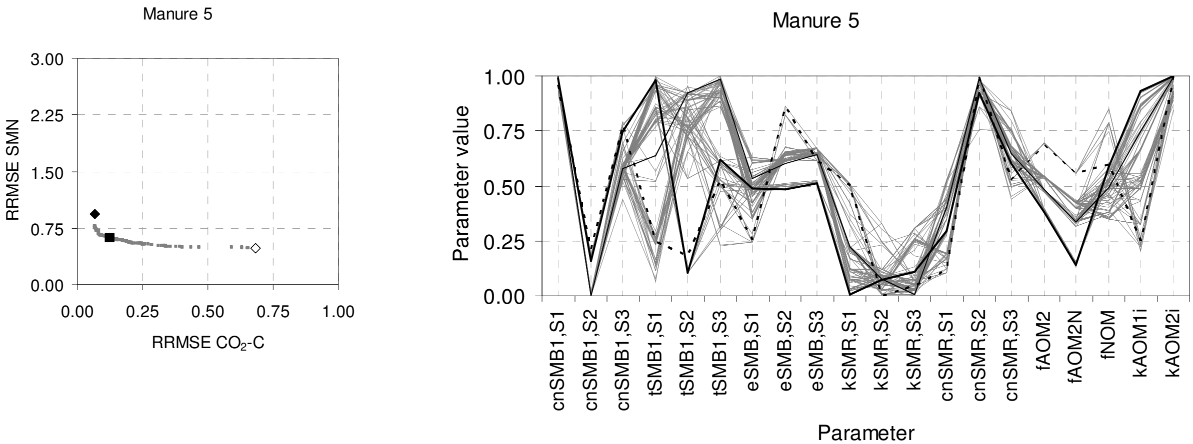


Figure 2.7. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #1 on three soils separately. Each solution is represented by a line connecting parameters, (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

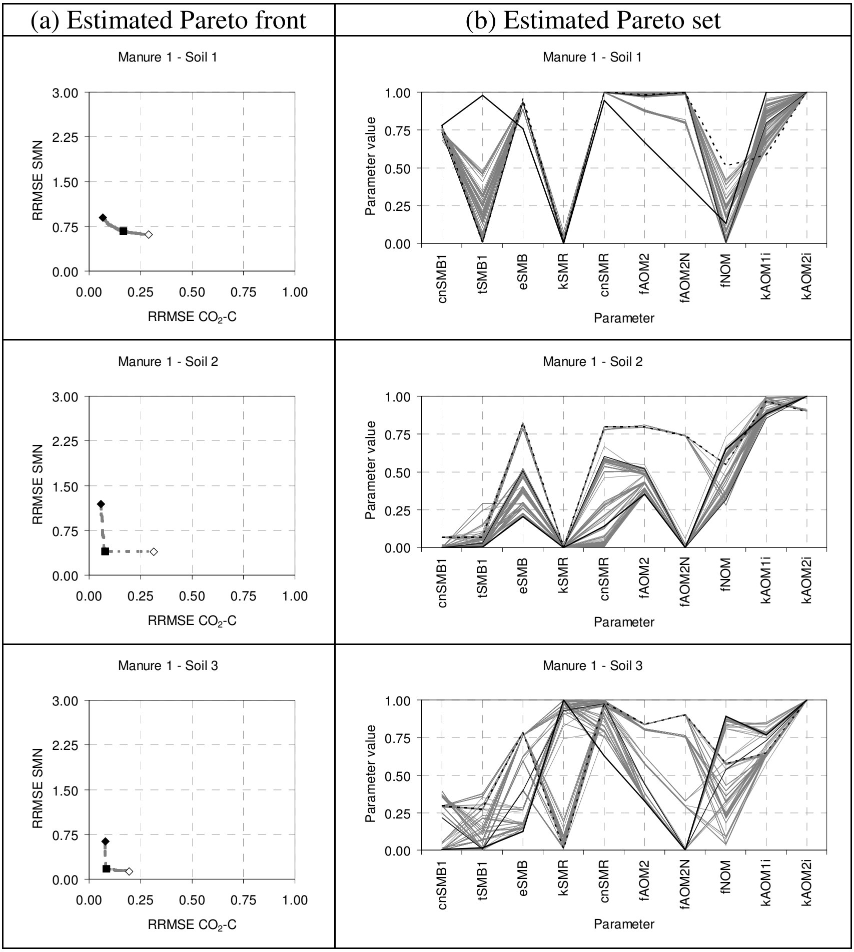


Figure 2.8. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #2 on three soils separately. Each solution is represented by a line connecting parameters, (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

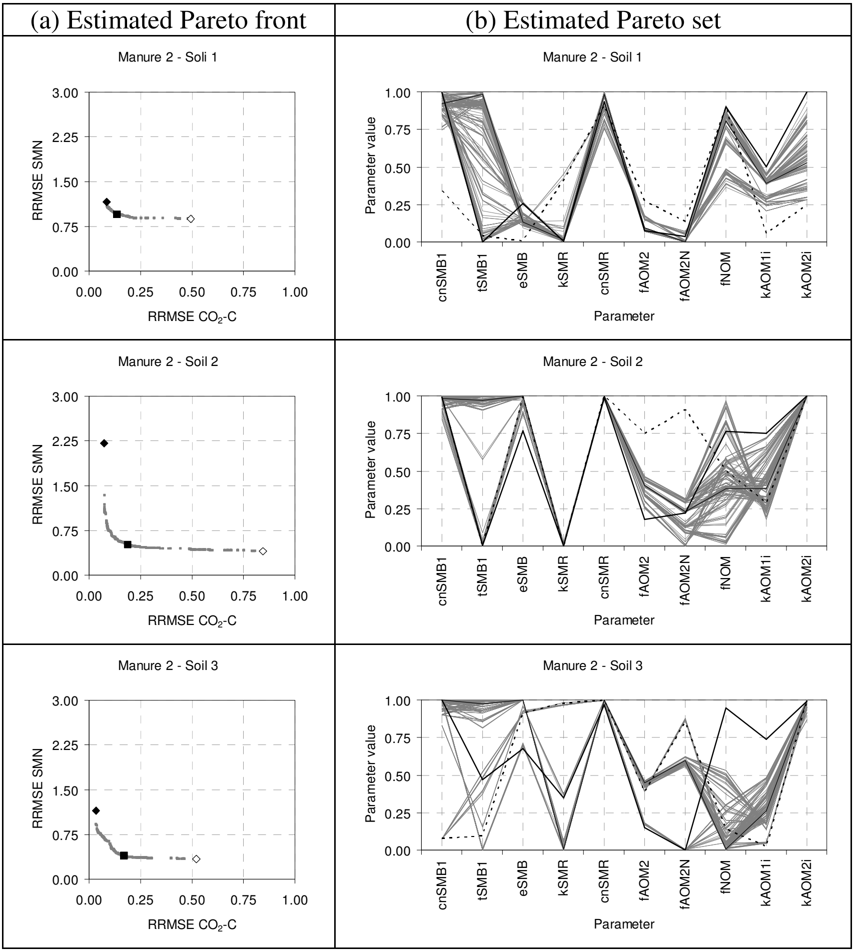


Figure 2.9. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #3 on three soils separately. Each solution is represented by a line connecting parameters, (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

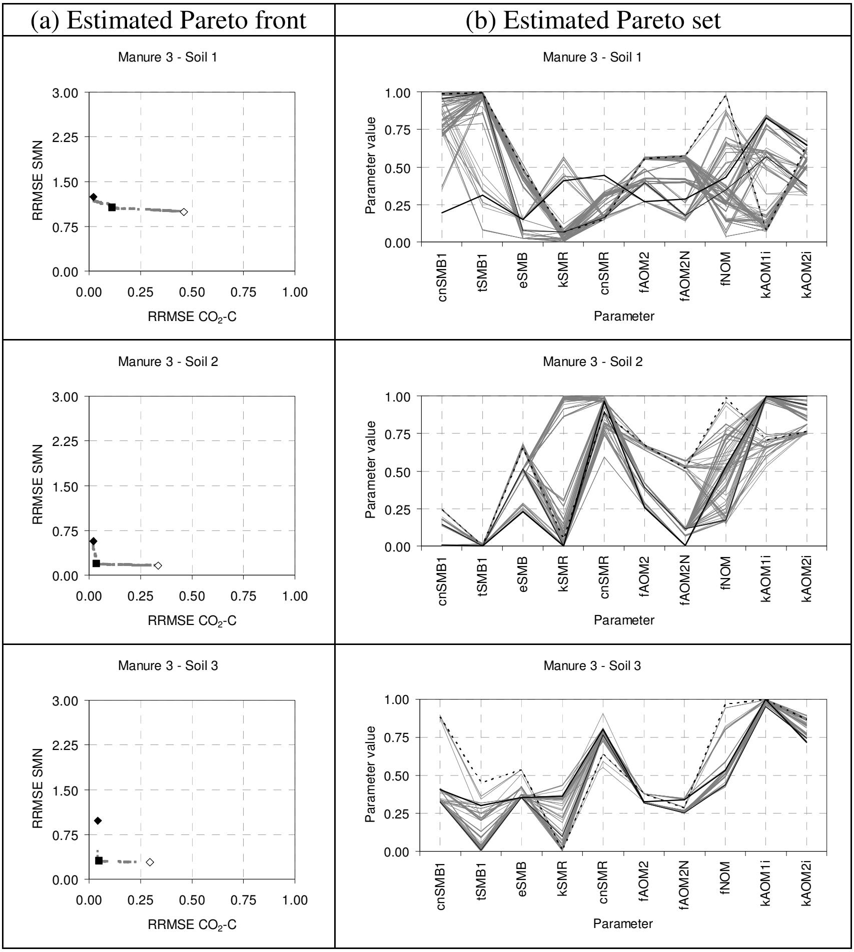


Figure 2.10. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #4 on three soils separately. Each solution is represented by a line connecting parameters, (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

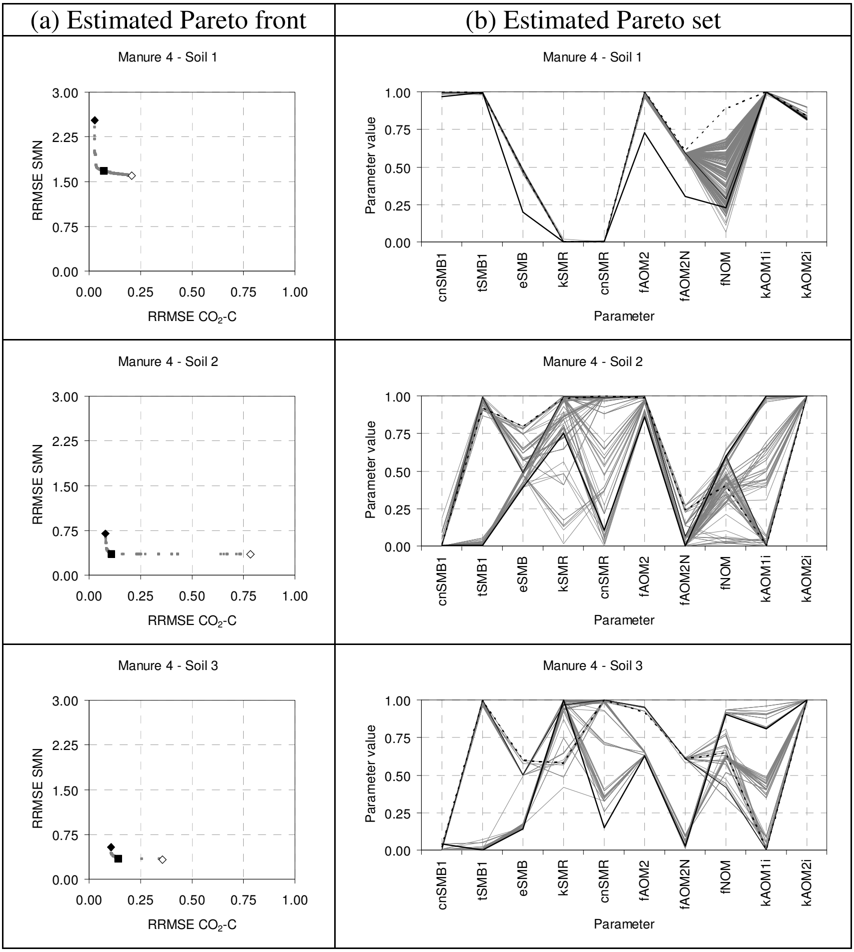


Figure 2.11. Estimated Pareto optimal front and Pareto optimal set for the simulation of Manure #5 on three soils separately. Each solution is represented by a line connecting parameters, (parameters values were normalized in the range 0 – 1; refer to Table 1.3 for the range of variation of parameters).

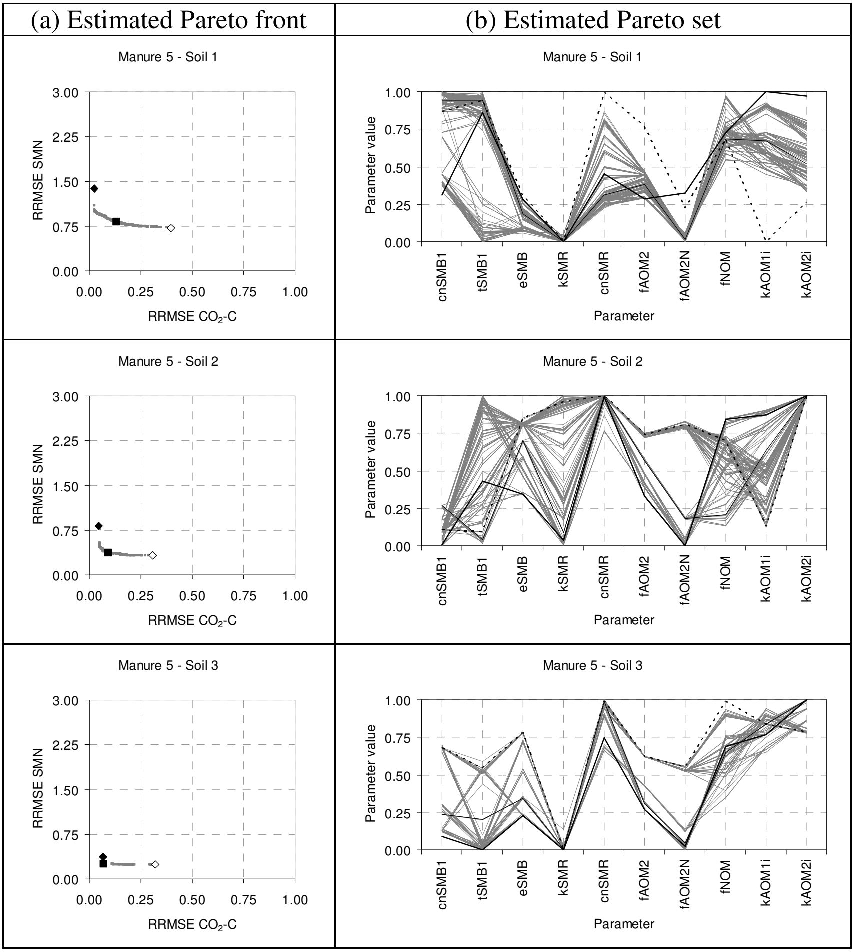
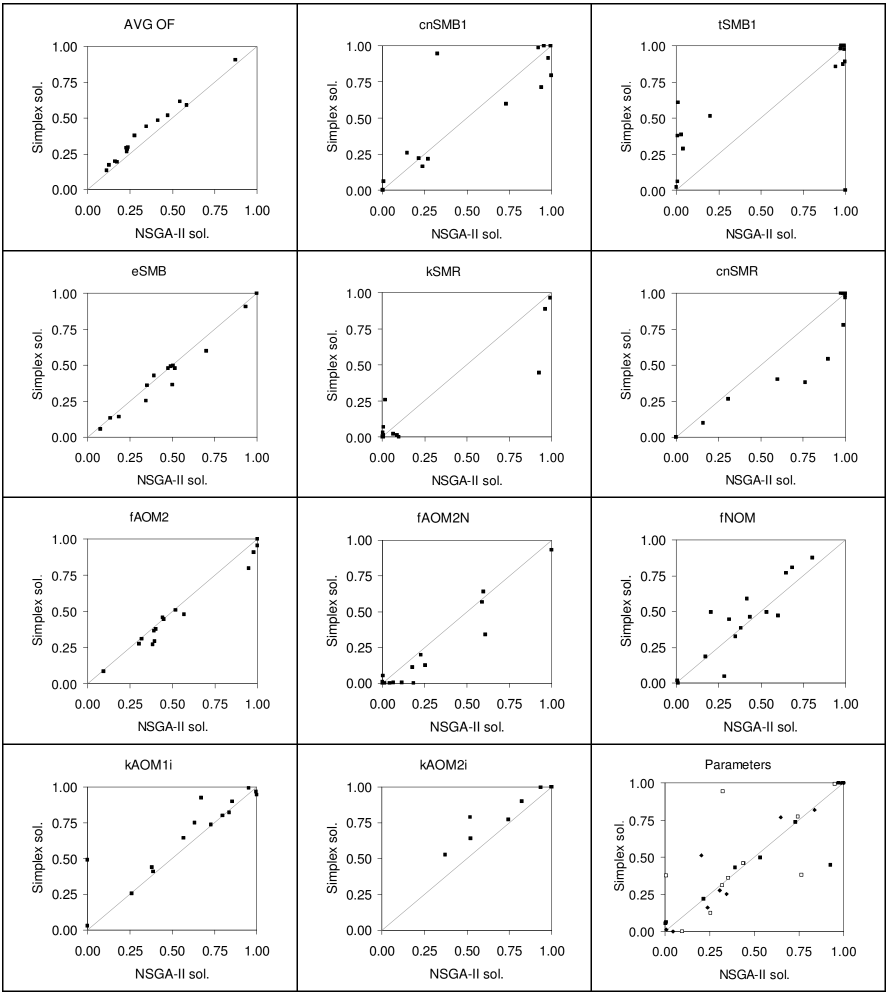


Figure 2.12. Comparison between optimized model parameter of SOL. 3 found with the NSGA-II algorithm and the best solution found with the downhill simplex method (Chapter 1), for the 5 manures on 3 soils. The last chart on the right bottom corner refers to parameters of Man.1-Soil 1, Man.3-Soil 3 and Man.5-Soil 3.



Discussion

The multi-objective approach used to perform the parameterization of the CN-SIM model enabled a better understanding of the trade-offs between the two objectives compared to the use of the downhill simplex. All the Pareto fronts show that the two objectives of interest (RRMSE CO_2–C and RRMSE SMN) are in conflict each other (Fig. 2.2a-2.11a). Solutions belonging to the Pareto fronts were rather well distributed between the two extreme solutions (SOL1 and SOL2). As demonstrated in many test cases (Deb 2004) solutions far from the others are preferentially selected during generations, allowing for a better exploration of the objective space.

The values of RRMSE CO_2–C were always lower compared to those of RRMSE SMN, showing that C respiration is simulated better than N dynamics with the current structure of the model. It is also evident that often a lower limit for RRMSE SMN occurs; this means that no parameter combinations can lead to an improvement in the simulation of SMN below a certain threshold. This can be partially due to the chosen parameter boundaries but could also be attributed to the current model structure that maybe does not implement all the processes needed for the correct joint simulation of C and N mineralization dynamics.

The ranges of variation of model parameters were chosen in order to be consistent with their bio-physical meaning. Should these values had been left free to vary in an unrealistic range, we would have hindered the model to be a useful instrument for understanding the processes of soil organic matter decomposition into the soil.

Better model performances (i.e. lower values of RRMSEs) were obtained when parameter calibration was performed separately for each soil × manure combination. RRMSEs values of SOL3 (the chosen compromise solution having the best average RRMSE between CO_2–C and SMN) ranged from 12 to 87% (on average 34%); higher values (in the range 30 – 88%, with an average of 50%) were obtained for SOL3 when optimizations were performed for the same manure on the three soils simultaneously. Lower performances in the second case were expected because the same set of optimized manure parameters were used on the three soil simultaneously. Also in this second case lower model performances can be attributed to the previously hypothesized causes (restricted parameters boundaries and lack in the implementation of some processes into the model) together with the higher number of model parameters subjected to calibration, that surely lead to a more difficult search both in the parameters and objective spaces.

These difficulties in model parameterization are in agreement with the results previously obtained using the downhill simplex (Chapter 1).

It is evident from the obtained Pareto sets that a model parameterization for the sole simulation of CO_2–C or SMN (SOL1 or SOL2) lead to a completely different estimation of model parameter values (Fig. 2.2a-2.11a and 2.2b-2.11b). Different calibrated parameters values were obtained for the compromise solution SOL3 and for the non-dominated solutions, indicating that a unique best combination of model parameters does not exist. Some non-dominated solutions belonging to the Pareto sets of different manure × soil combinations converged to the same restricted areas of the decision variables space (i.e. had the same parameter values); this convergence was mostly evident for the k_{AOM2i} parameter, which, in more than half of the treatments, reached a value close to its upper boundary. In order to gain an improvement in both the objectives (in fact all solutions converge to this value), the algorithm explored the area closed to the imposed parameter upper boundary; to understand why this was the only way to improve model performances, the same consideration previously done can be extended here. Moreover, as previously observed (Chapter 1), high k_{AOM2i} values arose because it was the only possibility for the model to simulate a C respiration and a decrease of SMN concentration as closed as possible to the experimentally measured CO_2–C and SMN. It is possible that other processes involving mineral nitrogen transformation into the soil (for example ammonium clay fixation) would improve model performances, enabling a sequestration of SMN without incorporation into the soil microbial biomass and other organic pools.

Conclusions

The results of the optimization indicated that, with the model and the data set used in this study for the simulation of C and N mineralisation of liquid dairy manures, the two objective functions (RRMSE CO_2–C and RRMSE SMN) are in conflict each other and consequently a single best solution does not exist. We clearly demonstrated that a good simulation of C respiration determines a poor fitting of SMN and vice versa, and that a unique solution allowing the joint simulation of C and N dynamics cannot be found. This conclusion is based on these results: 1) in many treatments the parameters reached the imposed boundaries and 2) under a certain threshold of RRMSE SMN, no improvements in the simulation of SMN could be achieved. These results confirmed those previously obtained with a different optimization methodology (the downhill simplex), and suggest that the current model structure can be improved by implementing the simulation of other N transformation in the soil (fixation of ammonium in the inter-layers of clay minerals; denitrification).

The multi-objective approach adopted in this study, apart from the optimized parameters values provided, demonstrated to be useful to get insights into the model behavior; many optimized parameter values giving different equivalent solutions indicated the limitations of the model structure in terms of system representation and that further improvements are necessary to avoid parameter values close to the boundaries of their allowed range of variation.

CHAPTER 3 - Incubation of two different manures on two soils of contrasting texture: effect of repeated manure applications on carbon and nitrogen dynamics in the soil.

Introduction

Repeated applications of animal manure to agricultural soils contribute not only to the short term fertility but determine also the residual effect, i.e. higher crop N availability in manured compared to unmanured soils. An improved understanding of the chemical and biological processes of the residual effect would help to improve nutrient management plans. After manure addition to soil, a decrease of soil mineral N is observed in the short term in the manured compared to the unfertilised control treatment, probably due to microbial immobilisation (e.g. Sørensen, 1998a; Bechini and Marino, 2009). Moreover, studies conducted using ^{15}N suggest that part of this immobilized N is stored in the soil in organic form, at least for a few years after manure addition (Sørensen, 2004). However a detailed analysis of N sinks under repeated manure applications has yet to be undertaken. Therefore, it is not clear what the role is of microbial immobilisation, clay fixation, denitrification and ammonia volatilisation in determining the observed soil mineral N dynamics after repeated manure additions. Moreover, it is not clear how different manure types (due to different decomposability of the organic fraction) and different soils (due to different clay content which may impact on microbial turnover and clay fixation) will interact with repeated manure applications.

The process of organic matter accumulation as a result of repeated manure applications has been studied in several field experiments (e.g. Schröder et al., 2005 and 2007). However, in field experiments it is very difficult and expensive to study in detail the flows of N in different compartments, due to the contemporary processes of crop N uptake, N loss, and mineralisation of native and added organic matter. Laboratory experiments permit the measurement of the net N mineralisation of manures, without the confounding effect of other inputs or outputs, and the study of the fate of added N in different compartments (e.g. Sørensen, 1998a; Van Kessel and Reeves, 2002; Bechini and Marino, 2009).

The aim of the experiment described here is to study in the laboratory, under constant conditions of soil temperature and water content, the dynamics of C and N following the repeated application of two different liquid dairy manures to two agricultural soils of contrasting texture.

Materials and methods

In the incubation experiment, 32 treatments were established, obtained as the full combination of two soils, four materials (two liquid dairy manures, and two controls: ammonium sulphate and water) and four applications (every 84 days).

Soils

In order to select the two soils needed for the incubation experiment, five candidates soils were obtained from the Pioneer Hi-Bred Italia soils database (40000 samples) using the following criterions: very different clay content (4% and 30%), pH < 6.8 (to minimise ammonia volatilisation), and similar C and N concentration and C/N ratio (to minimise the differences between control treatments). We excluded soils that had received organic fertilisers during the last 10 years, to minimize C and N mineralisation from control treatments. These soils, belonging to cereal farms in northern Italy, were resampled and reanalysed during summer 2009. Samples were collected in the 5–30 cm layer of the soil profile, air dried and visible roots residues were removed before sieving at 2 mm.

Analyses were performed according to the Italian official methods for chemical analyses of soil (Italian Ministry for Agriculture and Forestry Policy, 2000). Soil pH was potentiometrically determined in a 1:2.5 soil/water suspension using a Crison GLP 21+ pH-meter (Crison S.A, Spain). Particle-size classes (sand, silt, and clay concentrations) were determined by dispersing the samples with sodium hexametaphosfate and following determination with the pipette method. Organic C and total N were determined by dry combustion using a ThermoQuest NA1500 elemental analyzer (Carlo Erba, Milano, Italy). The gravimetric soil water content at the potential of -0.05 MPa was determined by a Richard apparatus (Soil Moisture Equipment Corp., Santa Barbara, CA) according to Italian official methods for physical analyses of soils (Italian Ministry for Agriculture and Forestry Policy, 1997). The mineralogical analyses were carried out on the bulk sample and the <2 μm fraction of the clay loam soil by X-ray diffraction (XRD), using a Philips PW 1810/30 diffractometer and Cu Kα radiation. The <2 μm fraction of the soil was collected from the first 5 cm of a soil-water suspension (30 g of soil:1 l of distilled water) after 4 hours of sedimentation. This analysis was carried out by Prof. Massimo Setti (Dip. Di Scienze della Terra, University of Pavia, Italy). Composition of the two selected soils are reported in Table 3.1.

Table 3.1. Characteristic of the two soils used in the incubation experiment.

Variable	Unit	Sandy loam soil	Clay loam soil
Sand	%	67	45
Silt	%	29	25
Clay	%	4	30
Total Carbon	%	1.33	1.16
Total Nitrogen	%	0.14	0.14
CEC	$cmol^+$ 100 g^{-1}	0.65	2.52
Exc. Ca	mg Ca kg^{-1}	340	2954
Exc. K	mg K kg^{-1}	53	110
Exc. Mg	mg Mg kg^{-1}	56	356
P (Olsen)	mg P kg^{-1}	--	31
pH	--	6.7	6.8
Mineralogical analysis			
Bulk sample			
Plagioclase	%	--	12
K-Feldspar	%	--	6
Quartz	%	--	21
Chlorite	%	--	18
Kaolinite	%	--	12
Mica/Illite	%	--	15
Smectite	%	--	16
Clay fraction (<2µm)			
Plagioclase	%	--	4
K-Feldspar	%	--	3
Quartz	%	--	12
Chlorite	%	--	19
Kaolinite	%	--	10
Mica/Illite	%	--	16
Smectite	%	--	36

Applied materials

In the incubation experiment four types of materials were added to the soils: 1) water (CON); 2) a solution of ammonium sulphate 1400 mg N l^{-1} (AS); 3) a heifer manure (HEI) and 4) a dairy cow manure (COW). Our objective during the selection of the manures was to obtain two very different materials. Manures were sampled looking mostly on farms adopting different animal diets, with different milk productions and different housing systems. The two manures were selected within a group of eight: four of them were sampled in commercial farms, according to the previous mentioned criteria, while four were experimentally obtained by collecting and mixing faeces and urine of 20 cows undergoing a feeding experiment that included two milk production levels (16–30 kg milk $animal^{-1}$) and two starch/protein ratios (1.1–1.6 g starch g $protein^{-1}$). The mix urine–faeces was added of water corresponding to 20% of the total weight of the mix; moreover, an aliquot (0.42 ml kg $manure^{-1}$) of the supernatant of a centrifuged dairy cow manure was added to the mix as inoculum (Sørensen, 1998a). Because of different manure ages at the time of sampling, every collected manure was stored in tank until reaching 90 days of storage. After this period manures were homogenised using a Ultra Turrax T-25 disperser (IKA Werke GmbH & Co. KG, Germany) and stored in plastic bottles at -20°C until they were analysed for chemical composition. Dry matter was determined by oven drying at 85°C according to Sørensen (1998a), and ash content by muffle combustion at 550°C for 4 hours. Manure pH was potentiometrically determined by a Crison GLP 21+ pH-meter (Crison S.A, Spain). Total N was determined according to the Kjeldahl method, using a Büchi 426 digestion unit and a Büchi 315 distillation unit (BÜCHI Labortechnik AG, Switzerland). Ammonium concentration was determined by steam distillation of fresh manure in presence of excessive MgO and subsequent titration using the same distillation unit used for total Kjeldahl N determination (Clesceri et al., 1998). Total C was determined on oven-dried samples (85°C) by the dry combustion method using a ThermoQuest NA1500 elemental analyzer (Carlo Erba, Milano, Italy). To determine water soluble C and N, manures were diluted to 1% (w/w) DM content, agitated for 1 h by a rotating shaker, centrifuged at 1610 relative centrifugal force (RCF; 3000 rpm) for 5 min, and filtered through 0.22-µm pore size plastic filters. Carbon and N content of the extracts were then determined with the previously mentioned elemental analyzer. Volatile Fatty Acid (VFA–C) content was determinated using the method reported in (Sørensen, 1998a); analysis were carried out by Dot.ssa Rizzi (Laboratory of ARAL, Crema, Italy).

After storage all manures were subjected to a preliminary incubation of 11 days, during which CO_2 respiration was measured on Day 1, 2, 4, 8 and 11, while exchangeable soil mineral nitrogen (SMN) was measured on Day 0 and 11. The two manures with the two most contrasting behaviour during the preliminary incubation were then selected for the experiment. After the preliminary incubation, the two selected manures were kept frozen (-20°C) until the laboratory incubation begun.

Characteristics of the two selected manures are reported in Table 3.2.

Table 3.2. Characteristic of the two manures used in the incubation experiment.

Variable	Untit	Heifer manure	Dairy cow manure
DM	%	3.9	8.2
Ash	% DM	10	13
Total Carbon	g C kg^{-1}	13.9	34.9
Water solubile C	g C kg^{-1}	2.2	10.5
Volatile Fatty Acids	g C kg^{-1}	0.0	4.2
Total Nitrogen	g N kg^{-1}	1.3	3.9
NH_4^+	g N kg^{-1}	0.3	1.9
Soluble N	g N kg^{-1}	0.5	2.5
Na	g Na kg^{-1}	0.1	0.5
Mg	g Mg kg^{-1}	0.2	0.6
K	g K kg^{-1}	2.5	1.9
Ca	g Ca kg^{-1}	0.9	1.8
pH	--	8.8	8.0

Incubation experiment

The incubation experiment was conducted according to the "nursery method" reported by Thuriès et al. (2000) which requires to set up sets of experimental units as numerous as the number of destructive sampling dates. As a consequences of 11, 8, 7 and 10 destructive samplings, respectively after the first, the second, the third and the fourth materials application, 35 experimental sets were prepared. Each set consisted of 24 experimental units, as a combination of 2 soils × 4 materials × 3 replications, arranged following a completely randomized experimental design. Totally, 840 experimental units were prepared. Each experimental unit was obtained by mixing proper doses of the different materials to an amount of moistened and pre-incubated soil equivalent to 100 g of dry weight. If necessary, water was added to reach a final soil water potential of -50 kPa. Experimental units that received materials more than once (i.e. the sets of experimental units belonging to applications 2, 3 and 4), were partially air dried for about 3–5 days (in an oven at 20°C) before materials were applied again on the same experimental unit; this was necessary in order to guarantee a constant soil water potential of -50 kPa during subsequent application events and so to avoid excessive water addition to the soil.

Mixing was performed by hand, with the help of a spatula, on a plastic sheet and when the mix appeared homogeneous it was transferred into a 250 ml large-neck plastic bottle for incubation.

Doses of manures and ammonium sulphate (applied as a solution containing 1400 mg N l^{-1}) were calculated to give an addition of 100 mg N kg^{-1} of dry soil, corresponding to a fertilisation of about 390 kg N ha^{-1} (calculated on the base of a soil layer deep of 0.3 m and a bulk density of 1.2 t m^{-3}). During application events the bottles containing the manures were kept in an ice bath and stirred during sampling in order to avoid sedimentation and to guarantee uniform materials application to the different experimental units.

During each application event, a sample of manures was collected at the beginning and at the end of the session and analyzed for total N and NH4 content, in order to check consistencies with original analysis performed before the incubation begun.

Before the first application, a 1-week pre-incubation of remoistened bulk sieved soil was carried out to allow mineralisation of labile pools eventually present in the soil after desiccation and sieving.

Samplings and measurements

For each sampling date, 24 experimental units (2 soils × 4 materials × 3 replicates) were put in 3-liters sealed jars together with a plastic bottle containing 30 ml of water and a beaker containing 10 ml of 0.5 M NaOH to trap evolved CO_2 from the soil. At the end of the interval, measurements of respired C, soil mineral nitrogen concentration and microbial biomass C content were carried out analyzing the experimental units incubated in the jars. Respired CO_2 was measured by titration with 0.25 M HCl of residual NaOH in the trap, using phenolphtalein as indicator, following precipitation of carbonate with an excess of $BaCl_2$ (Stotzky, 1965). Soluble and exchangeable NH_4^+ ($NH_4^+{}_e$) and NO_3^- were extracted for 2 hours using a solution of 1 M KCl (extraction ratio 1:3), the suspension was filtered through Watman #2 filter paper (Whatman International Ltd, Maidstone, England) and stored at -20°C until analysis. Ammonium and nitrate concentrations in the soil extracts were determined by flow injection analysis and spectrometric detection (FIAstar 5000 Analyzer, Foss Tecator, Denmark). Analysis of NH_4^+ was done by the gas semi-permeable membrane method according to the ISO 11732 procedure (1997). Analysis of NO_3^- was done by the sulphanilamide-naphtylethylendiamine dihydrocloride method, after preliminary reduction of NO_3^- to NO_2^- by a copper-cadmium reductor column, according to the ISO 13395 procedure (1996). Microbial biomass C (SMB-C) content was estimated following the fumigation-extraction method. Carbon and nitrogen in both unfumigated and chloroform-fumigated soil samples were extracted for 30 minutes using a solution of 0.5 M K_2SO_4 (extraction ratio 1:2) and subsequent filtration through Watman #42 filter paper (Whatman International Ltd, Maidstone, England). Fumigation was carried out in the dark for 24 hours in a desiccation vacuum saturated with CH_3Cl; after this time, the beaker containing the fumigant was removed from the vacuum and chloroform was removed from samples with 6 cycles of aeration and desiccation. Determination of C concentration in the extracts was performed analysing dried extracts using a ThermoQuest NA1500 elemental analyzer (Carlo Erba, Milano, Italy). Dried extracts were obtained by oven-drying (35°C max) aliquots of 250 µl of K_2SO_4 extracts in tin capsules for three times. In order to ensure that no significantly amount of carbon was lost during the drying procedure, 18 different soil extracts were analyzed in triplicates after drying and as fresh samples (liquid injection) using an Elemental analyzer FLASH-2000 (Thermo Fisher). Analysis conducted either on liquid or dried samples gave very similar results ($R^2 = 0.97$), showing no consistent volatilization of C during sample desiccation, both in fumigated and unfumigated soil samples. The total amount of biomass carbon concentration was estimated by subtracting C concentration in fumigated samples from unfumigated and multiplying the difference by the correction factor of 2.64. Non-exchangeable NH_4^+ ($NH_4^+{}_f$) was determined following the method of Silva and Bremner (1966). Soil sample were oven-dried (25°C max) and sieved at 1 mm. Samples were treated with an alkaline potassium hypobromite solution (KOBr-KOH) to remove both exchangeable ammonium and organic N. Residues from this pretreatment were washed three times with 0.5 M KCl and shacked for 24 hours with an acid solution (5 M HF:1 M HCl) in order to decompose soil silicates. The ammonium concentration in the acid extract was then determinated by steam distillation and titration. Distillation was performed using a Büchi K–350 distillation unit

(BÜCHI Labortechnik AG, Switzerland) after preliminary alkalinisation of the extracts with 50 ml of a 32% NaOH solution. The distillate, collected in a beaker containing 10 ml of 4% H_3BO_3 solution and 90 ml of distilled water, was then titrated with HCl 0.005 M using a G20 Compaq Titrator (Mettler-Toledo AG, Analytical, Switzerland). Soil pH was potentiometrically determined in a 1:2.5 soil/water suspension using a Crison GLP 21+ pH-meter (Crison S.A, Spain).

Measurements of carbon respiration, exchangeable NH_4^+ and NO_3^- concentrations and soil pH were carried out in all 35 incubation intervals. SMB-C was measured on days 0, 1, 3, 15, 28 and 84 during application 1 and on days 0, 1, 3, 15 and 84 during application 4. Non-exchangeable ammonium concentration was measured on the clay loam soil on days 0, 1, 3, 6, 29, 60 and 84 during application 1, at the beginning (on day 0) and at the end of the incubation interval (day 84) during applications 2 and 3, and on days 0, 1, 6, 15, 29 and 84 during application 4.

Calculations and statistical analysis

All results are expressed on an oven dry bases (105°C for soils and 85°C for manures).

CO_2 respiration measured in each incubation interval was summed to obtain the accumulated respiration, or divided by the duration of each interval to calculate the rate of respiration. For each incubation interval, the net CO_2-C respiration of manure C was determined by subtracting the CO_2-C respiration of the control from the CO_2-C respiration of the amended soil (assuming no priming effect from the manure); these values were then expressed as a fraction of added manure C.

The residual effect of added manure C (CRE) of the i^{th} application, was calculated by subtracting, for each incubation interval, the cumulated respired carbon in the first application from the cumulated respired C of the i^{th} application.

On both soils, soil mineral nitrogen (SMN) concentration was calculated as the sum of exchangeable + soluble NH_4^+ ($NH_4^+{}_e$) and NO_3^-. On the clay loam soil, SMN was also calculated including the non-exchangeable fraction of the soil ammonium, in order to take into account for the fluxes into and out from -the clay fixed ammonium pool ($NH_4^+{}_f$).

Recovery of added NH_4^+ as exchangeable ammonium on day 0 was calculated as:

$$NH_4^+ \text{ rec}_{\text{app\_i}} = \frac{\left(NH4_{day=0}^{app\_i} - NH4_{day=84}^{app\_i-1}\right)_T - \left(NH4_{day=0}^{app\_i} - NH4_{day=84}^{app\_i-1}\right)_C}{NH4_{ADDED}^{app\_i}}$$

Where $NH4_{ADDED}^{app\_i}$ is the concentration of added ammonium during application *i*; $NH4_{day=0}^{app\_i}$ is the $NH_4^+{}_e$ concentration of the treated (*T*) or untreated (*C*) soil on day 0 of application *i*, $NH4_{day=84}^{app\_i-1}$ is the $NH_4^+{}_e$ concentration of the treated (*T*) or untreated (*C*) soil during application *i-1* (the previous application; during the first application $NH4_{day=84}^{app\_i-1}$ represents the initial ammonium concentration of the soil).

The recovery of added NH_4^+ as non-exchangeable ammonium on day 0 was calculated using the previous equation, substituting $NH_4^+{}_e$ concentrations with $NH_4^+{}_f$ concentrations.

Also the recovery of added N as SMN at the beginning of each incubation interval was calculated using the previous equation substituting ammonium concentrations of treated and untreated soil with SMN concentrations, and $NH4_{ADDED}^{app\_i}$ with $N_{ADDED}^{app\_i}$ (the total N applied during application *i*).

The recovery of added N at the end of each incubation interval was calculated using the following equation:

$$\text{N rec}_{\text{app\_i}} = \frac{\left(SMN_{day=84}^{app\_i} - SMN_{day=84}^{app\_i-1}\right)_T - \left(SMN_{day=0}^{app\_i} - SMN_{day=84}^{app\_i-1}\right)_C}{N_{ADDED}^{app\_i}}$$

Where $SMN_{day=84}^{app\_i}$ is the SMN concentration of the treated/untreated soil on day 84 of application *i* and $SMN_{day=84}^{app\_i-1}$ is the SMN concentration of the treated/untreated soil during application *i-1* (the previous application.

Net nitrogen mineralization (NNM) rates on day *j* was calculated as a percentage of added manure organic N using equation:

$$NNM_{\text{app\_i}} = \frac{\left\{ \begin{array}{l} \left[\left(SMN_{day=j}^{app\_i} - SMN_{day=84}^{app\_i-1}\right)_T - \left(SMN_{day=j}^{app\_i} - SMN_{day=84}^{app\_i-1}\right)_C\right] - \\ \left[\left(SMN_{day=j-1}^{app\_i} - SMN_{day=84}^{app\_i-1}\right)_T - \left(SMN_{day=j-1}^{app\_i} - SMN_{day=84}^{app\_i-1}\right)_C\right] \end{array} \right\}}{N_{ADDED}^{app\_i}}$$

and by dividing NNM_{app_i} by the duration of the interval (j-j_{-1}). expressed in days.

Residual effect of manure N (NRE) of the i^{th} application, was calculated subtracting, for each incubation interval, the SMN concentration of the first application from the SMN concentration of the i^{th} application.

ANOVA was carried out using a completely randomized design with one experimental factor and by conducting mean separation with Dunnet's T3 test (Day and Quinn, 1989), separately for each sampling date; this choice was necessary because variances were not homogeneous when evaluated on the entire data set (Levene test, $P <$ 0.05). The experimental factor used for ANOVA was obtained by recoding the 2 × 2 × 4 factor structure (2 soils × 2 manures × 4 application events) into 16 levels of a unique factor. SPSS 16.0.2 was used for ANOVA.

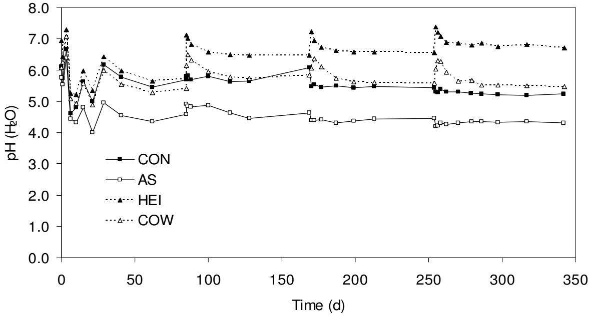
Results

Soil pH

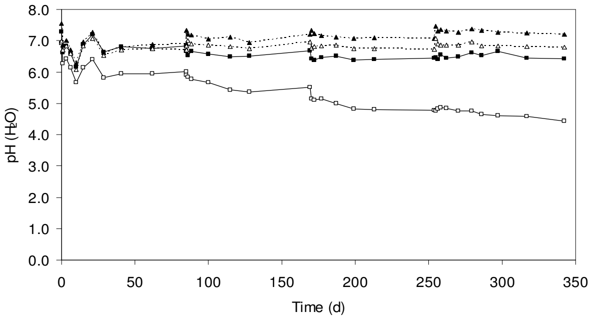
In all material × soil combinations, soil pH decreased from day 0 to day 84 during all application intervals (Fig. 3.1). In the unamended soil (CON treatment) soil pH at the beginning of the incubation was 6.1 and 7.3 on the sandy loam and clay loam soil, respectively; while on day 342, pH decreased to 5.2 and 6.4 on the two soils, respectively. In the ammonium sulphate treatment (AS), initial soil pH was similar to CON (5.8 and 6.9 on the sandy loam and the clay loam soils, respectively) but, on both soils, as the incubation continued, it decreased more than in CON. On the sandy loam soil pH measured on day 84 was 4.6 and decreased until 4.3 on day 342. On the clay loam soil, the final soil pH measured on day 342 was 4.4. In the manured soils pH increased immediately after manure applications and then decreased during each incubation interval (from day 0 to day 84). The initial increase of soil pH was more consistent in the heifer manure-amended soils (HEI) compared to the dairy cow manure-amended soils (COW); moreover it was more consistent on the sandy loam soil compared to the clay loam soil. At the end of incubation, on the sandy loam soil, pH reached a value of 6.7 and 5.5 in the HEI and COW treatments, respectively. On the clay loam soil, pH values measured on day 342 were 7.2 and 6.8 in HEI and COW, respectively.

Figure 3.1. Soil pH measured during the incubation experiment.

(a) Sandy loam soil



(b) Clay loam soil



Carbon dynamics

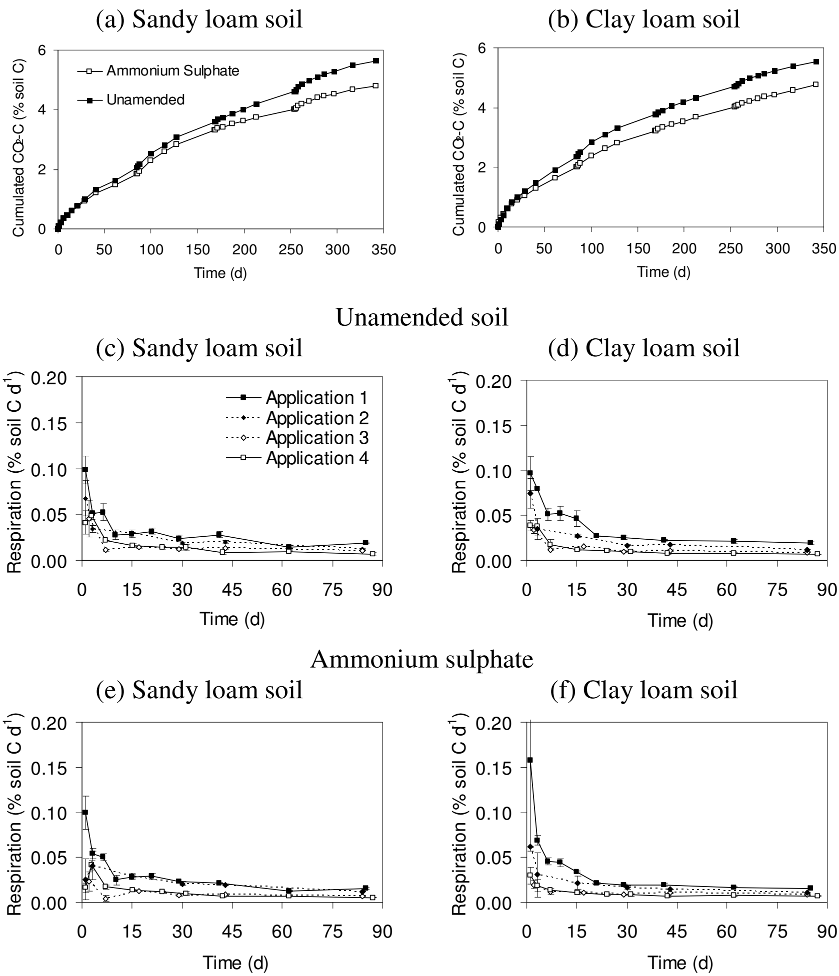
Control soil and ammonium sulphate-amended soil

Carbon respiration

On both soil types, accumulated C respiration of the two control treatments (CON and AS) was characterized by an initial phase with faster respiration rate after each application event (after days 0, 84, 168 and 250), followed by a second phase of slower and constant respiration rates until the subsequent application event. Accumulated CO_2 evolved from the unamended sandy loam soil (Fig. 3.2a) was 237, 415, 536 and 654 mg CO_2-C kg^{-1} after 84, 168, 250 and 342 days, respectively, corresponding to 2.0, 3.6, 4.6 and 5.6% of the initial (native) soil C content. Similar values were found on the unamended clay loam soil (Fig. 3.2b) where 2.4, 3.8, 4.7 and 5.5% of initial soil C content was respired, corresponding to 314, 502, 624 and 737 mg CO_2-C kg^{-1}. Accumulated C respiration for AS was lower than for CON, especially after the second application event. Accumulated respired C for AS on the sandy loam soil (Fig. 3.2a) was 212, 386, 467 and 557 mg CO_2-C kg^{-1} after 85, 168, 250 and 342 days, respectively, corresponding to 1.8, 3.3, 4.0 and 4.8% of initial soil C content. On the clay loam soil (Fig. 3.2b), these amounts were

266, 430, 535 and 632 mg CO_2-C kg^{-1} (corresponding to 2.0, 3.2, 4.0 and 4.8% of initial soil C).

Figure 3.2. Accumulated carbon respiration and respiration rates in the unamended and ammonium-sulphate amended soils.



Statistical analysis showed significant differences among respiration rates of different application events of CON in both soils. On the sandy loam soil (Fig. 3.2c), respiration rates (expressed as a percentage of soil initial C content) measured during the first application were significantly higher ($P < 0.05$) than respiration rates measured during applications 3 and 4 in all the period day 15 – day 84. In the same time period, C respiration decreased passing from application 2 to applications 3 and 4, and from application 3 to application 4 ($P < 0.05$). Similar results were obtained for CON on the clay loam soil (Fig. 3.2d), where C respiration decreased after each application events. Statistical differences were found in the period after day 28 until day 84 among applications ($P < 0.05$) with the exception of application 1 and application 2 that were not statistically different. As observed for the CON treatment, AS amended soils respired more during application 1 compared the other applications (Fig. 3.2e and 3.2f). On both soils, application 1 respired more than applications 3 and 4 after the first two weeks of incubation until day 84 ($P < 0.05$). In the same period (day 15 – day 84) respiration rates were also higher in application 3 with respect to application 4 ($P < 0.05$). In the first week of incubation, statistical differences were found only between application 1 and application 4, and between application 3 and application 4, with lower C respiration measured during the last application ($P < 0.05$).

On the sandy loam soil no significant differences were found between respiration rates in the CON and AS treatments. On the clay loam soil differences were found during application 1, in the period day 15 − day 21, with higher respiration rates measured in the CON treatment with respect to rates measured in the AS treatment (P < 0.05). Other significant differences were found during application 3 from day 0 to day 1 and from day 10 to day 15 with higher respiration rates always measured in the CON treatment in both intervals ($P < 0.05$).

Soil microbial biomass C

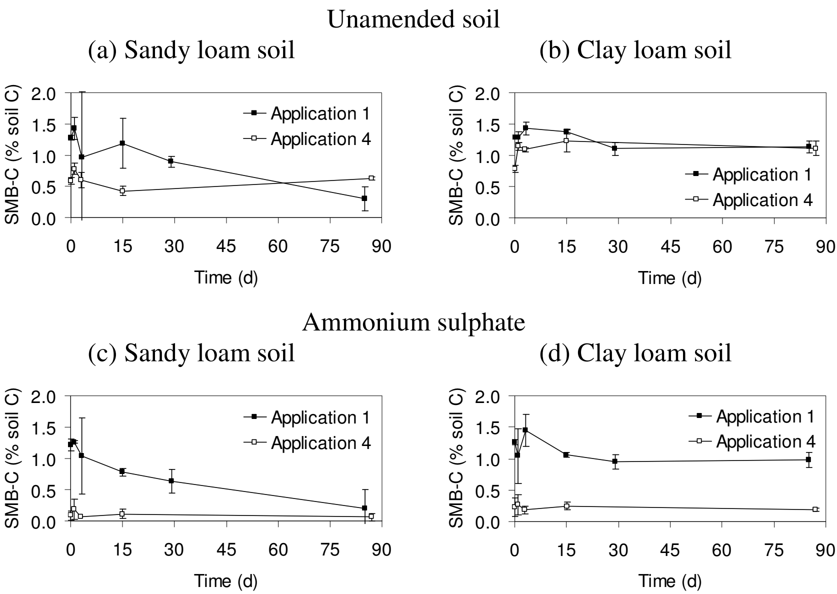
After 1 week of pre-incubation, the fraction of soil C estimated as microbial biomass (SMB) in the CON treatment was similar in both soils; in the sandy loam soil (Fig. 3.3a), SMB carbon (SMB–C) concentration was 149 mg C kg^{-1} (1.28% of the soil C content), while on the clay loam soil (Fig. 3.3b), SMB–C concentration was 170 mg C kg^{-1}, representing also in this case, 1.28% of the soil C content. On day 0 after the manure application, SMB-C concentration in both soils was lower in application 4 compared to application 1 (0.58 and 0.78% of soil C content). On the sandy loam soil (Fig. 3.3a), during application 1, SMB–C constantly decreased and, on day 84, it represented only 0.29% of the soil C content. In the same time period (day 0 – 84), during application 4, SMB–C concentration was quite stable until the end of the incubation interval, with a final C concentration representing 0.62% of the soil C content. On the clay loam soil (Fig. 3.3b), during application 1, SMB concentration only slightly decreases until 151 mg C kg^{-1} while, after the 4^{th} application event, it rapidly increased and, on day 84, it represented 1.11% of the soil C content.

Statistical analysis showed significant differences between application events only on day 1 when, on the sandy loam soil, SMB concentration was higher during application 1 compared to application 4 ($P < 0.05$).

In the AS treatment, after application 1, SMB concentration measured on day 0 was 141 mg C kg^{-1} (1.21% of the soil C content) and 168 mg C kg^{-1} (1.26% of the soil C content) on the sandy loam and the clay loam soils, respectively (Fig. 3.3a-b).

As observed in the CON treatment, the application 1 of AS treatment on the sandy loam soil resulted in a constant decreasing of SMB-C until day 84, and thereafter its concentration remained low, without any significant increase also during application 4 (Fig. 3.3c). On the clay loam soil (Fig. 3.3d), during application 1, SMB–C concentration remained stable until day 84 and, at the end of the incubation interval, it represented 0.97% of the soil C content. Immediately after the 4^{th} application event, on the same soil, SMB–C concentration was lower compared to the initial value measured during application 1 and, as observed for the sandy loam soil, did not increased in the days after the application event. Statistical analysis showed significantly higher SMB–C concentration during applications 1 compared to application 4, on both soils, in the period from day 3 to day 84.

Figure 3.3. Soil microbial biomass C in unamended and ammonium sulphate amended soils.

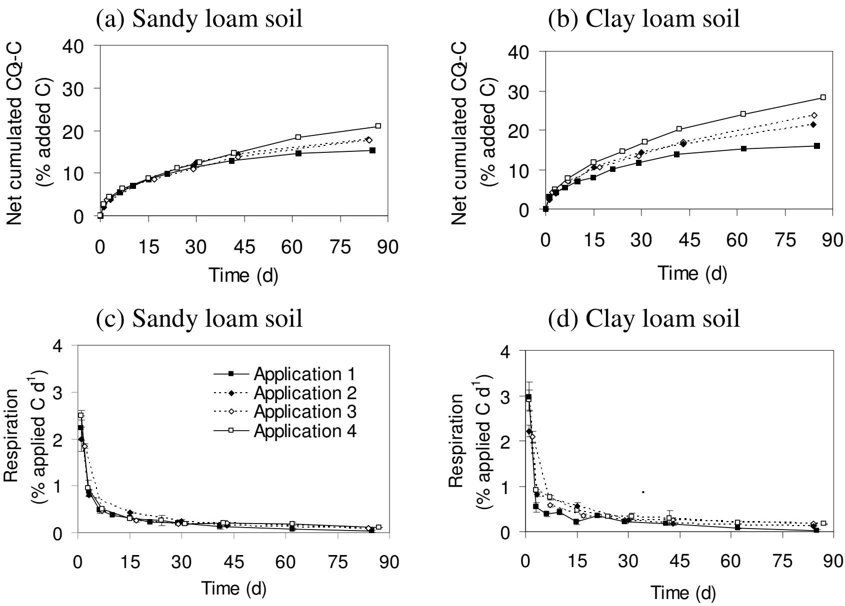


Manured soils

Carbon respiration

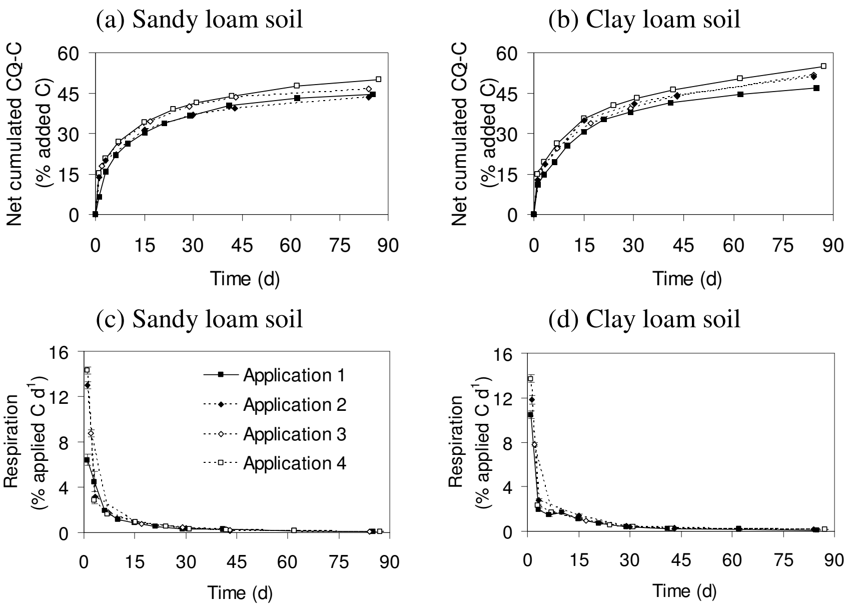
Net manure C respiration (expressed as percentage of C applied with the manure) showed similar pattern for both manures in all applications and in both soil types. Respiration rate was faster immediately after manure application (in the first 15 days) and slower thereafter (Fig. 3.4c-d and Fig. 3.5c-d). In all combinations of soil type × application event, COW respired much more than HEI. Therefore, accumulated C respiration at the end of the incubation was higher for COW compared to HEI (Fig. 3.4a-b and 3.5a-b). The total amount of C respired in HEI during the first 15 days was 8.6 and 11.4% of the added C (average of four application events) respectively on the sandy loam and clay loam soil; in the same time interval, COW respired three times more C than HEI (corresponding to 32.6 and 35.3% of the added C). The HEI treatment respired 15.4, 17.9, 17.8 and 21.1% of the added manure C on days 84, 168, 252 and 342 on the sandy loam soil (Fig. 3.4a). These percentages were 16.0, 21.5, 23.9 and 28.3% on the clay loam soil (Fig. 3.4b). Accumulated respiration in COW was 44.6, 43.5, 46.7 and 50.0% of the added manure C on the sandy loam soil (Fig. 3.5a) and 46.9, 51.0, 51.8, 54.7% on the clay loam soil (Fig. 3.5b).

Figure 3.4. Accumulated carbon respiration and respiration rates in the heifer manure amended soils.



Respiration rates measured in the HEI treatment on the sandy loam soil (Fig. 3.4c) were not statistically different among application events in all incubation intervals with the exceptions of the first incubation interval (from day 0 to day 1, when application 4 respired more than application 3), from day 10 to day 15 (when application 2 respired more than application 4) and from day 60 to day 84 (when application 4 respired more than application 2) ($P < 0.05$). For the HEI treatment on the clay loam soil (Fig. 3.4d), in the same time period (the last 30 days of incubation) and in the period day 3 – day 6, higher respiration rates were measured during application 4 with respect to applications 1 and 3; application 4 respired more than application 1 also from day 40 to day 60 ($P < 0.05$).

Figure 3.5. Accumulated carbon respiration and respiration rates in the dairy cow manure amended soils.



Carbon respiration rate in COW showed many significant differences in the first day after manure application in both soils (Fig. 3.5c-d): in this period (day 0 – day 1), the order of respiration was application 4 > application 2 > application 3 > application 1 on the sandy loam soil ($P < 0.05$), while on the clay loam soil the order was application 4 > application 2 > application 1 > application 3 ($P < 0.05$). On the sandy loam soil, in the period day 3 – day 6, higher respiration rates were measured during application 4 compared to application 1 ($P < 0.05$). At the end of incubation significantly lower C was respired during application 1 compared to application 2 and application 3 on the two soils respectively ($P < 0.05$).

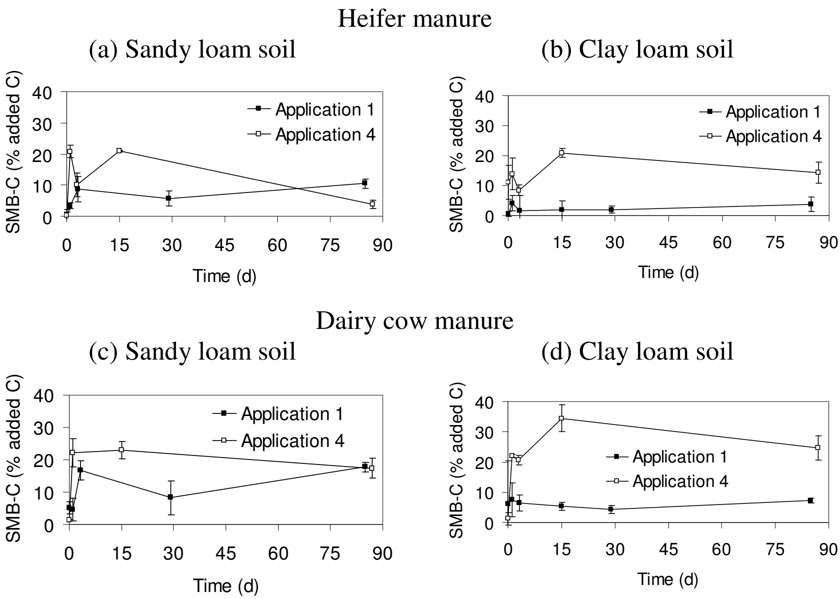
On both soils, during applications 1, 3 and 4, significant lower respiration rates were measured in the HEI treatment compared to the COW treatment in the first 4 weeks of incubation (from day 0 to day 28). During application 2, the COW treatment respired more C only in the period day 0 – day 3 ($P < 0.05$); thereafter no significant differences were found between respiration rates in the two manures-amended soils.

Soil microbial biomass C

On both soils, SMB–C concentration measured in the HEI treatment, increased after applications 1 and 4 (Fig. 3.6a-b). This increase was more consistent on the sandy loam soil during application 1, when, at the end of the incubation interval (on day 84), SMB–C concentration was 111 mg C kg^{-1} (corresponding to 11.10% of the added manure C) higher with respect to the initial value measured on day 0; on the same soil, during application 4, and on the clay loam soil, during applications 1 and 4, the increase of SMB–C concentration between day 0 to day 84, was in the range 30.60 – 35.79 mg C kg^{-1}, representing on average 3.31% of the added manure C. On the sandy loam soil, SMB–C concentration (expressed as a percentage of added manure C) measured on day 1 was significantly higher (*P* < 0.05) during application 4 compared to application 1 (20.81 and 3.39% of added manure C, respectively). On the clay loam soil, significantly higher SMB–C concentration was measured on day 15 during application 4 compared to application 1 (1.88 and 20.87% of the added manure C, respectively).

As observed for HEI, also in the COW treatment, SMB rapidly increased after manure applications to the soil, with the exception of application 1 on the clay loam soil, where SBC–C concentration only slightly increased in the period day 0 – 84 (Fig. 3.6c-d). On the sandy loam soil, at the end of the incubation interval (day 84), the net increase of SMB–C represented 12.61 and 16.12% of the added manure-C during applications 1 and 4, respectively. On the clay loam soil, the net increase of SMB–C, on day 84, represented 1.18 and 23.49% of the added manure-C during the first and the last application, respectively. On the sandy loam soil, SMB–C concentration measured during application 4 was significantly higher compared to application 1 only on day 1 (22.22 and 4.66% of the added manure-C, respectively) while on the clay loam soil, significantly higher SMB–C concentration was measured during application 4 compared to application 1 on days 3, 15 and 84 (*P* < 0.05).

Figure 3.6. Soil microbial biomass C in heifer manure and dairy cow manure amended soils.



Residual effect of added manure C

Residual effect of added manure C (CRE) was calculated as the difference between net cumulated CO_2 (expressed as a percentage of added manure C) of the i^{th} application and net cumulated CO_2 of application 1. Because respired carbon is accumulated in the entire period (from day 0 to day 84 after material applications) this calculated residual effect takes into account for respiration differences that occurred both in the first period and in the last period after material applications; thus higher respiration rates measured in the first days after material application contributed to a higher estimated C residual effect also in the following days of incubation. Anyway, in many soil × manure × application combinations, the slope of the line connecting two subsequent dates was not 0, indicating that each incubation interval contributes to a positive or negative C residual effect. While manures were repeatedly added to the soils (that is after each application event), more C was progressively respired in all soil × manure combinations with the exception of COW on the sandy loam soil during application 2, when much manure C was respired during application 1 compared to application 2 (Fig. 3.7a-b and Fig. 3.8a-b). In general, the residual effect of added manure-C was more evident on the clay loam soil for both manure treatments, and for the HEI treatment compared to COW on both soils.

For the HEI treatment on the sandy loam soil (Fig. 3.7a), C residual effect, calculated on day 84, ranged from 2.52 to 5.66% of the added manure-C after applications 2 and 4, respectively. At the end of incubation, on the clay loam soil (Fig. 3.7b), the increment of manure-C respiration ranged from 5.53, after application 2, to 12.31% of the added manure-C after application 4.

For the COW treatment on the sandy loam soil (Fig. 3.8a), the increment of C respiration was consistent only after the third application, when, on day 84, 2.12 and 5.48% more added manure-C was respired during applications 3 and 4 compared to application 1.

On the other soil (Fig. 3.8b), as observed for HEI, the residual effect of added manure-C was more evident and increased constantly after the second manure application until the last application; the calculated respiration increment was 4.10, 4.94 and 7.86% of the added manure-C after applications 2, 3 and 4, respectively.

Figure 3.7 Residual effect of added manure C (CRE) in heifer manure amended soils

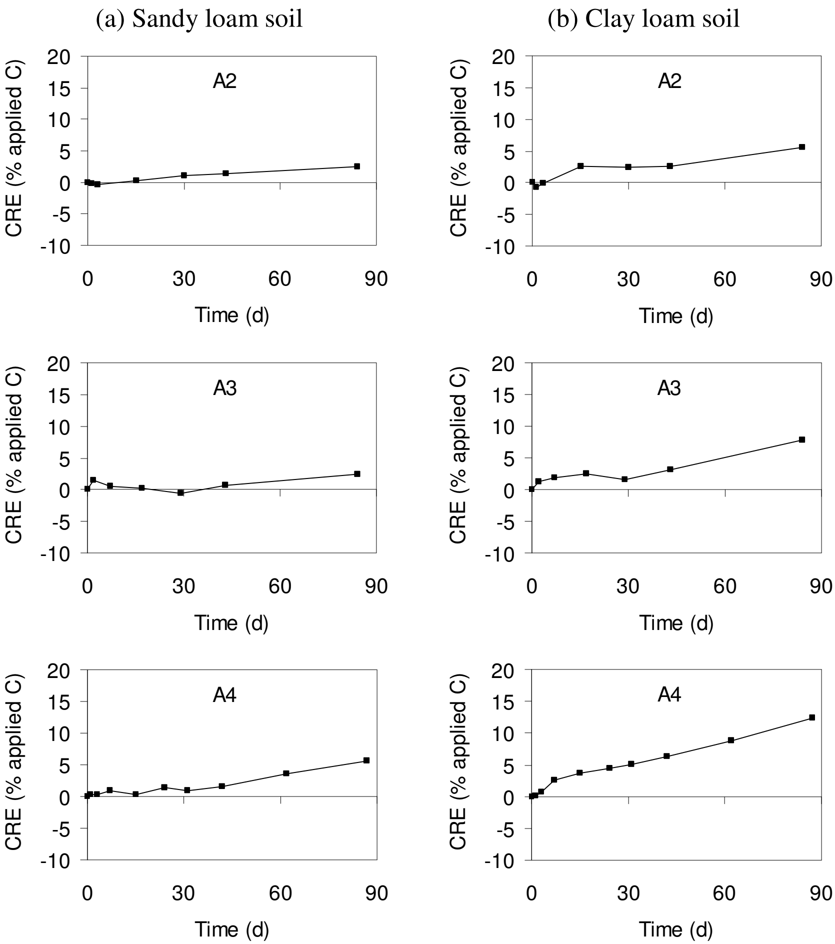
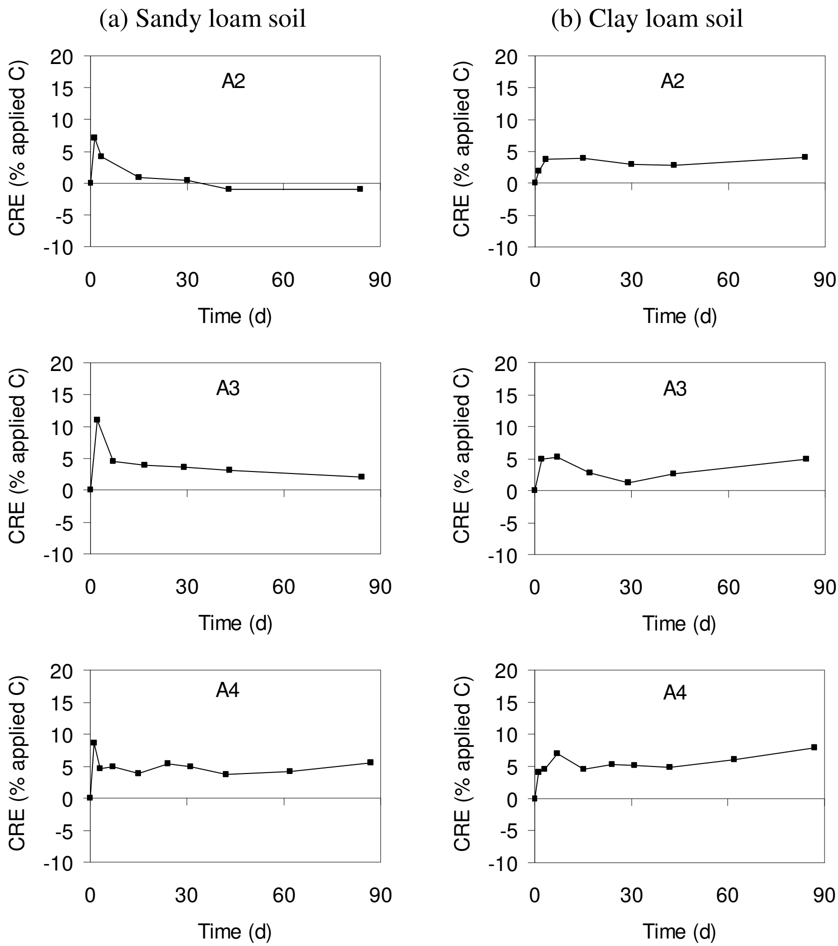


Figure 3.8 Residual effect of added manure C (CRE) in dairy cow manure amended soils



Nitrogen dynamics

Control soils

Ammonium dynamics

At the beginning of the incubation, total exchangeable plus soluble mineral N (SMN) in the unamended treatment (CON) was mostly in the form of NO_3^- (16 and 25 mg N kg^{-1} for the sandy loam and the clay loam soil, respectively), while exchangeable ammonium ($NH_4^+{}_e$) was substantially lower (2 mg N kg^{-1}; Fig. 3.9a-b). $NH_4^+{}_e$ remained low during the entire incubation period in both CON soils, and did not exceed 5 and 3 mg N kg^{-1} on the sandy loam and the clay loam soil, respectively.
Despite this, ANOVA showed significant differences of $NH_4^+{}_e$ concentration among application events in CON on both soils. In the sandy loam soil, $NH_4^+{}_e$ (Fig. 3.9a) was higher during application 1 with respect to applications 3 and 4 on day 2 and with respect to applications 2 and 4 on day 3 ($P < 0.05$). After 84 days of incubation, $NH_4^+{}_e$ was higher during application 2 compared to applications 1 and 4 (5, 3 and 2 mg N kg^{-1}, respectively). In the clay loam soil, a lower $NH_4^+{}_e$ (Fig. 3.9b) concentration was measured during application 3 with respect to applications 1 and 2 immediately after soil re-mixing ($P < 0.05$). Thereafter $NH_4^+{}_e$ was significantly higher during the first application compared to application 3 on day 21, to

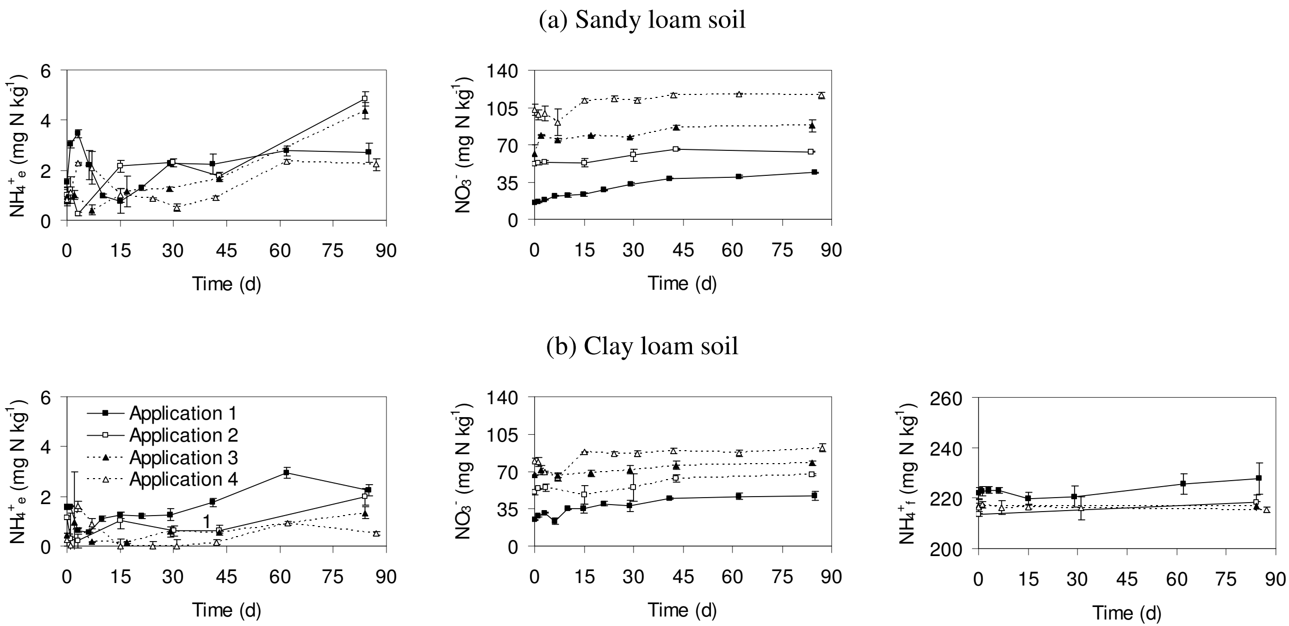
applications 2 and 3 on day 40, and to application 4 in all dates until day 84 ($P < 0.05$).

Non-exchangeable ammonium ($NH_4^+{}_f$) concentration measured in the clay loam soil (Fig. 3.9b), at the beginning of the incubation, was 222 mg N kg^{-1}, corresponding to 15.9% of the total soil N content. During the first application interval (from day 0 to day 84), $NH_4^+{}_f$ concentration slightly increased until 228 mg N kg^{-1} on day 84; thereafter, from the beginning of the second application, $NH_4^+{}_f$ concentration was quite lower compared to application 1 and remained stable until the end of the incubation (day 342). Statistical analysis showed significantly differences among applications only on day 0, when a higher $NH_4^+{}_f$ concentration was measured during application 1 with respect to applications 2 and 3 ($P < 0.05$).

Nitrate dynamics

Nitrate concentration (Fig. 3.9a-b) increased during the entire incubation period (from day 0 to day 84) after all application events; at the end of the incubation (day 342), NO_3^- concentration on the sandy loam soil was 116 mg N kg^{-1}, while on the clay loam soil it was 92 mg N kg^{-1}, representing 7.1 and 4.0% of the initial soil organic N content of the two soils. On the sandy loam soil, NO_3^- concentration (Fig. 3.9a) was significantly lower in all dates, with few exceptions, during application 1 compared to applications 2 (with the exception of day 28), application 3 (with the exception of day 1) and application 4 (with the exception of day 6) (P < 0.05). During the whole incubation period (from day 0 to day 84) NO_3^- concentration was higher during application 4 with respect to application 2. Applications 3 and 4 differentiated only on days 21, 28 and 40, with higher NO_3^- concentration measured during the last application. On the clay loam soil application 1 showed significantly lower NO_3^- concentration (Fig. 3.9b) during the entire incubation period compared to applications 3 and 4 (P < 0.05). On day 0, immediately after soil re-mixing, the rank of NO_3^- concentrations was application 1 (25 mg N kg^{-1}) < application 2 (50 mg N kg^{-1}) < application 3 (67 mg N kg^{-1}) < application 4 (80 mg N kg^{-1}).

Figure 3.9. Soil concentration of exchangeable ammonium ($NH_4^+{}_e$), nitrate (NO_3^-) and non-exchangeable ammonium ($NH_4^+{}_f$) in two soils subjected to repeated applications of water.



Ammonium sulphate-amended soils

Ammonium dynamics

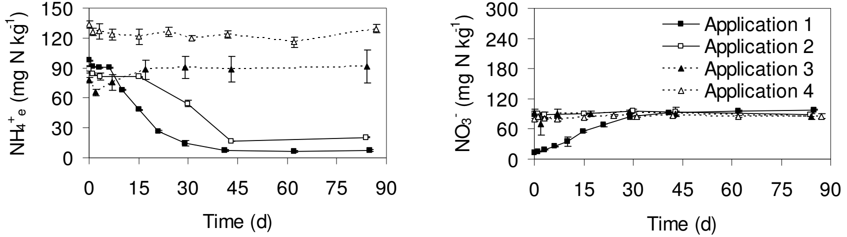
On both soils, $NH_4^+{}_e$ increased immediately after the addition of ammonium sulphate (Fig. 3.10a-b). The recovery of applied NH_4^+ (Fig. 3.11a), calculated by subtracting the $NH_4^+{}_e$ concentration measured in the unamended treatment (CON) from the $NH_4^+{}_e$ concentration measured in the amended treatment (AS), was on average for applications 1–3 89.4% of the added NH_4^+ in the sandy loam soil, showing no significant losses of ammonium during the addition of AS, while it was 58.6% for application 4. On the contrary, on the clay loam soil (Fig. 3.11d), the recovery of applied NH_4^+ ranged from 58.5 to 67.9%, highlighting that only a part of the added NH_4^+ was in the soluble + exchangeable form immediately after the addition of AS (≈ 2 h after material application). On the same soil, the amount of added NH_4^+ recovered as non-exchangeable ammonium (Fig. 3.11d) ranged from 74.7 to 48.7% of the added NH_4^+ after the first an the last AS application, respectively, and was significantly higher during application 1 compared to application 4, and during application 2 compared to applications 3 and 4 ($P < 0.05$). On the sandy loam soil (Fig. 3.10a) after the first application of AS, $NH_4^+{}_e$ progressively decreased during incubation, reaching, after 84 days, a concentration similar to CON (7 mg N kg^{-1}). After application 3 and application 4, $NH_4^+{}_e$ did not decrease and remained stable at the value measured after the addition of AS (around 83 and 125 mg N kg^{-1} for applications 3 and 4, respectively), presumably due to a strong reduction of the nitrification process. An intermediate behaviour was observed during application 2, where $NH_4^+{}_e$ concentration slightly decreased until day 15 and thereafter strongly decreased until a concentration of 20 mg N kg^{-1}, but never reached values closed to that measured in the CON treatment as observed during the first application. ANOVA showed significant differences among application 3 and applications 2 and 4 in the first week after addition of AS, when lower concentrations of $NH_4^+{}_e$ were measured during application 3 with respect to the other applications ($P < 0.05$). After the first 28 days of incubation, $NH_4^+{}_e$ concentration measured during application 2 was intermediate between application 1 and application 4 ($P < 0.05$).

On the clay loam soil (Fig. 3.10b), as observed on the sandy loam soil during application 1, $NH_4^+{}_e$ concentration progressively decreased during incubation, and reached, after 84 days, a concentration similar to CON (on average 5 mg N kg^{-1}). The decrease of $NH_4^+{}_e$ occurred fast until day 21 during the first application and until day 40 during applications 2–4; thereafter the reduction was slower with no significant differences among applications, with the exception of application 1 and application 4 on day 60 (3 and 12 mg N kg^{-1} respectively), and application 1 and application 3 on day 84 (3 and 6 mg N kg^{-1} respectively). Significant differences ($P < 0.05$) among applications were observed in the first period of incubation (until day 40), when $NH_4^+{}_e$ was lower during application 1 with respect to application 4, and respect to application 3 on days 6, 21 and 40. $NH_4^+{}_e$ was also significantly lower during application 2 with respect to application 4 on days 3, 28 and 40.

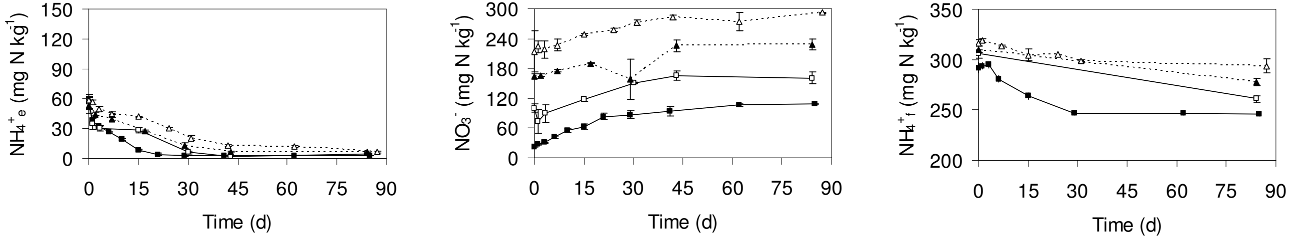
As observed for $NH_4^+{}_e$, on the clay loam soil, $NH_4^+{}_f$ concentration increased after each application event (Fig. 3.10b); thereafter, clay fixed ammonium was released as the incubation continued. During the first application $NH_4^+{}_f$ concentration rapidly decreased from day 1 until day 29 and thereafter remained stable until day 84. During application 4 the release of $NH_4^+{}_f$ was slower compared to the first application, and prolonged until the end of the incubation period. After repeated material applications, the amount of NH_4^+ held in a non-exchangeable form by the soil clay minerals increased and, on day 84, measured $NH_4^+{}_f$ concentration was 246 and 294 mg N kg^{-1} during applications 1 and 4, respectively, indicating an increase of 50 mg N kg^{-1} of $NH_4^+{}_f$ from the first to the last application. Statistical analysis showed that higher $NH_4^+{}_f$ concentration were measured during application 4 compared to application1 in the period from day 3 to day 28; and that, on day 84, the order of $NH_4^+{}_f$ concentration in the AS treatment was application 3 > application 2 > Application 1.

Figure 3.10. Soil concentration of exchangeable ammonium ($NH_4^+{}_e$), nitrate (NO_3^-) and non-exchangeable ammonium ($NH_4^+{}_f$) in two soils subjected to repeated applications of ammonium sulphate.

(a) Sandy loam soil



(b) Clay loam soil



Nitrate dynamics

On the sandy loam soil during application 1, while $NH_4^+{}_e$ decreased, NO_3^- progressively increased (Fig. 3.10a). The increase of NO_3^- occurred rapidly until day 28, and more slowly from day 28 until the end of the incubation period. After the second application, NO_3^- stabilized around 90 mg N kg^{-1} with no significant differences among applications at all dates. Significant differences ($P < 0.05$) were found only between application 1 and applications 4 during the first two weeks of incubation, when higher NO_3^- concentrations were measured during the last application. On the clay loam soil, NO_3^- always increased after each application event (Fig. 3.10b). As observed for the decrease of $NH_4^+{}_e$, nitrification probably occurred faster during the first 21 days of incubation during application 1; this initial phase lasted until day 40 during the other applications. At the end of incubation (day 342), NO_3^- concentration reached 292 mg N kg^{-1}. The NO_3^- concentration measured during application 1 was significantly lower compared to that measured during application 2 (until day 40) and application 3 in all dates with the exception of day 28. ANOVA showed also significant differences between application 2 and application 4, with lower NO_3^- concentration measured during application 2 with respect to application 4 during all the incubation period (from day 0 to day 84).

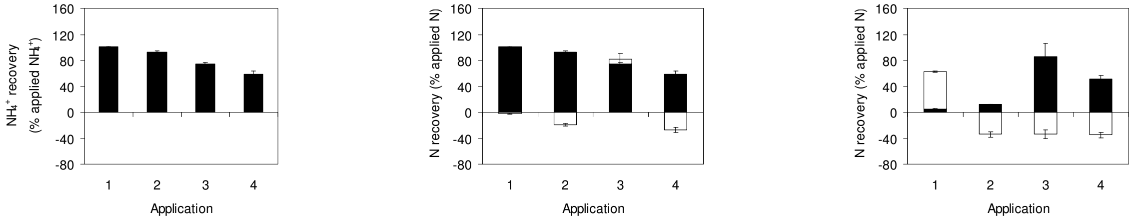
Recovery of added N

On the sandy loam soil, after AS applications, net variation of soil mineral nitrogen (SMN) concentration accounted for 98.9 to 31.4% of the added N (Fig. 3.11b), decreasing from application 1 to application 4. Net SMN concentration measure during the last application was significantly lower compared to the other applications ($P < 0.05$). The recovery of applied N in the clay loam soil (Fig. 3.11e) was higher than 100% during applications 1, 2 and 3 and 93% during the last application, with no significantly differences among applications. Considering the forms of N contributing to the recoveries, in the case of the sandy loam soil mostly contributed $NH_4^+{}_e$ while in the case of the clay loam soil beside $NH_4^+{}_e$ also $NH_4^+{}_f$ was relevant. Some negative values of NO_3^- resulted from calculations as a consequence of lower nitrate concentration measured on the first day of the i^{th}- application compared to nitrate concentration measured on day 84 of the $(i\text{-}1)^{th}$ application. On the sandy loam soil, at the end of the incubation intervals (on day 84), applied N was mostly in the form of nitrate, during application 1, and in the form of exchangeable ammonium during the other applications (Fig. 3.11c). The recovery of applied nitrogen at day 84 after material application was significantly higher in the application 1 (65.0% of applied N) compared to applications 2 and 4 ($P < 0.05$), and lower in application 2 compared to application 4 ($P < 0.05$). On the clay loam soil, on day 84, from 85.2 to 94.0% of the added N was recovered as sum of NO_3^- and $NH_4^+{}_f$ with no significant differences among applications (Fig. 3.11f).

Figure 3.11. Added NH_4^+ recovery after material applications and added N recovery at the beginning (day 0) and at the end of each incubation interval (day 84) in two soils subjected to repeated applications of ammonium sulphate.

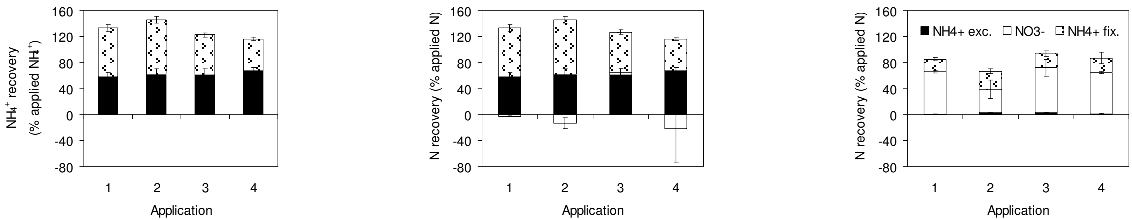
Sandy loam soil

(a) Recovery of added NH_4^+ (b) Recovery of added N on day 0 (c) Recovery of added N on day 84



Clay loam soil

(d) Recovery of added NH_4^+ (e) Recovery of added N on day 0 (f) Recovery of added N on day 84



Manured soils

Ammonium dynamics

As observed for the AS treatments, $NH_4^+{}_e$ concentration increased immediately after manure application on both soils, due to the fraction of added manure N in the form of NH_4^+ (Fig. 3.12a-b and Fig. 3.13a-b). On the sandy loam soil (Fig. 3.14a and Fig.3.15a), recovery of added NH_4^+ ranged from 71.9 to 114.8% of the manure NH_4^+ in the HEI treatment and from 76.9 to 105.4% in the COW treatment. On the clay loam soil (Fig. 3.14d and Fig. 3.15d), the recovery of manure NH_4^+ was lower compared to the sandy loam soil for both HEI and COW; on average 39.4% of the added manure NH_4^+ was recovered in the HEI treatment, and 45.7% in the COW treatment. This indicates, as observed also for AS on the clay loam soil, that a consistent fraction of applied manure NH_4^+ becomes not extractable from the soil matrix in less than two hours after manure application. In both HEI and COW treatments, added manure-NH_4^+ recovered as $NH_4^+{}_f$ decreased after repeated manure applications (Fig. 3.14d and Fig. 3.15d) and was significantly higher during application 2 compared to application 4, in HEI, and during the first two applications with respect to applications 3 and 4, in COW ($P < 0.05$).

In all soil × manure combinations (Fig. 3.12a-b and Fig. 3.13a-b), $NH_4^+{}_e$ concentration decreased quickly and, in less than 15 days on the sandy loam soil and less than 3 days on the clay loam soil, it reached concentrations lower than 4-8 mg N kg^{-1}. Thereafter $NH_4^+{}_e$ remained low until the subsequent application event. In the HEI treatment on the sandy loam soil (Fig. 3.12a) ANOVA showed no significant differences among applications with the exception of higher $NH_4^+{}_e$ concentration measured on day 3 during application 1 with respect to application 2 and on day 84 during application 2 with respect to application 1 . Also for the COW treatment on the same soil (Fig. 3.13a) only few significant differences of $NH_4^+{}_e$ ($P < 0.05$) were found among applications; in particular $NH_4^+{}_e$ was lower during application 4 with respect to applications 2 and 3 on day 28 and with respect to applications 1 and 3 on day 84. On the clay loam soil in the HEI treatment (Fig. 3.12b) $NH_4^+{}_e$ was significantly different between application 1 and application 3 on day 0 (immediately after manure application); for the same treatment, $NH_4^+{}_e$ was lower during application 4 compared to the other applications on day 84. In the COW treatment on the clay loam soil (Fig. 3.13b) $NH_4^+{}_e$ in application 1 was higher ($P < 0.05$) than application 3 on day 1 and higher than in all the other applications on day 40.

As observed for exchangeable ammonium, $NH_4^+{}_f$ concentration increased after each application event. In HEI (Fig. 3.12b), during application 1, only a small fraction of initially clay fixed ammonium was released as the incubation continued, while after all the other application events $NH_4^+{}_f$ concentration slightly increased or remained stable until the end of the application interval (day 84). In the HEI treatment, as observed for AS, $NH_4^+{}_f$ concentration on day 84 progressively increased when more manure-N was added to the soil following repeated applications. At the end of the incubation (on day 342) the concentration of $NH_4^+{}_f$ was 68 mg N kg^{-1} higher compared to the unamended soil. In the COW treatment (Fig. 3.13b), the first manure application showed a different dynamic with respect to the others. After manure application, about 60% of the initially clay fixed NH_4^+ was released in the first week of incubation and thereafter $NH_4^+{}_f$ concentration remained constant until day 84. After the second application event, no release of clay fixed ammonium was observed in the whole period day 0 – day 84, resulting in a progressive increase of $NH_4^+{}_f$ concentration as incubation continued. On day 342, the amount of clay fixed ammonium in COW was 74 mg N kg^{-1} higher compared to CON. ANOVA showed, for both HEI an COW, significantly higher $NH_4^+{}_f$ concentration during application 4 compared to application 1 in the entire period from day 0 to day 84 ($P < 0.05$). Moreover, the concentration of $NH_4^+{}_f$ measured on day 84 was significantly lower during applications 1 and 2 with respect to applications 3 and 4.

Figure 3.12. Soil concentration of exchangeable ammonium ($NH_4^+{}_e$), nitrate (NO_3^-) and non-exchangeable ammonium ($NH_4^+{}_f$) in two soils subjected to repeated applications of heifer manure.

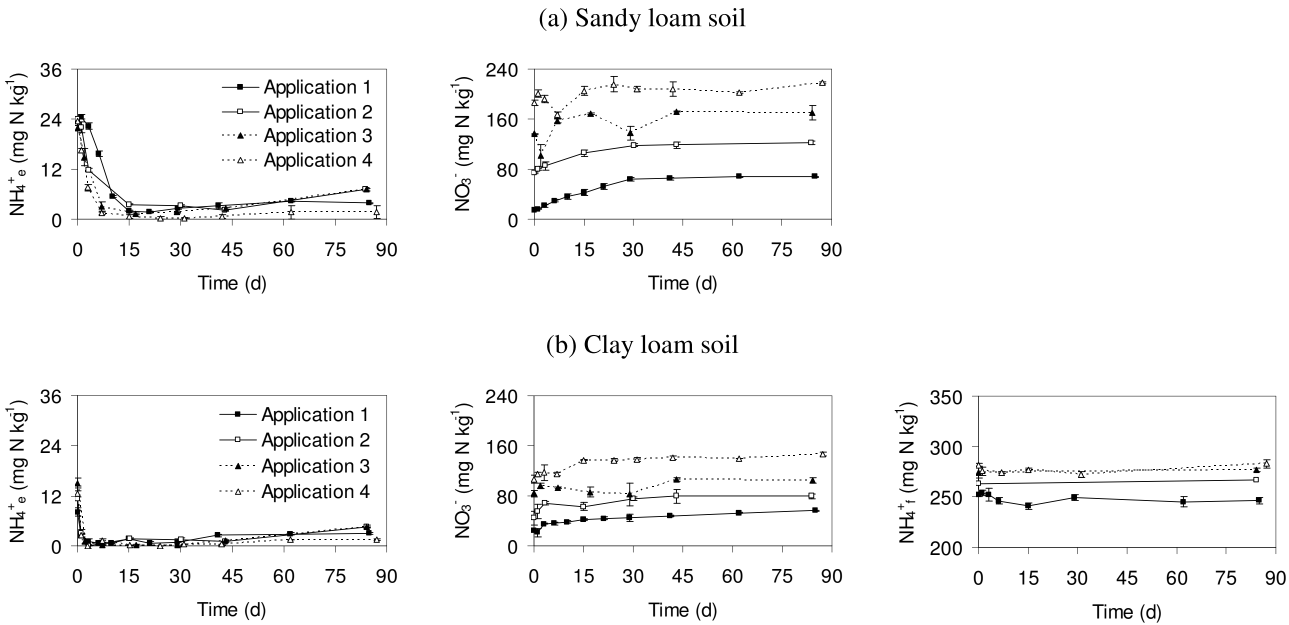
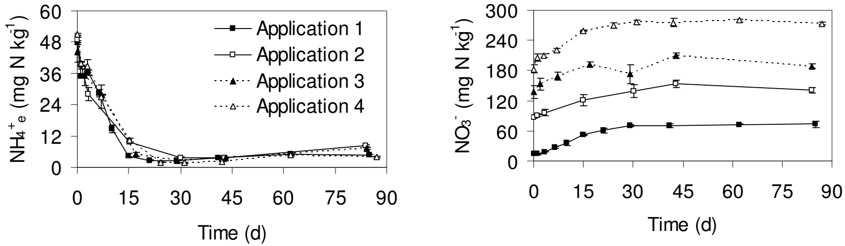
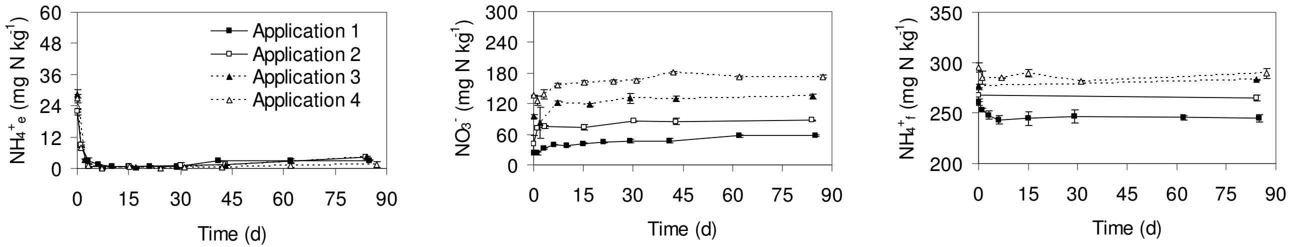


Figure 3.13. Soil concentration of exchangeable ammonium ($NH_4^+{}_e$), nitrate (NO_3^-) and non-exchangeable ammonium ($NH_4^+{}_f$) in two soils subjected to repeated applications of dairy cow manure.

(a) Sandy loam soil



(b) Clay loam soil



Nitrate dynamics

After each application event NO_3^- concentration increased constantly in all soil × manure combinations (Fig. 3.12a-b and Fig. 3.13a-b). As observed in AS, also in the manured treatments nitrification was sometimes (for example HEI and COW on the sandy loam soil during applications 1 and 2) characterized by two distinct phases. Nitrification was more consistent on the sandy loam soil with respect to the clay loam soil for both HEI and COW. At the end of incubation (day 342), NO_3^- concentration in HEI was 218 mg N kg^{-1} on the sandy loam soil (Fig. 3.12a) and 147 mg N kg^{-1} on the clay loam soil (Fig. 3.12b). On the same date, NO_3^- concentration in COW was 273 (Fig. 3.13a) and 173 mg N kg^{-1} (Fig. 3.13b) on the two soils, respectively. On the sandy loam soil for the HEI treatment (Fig. 3.12a) ANOVA showed significant differences ($P < 0.05$) in all dates between application 1 and application 2, between application 1 and application 3 (with the exception of day 1), and between application 1 and application 4, with lower NO_3^- concentration measured during the first application. NO_3^- concentration was also significantly higher during application 3 with respect to application 2 on days 0, 40 and 84. At the beginning of the incubation (on day 0) and on day 28, application 4 was different from application 3. In the COW treatment on the same soil (Fig. 3.13a) NO_3^- was lower at all dates during application 1 compared to application 2 (with the exception of days 15 and 28), to application 3 (with the exception of day 15) and to application 4. Application 4 had also higher NO_3^- concentration compared to application 2 during the entire period (from day 0 to day 84) and, compared to application 3, in all dates after day 3. So, with few exceptions, NO_3^- concentration significantly increased from application 1 to application 2, 3, and 4.

On the clay loam soil NO_3^- concentration in the HEI treatment (Fig. 3.12b) was significantly ($P < 0.05$) lower in application 1 compared to application 4 in all incubation dates, and in application 1 compared to application 3 from day 0 to day 6 and from day 40 to day 84. Significantly higher NO_3^- concentrations were also measured during application 4 with respect to application 2 (on days 0, 15, 28 and 84) and application 3 (on day 6, 21, 40 and 84). On the same soil NO_3^- concentration in the COW treatment (Fig. 3.13b) was significantly ($P < 0.05$) lower during application 1 compared to application 2, with the exception of days 0 and 15. Application 4 significantly differed from applications 2 and 3 in many days of incubation: higher NO_3^- concentrations were measured during application 4 compared to application 2 on days 3, 15, 28, 40 and 84, and during application 4 compared to application 3 on days 6, 21, 40 and 84.

Recovery of added N

On day 0 after HEI treatment application on the sandy loam soil, the recovery of N, as SMN, (Fig. 3.14b) varied from 19,8% to 34,6% without relation with increasing the application number; recovery observed in application 3 resulted significantly higher compared to that in applications 1 and 2 ($P < 0.05$). On the clay loam soil less added N was recovered as repeated manure applications were done (Fig. 3.14e). Net SMN accounted for 37.0 and 13.7% of the added N during applications 1 and 4 respectively. Despite this, statistical analysis showed significantly differences only between applications 3 and 4, with a higher N recovery during the former ($P < 0.05$).

In COW, on the sandy loam soil, from 60.0 (application 2) to 26.8% (application 4) of the added N was recovered as SMN on day 0, with no significant differences among applications (Fig. 3.15b). On the other soil, net SMN accounted for 59.5, during application 1, and 35.0%, during application 4, of the added manure-N (Fig. 3.5e). Application 1 gave a recovery significantly higher than that of applications 3 and 4 ($P < 0.05$).

At day 0 after manure addition, as observed for AS, most of the added manure-N was recovered as $NH_4^+{}_e$ and, only on the clay loam soil, as $NH_4^+{}_f$, with a negligible contribution of NO_3^- to the net SMN concentration.

At the end of the incubation intervals, on day 84, nitrate represented from 38.9 (application 2) to 15.8% (application 4) of the applied manure-N in the case of HEI treatment applied on sandy loam soil (Fig. 3.14c). A lower N recovery was measured during the last manure application compared to applications 1 and 2 ($P < 0.05$). On the clay loam soil, from 37.3 (application 2) to 25.8 (application 3) % of applied N was recovered as SMN on day 84, with prevailing contribution of fixed NH_4^+ during applications 1 and 2, and of NO_3^-, during applications 3 and 4 (Fig. 3.14f). In COW, on the sandy loam soil, the recovery of added manure N on day 84 (Fig. 3.15c) were significantly higher during applications 2 and 4 (55.3% of added N) compared to application 1 (32.5% of added manure N) ($P < 0.05$). On the other soil, net SMN concentration on day 84 accounted for 56.3 – 29.2% of added N, during applications 3 and 1, respectively (Fig. 3.15f), with a significant difference between the two applications ($P < 0.05$).

Figure 3.14. Added NH_4^+ recovery after material applications and added N recovery at the beginning (day 0) and at the end of each incubation interval (day 84) in two soils subjected to repeated applications of heifer manure.

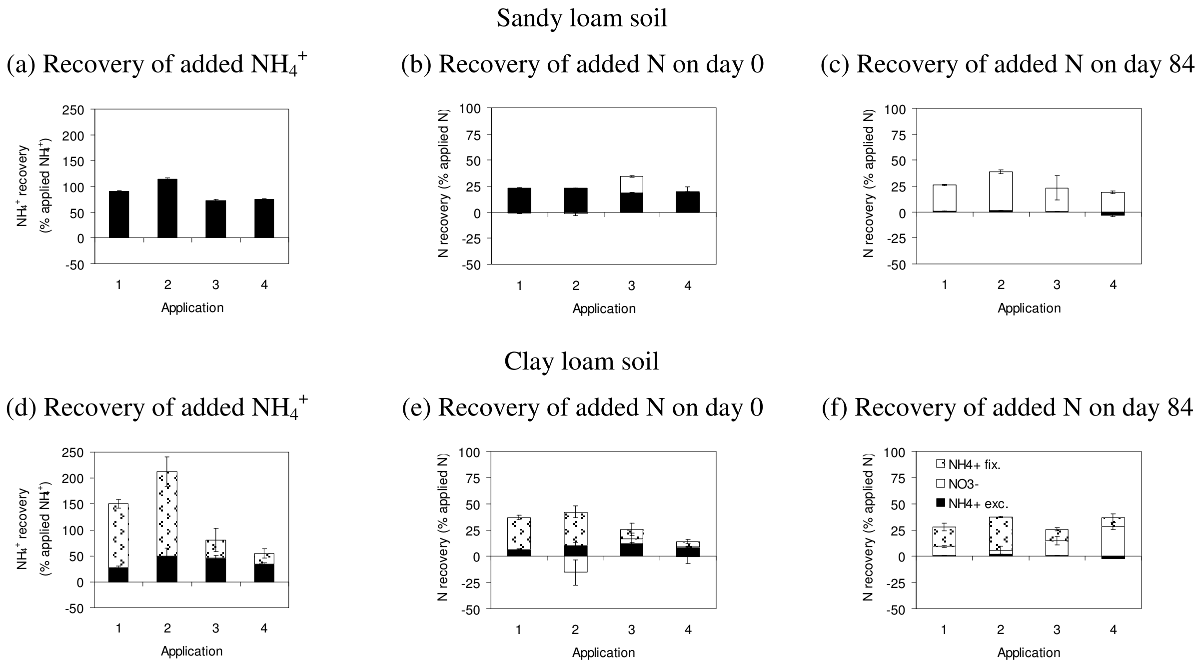
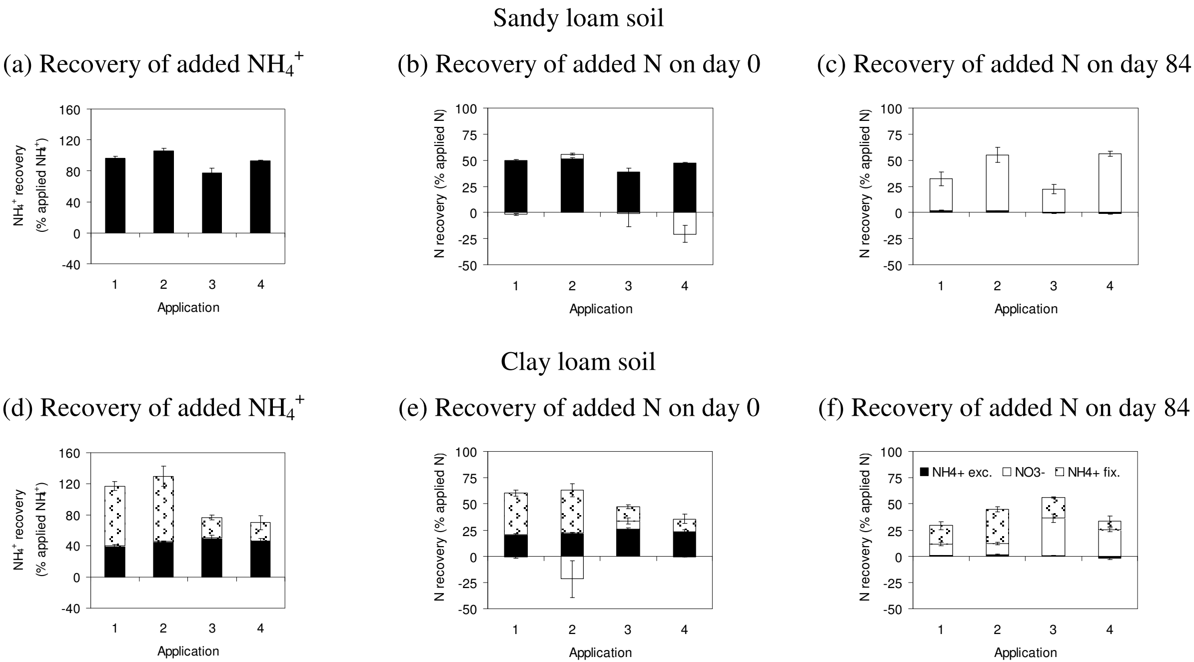


Figure 3.15. Added NH_4^+ recovery after material applications and added N recovery at the beginning (day 0) and at the end of each incubation interval (day 84) in two soils subjected to repeated applications of dairy cow manure.



Manure organic nitrogen mineralization

Manure organic nitrogen mineralization rates were higher (in their absolute values) during the first two weeks of incubation in all manure × soil combinations, and were also higher in the COW treatment compared to HEI (Fig. 3.16a-c and Fig. 3.17a-c). As incubation continued, mineralization rates were lower but applications preserved some differences each other, leading to different net manure organic N mineralization (NNM) dynamics (Fig. 3.16a-c and Fig. 3.17a-c).

In the combination HEI treatment x sandy loam soil (Fig. 3.13a), during the first week after manure application to the soil, no NNM occurred during applications 1 and 2, while applications 3 and 4 differentiated each other with a significantly lower NNM, on day 1, and higher NNM on day 6, during application 3 compared to application 4 ($P < 0.05$). In the period day 11 – day 28, no significant difference were found among applications. On day 84, higher NNM occurred in application 4 compared to applications 1 and 2 ($P < 0.05$), when application 1 immobilized a net amount of SMN corresponding to 6.3% of the added organic manure N, and application 4 mineralized 21.7% of the added manure organic N.

In the clay loam soil, the mineralization dynamics of manure organic N resulted different if $NH_4^+{}_f$ was considered or not in the calculation of SMN. When NNM was calculated excluding this fraction (thus considering only the soluble + extractable fraction of soil mineral nitrogen), significant differences among applications were found on day 15 (Fig. 3.16b), when application 4 immobilized much nitrogen compared to applications 1 and 2 and, on day 40, when application 3 mineralized more manure organic N with respect to applications 1 and 4 ($P < 0.05$). At the end of the incubation interval (day 84) NNM was higher (5.0% of added organic N) during application 4 compared to applications 3, when 1.4% of added organic N was immobilized.

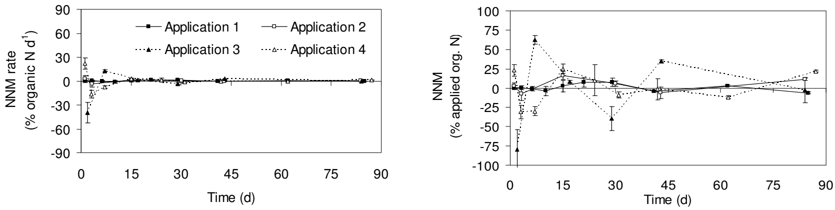
Including the non-exchangeable ammonium fraction into the calculation of NNM (Fig. 3.16c), resulted in a significantly higher NNM on day 84 during application 4 compared to application 1. In this case, at the end of the incubation interval, applications 1 and 4 showed a NNM of 3.1 and 24.4% of the added organic N, respectively ($P < 0.05$).

In COW, on the sandy loam soil, within 1 day after manure application, an amount of SMN corresponding (on average) to 25% of the manure organic N was immobilized during applications 1 – 3 (Fig. 3.17a), with a significantly higher NNM during application 1 compared to application 2 ($P < 0.05$). During the following incubation time, significant differences among applications were found only on day 40, when much manure organic N was mineralized during application 3 with respect to application 1 ($P < 0.05$). On day 84 all applications showed a net immobilization of soil mineral nitrogen ranging from 39.1 to 7.3% of added manure organic N, with no significant differences among applications.

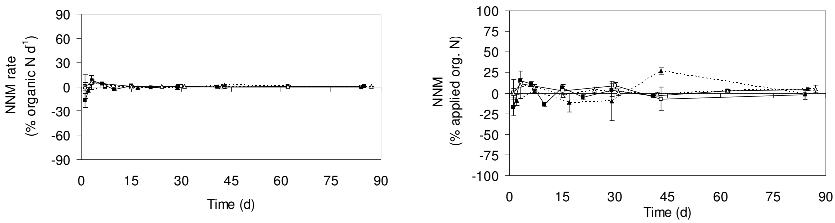
In the case of COW treatment applied onthe clay loam soil, the results obtained excluding $NH_4^+{}_f$ from calculations (Fig. 3.17b), show that, on day 1, application 2 mineralized more N compared to application 1, when a net immobilization of SMN, corresponding to 38.7% of the added manure N, occurred. As incubation continued, significant differences among applications were found on day 15 and on day 60 when application 4 showed a net N immobilization and application 1 a positive NNM ($P < 0.05$). After 84 days of incubation, all applications immobilized an amount of SMN ranging from 39.06 to 7.32% of added manure organic N, with no significant differences among applications. Results obtained including $NH_4^+{}_f$ into calculations (Fig. 3.17c) showed that at the end of incubation, on day 84, a significant percentage of manure organic N was mineralized during application 4 (25.3% of added organic N) compared to application 1 (4.13% of added organic N).

Figure 3.16. Net organic nitrogen mineralization (NNM) rates and NNM in two soils subjected to repeated applications of heifer manure. In (a,b) NNM included the effect of $NH_4^+{}_f$. In (c) NNM excluded the effect of $NH_4^+{}_f$.

(a) Sandy loam soil



(b) Clay loam soil



(c) Clay loam soil

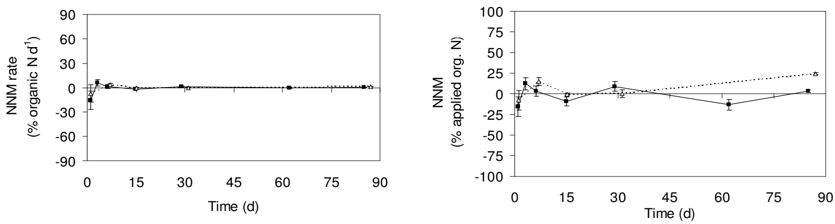
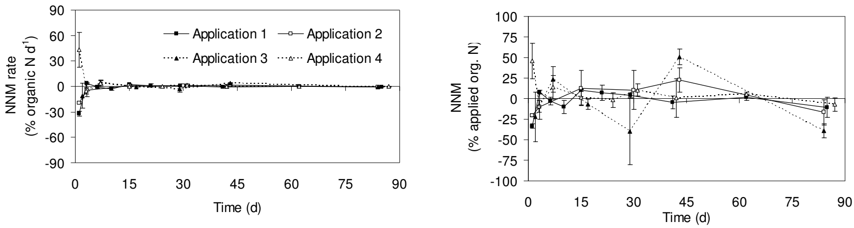
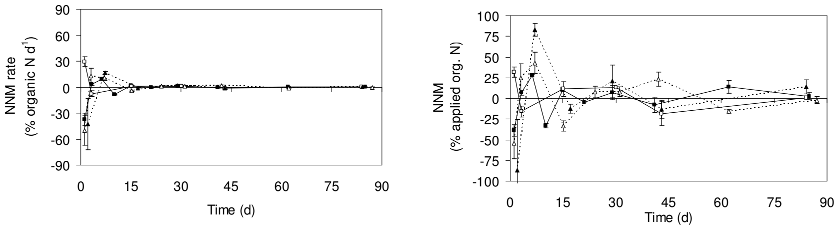


Figure 3.17. Net organic nitrogen mineralization (NNM) rates and NNM in two soils subjected to repeated applications of dairy cow manure. In (a,b) NNM included the effect of $NH_4^+{}_f$. In (c) NNM excluded the effect of $NH_4^+{}_f$.

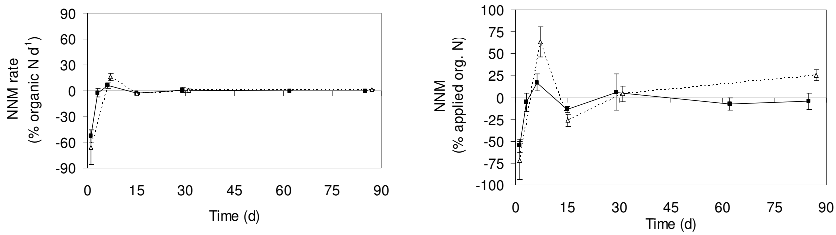
(a) Sandy loam soil



(b) Clay loam soil



(c) Clay loam soil



Manure N residual effect

Residual effect of added manure N (NRE) was calculated as the difference between net SMN (expressed as a percentage of added manure N) of the i^{th} application and net SMN of application 1.

For application4, only in the case of manures applied on the clay loam soil, residual effect was also calculated considering the non-exchangeable ammonium in the estimation of SMN, in order to take into account the fluxes into and out from the $NH_4^+{}_f$ pool.

HEI treatment on the sandy loam soil gave decreasing NREs after repeating manure applications (Fig. 3.18a); only application 2 had a positive NRE on day 84 (12.5% of the added manure N), while applications 3 and 4 showed a negative NRE corresponding to (on absolute value) 2.8 and 10.5% of the added manure N, respectively. Repeating the application of HEI tratment on the clay loam soil (Fig. 3.18b), induced increasing values of NRE. After the last application, on day 84, there was an increase of SMN corresponding to 17% of the applied manure N. The residual effect of added manure N was lower when SMN included the non-exchangeable ammonium fraction; in this case the estimated NRE during application 4 corresponded, on day 84, to 7.0% of the added manure N (Fig. 3.20a).

In the case of COW treatment applied on the sandy loam soil (Fig. 3.19a), NRE calculated on day 84 was equal for applications 2 and 4, corresponding to 22.8% of the added manure N. On the clay loam soil (Fig. 3.19b), NRE on day 84 ranged from 13.8 to 24.6% of the N added with applications 1 and 3, respectively. As observed for HEI, when $NH_4^+{}_f$ was considered in the calculation of the residual effect (Fig. 3.20b), NRE of application 4 (2.4% of the added manure N) was lower compared to NRE calculated excluding the clay fixed fraction of ammonium (11.5% of the added N).

Figure 3.18. Residual effect of added manure N (NRE) in heifer manure amended soils.

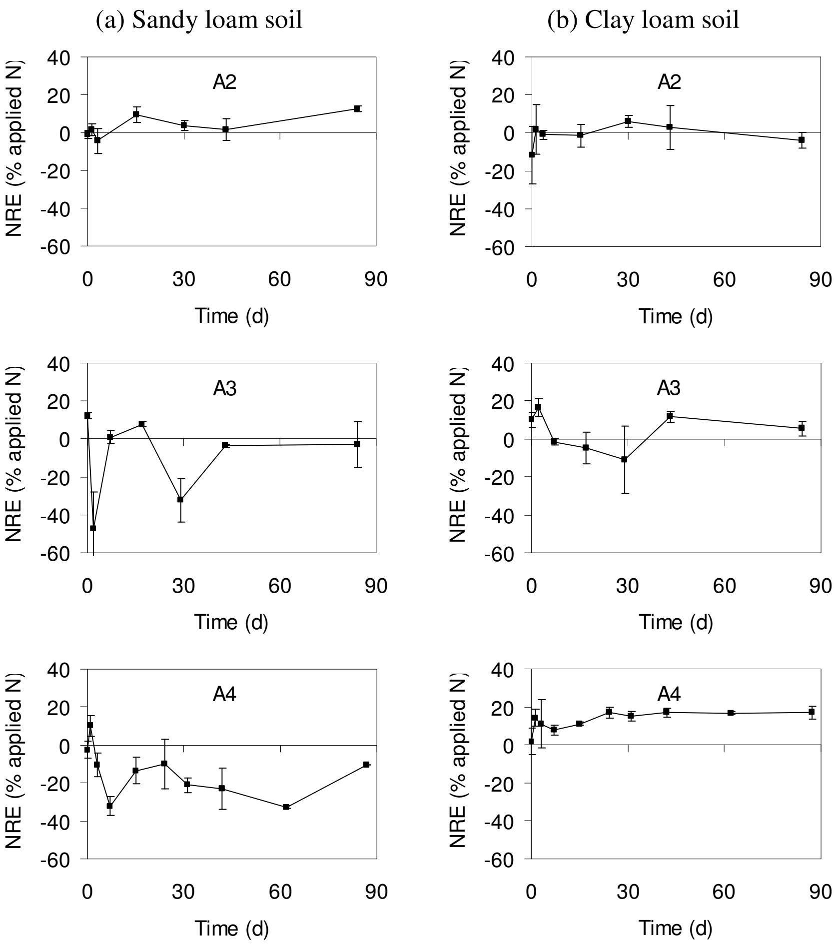


Figure 3.20. Residual effect of added manure N (NRE) in dairy cow manure amended soils.

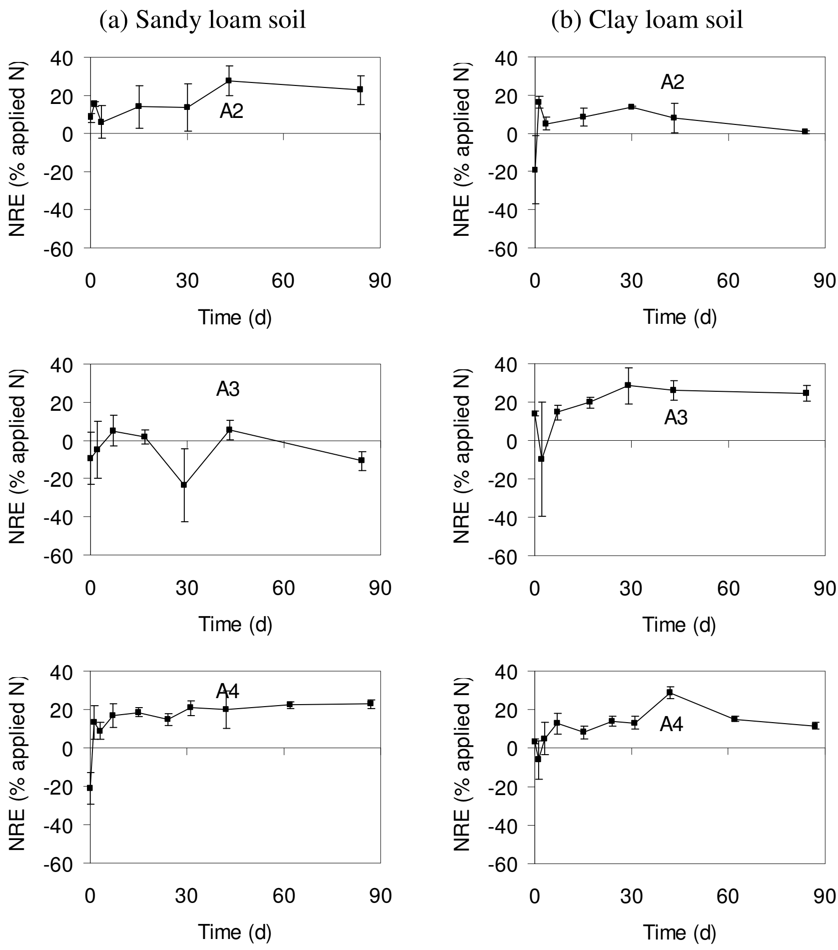
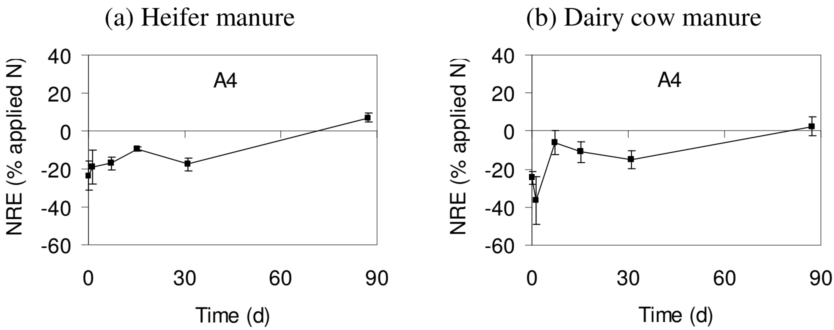


Figure 3.21. Residual effect of added manure N (NRE) including NH4+f into calculations in a clay loam soil subjected to repeated heifer and dairy manure applications.



Discussion and conclusions

Soil pH

Soil pH measured in the unamended treatment (CON) decreased during incubation (Fig. 3.1a-b) until it reached a value of 5.2 and 6.4 on the sandy loam and the clay loam soil, respectively. Also in the ammonium sulphate treatment (AS) soil pH decreased until values of 4.3 and 4.6 on day 342, on the two soils (Fig. 3.1a-b). The observed decrease of pH could have been due to the nitrification of added ammonium. The low values of pH measured in AS on the two soils could have affected microbial activity and growth, resulting in lower SMB-C concentrations in AS (Fig. 3.3a-b) compared to CON. Low pH could also have determined, on the sandy loam soil, a suppression of nitrification starting from the second application event (Fig. 3.10a).

In the manure-amended treatments (HEI and COW), soil pH increased after each application event (Fig. 3.1a-b). This increase of soil pH was more consistent in the heifer manure-amended soils (HEI) compared to the dairy cow manure-amended soils (COW) and was probably due to the high pH of the added materials and, in the case of COW, to a rapid decarboxylation of volatile fatty acids (VFA; Sørensen, 1998a). The increase of pH occurring after manure addition was more consistent on the sandy loam soil compared to the clay loam soil, may be due to a lower buffering capacity of the sandy loam soil compared to the clay loam soil. As pH increased after manure application, it is possible that some of the applied NH_4^+ volatilised as NH_3 when the manure was mixed with the soil, resulting in recovery of manure NH_4^+ lower than 100% after some application events (Fig. 3.14a-d and Fig. 3.15a-d). During the first weeks of incubation, as nitrification of added ammonium occurred (Fig. 3.12a-b and Fig. 3.13a-b), soil pH progressively decreased.

Carbon dynamics

In CON and AS treatments, accumulated carbon respiration in the period 0 – 84 days decreased after repeated applications possibly due to a decrease in the content of easily-decomposable organic matter with time (Fig. 3.2c-f). After each application event, on both CON and AS treatments, the action of manually mixing soil and manure temporary stimulated carbon respiration, as testified by higher respiration rates in the first days after each application event, compared to the remaining period until the subsequent application (Fig. 3.2c-f). Initially higher respiration rates can be attributed also to the partially drying of samples before materials were applied to the soil; this procedure could have killed part of the soil microbial biomass that became a readily decomposable C substrate for the survived microbial biomass once the soil was re-wetted after the application event. Despite this, it was necessary to partially dry samples before each application event in order to guarantee always a constant soil water content corresponding to a soil water potential of -50 kPa, due to the water content of the applied manures.

At the end of incubation (day 342) the accumulated C respiration of the two unamended soils was about 6% of the initial soil C content (Fig. 3.2a-b). Many authors reported higher respirations on sandy compared to clay soils (Thomsen and Olesen, 2000; Thomsen et al., 2003). In our experiment the respiration of C was higher in the clay loam compared to the sandy soil. When materials were applied to the soil, they were finely mixed with the soil, possibly leading to an increase of aeration of the clay loam soil that might explain its higher respiration rate.

At the beginning of incubation soil microbial biomass C (SMB-C) in CON accounted for 1.3% of the soil C content on both soils (Fig. 3.3a-b). On the sandy loam soil, during the period following the first application event, SMB-C concentration decreased, while, after the last application event, it remained stable until day 84. On the clay loam soil, SMB-C concentration only slightly decreased during the first application and rapidly increased after the last application event, when it reached a value close to that measured at the beginning of the incubation (1.1% of the soil C content). In the AS treatment, SMB-C concentration during the first application was similar to that measured in the CON treatment on both soils (Fig. 3.3c-d); after the last application event, SMB-C concentration was lower compared to

CON on both soils, may be due to the lower pH values of AS-amended soils compared to the unamended soils.

Carbon respiration in manured soils was characterized by faster respiration rates immediately after manure application (in the first 15 days) and slower respiration rates thereafter (Fig. 3.4c-d and Fig. 3.5c-d). This is a pattern commonly found in similar incubations (e.g. Thomsen and Olesen, 2000; Calderón et al., 2004; Morvan et al., 2006). In all combinations of soil type × application event, COW respired much more than HEI, resulting in a higher accumulated C respiration at the end of the incubation (Fig. 3.4a-b and 3.5a-b). The total amount of C respired in HEI during the first 15 days was 8.6 and 11.4% of the added C (average of four application events), respectively on the sandy loam and clay loam soil, corresponding to 54 and 71% of the manure-soluble C (Fig. 3.4a-b). At the end of the incubation interval (day 84), HEI respired 18.1 and 22.4% of the added C (average of four application events) on the two soils, indicating that only a small fraction of manure-insoluble C was mineralized within 84 days (Fig. 3.4a-b). In COW treatments in the first three days of incubation accumulated C respiration in the two soils was equivalent to the manure VFA-C content, while in the first 15 days of incubation accumulated C respiration corresponded to 32.6 and 35.3% of the added C on the two soils, corresponding to about 100% of the water-soluble C of the manure (Fig. 3.5a-b). At the end of the incubation interval (day 84), accumulated respiration in COW was 46.2% of the added manure C on the sandy loam soil

(Fig. 3.5a) and 51.1% on the clay loam soil (Fig. 3.5b), indicating that about 20% of the added manure insoluble C was mineralized in the period 15-84 day. Similar results can be found in the literature describing incubation experiments with animal manure. Sørensen (1998b), in an 84-d incubation at 23°C, measured respiration of 49 and 60% of added C, for stored and fresh cattle manure, respectively. For a longer incubation (224 d) at a lower temperature (15°C), Morvan et al. (2006) reported variable respiration for cattle manures (composted or not), ranging from approximately 15 to 58%. While our results for the COW manure are rather in agreement with most values found in the literature, those of the HEI manure are rather low and indicate an extremely resistant type of manure. Application of manure C to the soil increased soil microbial biomass carbon (SMB-C) concentration in both soils after the first and fourth application events (Fig. 3.6a-d). SMB-C increased immediately after manure application and, in general, was more consistent on the sandy loam soil compared to the clay loam soil, with the exception of COW on the clay loam soil. Moreover, SMB-C concentration in the clay loam soil was higher during the last manure application compared to the first one in both HEI and COW. At the end of the incubation interval (day 84), 4 to 14% of the added manure C was found as SMB-C in HEI, and 7 to 25% in COW.

The residual effect of added manure C (CRE) on day 84 was always positive in each soil × manure combination, with the exception of COW on the sandy loam soil during application 2 (Fig. 3.7a-b and

Fig. 3.8a-b), indicating that more C was mineralized following repeated manure applications to the same soil. The residual effect was more evident on the clay loam than in the sandy soil for both manure treatments, and for the HEI treatment compared to COW on both soils; this can be due the presence of more recalcitrant components in the HEI manure that required more time to be mineralized. In HEI on the sandy loam soil (Fig. 3.7a) the CRE of application 4 calculated on day 84 was 5.7% of the added manure-C, that is, about 6% more manure C was respired during the last application compared to the first. On the clay loam soil (Fig. 3.7b), the increment of manure-C respiration of the fourth application compared to the first, was two times higher compared to the sandy loam soil. For the COW treatment, residual effects of application 4 (Fig. 3.8a-b) corresponded to 5.5 and 7.9% of the added manure C on the sandy loam and clay loam soil, respectively.

Control soils

In both unamended soils (CON) the concentration of exchangeable ammonium ($NH_4^+{}_e$) was lower than 5 mg N kg^{-1} during all the incubation duration (from day 0 to day 342) (Fig. 3.9a-b). After each application event the concentration of nitrate increased constantly in both control soils, until it reached a value of 116 and 92 mg N kg^{-1} on the sandy loam and clay loam soil, respectively (Fig. 3.9a-b). Non-exchangeable ammonium ($NH_4^+{}_f$) concentration measured in the clay loam soil (Fig. 3.9b) at the beginning of the incubation was 216 mg N kg^{-1} (average of four applications). The concentration of $NH_4^+{}_f$ measured in the clay loam soil was consistent with values reported by other authors (Gouveia and Eoudoxie, 2007; Scherer and Ahrens, 1995; Steffen and Sparks, 1997; Stevenson, 1959).

The concentration of $NH_4^+{}_f$ remained constant during all the incubation period in the control treatment of the clay loam soil (from day 0 to day 342), with no consistent net release of $NH_4^+{}_f$ or net fixation of $NH_4^+{}_e$ as the incubation continued.

Ammonium sulphate-amended soils

Immediately after AS was applied to the sandy loam soil, $NH_4^+{}_e$ concentrations increased and, on the clay loam soil, an increase of $NH_4^+{}_f$ also occurred, indicating that a consistent part of the added NH_4^+ was rapidly fixed by the soil clay minerals and could not be extracted by neutral salt solutions. The mineral composition of clay-size fraction of the clay loam soil was characterized by a high percentage of high fixing clay minerals (Table 3.1) that can immobilize ammonium in a non-exchangeable form in few hours or few days after ammonium application to the soil. On the sandy loam soil the recovery of $NH_4^+{}_e$ (Fig. 3.11a) decreased as more AS was applied to the soil (i.e. moving from the first to the fourth application event), while on the clay loam soil the recovery slightly increased from application 1 to application 4. On the clay loam soil the amount of NH_4^+ recovered as $NH_4^+{}_f$ (Fig. 3.11b) ranged from 74.7 to 48.7% (first to fourth application event) of the added ammonium and decreased when more AS was applied to the soil, possibly due to a progressive saturation of the fixing complex of soil clay minerals after repeated AS applications.

On the sandy loam soil after the first application event, while $NH_4^+{}_e$ concentration decreased (reaching, at the end of the incubation interval -day 84- a concentration similar to CON), the nitrate concentration increased. However, on the sandy loam soil after the third application, $NH_4^+{}_e$ and NO_3^- concentrations remained constant during all the incubation intervals, presumably due to a strong reduction of the nitrification process due to the low soil pH value.

On the clay loam soil (Fig. 3.10b), $NH_4^+{}_e$ concentration always decreased during incubation, and reached after 84 days a concentration similar to CON (on average 5 mg N kg^{-1}). In the same time, a fraction of the initially fixed ammonium was progressively released into the soil solution. As more AS was applied to the soil, $NH_4^+{}_f$ concentration increased, due to a lower release of clay fixed ammonium after repeated applications. On the clay loam soil, nitrification of $NH_4^+{}_e$ occurred during all incubation intervals, leading to a net increase of NO_3^- concentration after each AS application.

Manure-amended soils

As observed for AS, in the manured treatments $NH_4^+{}_e$ concentration increased immediately after manure application on both soils, due to the fraction of added manure N in the form of NH_4^+ (Fig. 3.12a-b and Fig. 3.13a-b). On the sandy loam soil (Fig. 3.14a and Fig.3.15a), recovery of added NH_4^+ ranged from 71.9 to 114.8% of the manure NH_4^+ in the HEI treatment and from 76.9 to 105.4% in the COW treatment. The lower recovery of added manure NH_4^+ as $NH_4^+{}_e$ on the clay loam soil (Fig. 3.14d and Fig. 3.15d) was due to a fast fixation by the soil clay minerals of a fraction of the added manure NH_4^+.

In all soil × manure combinations (Fig. 3.12a-b and Fig. 3.13a-b), $NH_4^+{}_e$ concentration decreased quickly and, in less than 15 days on the sandy loam soil and less than 3 days on the clay loam soil, it reached concentrations similar to CON (on average 6 mg N kg^{-1}). Thereafter $NH_4^+{}_e$ remained low until the subsequent application event.

In HEI (Fig. 3.12b) after the first application event only a small fraction of initially clay fixed ammonium on the clay loam soil was released as the incubation continued, while after all the other application events $NH_4^+{}_f$ concentration slightly increased or remained stable until the end of the application interval (day 84), resulting in a progressive increase of $NH_4^+{}_f$ when more manure-N was added to the soil following repeated applications. In the COW treatment (Fig. 3.13b), during application 1 about 60% of the initially clay fixed NH_4^+ was released in the first week of incubation and thereafter $NH_4^+{}_f$ concentration remained constant until day 84. After the second application event, as observed also in HEI, no release of clay fixed ammonium was observed in the whole period 0 –84 day, resulting in a progressive increase of $NH_4^+{}_f$ concentration as incubation continued.

After manure application NO_3^- concentration increased constantly in all soil × manure combinations (Fig. 3.12a-b and Fig. 3.13a-b).

Manure N residual effect

In HEI on the sandy loam soil the residual effect of manure N (NRE) decreased after repeated manure applications (Fig. 3.18a); after four manure applications, a negative NRE was found, corresponding (on absolute value) to 10.5% of the added manure N. This means that in this soil the repeated application of the HEI manure did not lead to a build up of an organic N pool that is slowly mineralised.

On the clay loam soil (Fig. 3.18b), as more HEI manure was applied to the soil, the NRE increased. After the last application, on day 84, there was an increase of SMN corresponding to 17% of the applied manure N. When the non-exchangeable ammonium was included in the calculation of NRE (that is calculating SMN as the sum of $NH_4^+{}_e$

+ NO_3^- + $NH_4^+{}_f$), the estimated increase of SMN from application 1 to application 4 corresponded, on day 84, to 7.0% of the added manure N (Fig. 3.20a), indicating that part of the exchangeable ammonium of the soil came from the clay fixed ammonium pool, i.e. part of the residual effect is due to release of ammonium from the clay minerals.

In COW on both soils NRE was always positive. On the sandy loam soil (Fig. 3.19a), the NRE of application 4, calculated on day 84, corresponded to 22.8% of the added manure N. On the clay loam soil (Fig. 3.19b) the NRE of added manure N during application 4, on day 84, corresponded to 11.5% of the added N. Also in this case, the NRE of application 4 was lower when $NH_4^+{}_f$ was considered in the calculations (2.4% of the added manure N).

CHAPTER 4 - Modification of the CN-SIM model for the simulation of ammonium fixation and release in the soil.

Introduction

The conclusions previously reported in Chapter 1 highlighted that the parameterisation of the CN-SIM model (Petersen et al, 2005a) for a simultaneous simulation of manure C and N mineralization in a period of 180 days under laboratory conditions lead to a marked overestimation of soil mineral nitrogen (SMN) concentration after the first six weeks of incubation, due to the re-mineralization of initially immobilized N.

When calibrating model parameters using the data-set reported in Chapter 1, the optimization algorithm often found optimal manure parameters combinations leading to manures characterized by a fast (high values of $kAOM_{2i}$) and N-poor (low value of $fAOM_{2N}$) AOM_2 pool and slower and N-rich AOM_1 pool (Tab. 1.5). With the actual model structure, this was the only possibility for the optimization algorithm to match the experimentally measured decrease of SMN concentration within few hours after manures application to the soil. Despite a good initial fit of experimental data, immobilized N was re-mineralized more or less faster, depending on the turnover of the organic pools, due to the respiration of manure added C. The more C was respired, in order to maintain C respiration rates congruent with the experimental measured rates, more organic N was re-mineralized as the incubation continued.

Moreover, the conclusion reported in Chapter 2, demonstrated that a satisfying simulation of C respiration or N mineralization occurs with different manure calibrated parameters sets, due to a strong conflict between the two objectives of interest (the simulation of C and N mineralization). Even when more equivalent model parameter combinations were available for the simulation of C and N dynamics in the manured soils, in many soil × manure combinations, N mineralization was never simulated well, as indicated by the high values of RRMSE-SMN reported in Chapter 2.

For these reason it was hypothesised that the processes currently implemented in the model were not sufficient for a complete interpretation of incubation experiments with liquid dairy manures, and that other processes should be taken into account for a better synchronous simulation of C and N dynamics.

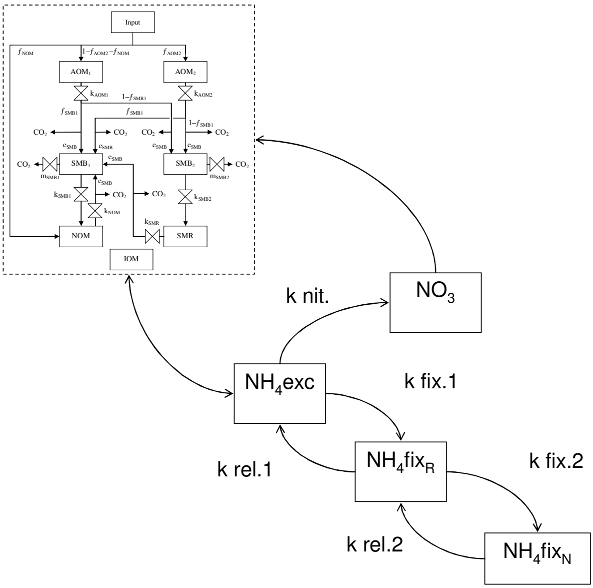
Based on this conclusion, the objective of the work described in this chapter was to modify the structure of the CN-SIM model in order to provide a first implementation of the processes of clay fixation and release of NH_4^+ in the soil. We decided to incorporate ammonium fixation/release and not other processes (such as ammonia volatilization or denitrification), because during the incubation experiment described in Chapter 3 non-exchangeable ammonium dynamics were measured in a period of 342 days, after repeated applications of three different sources of NH_4^+ (two manures and ammonium sulphate) to a clay loam soil able to fix consistent amounts of added NH_4^+.

Materials and methods

The original CN-SIM model (Fig. 1.1) was modified by the addition of two new mineral pools of ammonium (Fig. 4.1), conceptually corresponding to the native clay fixed ammonium (NH_4fix_N) and to the more recently fixed ammonium of the soil (NH_4fix_R). The representation of total soil clay-fixed ammonium with two pools is in agreement with the results obtained in many field and laboratory experiments. According to these results, the native $NH_4^+{}_f$ is more strongly retained by the soil clay minerals and consequently less accessible to plants and microorganisms, and less released compared to the recently fixed ammonium. Recently fixed ammonium concentration often increases after ammonium fertilizers are applied to the soil and, as ammonium is depleted from the soil solution via nitrification or microbial immobilization, it is easier released by the clay minerals compared to the native $NH_4^+{}_f$ (Feigenbaum et al., 1994; Steffens and Sparks, 1997, Gouveia and Eudoxie, 2007).

$NH_4^+{}_f$ flows into and out from the pools NH_4fix_N and NH_4fix_R were described by four first order kinetics (two for ammonium release and two for ammonium fixation, Fig. 4.1). First order kinetics were used to make this first implementation of the process easier; in future developments, other more appropriated equations (such as Langmuir-type equations) could be used to better represent the fixation and release of NH_4^+.

Fig. 4.1. Structure of the modified CN-SIM model (CN-SIM-FIX) including two new NH4 pools (NH_4fix_N and NH_4fix_R) for the simulation of ammonium clay fixation and release. Parameters k_{Rel1}, k_{Rel2}, k_{Fix1} and k_{Fix2} are the first order equations parameters used to calculate ammonium fixation and release rates.



As a support to model parameters calibration, a sensitivity analysis (SA) was carried out to assess the contribution of each model parameter in determining model output variation, following the same approach adopted in Chapter 1. Briefly, sensitivity analysis was performed in two steps: a first analysis was done using the method of Morris with the aim of fixing the value of those parameters that do

not significantly influence model outputs; a second analysis was carried out with the purpose of ranking parameters in order of importance on impacting model output variance, to diminish the number of parameters subjected to optimisation (method of Sobol').
In this work, differently from the second SA performed in Chapter 1, model sensitivity was explored for one manure type on a single soil type. Model outputs for which sensitivity was evaluated, were net cumulated CO_2 and net SMN concentration of the soil for both CN-SIM and CN-SIM-Fix models. In order to take into account for the effect of parameters on model output variation, both during the first manure application, and after repeated manure applications, model sensitivity was explored in the interval day 0-84 and day 245-342, after four manure applications. This two time periods corresponded to the first and last manure application intervals of the incubation experiment described in Chapter 3.

After SA, most sensitive parameters were subjected to calibration using the downhill simplex method (Nelder and Mead, 1965) ,while non-sensitive parameter values was fixed at the optimized value reported in Petersen et. al, 2005a, 2005b (Table 1.3).

Experimental data used to perform models parameters calibration were those related to the cumulated net CO_2, net SMN concentration and, only for CN-SIM-FIX , net $NH_4^+{}_f$ concentration measured on the clay loam soil amended with the dairy cow manure in the period 0-342 days (Chapter 3).

In the case of CN-SIM, the objective function was the average of the relative root mean squared errors (RRMSEs) between simulated and measured net CO_2–C (RRMSE–CO_2) and net SMN (RRMSE–SMN), while, in the case of CN-SIM-FIX it was the average of the relative root mean squared errors (RRMSEs) between simulated and measured net CO_2–C (RRMSE–CO_2) and net SMN (RRMSE–SMN) and net $NH_4^+{}_f$ (RRMSE–NH4fix).

During SA and parameters optimization, common models parameters boundaries were those already reported in Table 1.3; For the additional CN-SIM-FIX model parameters (k_{Rel1}, k_{Rel2}, k_{Fix1} and k_{Fix2}), boundaries were set in the range 0.0001 – 0.1.

Because this was a preliminary implementation of the clay fixation process, two empirical equations, based on the experimental recoveries of added manure NH_4^+ as $NH_4^+{}_e$ (exchangeable) or $NH_4^+{}_f$ (non-exchangeable), were used to calculate, for each manure application event, the amount of manure NH_4^+ immediately fixed after each material application.

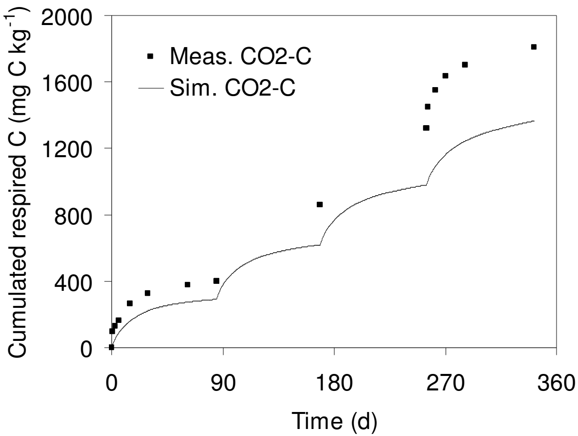
Preliminary results and further work

Results of the first sensitivity analysis (method of Morris) confirmed the previously obtained results in Chapter 1. Model parameters k_{AOM1s}, k_{AOM2s}, f_{IOM} and CN_{AOM} did not affected net model outputs and were therefore fixed at the optimized value reported in Table 1.3 (data not shown). After the second SA, among the model parameters that were mostly responsible for model output variations there were both manure parameters (k_{AOM1i}, k_{AOM2i}, f_{AOM2}, f_{Part}), and soil parameters (e_{SMB}, CN_{SMB1}). SA results for the CN-SIM-FIX model, together with the previously identified sensitive parameters, identified also k_{Rel1}, and k_{Fix1} as important parameters affecting SMN concentration. Even if k_{Rel2}, and k_{Fix2} were not identified as sensitive parameters, they were included in the preliminary optimization in order to achieve an optimized value for further simulations.

Carbon and nitrogen simulations were carried out with the best optimized parameters combination found with the downhill simplex method (Fig. 4.2 and Fig. 4.3) while optimized model parameters are reported in Table 4.1.

Fig. 4.2. Measured and simulated net accumulated C respiration on a clay loam soil subjected to four repeated dairy cow manure applications. a) simulation performed using the CN-SIM model; b) simulation performed using the modified version of the CN-SIM model, including the ammonium fixation and release processes.

(a)



(b)

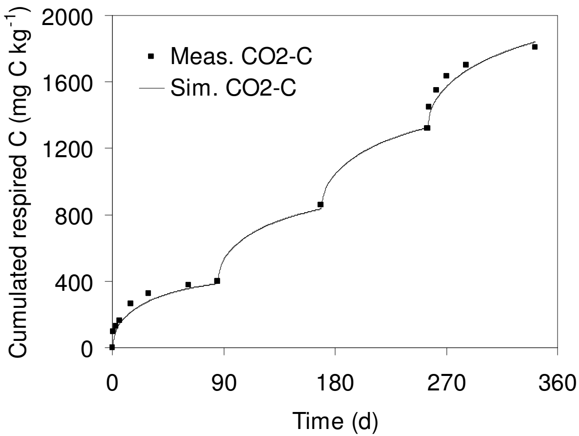
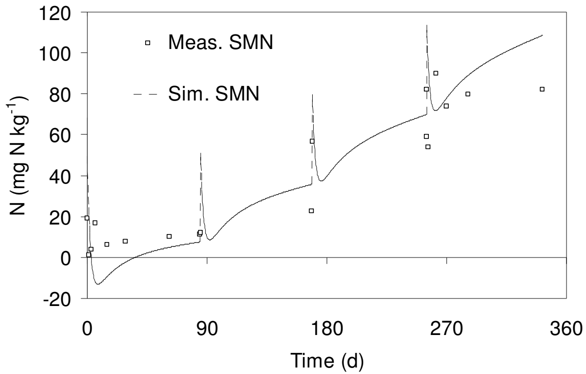


Fig. 4.3. Measured and simulated net SMN concentration (a-b) and net non-exchangeable ammonium concentration (b) on a clay loam soil subjected to four repeated dairy cow manure applications. a) simulation performed using the CN-SIM model; b) simulation performed using the modified version of the CN-SIM model, including the ammonium fixation and release processes.

(a)



(b)

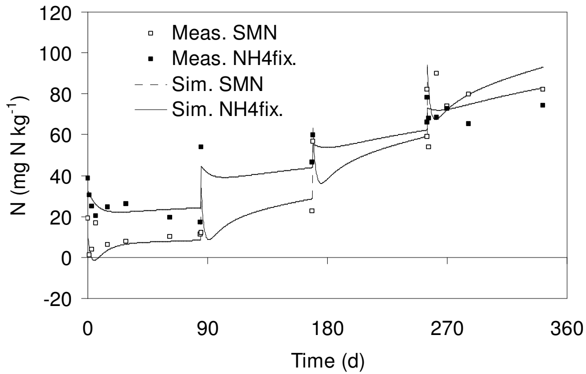


Table 4.1. Optimized parameters values for the simulation of C respiration and SMN (a-b) and non-exchangeable ammonium (b) in a clay loam soil subjected to four repeated dairy cow manure applications. a) optimized values found for the CN-SIM model; b) optimized values found for the modified version of the CN-SIM model, including the ammonium fixation and release processes.

Model parameter	(a)	(b)
k_{Fix1}	--	0.0094
k_{Fix2}	--	0.099
k_{Rel1}	--	0.055
k_{Rel2}	--	0.00011
e_{Smb}	0.90	0.78
CN_{Smb1}	7.97	8.89
f_{Aom2}	0.66	0.60
f_{Nom}	0.20	0.14
f_{Aom2N}	0.01	0.64
k_{Aom1i}	0.000043	0.017
k_{Aom2i}	0.42	0.42

As previously observed in Chapter 1, in order to contain an excessive overestimation of SMN (Fig. 4.3a), optimized CN-SIM parameters values lead to a decrease in C respiration (Table 4.1a), resulting in an underestimation of cumulated C as more manure was applied to the soil (Fig. 4.2). Including ammonium fixation into the model structure enabled a better synchronous simulation of both N mineralization and C respiration, also after repeated manure applications (Fig. 4.2b and Fig. 4.3b).

These preliminary results shows that better model performances can be achieved with the implementation of other processes involving mineral nitrogen transformation in the soil, but not directly related to the decomposition of soil C.

Further work will be necessary to better represent the equibrium soluble-exchageable and clay-fixed ammonium; moreover it will be necessary to perform further investigations in order to estimate the amount of nitrogen that is incorporated in the non-exchangeable pools following the application of NH_4^+-containing materials, ammonium fixation should also be related to the amount of added NH_4^+, the concentration of $NH_4^+{}_f$ and possibly to the concentration of other added competing ions (such as K^+).

GENERAL CONCLUSIONS

A published SOM model (CN-SIM) was applied to the results of an incubation experiment carried out before the start of this thesis (five manures incubated on three soils for six months) to test its performance when simulating the decomposition of manure after soil incorporation. The original version of the CN-SIM model, which does not include the simulation of clay fixation of ammonium, was coupled with two different automatic optimization methods (the downhill simplex and NSGA-II, a genetic algorithm). Both methods were unable to reach a satisfactory agreement between measured and observed CO_2 accumulated respiration and soil mineral N concentration. In most cases we observed a trade-off between a good simulation of CO_2 respiration and a good simulation of soil mineral N. In particular, we frequently observed the overestimation of soil mineral N due to the remineralisation of microbial immobilized N. This suggested that a soil process (not linked to C respiration) that decreases the concentration of soil mineral N was not represented in the model and thus was responsible for its lack of performance. A candidate process was the fixation of ammonium due to clay.

Therefore an incubation experiment of soil + manure mixtures (with repeated applications of manure to the same experimental units) was carried out. The experiment confirmed that the type of manure substantially influences the dynamics of C and N after manure incorporation in the soil. The heifer manure used in this experiment was more resistant to decomposition than the dairy cow manure. The heifer manure respired only about 20% of C applied, while this percentage was about 50% for the cow manure. The experiment also confirmed that the effect of soil on C respiration is less pronounced than that of manure.

In general the residual effect of added manure C measured after four additions of manure to soil was rather low (5-12%), idicating that only a small fraction of manure recalcitrant components were mineralized with subsequent applications.

In the incubation experiment, the two manured soils showed different mineral nitrogen dynamics. After repeated manure applications, nitrate concentration increased more on the sandy loam soil compared to the clay loam soil, mostly due to a lower exchangeable ammonium concentration in the former. The incubation experiment has also shown the extremely important role of clay fixation of added ammonium. In all treatments (ammonium sulphate- and manure-amended treatments) substantial fractions of added ammonium were fixed in soil in few hours after the addition of manure or ammonium sulphate, thus making it temporary not available for plants and microorganisms. The fixed ammonium ($NH_4^{+}{}_f$) was then partly released over time as exchangeable and then soluble ammonium. Repeated manure applications have decreased the amount of added NH_4^+ fixed by clay minerals immediately after materials applications; despite this, as more manure was applied to the clay loam soil, less clay-fixed ammonium was released in the soil solution during the subsequent incubation interval, leading to a progressive increase of the $NH_4^+{}_f$ concentration during incubation.

Using the data obtained in the experiment, we have run a preliminary calibration of a new version of CN-SIM that includes the simulation of ammonium fixation. Preliminary results confirm that the introduction of carbon-independent processes of mineral nitrogen transformation into the soil can improve the simulation of C and N mineralization.

Further work to be carried out for a mechanistic understanding of N dynamics after manure application to soil includes: i) to measure C and N dynamics after 10-20 repeated applications of manure, to have a more sizeable residual effect; ii) to improve the dynamic simulation of ammonium fixation in soil; iii) to simulate the ammonium fixation occurring immediately after manure addition, also in relation to other factors, such as the added potassium and the amount of clay fixed ammonium, that can influence the fixation process.

REFERENCES

Acutis, M., Confalonieri, R., 2006. Optimization algorithms for calibrating cropping systems simulation models. A case study with simplex-derived methods integrated in the WARM simulation environment. Ital. J. Agromet. 11, 26–34.

Bechini, L., Marino, P., 2009. Short-term nitrogen fertilizing value of liquid dairy manures is mainly due to ammonium. Soil Sci. Soc. Am. J. 73, 2159–2169.

Bellocchi, G., Rivington, M., Donatelli, M., Matthews, K., 2009. Validation of biophysical models: issues and methodologies. A review. Agron. Sustain. Dev. 30, 109–130.

Calderon, F.J., McCarty, G.W., Van Kessel, J.A.S, Reeves III, J.B., 2004. Carbon and nitrogen dynamics during incubation of manured soil. Soil Sci. Soc. Am. J. 68, 1592–1599.

Calderón F.J, McCarty G.W, Reeves III J.B, 2005. Analysis of manure and soil nitrogen mineralization during incubation. Biol Fertil Soils 41, 328–336.

Campolongo, F., Cariboni, J., Saltelli, A., 2007. An effective screening design for sensitivity analysis of large models. Environ. Modell. Soft. 22, 1509–1518.

Cariboni, J., Gatelli, D., Liska, R., Saltelli, A., 2007. The role of sensitivity analysis in ecological modelling. Ecol. Model. 203, 167–182.

Causarano, H.J., Shaw, J.N., Franzluebbers, A.J., Reeves, D.W., Raper, R.L., Balkcom, K.S., Norfleet, M.L., Izaurralde, R.C., 2007. Simulating field-scale soil organic carbon dynamics using EPIC. Soil Sci. Soc. Am. J. 71, 1174–1185.

Chan, K., Saltelli, A., Tarantola, S., 1997. Sensitivity analysis of model output: variance-based methods make the difference. In the Proceedings of the 1997 Winter Simulation Conference. Atlanta, Georgia, United States. Eds. Andradottir, S., Healy K.J., Withers, H., Nelson, B.L., 261–268.

Chantigny, M.H., Angers, D.A., Morvan, T., Pomar, C., 2004. Dynamics of pig slurry nitrogen in soil and plant as determined with 15N. Soil Sci. Soc. Am. J. 68, 637–643.

Chatskikh, D., Olesen, J., Berntsen, J., Regina, K., Yamulki, S., 2005. Simulation of effects of soils, climate and management on N2O emission from grasslands. Biogeochemistry 76, 395–419.

Coello, C.,C.,A., 2006. Evolutionary multi-objective optimization: a historical view of the field. IEEE Computational intelligence magazine, 29-36.

Confalonieri, R., 2010. Monte Carlo based sensitivity analysis of two crop simulators and considerations on model balance. Eur. J. Agron. 33, 89–93.

Day, R.W., Quinn G.P., 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. 59, 433–463.

Deb, K., Pratap, A., Agarwal, S., Meyarivan, T., 2002. A fast and elitist multiobjective genetic algorithm: NSGA-II. Evolutionary Computation, IEEE Transactions on 6, 182-197.

Deb, K., 2004. Multiobjective optimization using evolutionary algorithms. John Wiley & Sons.

Duan, Q., Sorooshian, S., Gupta, V.K., 1992. Effective and efficient global optimization for conceptual rainfall-runoff models. Water Resour Res 28, 1015-1031.

Feigenbaum, S., Hadas, A., Sofer, M., 1994. Clay-Fixed Labeled Ammonium as a Source of Available Nitrogen. Soil Sci Soc Am J 58, 980-985.

Grignani, C., Bassanino, M., Sacco, D., Zavattaro, L., 2003. Il bilancio degli elementi nutritivi per la redazione del piano di concimazione. Rivista di agronomia 37, 155-172.

Gouveia, G.A., Eudoxie, G.D., 2007. Distribution of fertiliser N among fixed ammonium fractions as affected by moisture and fertiliser source and rate. Biol Fertil Soils 44, 9–18.

Heinen, M., 2006. Simplified denitrification models: overview and properties. Geoderma 133, 444–463.

Homma, T., Saltelli, A., 1996. Importance measures in global sensitivity analysis of model output. Reliab. Eng. Syst. Safe. 52, 1–17.

Konak, A., Coit, D.W., Smith, A.E., 2006. Multi-objective optimization using genetic algorithms: a tutorial. Rel. Eng. Sys. Saf. 91, 992-1007.

Loague, K., Green, R.E., 1991. Statistical and graphical methods for evaluating solute transport models: overview and application. J. Contam. Hydrol. 7, 51–73.

Maes, F., Vandermeulen, D., Suetens, P., 1999. Comparative evaluation of multiresolution optimization strategies for multimodality image registration by maximization of mutual information. Medical Image Analysis 3, 373–386.

Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: Theory and models across scales. Soil Biol. Biochem. 41, 1355–1379.

Marino, P., De Ferrari, G., Bechini, L., 2008. Description of a sample of liquid dairy manures and relationship between analytical variables. Biosyst. Eng. 100, 256–265.

Masse, D., Cambier, C., Brauman, A., Sall, S., Assigbetse, K., Chotte, J., 2007. MIOR: an individual-based model for simulating the spatial patterns of soil organic matter microbial decomposition. Eur. J. Soil Sci. 58, 1127–1135.

Monaco, S., Hatch, D.J., Sacco, D., Bertora, C., Grignani, C., 2008. Changes in chemical and biochemical soil properties induced by 11-yr repeated additions of different organic materials in maize-based forage systems. Soil Biology and Biochemistry 40, 608-615.

Morris, M.D., 1991. Factorial sampling plans for preliminary computational experiments. Technometrics 33, 161–174.

Morvan, T., Nicolardot, B., Péan, L., 2006. Biochemical composition and kinetics of C and N mineralization of animal wastes: A typological approach. Biol. Fertil. Soils 42:513–522.

Muñoz, R, Powell, J.M, Kelling, K.A, 2003. Nitrogen budget and soil N dynamics after multiple applications of unlabeled or ^{15}Nitrogen-enriched dairy manure. Soil Science Society of America Journal 67, 817-825.

Nelder, J.A., Mead, R., 1965. A simplex method for function minimization. Comput. J. 7, 308–313.

Petersen, B.M, Berntsen, S., Hansen, S., Jensen, L.S., 2005a. CN-SIM—a model for the turnover of soil organic matter. I. Long-term carbon and radiocarbon development. Soil Biol. Biochem. 37, 359–374.

Petersen, B.M., Jensen, L.S., Hansen, S., Pedersen, A., Henriksen, T.M., Sørensen, P., Trinsoutrot-Gattin, I., Berntsen, S., 2005b. CN-SIM: a model for the turnover of soil organic matter. II. Short-term carbon and nitrogen development. Soil Biol. Biochem. 37, 375–393.

Powell, J.M, Kelling, K.A, Muñoz, R, Cusick, P.R, 2005. Evalutation of dairy manure nitrogen enrichment methods on short-term crop and soil nitrogen budgets. Agronomy Journal 97, 333-337.

Probert, M.E., Delve, R.J, Kimani, S.K, Dimes, J.P., 2005. Modelling nitrogen mineralization from manures: representing quality aspects by varying C:N ratio of sub-pools. Soil Biol. Biochem. 37, 279–287.

Richter, G., Acutis, M., Trevisiol, P., Latiri, K., Confalonieri, R., 2010. Sensitivity analysis for a complex crop model applied to Durum wheat in the Mediterranean. Eur. J. of Agron. 32, 127–136.

Saltelli, A., 2002. Sensitivity analysis for importance assessment. Risk Anal. 22, 579–590.

Saltelli, A., Tarantola, S., Campolongo, F., Ratto, M., 2004. Sensitivity analysis in practice. A guide to assessing scientific models. John Wiley & Sons.

Saltelli A, Ratto M, Andres T, Campolongo F, Cariboni J, Gatelli D, Saisana M, Tarantola S, 2008. Global Sensitivity Analysis The Primer. John Wiley & Sons (ISBN: 978-0-470-05997-5).

Sherer, H.W., Ahrens, G., 1996. Depletion of non-exchangeable NH_4-N in the soil-root interface in relation to clay mineralogical composition and plant species. Eu. J. Ag. 5. 1-7.

Schröder, J., Jansen, A.G., Hilhorst., G.J., 2005. Long-term nitrogen supply from cattle slurry. Soil Use and Management. 21, 196-204.

Silva, J. A., Bremner, J.M., 1966. Determination and isotope-ratio of dofferent forms of nitrogen in soils: 5. Fixed ammonium. Soil Sci. Soc. Amer. Proc. 30, 597-594.

SIMLAB (2004) Version 2.2 Simulation Environment for Uncertainty and Sensitivity Analysis, developed by the Joint Research Centre of the European Commission.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555-569.

Sobol', I.M., 1993. Sensitivity estimates for nonlinear mathematical models. Math. Modell. Comput. Exp. 1, 407–414.

Sobol', I.M., Kucherenko, S.S., 2005. Global sensitivity indices for nonlinear mathematical models. Review. Wilmott Magazine 2, 2–7.

Sørensen, P., 1998a. Effects of storage time and straw content of cattle slurry on the mineralization of nitrogen and carbon in soil. Biol. Fert. Soils 27, 85–91.

Sørensen, P., 1998b. Carbon mineralization, nitrogen immobilization and pH change in soil after adding volatile fatty acids. Eur. J. Soil Sci. 49, 457–462.

Sørensen, P., 2004. Immobilisation, remineralisation and residual effects in subsequent crops of dairy cattle slurry nitrogen compared to mineral fertiliser nitrogen. Plant Soil 267, 285–296.

Sørensen, P., Amato, M., 2002. Remineralization and residual effects of N after application of pig slurry to soil. European Journal of Agronomy 16, 81-95.

Srinivas, N., Deb, K., 1994. Multiobjective optimization using nondominated sorting genetic algorithms. Evolutionary Computation 3, 221–248.

Steffens, D., Sparks, D.L., 1997. Kinetics of Nonexchangeable Ammonium Release from Soils. Soil Sci. Soc. Am. J, 61, 455-462.

Stevenson F. J, 1982. Nitrogen in agricultural soils.

Stotzky, G., 1965. Microbial respiration. p. 1150–1572. In C.A. Black et al (ed.) Methods of soil analysis. Part 2. 1st ed. Agron. Monogr. 9. ASA, Madison, WI.

Thiet, R.K, Frey, S.D, Six, J., 2006. Do growth yeld efficiencies differ between soil microbial community differing in fungal:bacterial ratios? Reality check and methodological issues. Soil Biology and Biochemistry 38, 837-844.

Thomsen, I.K., Olesen, J.E., 2000. C and N mineralization of composted and anaerobically stored ruminant manure in differently textured soils. J. Agric. Sci. Camb. 135, 151–159.

Thomsen, I.K., Schjønning, P., Christensen, B.T., 2003. Mineralisation of 15N-labelled sheep manure in soils of different texture and water contents. Biology and Fertility of Soils 37, 295-301.

Thuriès, L., Larre-Larrouy, M.C., Pansu, M., 2000. Evaluation of three incubation designs for mineralization kinetics of organic materials in soil. Communications in Soil Science and Plant Analysis 31, 289-304.

Van Kessel, J.S., Reeves III, J.B., Meisinger, J.J., 2000. Nitrogen and carbon mineralization of potential manure Components. J. Environ. Qual 29, 1669-1677.

Vrugt, J.A., Gupta, H.V., Bastidas, L.A., Bouten, W., 2003. Effective and efficient algorithm for multiobjective optimization of hydrological models. Water resources research, 39, 1-19.

Vrugt, J.A, Robinson, B.A., 2007. Improved evolutionary optimization from genetically adaptive multimethod search. PNAS, 104, 708-711.

Wöhling, T., Vrugt, J.A., Barkle, G.F., 2008. Comparison of three multiobjective optimization algorithms for inverse modelling of Vadose zone hydraulic properties. Soil Sci. Soc. Am. J., 2, 305-319.

Zitzler, E., Laumanns, M., Bleuler, S., 2004. A tutorial on evolutionary multiobjective optimization. In Xavier Gandibleux et al., editor, Metaheuristics for Multiobjective Optimisation, pp. 3–37, Berlin, Springer. Lecture Notes in Economics and Math-ematical Systems Vol. 535.