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Individual-based modelling of carbon and nitrogen dynamics in soils: Parameterization and sensitivity analysis of microbial components

Anna Gras[a,*], Marta Ginovart[b], Joaquim Valls[c], Philippe C. Baveye[d,e]

[a] Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, Campus Baix Llobregat, Esteve Terradas 8, 08860, Barcelona, Spain
[b] Department of Applied Mathematics III, Universitat Politècnica de Catalunya, Campus Baix Llobregat, Esteve Terradas 8, 08860, Barcelona, Spain
[c] Department of Physics and Nuclear Engineering, Universitat Politècnica de Catalunya, Campus Baix Llobregat, Esteve Terradas 8, 08860, Barcelona, Spain
[d] SIMBIOS Centre, Abertay University, 40 Bell Street, Kydd Building, Dundee DD1 1HG, UK
[e] Laboratory of Soil and Water Engineering, Department of Civil and Environmental Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

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ABSTRACT

The fate of soil carbon and nitrogen compounds in soils in response to climate change is currently the object of significant research. In particular, there is much interest in the development of a new generation of micro-scale models of soil ecosystems processes. Crucial to the elaboration of such models is the ability to describe the growth and metabolism of small numbers of individual microorganisms, distributed in a highly heterogeneous environment. In this context, the key objective of the research described in this article was to further develop an individual-based soil organic matter model, INDISIM-SOM, first proposed a few years ago, and to assess its performance with a broader experimental data set than previously considered. INDISIM-SOM models the dynamics and evolution of carbon and nitrogen associated with organic matter in soils. The model involves a number of state variables and parameters related to soil organic matter and microbial activity, including growth and decay of microbial biomass, temporal evolutions of easily hydrolysable N, mineral N in ammonium and nitrate, CO_2 and O_2. The present article concentrates on the biotic components of the model. Simulation results demonstrate that the model can be calibrated to provide good fit to experimental data from laboratory incubation experiments performed on three different types of Mediterranean soils. In addition, analysis of the sensitivity toward its biotic parameters shows that the model is far more sensitive to some parameters, i.e., the microbial maintenance energy and the probability of random microbial death, than to others. These results suggest that, in the future, research should focus on securing better measurements of these parameters, on environmental determinants of the switch from active to dormant states, and on the causes of random cell death in soil ecosystems.

1. Introduction

In the last few years, a number of critical reviews of the literature have highlighted the fact that current models of C and N dynamics in soils fail to reproduce observed measurements in a wide range of situations in which the temperature, the hydrological regime of soils, or both, vary significantly (Kirschbaum, 2006; Baveye, 2007; Gras et al., 2010). One perspective on this poor performance of models is that they are not describing processes at an appropriate scale, or at least do not contain upscaled information that is appropriate to satisfactorily account for the macroscopic emergence of microheterogeneous processes. From that viewpoint, a novel type of mathematical model is needed, which combines a pore-scale description of water and solute transport (e.g., via the Lattice-Boltzmann method), with a detailed account of microbial growth and metabolism.

A difficulty in trying to combine these two components is that the type of unstructured, population-level biokinetic equation (like Tessier's or Monod's) traditionally used to describe microbial growth in soils (e.g., Baveye and Valocchi, 1989; Seki et al., 2004; Thullner and Baveye, 2008) is not in principle applicable to the generally limited numbers of bacteria present in small pores. Therefore a different kind of microbial growth kinetic model is required, which accounts statistically for behavioural differences that may exist among individual microorganisms in a soil pore. Models with that scope have been the object of considerable research in general microbiology and population biology in the last decade (Bousquet and Le Page, 2004). In particular, individual-based models (IBMs) have received a lot of attention and met with considerable success in the description of microbial growth and metabolism under

* Corresponding author at: Technical University of Catalonia, School of Agricultural Engineering of Barcelona, Edifici ESAB, Campus Baix Llobregat Esteve Tarrades, 8, 08860, Barcelona, Spain. Tel.: +34 93 5521224; fax: +34 93 5521001.
E-mail address: anna.gras@upc.edu (A. Gras).

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a wide range of conditions (Grimm, 1999; Ferrer et al., 2008; Prats et al., 2008, 2010; Hellweger and Bucci, 2009; Ginovart et al., 2011).

The IBM INDISIM-SOM stems from an earlier model called INDISIM (for *IN*dividual *DI*screte *SIM*ulations) and described in detail by Ginovart et al. (2002). INDISIM resulted from the merging of a discrete approach to ecosystems through individual-based modelling with the formalism used to model molecular dynamics in fluids (Ferrer et al., 2008). In a nutshell, INDISIM settles and controls a group of microbial cells in a discrete space—a regular lattice consisting of a set of spatial units, subjected to appropriate boundary conditions. Then, INDISIM models the global evolution of the group of microbial cells by determining the individual behaviour of each bacterium and spatial unit in discrete events (time steps). The model uses stochastic rules and allows variability within the microbial population. Ginovart et al. (2005) were the first to adopt the IBM perspective to describe the fate of soil organic matter (SOM) with the model called INDISIM-SOM, describing in detail the many abiotic and biotic reactions controlling the dynamics of C and N. INDISIM-SOM encompasses a wide range of physical, chemical, and microbiological processes that regulate the short-term dynamics of soil C and N, namely decomposition, mineralization or immobilization of C and N, nitrification, and humification. In particular, in terms of microbiology, the model describes explicitly the uptake, metabolism, reproduction, death and lysis of microbial cells belonging to two broad metabolic groups, heterotrophic microorganisms (ammonifiers or decomposers), and nitrifying bacteria or autotrophs. Further details on the microbiological component of the model are provided by Ginovart et al. (2005) and Gras and Ginovart (2004, 2006).

Since Ginovart et al.'s (Ginovart et al., 2005) first implementation of an IBM in connection with soil microorganisms, significant additional research has been done in the area. Knudsen et al. (2006) developed a model of the hyphal growth of a biocontrol fungus, in which records of spatial location and branching hierarchy are maintained for individual hyphal nodes, so that the entire spatial structure of a fungal colony (hyphal network) can be explicitly reconstructed at any time. Masse et al. (2007) developed an IBM to analyze the effect of the spatial distribution of organic matter and microbial decomposers on soil carbon and nitrogen dynamics. With this model, two scenarios were tested according to the degrees of clustering of organic matter and microorganisms. The results of simulations highlighted the effect of the ratio of accessible organic carbon to microbial carbon on the dynamics of microbial biomass and CO_2 release. This ratio was determined by the number of contacts between one object representing the microbial decomposers and the surrounding objects representing the labile or soluble organic substrates. More recently, Falconer et al. (2008) presented an IBM of the growth and propagation of fungal hyphae, and with this model, demonstrated that the spatial heterogeneity of the pore space in soils could affect the level of interaction of distinct fungal colonies.

In this general context, a primary objective of this article is to analyze in detail the calibration of the parameters of the microbial submodel of the current implementation of the INDISIM-SOM model (involving several new features and model components, compared to the original model), using a larger soil data set than that used by Ginovart et al. (2005), and to determine if the fit of model outputs to experimental data of C and N dynamics is satisfactory. Because of the large number of parameters involved, and clear conceptual differences among abiotic and biotic ones, the analysis is carried out here only for the biotic components of the model, related to heterotrophic and nitrifier microorganisms. A parallel treatment for the abiotic parameters is provided by Gras et al. (2010). A second objective of this article is to assess the sensitivity of the model toward its biotic parameters, specifically the C to N microbial biomass ratio for the heterotrophs, maintenance energy and death probability for the two prototypes of microorganisms, and the ratio of nitrifier C to microbial C. Such a sensitivity analysis should prove particularly useful in the near future, in two different ways. The first will be when INDISIM-SOM will be integrated in a pore-scale, Lattice-Boltzmann model of water and solute transport in soils. A combined model of that sort is likely to be computationally demanding. Initial integration and calculations would be greatly facilitated if it turned out that, under some conditions, model predictions are far less sensitive to some components than to others. In this case, as a first approximation, a "lighter" version of INDISIM-SOM could be implemented, with only the most sensitive components, leaving the integration of the full model formulation to a later stage. In addition, and perhaps more importantly, by indicating which parameters of the model are most sensitive, the analysis carried out in the following suggests clearly where emphasis should be placed in experimental research.

2. Description of the individual-based model

2.1. Outline of the model

The different soil organic matter and mineral pools considered by this model comprise labile C and N and their polymers, humified organic matter, ammonium, nitrate, CO_2 and O_2 gas, and microbial biomass, the latter constituted by two different groups of microorganisms, the heterotrophs and the nitrifiers. Ginovart et al. (2005) showed that a previous and simpler version of this model could be calibrated in such a way that it satisfactorily reproduced measured patterns of C and N dynamics in two soils. Gras et al. (2010) describe explicitly the abiotic components of the model (types of substrates), the processes they undergo (hydrolysis reactions, adsorption and desorption of ammonium, and diffusion), and the three phases considered in the spatial model (solid, liquid and gaseous). Gras et al. (2010) also analyze in detail the parameterization and sensitivity of these abiotic components.

The core idea of the model is a division of the non-living soil organic matter into three distinct pools, defined on the basis of their decomposition rate. These pools are illustrated schematically in figure 1 of Gras et al. (2010). The first pool involves labile molecules, which can be distinguished by their C and N composition, and either contain nitrogen (e.g., amino acids or nucleic acid-like molecules) or not (e.g., loosely speaking, glucose-like molecules). The quantities of the former compounds present in the system are represented by the variables CN_L (with a molar C to N ratio equal to 3 to 1) and C_L (with a molar ratio C to N equal to 6 to 0), respectively. The second pool contains "polymerized" organic compounds, some of which result from polymerization of the labile compounds of the first pool. To parallel the notation adopted for the labile organics, the polymerized compounds are denoted quantitatively by the variables CN_P and C_P. Finally, the third pool contains humified organic matter with a C/N ratio typically near 10, and whose quantity in the system is denoted by the variable CN_H. This pool represents the SOM fraction most resistant to enzymatic activity and comprises all compounds that experience low decomposition in the short or medium term.

Besides these organic matter pools and the microbial pool, whose mass is quantified by the variable CN_{MIC} and by a carbon to nitrogen ratio assumed different for the two groups of microorganisms, the model also considers a number of inorganic components of soil ecosystems, specifically ammonium in soil solution (N_{NH_4}), nitrate (N_{NO_3}), carbon dioxide (C_{CO_2}) and O_2.

A web-based simulator version of INDISIM-SOM is available at https://aneto.upc.es/simulacio2/hoja-portada.html.

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2.2. Microbial submodel and parameterization

Succinctly, the microbial submodel involves a number of successive steps. The first one, at the top of the flowchart diagram of Fig. 1, is the uptake of one or more substrates by the microorganisms from their immediate surrounding. These substrates are then metabolised by the heterotrophs and the nitrifiers. In the case of the heterotrophs, maintenance energy requirements are initially satisfied with the C_L taken up, and if the energy thus derived is not sufficient (arrow with "no"), the cells turn successively to CN_L, then eventually may begin to degrade their own intracellular material, leading to a loss of biomass. A similar sequence of metabolic processes to satisfy maintenance energy requirements occurs in the case of the nitrifiers. If not enough energy can be achieved from ammonium oxidation, they degrade their own biomass. If the energy derived from the substrates is enough to cover the entirety of the maintenance, microorganisms can synthesize new biomass with the energy or C and N surplus (arrows with "yes"). If the level of biomass synthesized is such that the cell mass exceeds the minimum mass needed for reproduction, m_{REP}, bipartition takes place after a period of time equal to t_R, with the apparition of two new cells. In either case, the next decision the submodel has to make at that point is whether the cell, or cells resulting from reproduction, die and are lysed ("yes" arrow), or start the cycle anew with uptake ("no" arrow). These various steps of the microbial submodel are described in detail in the following subsections.

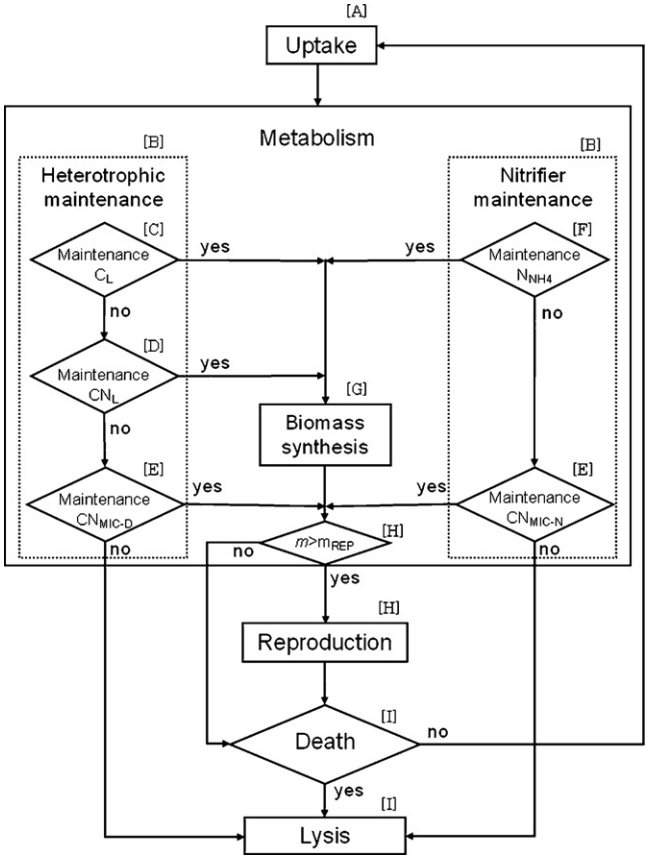

Fig. 1. Schematic diagram of the microbial activity of the heterotrophic and nitrifier populations, as described in the INDISIM-SOM model. In order to link this diagram of the model to the text, the section and equation numbers associated with the processes represented by the different boxes are as follows. In Section 2.2.2: [A] Eqs. (1)–(3); in Section 2.2.3: [B] Eq. (4); [C] Eq. (5); [D] Eq. (6); [E] Eq. (8); [F] Eq. (7); [G] Eqs. (9)–(25). Section 2.2.4: [H]. Section 2.2.5: [I].

2.2.1. Microbial biomass quantification

In the model, the individual microbial biomass, m, is expressed in molar units, so that each biomass pmol is composed by a_{0i} pmols of C and 1 pmol of N, where the subindex i stands for the type of microorganism ($i=1$ for heterotrophics and $i=2$ for nitrifiers). The values of carbon to nitrogen ratio for the biomass, a_{0i}, for the two microbial populations are selected to represent averages of the microbial C/N molar ratios typically found in soils. In microbial populations extracted from soils and cultured in artificial nutrient media, the observed C/N ratios range from 3 to 6 for bacteria, and from 7 to 12 for fungi (Anderson and Domsch, 1980). The range considered in the model for the microbial C/N ratio of the heterotrophs, a_{01}, varies between 5 and 12. Because nitrifiers consist mainly of bacteria, in this case a_{02} can be taken equal to 5 (Table 1). To determine the quantity of pmols that comprise an individual, the C content of microbial biomass is assumed equal to 0.47 g C g^{-1} (Jenkinson and Ladd, 1981; Tunlid and White, 1992).

The mass at reproduction, m_{REP}, and the minimum mass, m_{MIN}, are derived from the bacterial cell volume. In principle, the calculations should include the fungal biomass. However, there is a dearth of information about the volume/biomass relationship for fungi in soils, and therefore, as an approximation, only the bacterial biomass is taken into account in the calculations. To determine the cell volume, the average of the minimum and maximum cell sizes reported in the literature (Claus and Berkeley, 1986) for representative organisms is used. For the heterotrophs, the organisms selected are *Pseudomonas sp.*, *Arthrobacter sp.* and *Bacillus sp*, whereas for the nitrifiers data associated with *Nitrosomonas europaea* and *Nitrobacter winogradskyi* are adopted. The cell volume is calculated by assuming that each bacterial cell is cylindrical with a hemisphere at each end. The minimum cell volume calculated for heterotrophs under these conditions is 0.53 μm^3, and the average maximum cell volume is 1.56 μm^3, whereas for nitrifiers the corresponding numbers are 0.297 μm^3 and 0.976 μm^3, respectively. The minimum cell volume is considered to be correlated to the minimum mass, m_{MIN}, that an individual reaches before it dies, and the maximum cell size to the mass of the individual when it starts its reproduction by bipartition (m_{REP}) (Ginovart et al., 2005). Biomass is deduced from cell volume by assuming a biomass density of 1.1 g cm^{-3} and a ratio of dry to wet weight of 25%, following Jenkinson and Ladd (1981) and Griffiths et al. (1997). The resulting reproduction and minimum masses are listed in Table 1.

2.2.2. Uptake

In INDISIM-SOM, a microbial cell takes up substrate from its immediate surroundings. The uptake of each substrate depends on the microbial biomass composition, the uptake capabilities of each microbial cell and the substrate availability in the medium.

C/N microbial ratio for heterotrophs, the carbon to nitrogen ratio of the biomass, C/N_{MIC}, can change as a consequence of the storage of unmetabolised labile organic compounds. This ratio is controlled in each individual before it takes up compounds, and its value must remain close to a_{01} to establish the connection between the uptake of diverse sources of C and N. Whether C- or N- substrates are taken up depends on the individual C/N_{MIC}; we assumed that if C/N_{MIC} is smaller than 5 the cell cannot use N compounds, and if its value is greater than 12 it cannot use C_L. This allows the individual biomass C/N_{MIC} ratio to remain within the above range of values depending on the relative substrate uptake.

The maximum uptake, $(U_{max})_{ij}$, or potential uptake, is the amount of substrate j that a microorganism can use under non-limiting nutrient availability. It is defined as:

$$(U_{max})_{ij} = u_{ij} c_i m^{2/3}. \quad (1)$$

In this equation, the subindex i stands for the type of microorganism and $j=1,2,\ldots,6$ for C_L, CN_L, N_{NH_4}, N_{NO_3}, C_{CO_2} and O_2,

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Table 1
Numerical values, fixed from the onset or fitted during calibration, of a number of microbial parameters describing heterotrophs and nitrifiers. The numbers in parenthesis, for selected parameters, correspond to the parameter ranges considered in the sensitivity analysis of the model.

Parameter description	Symbol	Unit	Value		
			Heterotrophic ($i=1$)	Nitrifier ($i=2$)	
Reproduction mass	m_R	pmol	2400	21.5	Fixed
Minimum mass	m_{MIN}	pmol	1920	6.45	Fixed
Reproduction time	t_R	h	1	1	(Kreft et al., 1999)
Microbial biomass C/N ratio	a_{0i}	mol C mol^{-1} N	7 (5–12)	5	Fitted and fixed
Fraction of microbial C that belongs to nitrifiers	h_{02}	adimensional	–	0.0002 (0.00004–0.001)	Fitted
Maintenance energy	E_i^m	g C g^{-1} C_{MIC} h^{-1}	0.004 (0.002–0.006)	0.008 (0.004–0.01)	Fitted
Yield in biomass production	Y_i	g C_{MIC} g^{-1} C h^{-1}	0.6	See text	Fixed
Death probability	d_i	h^{-1}	0.005 (0.0–0.01)	0.01 (0.0–0.02)	Fitted
Uptake constant	c_i	μm^2 pmol$^{-2/3}$	0.062 (for $a_{01}=7$)	0.077 (for $a_{02}=5$)	Fixed
Maximum growth rate	μ_{MAX}	h^{-1}	0.225 (0.125–1)	0.125	Fitted
Substrate availability (A_{ij})	C_L	h^{-1}	0.025	–	(Gras et al., 2010)
	CN_L		0.025	–	
	N_{NH_4}		0.050	0.050	
	N_{NO_3}		0.050	–	
	O_2		0.500	0.500	
	CO_2		–	0.500	

respectively. The variable u_{ij} represents the quantity of substrate that could cross per unit of time, per unit area of the microbial cell surface, and the constant c_i relates the cellular surface of the microorganism to its biomass, m.

The determination of u_{ij} is a novel feature of the model, compared with the version presented by Ginovart et al. (2005). In principle, this determination requires detailed knowledge of the extent to which substrates are taken up through the cell membrane. However, since this information at the level required by the model is unavailable, approximate macroscopic parameters are used to estimate these microscopic uptake rates. Specifically, it is considered that the maximum growth rate is closely connected with the maximum cellular uptake rate. Henze et al. (2000) in their model of waste water treatments estimated a maximum growth rate between 0.125 and 0.250 h^{-1} depending on the temperature. Kreft et al. (1999) used a maximum growth rate equal to 1.23 h^{-1} for a bacterial culture. This latter value seems high for a model of a microbial system in a natural ecosystem, therefore values for μ_{MAX} within the range from 0.125 to 1 h^{-1} are adopted in INDISIM-SOM. Also, to calculate c_i, each cell is once again looked at as a cylinder with two hemispheres at the ends. Assuming a microbial density equal to 1.1 g cm^{-3}, dry weight equal to 25% and carbon content equal to 47%, one finds that the constant c_i, [μm^2 pmol$^{-2/3}$], can be expressed as a function of the C/N ratio a_{0i} for each microbial cell type i, by

$$c_i = 0.225 a_{0i}^{-2/3}. \quad (2)$$

The maximum substrate availability, $(A_{max})_{ij}$, is the maximum amount of substrate j that is available for a microorganism of type i in the medium surrounding the individual, in a given time step. This parameter, assumed to be directly related to soil characteristics and not to the types of microorganisms involved, is given by the

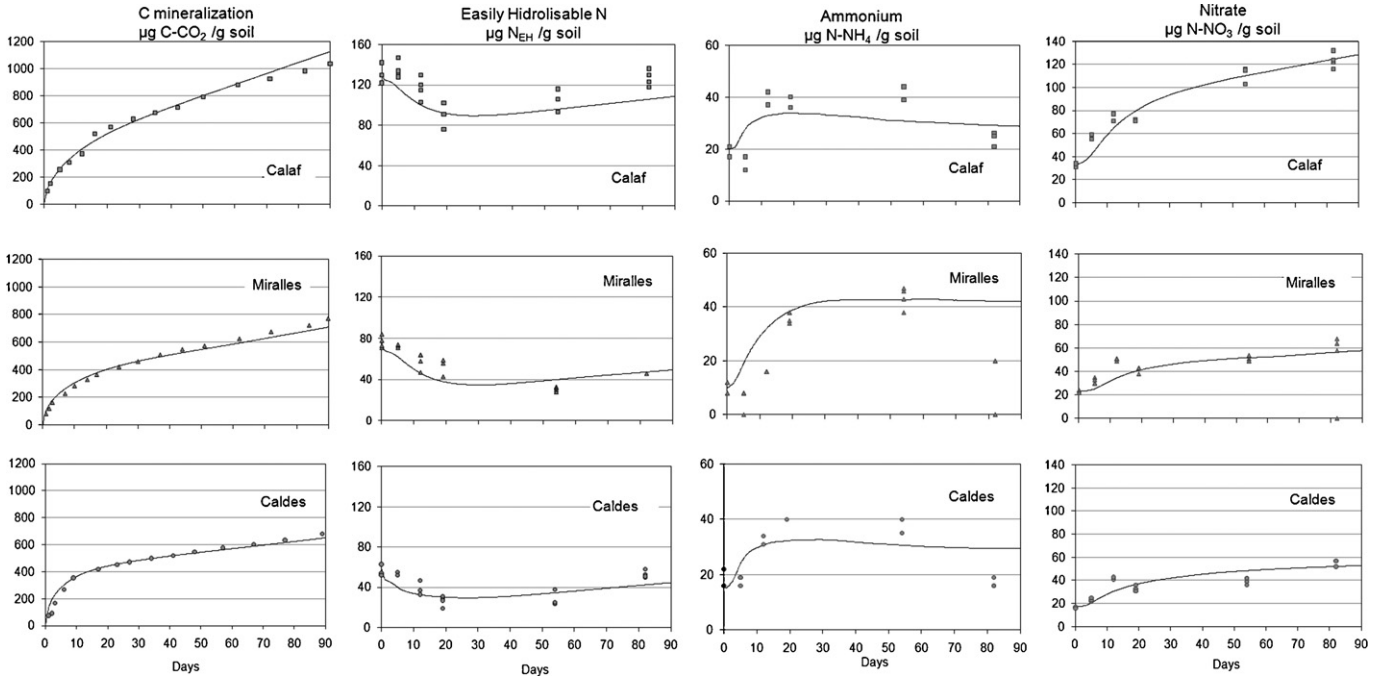

Fig. 2. Experimental data (points) and model predictions (lines) of cumulative C–CO_2 produced, easily hydrolysable N, ammonium and nitrate progress in the three soils (Calaf, Miralles and Caldes).

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following expression:

$$(A_{\max})_{ij} = A_{ij} s_j / n, \quad (3)$$

where A_{ij} represents the fraction of the quantity of substrate j available to cells of type i, s_j is the amount of substrate in the spatial unit where the individual cell is located, and n is the number of individuals living in the spatial unit.

If $(U_{\max})_{ij} \geq (A_{\max})_{ij}$, only the available substrate is taken up. Therefore, in this case, the real uptake of the cell is $U = (A_{\max})_{ij}$. On the other hand, if $(U_{\max})_{ij} < (A_{\max})_{ij}$, then the real uptake is $U = (U_{\max})_{ij}$.

2.2.3. Metabolism

Johnson et al. (1999) argued that hydrolysable N is the most important net source of N for the microorganism. Therefore, it is assumed in the model that the choice of the N source for biomass production will be labile N, CN_L, and afterwards, if C_L remains in the microbial store, the microorganism uses N_{NH_4} and later N_{NO_3} until the C source or the N source limits the biomass production for heterotrophs. Nitrifiers can use only ammonium as an N source and CO_2 as a C source. Microorganisms metabolise each substrate via specific pathways to derive the cellular maintenance energy and to synthesize new microbial biomass, as well as the energy required for this process. In INDISIM-SOM, microbial cells are assumed to only uptake low weight molecular organic compounds.

Maintenance energy: The individual requirement for maintenance energy, m_e, was assumed to be proportional to microbial biomass, m, as follows:

$$m_e = E_i^m m. \quad (4)$$

All the microorganisms in soil cannot be considered to be actively growing, because soils typically do not have enough resources to sustain such growth levels. Consequently, maintenance energy values (E_i^m) may drop below 0.002 g C g^{-1} C_{MIC} h^{-1} to achieve steady state (Smith and Paul, 1990). Other authors have considered that the maintenance energy is between 0.0042 and 0.021 g C g^{-1} C_{MIC} h^{-1} (Gignoux et al., 2001) or 0.003 g C g^{-1} C_{MIC} h^{-1} (Norton and Firestone, 1996). Input values between 0.0004 and 0.000075 g C g^{-1} C_{MIC} h^{-1} have been used in the Daisy model (Hansen et al., 1990; Mueller et al., 1998).

Therefore, to fit the INDISIM-SOM model, maintenance energy rate (E_1^m) values close to the highest aforementioned values are adopted. Tappe et al. (1999) in their work with *Nitrosomonas europaea* find that the maintenance requirements for nitrifier biomass are between 1.19 and 2.7 mmol NH_4^+ g^{-1} h^{-1}, so the maintenance energy for nitrifiers, E_2^m, is tested here for values from 0.004 to 0.01 g C g^{-1} $C_{MIC\text{-}N}$ h^{-1}.

Heterotrophic microorganisms obtain the cellular maintenance energy from labile C (Eq. (5)) and, if needed, from labile N (Eq. (6)) as well, whereas autotrophic microorganisms use N_{NH_4} for their cellular maintenance (Eq. (7)). In both cases, if the maintenance requirements are satisfied, microorganisms can synthesize new biomass. On the other hand, if the energy obtained from the catabolism of a given substrate is below its maintenance requirements, new biomass cannot be synthesized. In such a case, a microorganism could obtain energy from its own biomass (Eq. (8)) until, successive time steps using its own biomass, the microorganism reaches a minimum biomass (m_{MIN}) value (Table 1). The catabolic reactions modelled to obtain metabolic energy (ε_j) for the different energetic sources considered are written as follows:

$$C_L + 6O_2 \rightarrow 6C_{CO_2} + \varepsilon_1 \quad (5)$$

$$CN_L + 3O_2 \rightarrow N_{NH_4} + 3C_{CO_2} + \varepsilon_2 \quad (6)$$

$$N_{NH_4} + 2/3O_2 \rightarrow N_{NO_3} + \varepsilon_3 \quad (7)$$

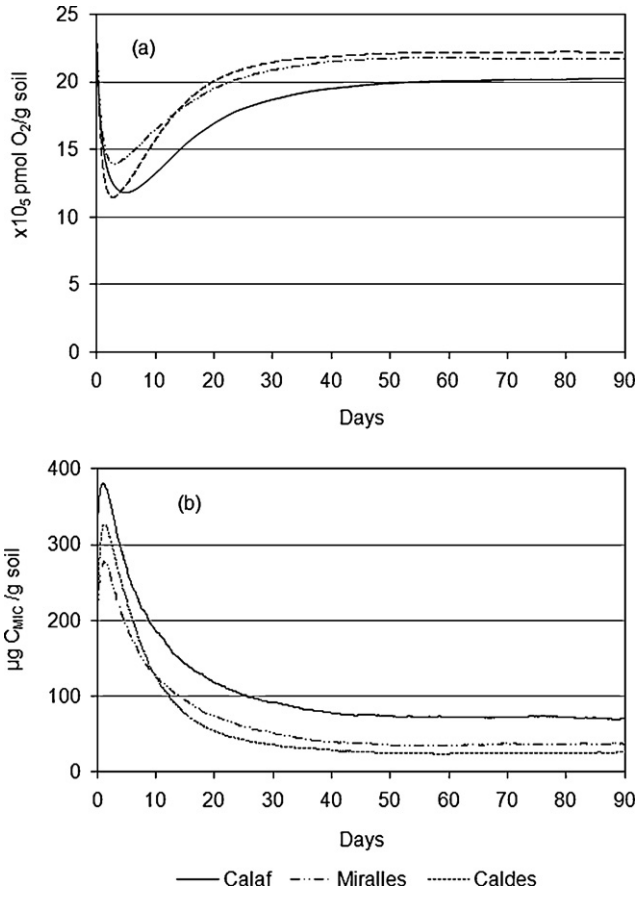

Fig. 3. Temporal evolution of oxygen and microbial C of the three simulated soils.

$$CN_{MIC} + a_{0i}O_2 \rightarrow a_{0i}C_{CO_2} + N_{NH_4} + \varepsilon_{0i}. \quad (8)$$

The stoichiometric coefficients for the metabolic reactions are based on a biochemical approach involving a set of assumptions. Each energy source provides a quantity of metabolic energy that is related to its C content. As most C compounds are catabolised throughout the tricarboxilic acid cycle, in which 15 ATP are obtained from one pyruvic acid (Cooper and Hausman, 2004), substrate used as energy source is assumed to be reduced to the maximum extent possible. So, for each unit of CO_2 released, five energy units are produced. As the complete oxidation of C_L occurs via glycolysis and the citric acid cycle, then the energy obtained from one C_L is 36 energy units. Also there is a net gain of 3 ATP from the deamination of an amino acid (Stryer et al., 2003), thus the deamination of one CN_L or one unit of biomass yields three energy units. Because the main polymers can be hydrolysed without using ATP, but some specific enzymes are required, some energy source must be associated with the hydrolysis of polymer bounds. If biomass hydrolysis has the same cost as protein polymerisation, which requires two or three energetic units (Harris, 1982), the energetic cost of cleaving the polymeric bounds can be taken equal to three energetic units. The value of ε_3 is equal to two units because, from ammonium oxidation, 2 ATP are produced. Taking

Table 2
Maximum growth and mortality rates observed in the course of the simulations, related to the heterotrophs population.

Soil	Maximum growth rate (h^{-1})	Maximum mortality rate (h^{-1})
Calaf	0.021	0.011
Miralles	0.031	0.014
Caldes	0.039	0.011

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into account all of these considerations, the simulated energies obtained from the reactions of Eqs. (5)–(8) are $\varepsilon_1 = 36$, $\varepsilon_2 = 18$, $\varepsilon_3 = 2$, and $\varepsilon_{0i} = 5a_{0i}$, respectively.

Synthesis of new biomass: This component of the model has been greatly expanded since Ginovart et al. (2005). In order to model the metabolisation of absorbed substrates in cells and for each kind of *j*-substrate, a set of rules or statements has been introduced. First, because a certain amount of energy is required for the synthesis of one unit of biomass, an adequate amount of energy-giving substrate, α_j, must be added for the reaction to be completed efficiently. The parameter α_j is related to the yield of biomass production (Y). So, the overall metabolic reactions are the result of coupling the anabolic reaction with its corresponding catabolic reaction, and the sum of both reactions may result in a balance between the energy yielded and the energy used. For a heterotrophic microorganism, the organic molecules that are not used up in a given time step remain in storage for the cell until they are used up in the subsequent step. This alters the C/N_{MIC} ratio of the cell. Unmetabolised mineral compounds are released to the medium.

In anabolic routes, C_L is always used as the energy source, whereas the source of N could be different for each case. Direct transfer of organic N from organic N pools into microbial biomass has been discussed in early tracer investigations and has been defined by the DIR (DIRect assimilation) hypothesis (Hadas et al., 1992). For INDISM-SOM, this is the main biomass synthesis pathway, expressed as:

$$CN_L + \left(\alpha_1 + \frac{a_{01} - 3}{6}\right) C_L + 6\alpha_1 O_2 \rightarrow 6\alpha_1 C_{CO_2} + CN_{MIC\text{-}D}, \quad (9)$$

which is the combination of the two reactions:

$$CN_L + \left(\frac{a_{01} - 3}{6}\right) C_L + E_1 \rightarrow CN_{MIC\text{-}D} \quad (10)$$

$$\alpha_1 C_L + 6\alpha_1 O_2 \rightarrow 6\alpha_1 C_{CO_2} + \alpha_1 \varepsilon_1 \quad (11)$$

and of the equality:

$$E_1 = \alpha_1 \varepsilon_1. \quad (12)$$

The MIT (Mineralization–Immobilization Turnover) hypothesis assumes that the N assimilated by the microbial biomass is in mineral form (N immobilization) (Hadas et al., 1992). Thus, in the model N_{NH_4} is immobilized only when there is not enough available labile N in proportion to the available labile C. This corresponds to the following equation:

$$\left(\alpha_2 + \frac{a_{01}}{6}\right) C_L + N_{NH_4} + 6\alpha_2 O_2 \rightarrow 6\alpha_2 CO_2 + CN_{MIC\text{-}D}. \quad (13)$$

As before, this reaction is the combination of two reactions and of an equality for E_2, as follows:

$$\frac{a_{01}}{6} C_L + N_{NH_4} + E_2 \rightarrow CN_{MIC\text{-}D} \quad (14)$$

$$\alpha_2 C_L + 6\alpha_2 O_2 \rightarrow 6\alpha_2 C_{CO_2} + \alpha_2 \varepsilon_1 \quad (15)$$

$$E_2 = \alpha_2 \varepsilon_1. \quad (16)$$

If some C_L remains, it is possible to immobilize the nitrate, N_{NO_3}, in the biomass, following the reaction:

$$\left(\alpha_3 + \frac{a_{01}}{6}\right) C_L + N_{NO_3} + 6\alpha_3 O_2 \rightarrow 6\alpha_3 CO_2 + CN_{MIC\text{-}D}, \quad (17)$$

which again is the combination of two reactions and of equality:

$$\frac{a_{01}}{6} C_L + N_{NO_3} + E_3 \rightarrow CN_{MIC\text{-}D} \quad (18)$$

$$\alpha_3 C_L + 6\alpha_3 O_2 \rightarrow 6\alpha_3 C_{CO_2} + \alpha_3 \varepsilon_1 \quad (19)$$

$$E_3 = \alpha_3 \varepsilon_1. \quad (20)$$

Nitrifiers use CO_2 and N_{NH_4} to synthesize new biomass, the anabolic reaction, where the synthesis of biomass is produced as a result of the assimilation of CO_2, through the Calvin cycle, and

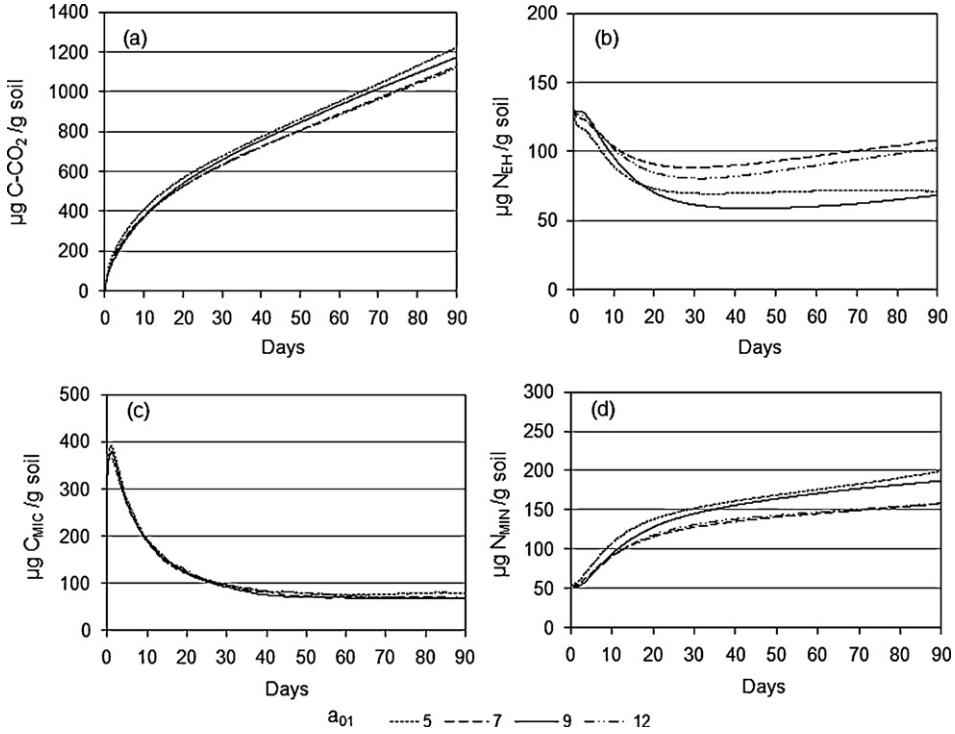

Fig. 4. Simulation results of the temporal evolution of: (a) cumulative C–CO_2, (b) easily hydrolysable N, N_{EH}, (c) microbial biomass carbon, C_{MIC}, and (d) mineral N, N_{MIN}, throughout Calaf soil simulations, for different values of the microbial C to N ratio, a_{01}.

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the immobilization of NH_4^+. The metabolic energy is just from the oxidation of ammonia (Eq. (21)),

$$5C_{CO_2} + (1+\alpha_4)N_{NH_4} + (2/3)\alpha_4 O_2 \rightarrow CN_{MIC\text{-}N} + \alpha_4 N_{NO_3}, \quad (21)$$

which is the combination of these two reactions and of the Eq. (24):

$$5C_{CO_2} + N_{NH_4} + E_4 \rightarrow CN_{MIC\text{-}N} \quad (22)$$

$$\alpha_4 N_{NH_4} + (2/3)\alpha_4 O_2 \rightarrow \alpha_4 N_{NO_3} + \alpha_4 \varepsilon_3 \quad (23)$$

$$E_4 = \alpha_4 \varepsilon_3. \quad (24)$$

For the anabolic reactions, the value assigned to the yield (Y) of newly produced biomass for the heterotrophs corresponds to the pathway that uses CN_L as N source (Eq. (9)). According to the MIT hypothesis for heterotrophs, or synthesis for nitrifiers, the addition of one ammonium to a carbon strain requires one ATP and one NADPH (Harris, 1982; Coyne, 2000). Thus the energy required for this addition is equivalent to four energy units. Also, for both prototypes, three energy units are required to add a monomer to a biomolecule. The values of α_i in the former reactions are dependent on the yield of biomass production. The reported yield values (Y) vary among authors and experimental tests. For instance, a value of 0.8 g C g^{-1} C_{MIC} has been estimated for soils amended with glucose (Sparling et al., 1981), whereas a value of 0.4 g C g^{-1} C_{MIC} was adopted by Gilmour et al. (1985) in order to obtain a good fit of their simulations to experimental data. Therefore an average Y value of 0.6 g C g^{-1} $C_{MIC\text{-}D}$ is adopted for decomposers. To reduce six molecules of CO_2, through the Calvin cycle, 54 ATP are needed (Prescott et al., 1999), so it is assumed for nitrifiers that to link five CO_2 in a biomolecule requires 45 energy units.

As a result of those assumptions, biomass synthesis by nitrifiers is described by the following reaction:

$$5C_{CO_2} + 44N_{NH_4} + 64.5O_2 \rightarrow CN_{MIC\text{-}N} + 43N_{NO_3}. \quad (25)$$

2.2.4. Reproduction

The reproduction component of the model is identical to that included in INDISIM (Ginovart et al., 2002; Ginovart et al., 2005). Because its parameters are not varied in the sensitivity analysis that constitutes the main objective of the research described in the present article, readers are referred to Ginovart et al. (2002) for further details.

2.2.5. Death and lysis

When a microbial cell cannot maintain its energetic requirements via the degradation of its mass because it has reached its minimum value (m_{MIN}), then the cell dies and lysis occurs, releasing its biomass in the medium. Following Nicolardot et al. (2001) and Ginovart et al. (2005), it is assumed that a fraction equal to half the biomass is transferred to humic compounds, CN_H. The turnover of the reminder of the microbial biomass is modelled by considering that this fraction is composed of polymeric compounds (Kuzyakov, 1996), which are returned to the environment as C_P and CN_P, and of a variety of unmetabolised and stored compounds, which are released into the medium in their original form.

Microbial cells die not just because of starvation, but also because of predation or phage lysis, among others causes. These processes are modelled as random events, and the model assumes a certain death probability, d_i, for microorganisms. There is relatively little information on what would be an acceptable range for d_i in soil ecosystems. Gignoux et al. (2001) observed values of 0.0002–0.002 h^{-1}, which are used in the model (Table 1).

3. Experimental data and methods of analysis

3.1. Experimental data

Experimental data used in the present research were obtained from Vidal (1995), and relate to soil samples collected in the Ap horizon (0–20 cm) of sandy loam soils samples at three different locations in Catalonia (see Gras et al., 2010 for detailed physical and chemical characterization). Two of the soils, Calaf and Miralles, were considered already by Ginovart et al. (2005). The third soil, located in Caldes, has a similar pH, lower organic matter content (0.91% versus 2.17 and 1.16, respectively, for Calaf and Miralles), lower nitrogen content, and a slightly lower clay content (9% instead of 13 and 16, respectively), which leads to a lower cation exchange capacity.

Prior to monitoring experiments, soil samples were air dried, sieved and rewetted to a moisture content equivalent to 80% water-holding capacity. They were then placed in a dark room for 90 days at a constant temperature of 30 °C. Initially during these 90 days, the samples were aerated frequently to ensure aerobic conditions during the phase of rapid transformations, but aeration eventually was carried out on a daily basis. At the same time, samples were rewetted to maintain moisture at a constant level. Analytical determinations were carried out in triplicate at different time intervals. Total soil N was determined via the regular N-Kjeldahl method (Bremner and Mulvaney, 1982). To determine the easily hydrolysable N (N_{EH}), samples were distilled in alkaline medium, and the distillate fraction was then collected in an acidic solution (Vapodest 12/Gerhardt). Carbon dioxide production was measured

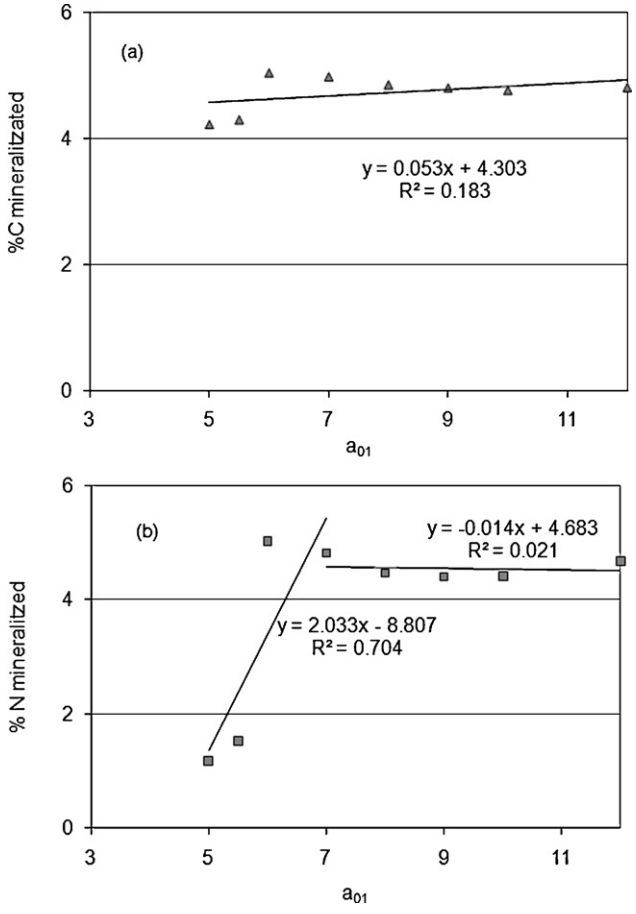

Fig. 5. Relationship between the ratio of microbial C to N, a_{01}, and the (a) percentage of C mineralization (b) percentage of N mineralization. Each point is the value reached at the end of a given simulation.

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using the alkali absorption method, according to which moist soil samples are incubated in closed receptacles. The CO_2 produced by the soil was absorbed by an NaOH trap, and the free NaOH was then determined by back titration with HCl (e.g., Dumestre et al., 1999).

3.2. Model parameters held fixed

Several parameters related to microbial growth and metabolism were kept constant throughout the simulations, at values based directly on, or calculated from, data available in the literature (Table 1). These parameters are the reproduction mass, the minimum mass, the reproduction time equal to 1 (Kreft et al., 1999), the biomass yield, the uptake constant, and the different substrate availabilities. In addition, initial values were set for a number of composition parameters at the onset of the simulations, based on measured soil analysis data (Vidal, 1995), and were not varied thereafter.

3.3. Parameter calibration

The variable parameters of the model were calibrated by optimizing model fits, in priority to available experimental data for CO_2 evolution and mineral N, N_{MIN}, then to other measured data as well. N_{MIN} was selected to calibrate the model because in heterogeneous field soil samples, analytical determinations of organic matter fractions are much less accurate than those of mineral N determinations. Parameter values were independently adjusted, by trial and error, until a satisfactory qualitative (visual) fit was obtained between the simulated data and the experimental ones (Gras et al., 2010). In the past, this approach has been adopted, for example, by Molina et al. (2001).

3.4. Sensitivity analysis

After parameterization and calibration of the variable parameters of the model, a simple local sensitivity analysis was carried out by varying biotic constants individually and determining the extent to which the model input data affect model outputs, in the case of the Calaf soil. The model outputs that were used as indicators of the sensitivity of the model to its input parameters are the evolution of C–CO_2, easily hydrolysable N, microbial biomass carbon and net mineral N production. The sensitivity analyses are performed for the parameters that are modified in order to obtain a calibration of the model to experimental data, namely the heterotrophic biomass C/N ratio (a_{01}), the rate of maintenance energy (E_i^m), the death probability (d_i), the maximum growth rate (μ_{MAX}), and the fraction of microbial C that belongs to nitrifier microorganisms (h_{02}). The range of values of these parameters considered during the sensitivity analysis are listed (in brackets) in Table 1.

4. Results and discussion

4.1. Calibration results and fit to experimental data

The calibration of the model with available experimental data is described in detail in Gras et al. (2010). The discussion here concentrates on aspects directly relevant to the microbial parameters of the model.

Experimental results obtained for the evolution of C mineralization, easily hydrolysable nitrogen, ammonium, and nitrate in the Calaf, Miralles and Caldes soils over time (Fig. 2) suggest the existence of two successive stages. These two stages are particularly clear in the case of the easily hydrolysable nitrogen and ammonium. The first stage seems to last approximately 20 days during which C mineralization increases sharply, easily hydrolysable N drops precipitously, the amount of ammonium roughly quadruples, and

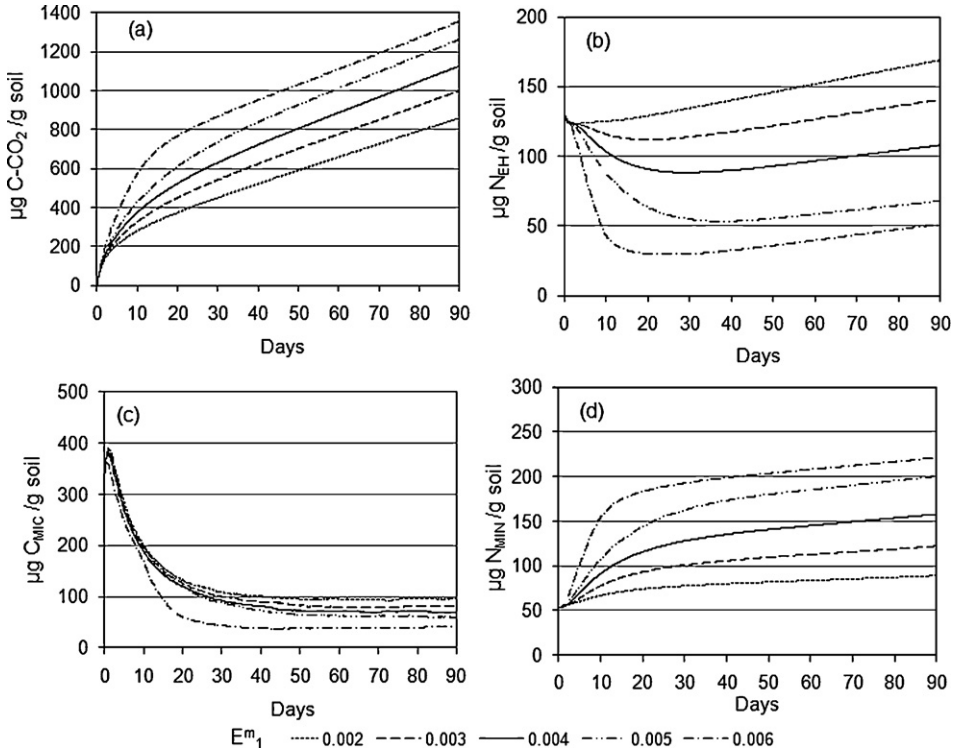

Fig. 6. Simulation results of the temporal evolution of (a) cumulative C–CO_2, (b) easily hydrolysable N, N_{EH}, (c) microbial biomass carbon, C_{MIC}, and (d) mineral N, N_{MIN}, throughout Calaf soil simulations, for different values of the microbial maintenance energy, E_1^m.

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nitrate also increases, even though noise in the data makes it less straightforward in this latter case to identify a clear effect. Whereas C mineralization starts immediately at a very high rate, N mineralization seems to be lagging in the first five days after initiation of the experiments. Easily hydrolysable N appears to remain constant for the first 5 days in the Calaf soil, and to decrease very slightly in the Miralles and Caldes soils. Ammonium does not exhibit any clear trend, upward or downward, during the same period. After 20 days, the rate of C mineralization and nitrate release appears to level off markedly, whereas the easily hydrolysable N stabilizes then progressively increases again. Ammonium also levels off then drops, between 60 and 80 days after the onset of the experiments.

Analysis of the results in Fig. 2 suggests that there is good agreement between predicted curves and experimental data in a number of cases. The fact that the model is able to reproduce very faithfully the evolved CO_2 data should not be too surprising, given that these data are used in priority in the calibration of the model. The fit to N_{MIN} is equally good, probably for the same reason. For nitrate, the model is able to reproduce the main qualitative elements of the observed curves, except for the fast rise during the first phase, which is consistently underestimated in the three soils. In the case of the easily hydrolysable nitrogen and ammonium, in spite of quantitative discrepancies, the model is able to reproduce several qualitative features of the data. Nevertheless, the sharp increases in easily hydrolysable N in the Calaf and Caldes soils at the last measurement time (82 days), and the drops in ammonium in all three soils, are largely missed, in spite of significant efforts to adjust the model parameters so that predictions would be more satisfactory in that timeframe. To some extent, it might be asking too much from the model. Indeed, it is possible that after 80 days into the experiments, other processes than those described by the model may have become significant. The rise in easily hydrolysable N and the drop in ammonium in the soil samples suggest that nitrogen may become lost by denitrification, for example. Other aspects of the results presented in Fig. 2, related to several abiotic components of the model, are discussed in more detail in Gras et al. (2010).

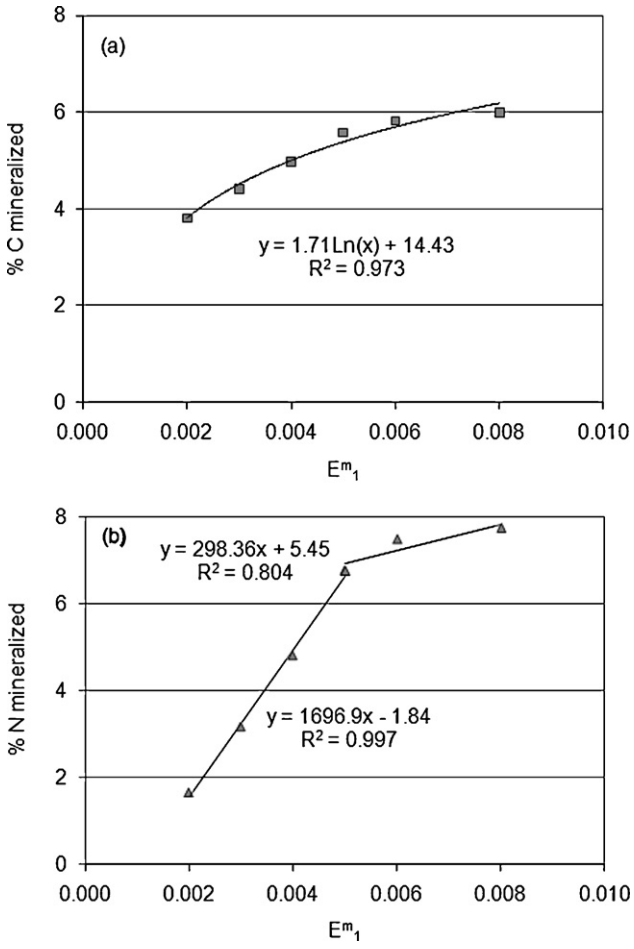

Fig. 7. Relationship between microbial maintenance energy, E_1^m, and (a) percentage of C mineralization (b) percentage of N mineralization. Each point is the value reached at the end of a given simulation.

4.2. Microbiological parameters

The calibrated values of the microbiological parameters that produced the best fit of simulation results to experimental data for the three Mediterranean soils (Fig. 2) are listed in Table 1. The maintenance rate that leads to the best fit for CO_2 production and N mineralization is equal to $0.004\,g\,C\,g^{-1}\,C_{MIC}\,h^{-1}$ for heterotrophic microorganisms. This value falls within the range of values considered by Gignoux et al. (2001), but it is higher than the average value considered by Smith and Paul (1990) or Grant et al. (1993). A possible reason for this may be that in INDISIM-SOM, the microorganisms that are involved in the mineralization process are predominantly those that are active. In other words, the microorganisms in latent states, with typically lower maintenance requirements, are not taken into account, which is likely to drive the mean maintenance energy requirement upward.

After calibration, it is possible to simulate the evolution of variables that were not experimentally measured, such as the simulated temporal evolution of oxygen in the medium and the microbial C (Fig. 3). In the simulations, as was the case in the original experiments (Vidal, 1995), the soils should in principle remain aerobic, preventing the occurrence of anaerobic metabolism. This indeed turns out to be the case in the simulations with the three soils. In all cases, the oxygen concentration in the medium decreases during the first 5–7 days until it reaches a level of 10–12% of O_2, depending on the soil. The oxygen level increases subsequently until the initial conditions are restored (i.e., a concentration of circa 21% O_2). This evolution is in agreement with the experimental results obtained by Yamaguchi et al. (1996), who detected a minimum oxygen concentration on the fifth day, with a value around 15%, and reported that initial conditions were restored around the 15th day. This drop in oxygen level is a consequence of a rise in microbial activity, with a concomitant peak in oxygen demand (Fig. 3).

Marumoto et al., 1982a,b concluded that the contribution of microbial N to N mineralization at the end of 28 days of soil incubations is 76% for a soil that has been dried with applied heat, and 56% for a soil dried without applied heat. The remaining percentages are associated with other organic fractions and from non-microbial biomass. These results are similar to those obtained in our simulations, which range from 41%, for Calaf soil, to 68%, for Caldes soil. The contribution of microbial N to mineral N production could be a direct result of microbial maintenance, or could occur through intermediate steps of microbial N turnover. When model simulations reach a steady state, the ratio of microbial C to organic C is 0.0031 for Calaf and Miralles, and 0.0028 for Caldes.

The simulated quantity of microbial biomass is much higher than the microbial biomass at steady state (Fig. 3). The curves for C and N mineralization show an initial flush due to high microbial activity (i.e., higher than in a non-disturbed soil). Probably, since the soil samples were subjected to pre-treatments of sieving, drying and rewetting, the aeration, soil moisture and substrate availability in the soil were very nearly optimal (Mueller et al., 1997). These conditions stimulate the growth and metabolism of the soil microorganisms, all of which are then able to use up the

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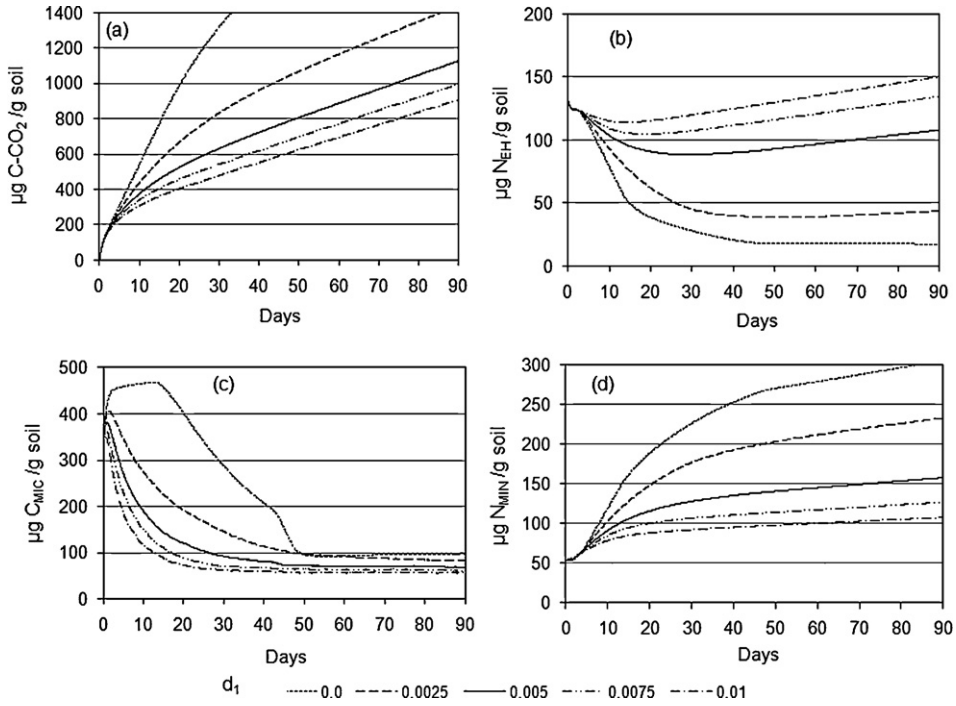

Fig. 8. Simulation results of the temporal evolution of: (a) cumulative C–CO_2, (b) easily hydrolysable N, N_{EH}, (c) microbial biomass carbon, C_{MIC}, and (d) mineral N, N_{MIN}, throughout Calaf soil simulations, for different values of the death probability, d_1.

nutritional resources in the soil, causing a fast mineralization. At the end of the simulation, the microbial biomass C reaches minimum levels, equal to 20%, 16% and 10% of the initial quantities for Calaf, Miralles and Caldes, respectively. These numbers are within the range of 10–40% found for the ratio of active to total microbial biomass in soils (Jenkinson and Ladd, 1981; Tunlid and White, 1992).

The highest values (Table 2) found during the simulation for the microbial growth rate (i.e., the rate of increase of microbial biomass) are in agreement with those, between 0.03 and 0.05 h^{-1}, observed in experiments involving a range of soil microbiota (Barros et al., 1995). Interestingly, the highest values found in the model simulations for the maximum growth rate and for the maximum mortality rate (i.e., the maximum decrease in the number of individuals in an hour) of the microbial population as a whole (Table 2) bear contrasting relationships with their individual counterparts. In the case of the mortality rate, the maximum value observed for the microbial population in the three soils is of the same order of magnitude as the individual rates for heterotrophic and nitrifier microorganisms (Table 1). However, the situation is very different for the population growth rate obtained during the simulations; in the three soils, this macroscopic parameter is much lower than the corresponding potential value assigned to each individual (μ_{MAX} = 0.225 h^{-1}). This suggests that it is substrate availability that limits microbial growth most under the conditions investigated, rather than the intrinsic capacity of each individual microorganism to uptake substrates.

4.3. Sensitivity analysis

From the sensitivity analysis, it appears that the maximum growth rate does not have a marked effect on the evolution of the measured variables (data not shown). This is consistent with data in the literature showing that significant changes in net C and N mineralization patterns are compatible with only minor differences in the microbial consumption and production processes (Verchot et al., 2001).

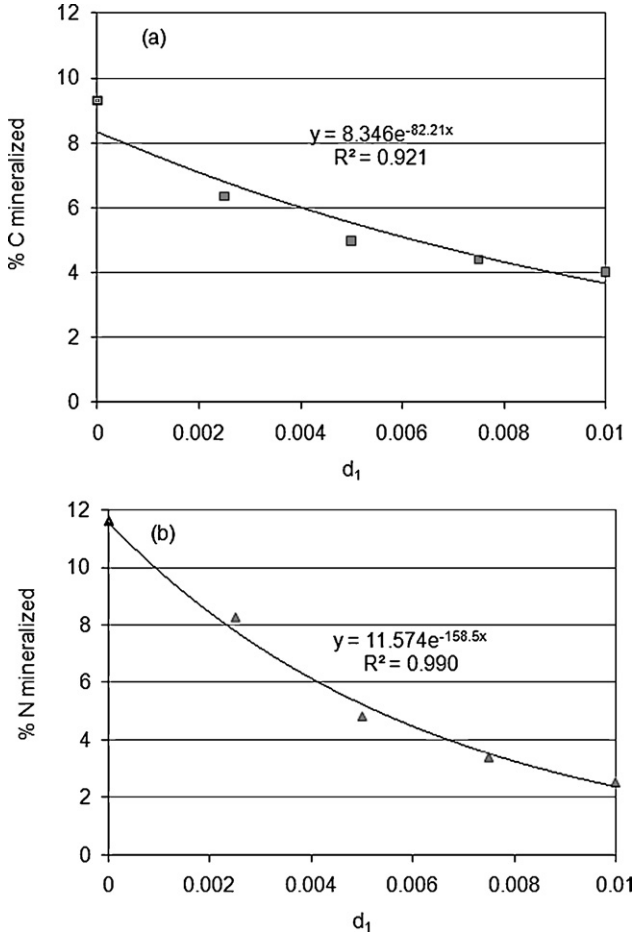

Fig. 9. Relationship between death probability, d_1, and (a) percentage of C mineralization (b) percentage of N mineralization. Each point is the value reached at the end of a given simulation.

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The microbial C to N ratio (a_{01}) for the heterotrophs has a large effect on the total mineralized N (Fig. 4); for values between 5 and 7, the mineral N produced is linearly related to the microbial C to N ratio, and the slope is high (=2.033), whereas for values of a_{01} between 7 and 12, the linear relationship between the two variables has a virtually zero slope (Fig. 5b).

Model simulation of all the measured variable appears highly sensitive to microbial maintenance energy values. The higher the maintenance energy, the higher are the quantities of C and N mineralized at the end of the simulation, and the lower is the quantity of microbial biomass that the soil can support at steady state (Fig. 6). This result is in agreement with the work of Smith and Paul (1990), who report that it is impossible to treat the entire soil microbial population as active because most soils do not have enough nutritional resources to satisfy microbial maintenance requirements unless the demand is lower than 2×10^{-3} kg C kg^{-1} C_{MIC} h^{-1}.

The amount of C and N mineralized at the end of the simulations correlated strongly with maintenance energy (Fig. 7). In the case of C mineralized (Fig. 7a), the relationship appears non-linear, and progressively levels off, as the maintenance energy requirement increases. In the case of the mineralized N (Fig. 7b), the overall response to changes in maintenance energy requirements is larger. Even though the relationship between the mineralized N and the maintenance energy could be described by some non-linear function, it can be described simply, with a high correlation coefficient, using two different linear relationships: the first, in the range of 2×10^{-3}–5×10^{-3} kg C kg^{-1} C_{MIC} h^{-1}, and the second, in the range of 5×10^{-3}–8×10^{-3} kg C kg^{-1} C_{MIC} h^{-1}. The model is more sensitive for values in the first range (Fig. 7b).

The modelled system is highly sensitive to changes in the value of the death probability for the heterotrophs (Fig. 8). Mortality rate, as a measure of the number of deaths in a given population, is the result of the effect of limiting energy sources on the microorganisms and this probability. This perspective is consistent with the use of an IBM: if the probability of accidental death for a microorganism is zero, the individual dies only once it reaches its minimum mass. This occurs after a period of auto-consumption of biomass for cellular maintenance, in which the evolution of microbial C influences the evolution of other variables (Fig. 8c). As in other simulations, the microbial biomass increases quickly during the first few days, and then continues to increase, but at a slower rate, until the 15th day. The latter period most likely encompasses simultaneous synthesis and consumption of biomass. Microbial C then decreases quickly until the 40th day, probably because the individuals consume their own biomass, and the medium becomes progressively depleted in energy resources (i.e., labile compounds) (Fig. 8b). After that the microbial biomass becomes stable. C and N mineralization occur in parallel to changes in biomass, and exhibit a constant increase after the 40th day (Fig. 8a and d). The net C and N mineralization have a strong exponential relation to death probability (Fig. 9).

Experience with the model shows that if an accidental mortality is not included into the model, predictions of organic matter mineralization tend to be too high and thus difficult to fit to experimental values. The fact that an individual can die only when it reaches its minimum mass does not provide enough flexibility to inactivate the microorganism. In the SOMKO model, inactive microorganisms are considered to have no maintenance requirements (Gignoux et al., 2001). In this respect, a further development of the INDISIM-SOM

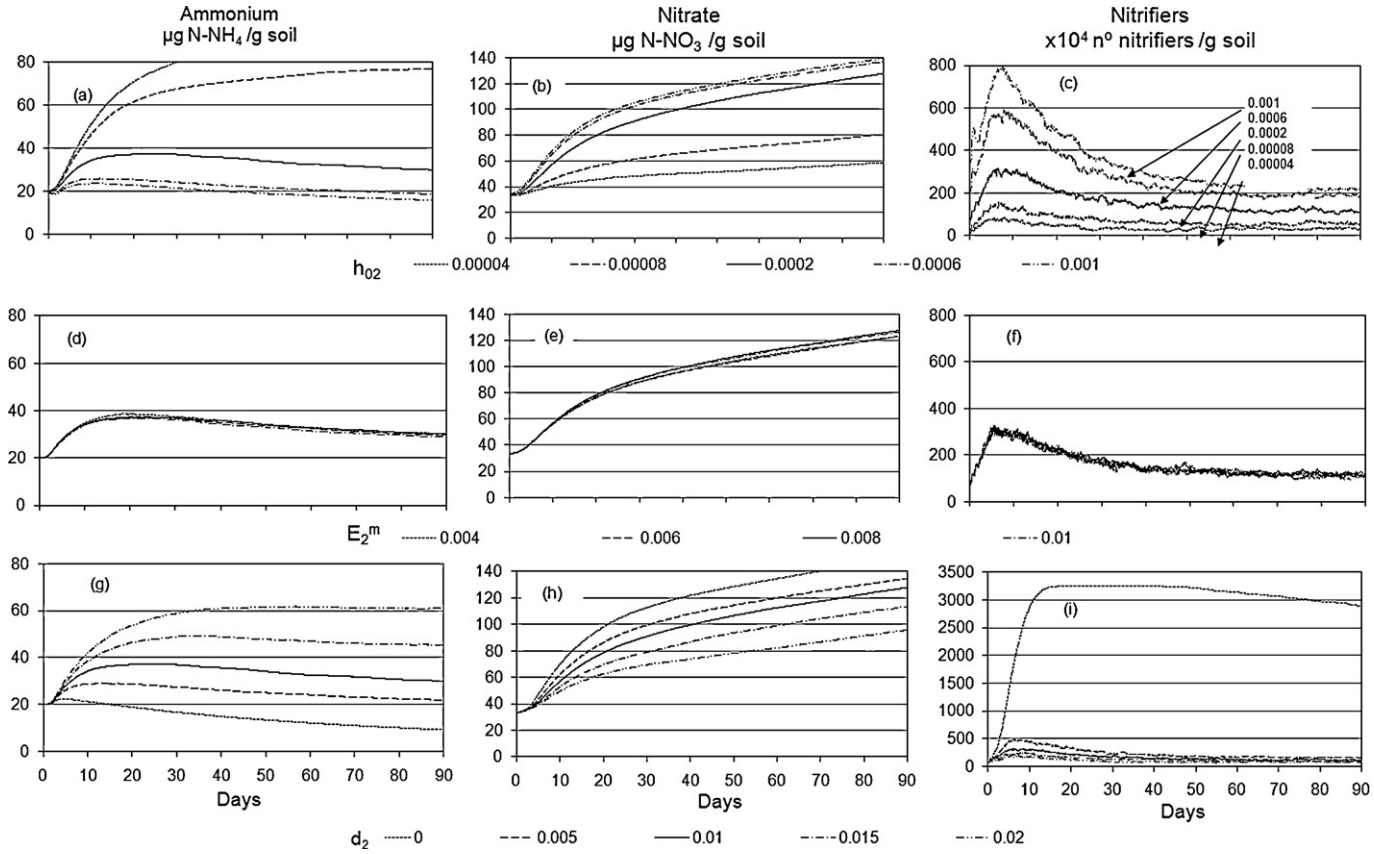

Fig. 10. Sensitivity analyses related to model parameters for nitrifiers. Temporal evolution of (a), (d) and (g) cumulative µg N-NH_4^+ g^{-1} soil; (b), (e) and (h) cumulative µg N-NO_3^- g^{-1} soil; (c), (f) and (i) number of nitrifier individuals, ×10^4 g^{-1} soil, throughout Calaf soil simulations modifying: (a)–(c) fractioning constant, h_{02}, that determines the microbial C associated to nitrifiers microorganisms; (d)–(f) the value of maintenance energy of nitrifiers, E_2^m; and (g)–(i) the value of death probability, d_2.

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model would be to distinguish active and non-active microorganisms.

The maximum individual growth rate, μ_{MAX}, enters into the calculation of the individual uptake rate (u_{ij}) and eventually of the maximum uptake for each substrate (Eq. (1)). Varying this parameter μ_{MAX}, one does not observe noticeable changes in model output. This result is not surprising because, as previously noted, low levels of labile C in the medium are the main growth-limiting factor considered in the model, regardless of the capacity of a microorganism to take up any specific substrate.

In relation with nitrifiers, the ratio h_{02}, or $C_{MIC\text{-}N}/C_{MIC}$, that characterizes the relative abundance of nitrifiers is the most sensitive model parameter (Fig. 10a–c). The more nitrifier biomass there is in the system, the more the nitrate production increases, up to values higher than $C_{MIC\text{-}N}/C_{MIC} = 0.006$, beyond which the nitrification rate remains virtually constant. This might be due to the fact that the levels of free ammonium are very low and that, at the same time, the desorption of fixed ammonium is not fast enough to allow the growth of nitrifiers. In contrast with heterotrophs, as much nitrifier biomass there is at the beginning of the simulation, there is more at the end. It is reasonable to think that if there is more biomass able to use ammonium before it is fixed, there would be more potential nitrification and the population would grow. When the maintenance energy is modified, there are no substantial changes on the different variables studied, which suggests that if the quantity of free ammonia is not modified, then the net nitrification is independent of this parameter (Fig. 10d–f). And in a manner similar to what happens with the heterotroph population, the mortality of the nitrifiers is essentially due to the death probability and not the fact of reaching the minimum mass. When death probability is assigned a value of zero, the maximum number of nitrifying individuals is approximately $3.25 \times 10^7\ g^{-1}$ (Fig. 10g–i). This value seems to be high, considering that Coyne (2000) suggests, for non-amended soils, values around 10^2 and $10^5\ g^{-1}$.

5. Conclusions

Results from variation of model parameters around their calibrated values demonstrate that the model is far more sensitive to some parameters than to others. The parameters that have the greatest effect on the evolution of C and N variables are microbial maintenance energy and death probability. The nitrification rate occurring in the soil, in particular, appears highly affected by the death probability. Both maintenance energy and death probability appear to be strongly related to the metabolic status (i.e., active, inactive, or dead) of individual microorganisms. Because of its individual-based approach, INDISIM-SOM can handle this type of dependence with great ease.

Information of this nature, on the relative preponderance of some parameters, is useful in two different contexts. Future attempts to couple the model INDISIM-SOM with water and solute transport in soils, which are expected to be computationally intensive, may benefit greatly from the knowledge that some components of the model are far less sensitive than others, and might therefore be simplified to speed up calculations. On the other hand, evidence, that, for example, the metabolic status of the microbial biomass present in a soil system at any given time is a crucial parameter in simulations, suggests that efforts should be devoted to evaluating this parameter with more accuracy than is currently achievable. It also indicates that the model would benefit from further refinements in this particular area, for example, through a description of the mechanisms by which an individual cell becomes dormant, of the factors that enable it to switch to an active state, and of the potentialities and requirements of dormant microorganisms.

Acknowledgement

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