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DENITRIFICATION IN A HOMOGENEOUS, CLOSED SYSTEM: EXPERIMENT AND SIMULATION

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A simulation model describing microbial respiration and denitrification was developed for a homogeneous (i.e., spatially uniform in all phases) soil layer, in which no transport processes occurred. Major processes included were growth and maintenance of biomass at the expense of glucose carbon and the concomitant reduction of nitrate to molecular nitrogen, via the intermediates nitrite and nitrous oxide. Growth of biomass was calculated by a first-order rate equation in which the relative growth rate was described by a double Monod equation consisting of rate-limiting factors for carbon and nitrogenous substrates. The Pirt equation was used to calculate the consumption rates of substrates.

As a starting point to parameterize the model, we compiled a data set from various literature sources and investigated the possibility of simulating experimental observations of the sequence of denitrification products by modifying some of these literature data within reasonable limits. We concluded that the model gives a reasonable description of the denitrification process, because the experiments reported in this paper and one from the literature could be simulated rather well.

The objective of this paper is to describe the model with the underlying assumptions and to compare some of its results with experimental data.

Microbial denitrification refers to the process in which nitrate (NO_3^-), nitrite (NO_2^-), and nitrous oxide (N_2O) serve as electron acceptors for essentially aerobic bacteria at low oxygen concentrations, with the result that molecular nitrogen (N_2) can be produced (Delwiche 1981; Knowles 1982). In this reductive pathway, nitric oxide (NO) may occur as intermediate between NO_2^- and N_2O, but its existence has not been assessed unambiguously (Firestone 1982). Experimental studies on denitrification comprise laboratory experiments using soil columns (e.g., Rolston and Marino 1976) and soil incubation flasks (Cooper and Smith 1963; Cho and Sakdinan 1978; Cho 1982; Lind 1980) and field experiments using different techniques (Hauck and Weaver 1986). Simulation studies on denitrification appear to have been confined largely to field soil models that integrate a number of physical processes with denitrification (Frissel and van Veen 1981; Tanji 1982). The evaluation of such models was then based on comparison with data obtained in field experiments. Thus, the comparison between experiment and the submodel of denitrification was indirect only: model results were the outcome of the combined submodels, rather than that of the denitrification submodel alone. It is desirable, however, that submodels be tested more rigorously (Tanji 1982). This applies particularly to denitrification, because, even in a system without transport processes, the overall process is still governed by complex interactions among bacteria, carbon substrate (electron donors), electron acceptors, and oxygen status of soil. More rigorous model testing implies testing of the denitrification submodel by comparing its results directly with simple laboratory incubation studies cited earlier. Denitrification models for homogeneous soil systems without transports have been proposed by Betlach and Tiedje (1981) and Cho and Mills (1979). The former model was used to describe incubation experiments. The latter model was not compared with experimental data. The major drawback of both models is that they do not incorporate microbial growth, though microbial growth strongly influences the nitrogen transformation kinetics.

The objective of this paper is to describe a denitrification model including microbial growth, in a homogeneous (i.e., spatially uniform in all phases) soil, incorporating the reductive pathway of $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$ and to

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compare model results with experimental data reported in this paper and obtained from the literature.

MATERIALS AND METHODS

Denitrification is but one of the many interrelated N transformations that occur in soil (Stevenson 1982; Legg and Meisinger 1982). To limit attention to denitrification, therefore, it is most appropriate to exclude as many N transformations from the system as possible, or at least minimize their influence on system behavior. Therefore, nitrogen inputs with rain, capillary rise, or organic manure were excluded from the experiment. Roots were absent; thus root uptake of nitrogen and exudation of carbohydrates (Barber and Lynch 1977) were also excluded. Minimization of ammonium fixation, volatilization, and nitrification was attained by supplying the anoxic soil with potassium nitrate. Furthermore, it is probable that dissimilatory reduction of nitrate and nitrite with ammonium as the major end product hardly occurs, because very anaerobic conditions are needed for this conversion (Knowles 1982). The major processes that occur in the soil system are then: denitrification, nitrate assimilation by bacteria, mineralization, immobilization, and diffusion of gaseous denitrification products from soil into the head space of the incubation container (Letey et al. 1980*a*; Cho 1982). In the present system, the influence of this diffusive transport on gas concentrations in the head space was minimized using Petri dishes (glass, internal diameter and height about 11 and 1.7 cm, respectively), because these permit the incubation of a thin (about 0.2 cm) soil layer that still contains enough material (about 20 g) for chemical analysis. Furthermore, the small container volume (about 160 ml) assured that small amounts of evolved gases yielded detectable concentrations in gas chromatographic analysis.

Soil

A loam soil from Herveld was taken from the upper 25-cm layer and stored under field-moist conditions. Some characteristics are: pH (measured in 4 g of soil suspended in 10 ml of liquid) in H_2O and KCl: 7.3 and 6.9, respectively; $CaCO_3$ (Scheibler's method described by Allison and Moodie 1965): 2.5%; organic carbon (Mebius 1960): 1.3%; total nitrogen (Novozamsky et al. 1984): 0.14%; CEC-$BaCl_2$ (Bascomb 1964): 22 cmol(+) per kg of soil and soil texture (pipette method described by Day 1965) <2 μm, <20 μm, and <50 μm: 22, 42, and 61%, respectively. Treatment of soil before use in the experiment has been described in Leffelaar (1986).

Soil container

Incubation vessels were constructed from Petri dishes, the rims of which were flattened by grinding. Pieces of windowpane equipped with septum caps (Subaseal) served as cover. Leak-proof connections were attained by greased (Dow Corning vacuum grease) viton rubber rings (0.1 cm thick) placed between the Petri dishes and covers.

Experimental procedure

About 23.5 g of soil with gravimetric moisture content of 0.22 g g^{-1}, was transferred to a Petri dish. Two milliliters of a solution containing potassium nitrate (KNO_3) and glucose ($C_6H_{12}O_6$) was added and thoroughly mixed with a spatula. Concentrations were chosen to obtain C and N additions of about 520 and 175 mg kg^{-1} dry soil, respectively. Total initial N content of the soil was then about 315 mg kg^{-1}, because some endogenous nitrate N was present. The incubation vessel was covered and air was replaced by neon (Ne) (Matheson Gas Products, Oevel, Belgium) by flushing through a needle pierced through the septum, while a second needle was installed to remove excess gas. Soil atmosphere was analyzed for (traces of) oxygen (O_2), carbon dioxide (CO_2), N_2O, N_2, and Ne by gas chromatography. Chemical analysis for NO_3^-, NO_2^-, and ammonium (NH_4^+) were performed at termination of incubation. All analytical procedures were described in Leffelaar (1986). Gas percentages were converted to milligrams of gas using the gas-filled volume of the incubation vessel and a correction for pressure buildup due to the evolved gases. The correction was calculated as the ratio of the percentages of Ne at time zero and at sampling time. Treatments were duplicated. Experiments were done in a constant-temperature room (22.7 ± 1.5°C).

DENITRIFICATION MODEL

Major processes that occur in the experimental system used to measure denitrification were given in the previous sections. In principle, these

processes also occur in the model. A model, however, remains a simplified representation of a system (de Wit 1982), and numerous choices and assumptions have to be made during its development. The choices made with respect to the state variables included in the model and with respect to the degree of detail of the description of their rates of change are reflected in the differential equations, Eqs. (1) through (16), given in Table 1. All symbols are defined in Table 2. State variables distinguished are: bacteria (B), glucose carbon (C), CO_2, O_2, NO_3^--N, NO_2^--N, N_2O-N, N_2-N, assimilated N (N_{ass}), mineralized carbon and nitrogen from dead biomass (C_{min} and N_{min}), and immobilized carbon and nitrogen in resistant organic matter (C_{imm} and N_{imm}). The equations in Table 1 show that the processes directly related to denitrification, i.e., growth of biomass and consumption of electron acceptors, were calculated in a detailed fashion, while other processes such as mineralization and immobilization of carbon and nitrogen were calculated more roughly. Furthermore, three main types of equations may be distinguished: first-order rate equations for biomass, Eqs. (2) and (3), double-Monod equations for relative growth rates, Eq. (5), and the Pirt equation for substrate and electron acceptor consumption, Eqs. (6) and (10). An account of the choices and assumptions made during the development of the model now follows on the basis of the differential equations.

Bacteria

Two groups of heterotrophic strictly aerobic bacteria are considered, i.e., bacteria that can grow only with oxygen as an electron acceptor (further called *strict aerobes*), and bacteria that can grow with oxygen as an electron acceptor under aerobic conditions or with nitrate, nitrite, and nitrous oxide as electron acceptors under anoxic conditions (further called *denitrifiers*). The number of microbial groups distinguished is kept small, because the model must be initiated for each group by quantitative data that are difficult to obtain (Focht and Verstraete 1977). Because denitrifiers usually form a portion of the total microbial population in soil (Focht and Verstraete 1977; Woldendorp 1981; Tiedje et al. 1982), however, the distinction of two groups represents the absolute minimum. The chemical composition of the bacteria, needed to calculate carbon nitrogen ratios (Eqs. (12), (14), and (16)), was set at $C_5H_{10.8}N_{1.5}O_{2.9}$, in accordance with data reported for *Paracoccus denitrificans* (van Verseveld and Stouthamer 1978). All bacteria are assumed to be active, and they are the only organisms that occupy the soil. The bacteria were homogeneously distributed in the model soil, and immobility of the organisms was assumed (Woldendorp 1981). Growth rates of both groups of bacteria are taken proportional to their respective amounts of biomass, Eq. (2), (van Veen and Frissel 1981; Schlegel 1972). Thus, it is assumed that the population densities of the bacteria never limit their growth, as would be presented by the logistic growth equation (Schmidt et al. 1985). Low population densities with respect to the carrying capacity of the soil surface area (0.1 to 0.2%) were reported by Woldendorp (1981). Relative growth rates depending on the concentrations of carbon and electron acceptor, were calculated by double-Monod kinetics, Eq. (5), which is a simple, mathematically continuous function to describe multiple-nutrient-dependent relative growth (Megee et al. 1972; Shah and Coulman 1978; Bader et al. 1975; Bader 1978). The double-Monod model assumes that the reductant (C) and the oxidant (O_2 or one of the N oxides) combine in the same organism. This assumption is based on the existence of different enzymes that catalyze the respective reductions within the same organism (Knowles 1982). Denitrifying enzymes were assumed to be already present or immediately induced after the onset of anaerobic conditions. This is supported by the relatively short (1 to 10 h) lag periods before denitrification started (Tiedje 1978; Smith and Tiedje 1979). Total simulation time lasted over 100 h. The total relative growth rate of the denitrifiers under anaerobic conditions is simply calculated as the sum of the single relative growth rates, Eq. (4). Equation (5) also shows that independency of relative growth rates with different electron acceptors was assumed, and that competition between the two groups of bacteria took place via the common carbon substrate and, if present, oxygen. When aerobic conditions prevail, both groups use oxygen as electron acceptor. Death rates of both groups of bacteria were taken proportional to their respective amounts of biomass, Eq. (3). Relative death rates were assumed constant and numerically equal to the product of maintenance

TABLE 1

Differential equations of the denitrification model

Net growth of bacteria

$$\frac{dB}{dt} = \left(\frac{dB}{dt}\right)_g - \left(\frac{dB}{dt}\right)_d \tag{1}$$

$$\left(\frac{dB}{dt}\right)_g = \mu B \tag{2}$$

$$\left(\frac{dB}{dt}\right)_d = m_c Y_c^{\max} B \tag{3}$$

Relative growth rates

$$\mu = \sum_i \mu_{E_i}, \qquad \text{for } i = 2, 3, 4 \tag{4}$$

$$\mu_{E_i} = \mu_{E_i}^{\max} \frac{[C]}{K_c + [C]} \frac{[E_i]}{K_{E_i} + [E_i]}, \tag{5}$$

$i = 1, 2, 3, 4$, refers to O_2, NO_3^--N, NO_2^--N, and N_2O-N, respectively

Consumption of glucose carbon

$$\left(\frac{dC}{dt}\right)_d = \left(\frac{\mu}{Y_c^{\max}} + m_c\right)B \tag{6}$$

Net rate of change of glucose carbon

$$\frac{dC}{dt} = \frac{dC_{\min}}{dt} - \left(\frac{dC}{dt}\right)_d \tag{7}$$

Production of carbon dioxide

$$\frac{d\mathrm{CO_2}}{dt} = \left(\left(\frac{dC}{dt}\right)_d - \left(\frac{dB}{dt}\right)_g\right) \Big/ M_c \tag{8}$$

Consumption of electron acceptor

$$E = \sum_i E_i, \qquad \text{for } i = 2, 3, 4 \tag{9}$$

$$\frac{dE_i}{dt} = \left(\frac{\mu_{E_i}}{Y_{E_i}^{\max}} + m_{E_i}\frac{E_i}{E}\right)B, \tag{10}$$

$i = 1, 2, 3, 4$ refers to O_2, NO_3^--N, NO_2^--N, and N_2O-N, respectively

Net rates of change of electron acceptors

$$\begin{aligned}
\frac{d\mathrm{O_2}}{dt} &= -\left(\frac{dE_1}{dt}\right) \Big/ M_{\mathrm{O_2}};\\
\frac{d\mathrm{NO_3^-N}}{dt} &= \frac{dN_{\min}}{dt} - \frac{dE_2}{dt} - \frac{dN_{\mathrm{ass}}}{dt};\\
\frac{d\mathrm{NO_2^-N}}{dt} &= \frac{dE_2}{dt} - \frac{dE_3}{dt};\\
\frac{d\mathrm{N_2O}}{dt} &= \left(\frac{dE_3}{dt} - \frac{dE_4}{dt}\right) \Big/ 2M_n;\\
\frac{d\mathrm{N_2}}{dt} &= \left(\frac{dE_4}{dt}\right) \Big/ 2M_n
\end{aligned} \tag{11}$$

TABLE 1—*Continued*

Nitrate assimilation

$$\frac{dN_{ass}}{dt} = \frac{F_{nb}}{F_{cb}}\left(\frac{dB}{dt}\right)_g \tag{12}$$

Carbon mineralization from dead biomass

$$\frac{dC_{min}}{dt} = F_c\left(\frac{dB}{dt}\right)_d \tag{13}$$

Nitrogen mineralization from dead biomass

$$\frac{dN_{min}}{dt} = F_n \frac{F_{nb}}{F_{cb}}\left(\frac{dB}{dt}\right)_d \tag{14}$$

Immobilization of carbon

$$\frac{dC_{imm}}{dt} = (1 - F_c)\left(\frac{dB}{dt}\right)_d \tag{15}$$

Immobilization of nitrogen

$$\frac{dN_{imm}}{dt} = (1 - F_n) \frac{F_{nb}}{F_{cb}}\left(\frac{dB}{dt}\right)_d \tag{16}$$

TABLE 2

List of symbols

Symbol	Meaning	Unit
B	Amount of bacterial carbon. Refers either to strict aerobes that cannot denitrify or to denitrifiers	kg C
C	Amount of glucose C	kg C
E, E_i	Total amount of electron acceptor (O_2 or nitrogenous compounds) and amount of individual electron acceptor, respectively. i = 1, 2, 3, 4 refers to O_2, NO_3^--N, NO_2^--N, and N_2O-N, respectively	kg O_2 or kg N
F_{cb}, F_{nb}	Mass fraction of carbon and nitrogen to biomass	–
F_{den}	Initial mass fraction of denitrifiers with respect to total bacterial biomass	–
F_c, F_n	Mass fraction of carbon and nitrogen that mineralizes from the dead biomass	–
K_c, K_{E_i}	Half-saturation value in Monod model with respect to carbon and electron acceptor, respectively	kg m^{-3} H_2O
m_c, m_{E_i}	Maintenance coefficients with respect to carbon and electron acceptor, respectively	kg kg^{-1} B s^{-1}
M_c, M_n, M_{O_2}	Molecular weight of carbon, nitrogen, and oxygen, respectively	kg mol^{-1}
t	Time	s
Y_c^{max}, $Y_{E_i}^{max}$	Maximum growth yield on glucose C and on electron acceptor E_i when no substrate would be used for maintenance	kg B kg^{-1}
ass	Subscript that refers to assimilated	
b	Subscript referring to bacteria	
c	Subscript referring to carbon substrate	
d, g	Subscripts indicating death or decrease, and growth, respectively	
h	Subscript referring to high critical level	
imm	Subscript that refers to immobilized	
l	Subscript referring to low critical level	
min	Subscript that refers to mineralized	
μ	Total relative growth rate on oxygen or on all N-electron acceptors together	s^{-1}
μ_{E_i}, $\mu_{E_i}^{max}$	Actual and maximum relative growth rate on electron acceptor E_i, respectively, with glucose C as carbon-limiting substrate	s^{-1}
$\sum$	Summation operator	
[ ]	Brackets around an amount convert it to a concentration, either with respect to dry soil (e.g., for bacteria) or with respect to volume of water (e.g., for substrates or electron acceptors)	

TABLE 3

Total concentration of biomass ([B]), *initial fraction denitrifiers* (F_{den}), *maximum relative growth rates* (μ), *half-saturation values* (K), *maximum growth yields* (Y), *and maintenance coefficients* (m), *with respect to carbon and different electron acceptors at 20°C*

Symbol	Unit	Substrate	Parameter values used in simulation runs		
			As derived directly from literature compilation Fig. 1	Modified values used for closer comparison with experiments	
				In this paper Fig. 2	In Cooper and Smith (1963) Fig. 3
$[B] \times 10^4$	$\frac{\text{kg C}}{\text{kg}}$		1	1	1
F_{den}	–		0.02	0.02	0.6
$\mu \times 10^6$	s^{-1}	NO_3^-	4.8	3.9	4.8
		NO_2^-	4.8	2.4	2.4
		N_2O	2.4	1.2	1.2
$K \times 10$	kg C	C	0.17	0.17	0.17
	or N	NO_3^-	0.83	0.83	0.83
	$\overline{m^3}$	NO_2^-	0.83	0.83	0.83
		N_2O	0.83	0.83	0.83
$Y \times 10^2$	$\frac{\text{kg B}}{\text{kg C}}$	C	50.3	50.3	50.3
	or N	NO_3^-	40.1	10.0	30.0
		NO_2^-	42.8	5.4	22.5
		N_2O	15.1	0.38	1.9
$m \times 10^5$	kg C	C	0.21	0.21	0.21
	or N	NO_3^-	2.5	2.5	2.5
	$\overline{\text{kg B s}}$	NO_2^-	0.97	0.97	0.97
		N_2O	2.2	2.2	2.2

coefficient and growth yield (Verstraete 1977). Cell decay is interpreted as lysis of the cell (Painter 1970). The products of the decay process are discussed in the section on mineralization.

Consumption of carbon substrate and electron acceptors

The consumption of carbon (glucose) is described by an equation by Pirt (1965), Eq. (6). The first term of this equation represents the use of substrate for cell synthesis and growth energy, whereas the second term represents the maintenance requirements of the organism for, for example, turnover of cell materials and osmotic work to maintain concentration gradients between the cell and the surroundings (Pirt 1975). The carbon substrate in the model serves both as carbon and energy source for the bacteria. Complete oxidation is assumed if it is used as energy source. Thus, carbon dioxide production can be calculated as the difference between the total amount of carbon consumed and the amount used for cell synthesis, Eq. (8). The consumption of electron acceptors was also calculated with Pirt's equation, i.e., Eq. (10). The maintenance coefficients in Eq. (10), however, were multiplied with the relative presence of each electron acceptor in the water phase. This correction was introduced because the maintenance data derived from the literature for each reductive step suffice to maintain the whole of the biomass. Without the correction, therefore, the bacteria would consume too much electron acceptor for their maintenance.

Nitrate assimilation

Besides the use of nitrate in denitrification, nitrate assimilation will also occur in the system. The process of assimilation goes via nitrite to ammonium at the rate required for the synthesis of organic nitrogen compounds.[3] This characteristic of the process is reflected by Eq. (12), through the stoichiometric relation of assimilation rate to the gross growth rate of the bacteria via the inverse of the carbon-nitrogen ratio.

[3] F. C. Boogerd, 1984, Energetic aspects of denitrification in *Paracoccus denitrificans*, Ph.D. thesis, Vrije Univ., Amsterdam, p. 132.

Enzymes that are active in the assimilatory process are different from those of the dissimilatory process, and they are not affected by oxygen (Focht and Verstraete 1977; Bryan 1981). Therefore, nitrate assimilation was similarly calculated under aerobic and anaerobic conditions for both groups of bacteria. The absence in the model of assimilation of nitrite and possibly nitrous oxide means that growth and maintenance cease as soon as nitrate is depleted.[1] A more complete description of nitrogen assimilation would be possible by incorporating bacterial growth on these nitrogenous compounds. However, this would also introduce new problems with respect to which nitrogenous form would be preferentially assimilated; therefore, it was not attempted.

Mineralization and immobilization

Products from cell decay (see section about bacteria) or mineralization will, partially, enter the surroundings and may be used again as substrates for growth and maintenance. Under aerobic conditions it was assumed that certain fractions of the carbon and nitrogen, F_c and F_n in Eqs. (13) and (14), respectively, were liberated from the dying cells. The carbon and nitrogen released were considered equivalent to glucose carbon and nitrate nitrogen, as expressed by Eqs. (7) and (11), where the glucose C pool and the nitrate N pool are replenished by these liberated products. Thus, it was implicitly assumed that both sequential processes in mineralization, i.e., ammonification and nitrification (Russell 1973), occurred. Under anoxic conditions nitrification cannot occur, because oxygen is needed for this process (Patrick 1982). Therefore, under anaerobic conditions mineralization was assumed not to occur, by taking F_c and F_n equal to zero. By these simplifications, model descriptions of ammonification, ammonium assimilation, nitrification, and inhibition of assimilatory nitrate reductase by ammonia (Bryan 1981; Payne 1973) were avoided. The remaining carbon and nitrogen from dying cells were added to the pools of immobilized carbon and nitrogen, C_{imm} and N_{imm} in Eqs. (15) and (16), respectively: this carbon and nitrogen did not participate any more in the dynamic processes.

Environmental conditions

Major environmental conditions affecting denitrification are concentrations of water-soluble carbon (Burford and Bremner 1975; Stanford et al. 1975*a*), and electron acceptor in the soil water where the bacteria live. These variables affect relative growth rates by means of Eq. (5). Growth of both groups of bacteria was similarly described by Eqs. (1) through (16). In the case of the strict aerobes, E_i in Eqs. (5) and (10) always refers to oxygen (i=1). In the case of the denitrifiers, three situations were distinguished. First, when ample oxygen is available, i.e., $[O_2] > [O_2]_h$, growth of denitrifiers was described similarly to that of the strict aerobes. Second, when the oxygen concentration is lower than a certain limit, $[O_2]_l$, the N oxides are used as electron acceptors, and i equals 2, 3, or 4 in Eqs. (5) and (10). The third situation occurs in the transition zone, where the oxygen concentration is between the lower and upper limits, i.e., $[O_2]_l < [O_2] < [O_2]_h$: both oxygen and N oxides are used as electron acceptors (Meiberg et al. 1980). Because the gaseous electron acceptors oxygen and nitrous oxide occur in both the soil water and gas phase, their concentrations and thus the pattern of denitrification will be affected by their solubility in the water phase and their pressures in the gas phase. Growth and maintenance of both groups of bacteria were assumed to cease whenever either the appropriate electron acceptor (oxygen for strict aerobes or oxygen and nitrogenous electron acceptors for denitrifiers) or nitrate and/or carbon for assimilation were depleted.

Other environmental conditions, e.g., soil acidity (Bremner and Shaw 1958; Stanford et al. 1975*a*), temperature (McKenney et al. 1984; Stanford et al. 1975*b*; Bremner and Shaw 1958), and soil water potential (Harris 1981; Griffin 1981) merely determine overall bacterial activity. Though empirical relationships between microbial activity and soil pH, temperature, and water potential could be incorporated in the model, we believe that these incorporations would not significantly contribute to a better understanding of the principal processes now reflected in Eqs. (1) through (16). Therefore, they were omitted. This, however, implies that the buffer capacity of soil was considered high and that pH would remain between 6 and 8, a range that is reported to have little effect on denitrification (Burford and Bremner 1975; Stanford et al. 1975*a*). Furthermore, data that were known to be temperature-dependent, e.g., relative growth rates, maintenance coefficients,

and solubility coefficients, were converted to 20°C, whereas water potential was assumed not to affect microbial activity: Griffin (1981) reported that bacterial activities in soil decrease sharply when the matric potential falls to values between −50 and −300 kPa. Though the assumption of a negligible effect of water potential on microbial activity seems reasonable at the water content used (about 0.32 g g^{-1}), no soil water characteristic was available for this loosely packed soil to substantiate this assumption. Therefore, effects of water potential may be hidden in the parameterization of the model with respect to relative growth rates.

Bacteria are subjected to the concentrations of substrates as these occur in the water phase of soil. Soil water content will thus have a profound influence on these concentrations. This has a number of consequences. First, different concentrations directly affect relative growth rates through Eq. (5). Second, substrate concentrations strongly affect the maintenance coefficients in the Pirt equation (Pirt 1975). Third, low water contents will decrease the rate of diffusion over short distances (microdiffusion) of nutrients to bacterial colonies or cells. It was already stated that low population densities with respect to the surface area of soil exist (Woldendorp 1981). Woldendorp also stated that the microorganisms on the soil particles were seen as isolated cells or small colonies. Thus, even when nutrients are homogeneously distributed, microdiffusion must occur to transport nutrients to these patches of growing cells. It is not feasible to model microdiffusion, however, and it seems unavoidable to incorporate its effect on bacterial activity through "effective" half-saturation values that have larger values than those measured in pure culture studies (Shieh 1979). Half-saturation values found in soil are indeed much higher than those obtained with pure cultures (Firestone 1982).

Computer program

Numerical calculations were done by a program written in Continuous System Modeling Program III (CSMP III) language (IBM 1975) and executed on a VAX machine. The program was developed and written with three targets in mind (apart from simulating the respiration and denitrification process): (1) to facilitate the communication of the model and the program to others; (2) to enable the author to incorporate the program in a very large program including transport processes of water, solutes, and gases in an unsaturated soil aggregate; (3) to minimize programming errors. Therefore, the calculation sequence has been summarized in terms of calls to (FORTRAN) subroutines in the main (CSMP) program. The main program contains three major sections: (1) a parameter section, summarizing all biological, soil physical, chemical, and run-time control parameters; (2) an initial section, mainly to calculate the amounts of the state variables at time zero, and to convert a number of parameters to SI units and to 20°C; all actual input parameters for the dynamic section are printed for control purposes; (3) a dynamic section, starting with the state variables in terms of amounts contained in integrals. The latter is followed by subsections to calculate: (a) derived quantities from the state variables (material balances and concentrations), (b) production terms, (c) gross rates of change of each integral value, and (d) net rates of change of each integral value. A last subsection contains the routines for printing results. The types of subroutines that are called from the dynamic section can be classified similarly to the subsections distinguished there. In addition, however, a subroutine that contains only the control structure to choose the correct calculation subroutines, i.e., on the basis of the actual environmental conditions, is distinguished. Thus, the extensively structured program enables one to get a quick overview of the calculations, whereas details may be studied in the separate subroutines. Care has been taken to maintain the recognizability of the rate equations in the subroutines. Units and abbreviations of variables have been given in a separate listing. A system to abbreviate variables was designed and applied to improve the recognizability of the variables, and the readability of the program.

All results presented have been obtained by the variable time-step-integration method of Runge-Kutta Simpson. Material balances of nitrogen and carbon were computed during the simulation runs and were found to be correct. To prevent adverse numerical effects of the occurrence in the simulations of slightly negative amounts or concentrations of substances that were consumed, small ($< 0.5\%$ with respect to the maximum of the variable) threshold values were introduced. Below these threshold values, the appropriate consumption rates were set to

zero. The program gives results in terms of rates of respiration and denitrification, concentrations of biomass (with respect to dry soil mass) and substrates (with respect to soil water), and concentrations and pressures of gases in the soil container.

Model parameters

The microbiological and physicochemical parameters that are needed to test the model should, ideally, originate from soil batch culture studies in which the parameters summarized in Eqs. (1) through (16) are reported. If the sequence of denitrification products could be simulated by using these parameters, it would be warranted to conclude that the model is a very reasonable representation of the soil biological system. Such data sets do not exist to date, however. Another way to come to a judgment about the model is to use data from different authors and to investigate whether it is possible to simulate the experimental sequence of denitrification products by modifying some of these data within reasonable limits, i.e., within the limits found for representative denitrifying organisms. For this purpose a data set was compiled from the literature (Table 3) and is discussed below.

Biomass contains about 1 to 2% of total soil carbon (Woldendorp 1981). When the organic carbon content of soil is about 1%, this results in 10^{-4} to 2×10^{-4} kg of biomass C kg^{-1} dry soil. The lower value has been used throughout this study.

The (initial) fraction of bacteria that is able to denitrify, F_{den}, may vary from decimal fractions of a percent to half of the soil bacterial flora, depending on the medium used in enumeration by the most-probable-number method (Focht and Verstraete 1977). Therefore, it seems not unreasonable to use this fraction to tune the model results to those of the experiments. Note, however, that this fraction is specified only at the start of the simulation, for it will change in time due to different growth rates of the strict aerobes and denitrifiers.

Maximum relative growth rates on three N-electron acceptors for an organism that can grow on glucose are needed in the model. Koike and Hattori (1975*a*) reported maximum relative growth rates for *Pseudomonas denitrificans* grown in liquid batch culture under aerobic and anaerobic conditions with glucose and glutamate ($C_5H_9NO_4$) as carbon source and oxygen or nitrate as electron acceptor. The organism could grow aerobically, but not anaerobically, on glucose; anaerobically it needed glutamate. The data for the maximum relative growth rates in Table 3 were derived as follows. The ratio of relative growth rates on glutamate under anaerobic conditions to that under aerobic conditions was 0.14/0.66 (Koike and Hattori 1975*a*, their Table 1). The aerobic relative growth rate on glucose was 5.694 10^{-5} s^{-1} at 20°C, using a Q_{10} value of 2. Assuming that the ratios of relative growth rates on nitrate, nitrite, and nitrous oxide equal those of the number of electrons accepted by the nitrogen atom in each reduction step, the value for, for example, the relative growth rate on nitrate being reduced to nitrite becomes $0.14/0.66 \times 5.694\ 10^{-5} \times 2/5$.

Half-saturation values for heterogeneous microbial populations obtained from soil extracts grown in continuous cultures with glucose and nitrate were reported by Shah and Coulman (1978) and are given in Table 3. The half-saturation values for nitrite and nitrous oxide nitrogen were taken equal to that of nitrate nitrogen, so that relative growth rates retain similar ratios as the maximum relative growth rates when equal electron acceptor concentrations would be present.

Maximum growth yields and maintenance coefficients on glucose and on three N-electron acceptors are needed in the model. Van Verseveld et al. (1977) reported values for *Paracoccus denitrificans* grown in continuous culture under anaerobic conditions with gluconate ($C_6H_{12}O_7$) as carbon source and nitrate as electron acceptor. Their data show some variation when gluconate or nitrate is the limiting growth factor. Therefore, mean values of Y_c^{max} and m_c were used. The data were converted to the units reported in Table 3 using the elementary composition of *Paracoccus denitrificans*, $C_6H_{10.8}N_{1.5}O_{2.9}$ (van Verseveld and Stouthamer 1978). Koike and Hattori (1975*b*) reported maximum growth yields and maintenance coefficients on glutamate and on all three nitrogenous electron acceptors for *Pseudomonas denitrificans* grown in continuous culture. Because the maximum growth yields on nitrate in the studies of both van Verseveld et al. (1977) and Koike and Hattori (1975*b*) differed but 10%, the electron acceptor data for growth yield from Koike and Hattori were used. The maintenance value from

Koike and Hattori for nitrate was about 3.7 times as high as the corresponding value of van Verseveld et al. The maintenance data are used, however, because they form part of a consistent data set with the maximum growth yields, and no other data are known to be reported.

The data for the maximum growth yields on the three electron acceptors as reported in Table 3 were derived as follows. The growth yields reported by Koike and Hattori on nitrate, nitrite, and nitrous oxide refer to the reduction of each electron acceptor to molecular nitrogen. Growth yields for each separate reduction step, as needed in the model, were therefore calculated as the difference between the values of two consecutive reductions. The resulting values were converted to the units reported in Table 3, using the elementary composition of *Paracoccus denitrificans.*

The data for the maintenance coefficients on the three electron acceptors reported in Table 3 were derived as follows. The maintenance coefficients on nitrate, nitrite, and nitrous oxide from Koike and Hattori again refer to the reduction of the electron acceptor to molecular nitrogen. In one time unit the amount of biomass maintained per unit nitrate electron acceptor that is reduced to molecular nitrogen is the inverse of the maintenance coefficient for nitrate as reported by Koike and Hattori. The same holds for nitrite and nitrous oxide. In analogy with the derivation of growth yields for each separate reduction step, the amount of biomass that is maintained when nitrate is reduced to nitrite, for example, will be the difference between the inverse maintenance coefficients: $1/m\mathrm{NO_3^-} = (1/m'\mathrm{NO_3^-}) - (1/m'\mathrm{NO_2^-})$, where m' values are those from Koike and Hattori expressed in kg N kg^{-1} biomass s^{-1} at 30°C, and $m\mathrm{NO_3^-}$ is the desired maintenance coefficient for the reduction of nitrate to nitrite. The latter value was converted to the units in Table 3 using the elementary composition of *Paracoccus denitrificans* and a Q_{10} value of 2.

The mineralization parameters, F_c and F_n, were taken as zero under anaerobic conditions for reasons outlined in the section about mineralization and immobilization.

Gas solubility values (m^3 gas m^{-3} water) at 1 atm and 20°C for nitrous oxide (0.6788) and molecular nitrogen (0.01686) were taken from Wilhelm et al. (1977).

The remaining parameters used to simulate the experiment, i.e., amount of soil, moisture content, and concentrations of added substrates, were taken according to the description in the section about materials and methods.

Parameter values for μ, K, Y, and m under aerobic conditions are not reported in this study, because no experimental data that give the full time course of the development of anaerobiosis and the subsequent reduction of nitrate to molecular nitrogen via the intermediates nitrite and nitrous oxide were found in the literature; as a consequence, no simulation runs under aerobic conditions were made.

The denitrification model will form part of an extended model that includes the dynamic interactions between denitrification and the physical transport processes of water, gases, and ions in a partially saturated soil aggregate that is surrounded by air. The extended model and the parameters needed for aerobic growth conditions will be described in a subsequent paper.

RESULTS AND DISCUSSION

Model results are presented in terms of the reduction sequence nitrate, nitrite, nitrous oxide, and molecular nitrogen. In Figs. 1, 2, and 3 the simulation results are compared with the experimental data obtained in this paper (Figs. 1 and 2) and those from Cooper and Smith (1963) (Fig. 3), without (Fig. 1) and with (Figs. 2 and 3) modifications of the data as compiled from the literature. Finally, Fig. 4 gives some results of a sensitivity analysis of the model. Values of the microbiological parameters used in the simulations are given in Table 3.

Figure 1 shows the experimental data from this study, with bars indicating one standard deviation, and the results of the simulation using the unmodified literature data. Comparing these simulated and experimental results makes clear that the model assumptions are not unreasonable: denitrification products appear in the right sequence, and the time course of the simulated and experimental denitrification process is rather similar. In particular the simulated nitrate and nitrite curves are close to the experimental curves. Major differences are the delayed start of the evolution of molecular nitrogen in the simulated results and the fact that no reduction of nitrite and nitrous oxide takes place when nitrate is depleted. The latter feature is found in all figures. It is the direct consequence of the "practical," but not necessary, assumption

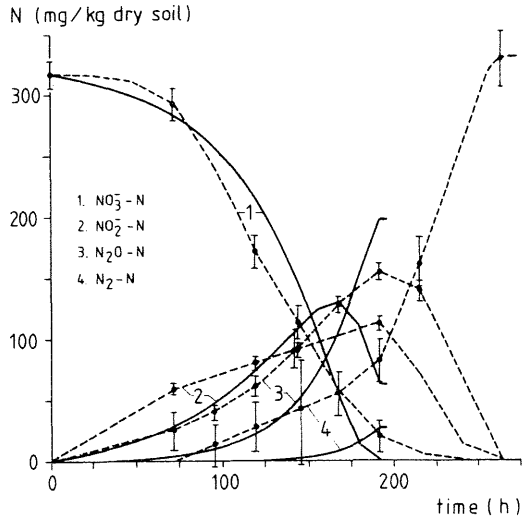

FIG. 1. Experimental (this paper, bars indicate one standard deviation; dashed curves) and simulated (based on unmodified literature data, Table 3, fourth column; continuous curves) concentrations of nitrate, nitrite, nitrous oxide, and molecular nitrogen as functions of time.

that growth and maintenance cease when nitrate for assimilation is depleted.

Figure 2 shows results of a simulation using modified literature data and again the experimental results from this paper (only the smoothed dashed curves are given for readability). The input data to obtain the simulated results in Fig. 2 were modified with respect to those from the literature compilation by factors of 0.5 and 0.8, for the maximum relative growth rates, and 0.25, 0.125, and 0.025, for the maximum growth yields on nitrate, nitrite, and nitrous oxide, respectively. The changes in the maximum relative growth rates are supported by data from Koike and Hattori (1975*a*), who reported such values for *Pseudomonas denitrificans* under anaerobic conditions on different substrates that differed by a factor of 0.43. The changes that were introduced for the maximum growth yields on nitrogenous electron acceptors could not be substantiated by data from the literature: only Koike and Hattori (1975*b*) have reported on these three parameters. The similarity between the simulated and experimental results is rather good. Especially the disappearance of nitrate and the evolution of molecular nitrogen are almost quantitatively described. The maxima of nitrite and nitrous oxide are about 25% too low compared with the experimental values, but they appear at about the right moment. It is likely that similar or even better results would have been obtained if the maintenance coefficients on nitrogenous oxides had been included in the modifications. Then, the modification of, for example, growth yield on nitrous oxide could have been smaller, because a larger maintenance value would take over part of the adaptation of the model results to those of the experiment. It must be stressed, however, that no parameter optimization has been carried out; rather it was investigated whether the model had the potential to describe experimental data. All modified parameters to obtain the simulated results are lower than in the compiled literature data. This is a promising result, for growth and yield of bacteria in soil will not be as efficient as in liquid-batch and continuous-culture studies.

Figure 3 shows experimental results from Cooper and Smith (1963, results at 20°C from their Fig. 1; no standard deviations were supplied) and results of a simulation using modified literature data. The remaining parameters used to simulate this experiment, i.e., amount of soil, moisture content, and concentrations of added substrates, were derived from the section about

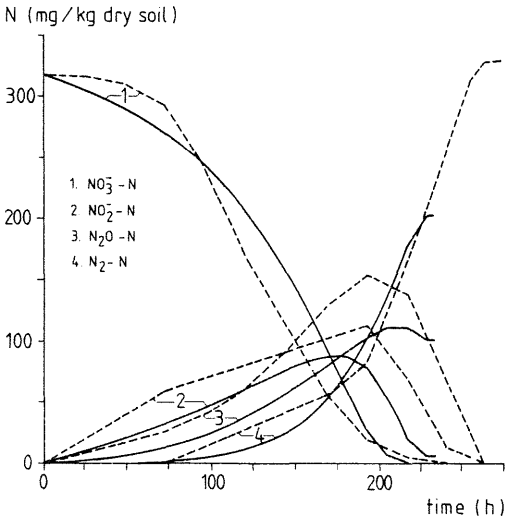

FIG. 2. Experimental (this paper, dashed curves) and simulated (based on modified literature data, Table 3, fifth column; continuous curves) concentrations of nitrate, nitrite, nitrous oxide, and molecular nitrogen as functions of time.

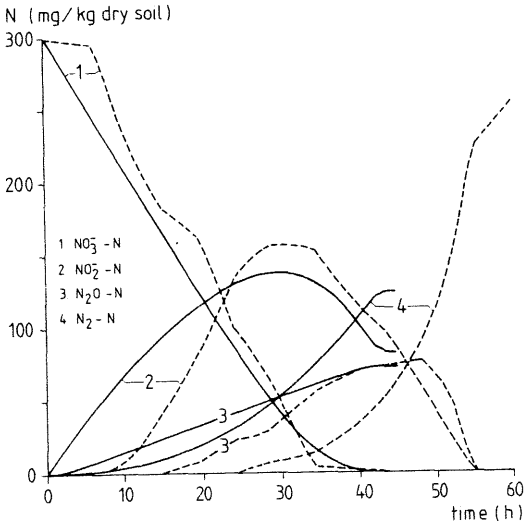

FIG. 3. Experimental (paper of Cooper and Smith, dashed curves) and simulated (based on modified literature data, Table 3 last column; continuous curves) concentrations of nitrate, nitrite, nitrous oxide, and molecular nitrogen as functions of time.

methods and procedures from Cooper and Smith. The data to obtain the simulated results in Fig. 3 were modified with respect to those from the literature compilation by factors of 0.5 for the maximum relative growth rates on nitrite and nitrous oxide, and 0.75, 0.53, and 0.125 for the maximum growth yields on nitrate, nitrite, and nitrous oxide, respectively. The initial fraction of bacteria that is able to denitrify, F_{den}, has been increased by a factor of 30 to 0.6. The agreement between the simulated and experimental results is satisfactory. Even the simulated maxima of nitrite and nitrous oxide are close to the experimental data. The evolution of molecular nitrogen deviates most from the experimental curve: it occurs too fast. The experimental results of nitrate indicate a time lag of about 4 to 6 h. It appears that the bacteria needed this period to recover their activity after the air-dried soil had been moistened. This time lag is not produced by the present simulation model, since it was assumed that such time lags would be too short to be taken into account, as compared with the total duration of the experiments. The parameter indicating the initial fraction denitrifiers (F_{den}), has a strong influence on the time course of the denitrification process. It determines how the nitrate curve departs from the y axis: when F_{den} is low, first a bacterial population need to be built up and as a consequence the initial decrease in nitrate is not appreciable. Then, the nitrate curve departs parallel to the time axis. When F_{den} is high, the decrease in nitrate is directly substantial and the curve departs from the y axis at an appreciable angle. Perhaps, therefore, this parameter could take over part of the time lag observed in the experimental data.

The process of denitrification in the experiment of Cooper and Smith takes place in 60 h, whereas that reported in this paper, Fig. 2, lasted 4.5 times longer. This difference in rates will mainly be caused by the pretreatment of the soils: Cooper and Smith used air-dried and previously stored soil, whereas we used moist soil. Wetting air-dry soil is known to cause a flush in soil microbial activity (Birch 1958, 1959; Fillery 1983) and to increase denitrification (MacGregor 1972). The simulations of both the slow (Fig. 2) and the fast (Fig. 3) denitrification process are of similar quality. This supports the view that no principal differences exist in the description of the denitrification process in initially air-dry or moist soil.

No other appropriate experimental data have been found in the literature to compare the simulation model with. For example, Cho and Sakdinan (1978) reported about the full sequence of denitrification products in soil samples with a layer thickness of at least 4 cm. Their data could not be used because transport processes occurred in these thick soil layers, and the exchange of gases between soil and the head space of the container was seriously hampered (Cho 1982). The assumption that the soil system must be homogeneous was thus not fulfilled. Cho (1982) used the same experimental setup as in his paper with Sakdinan in 1978, but he eliminated the influence of transport processes on denitrification by shaking. Unfortunately, however, no data on the full sequence of denitrification products were reported.

It is concluded that the model gives a reasonable description of the denitrification process in homogeneous soil. Therefore, some results of a sensitivity analysis of the model with respect to the parameters used to produce Fig. 2, are presented. The influence of the container volume on the simulated results was investigated.

Figure 4 depicts the effect of halving the container volume on the time course of the devel-

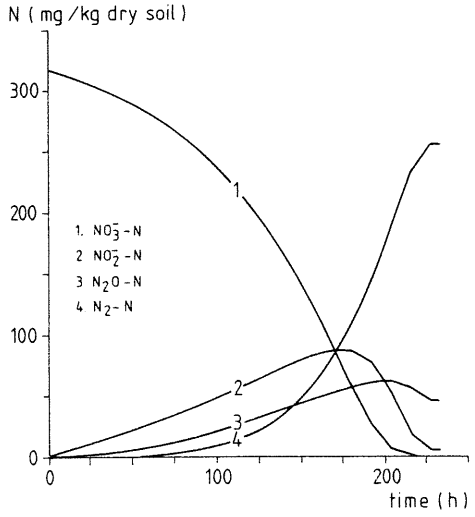

FIG. 4. Simulated concentrations of nitrate, nitrite, nitrous oxide, and molecular nitrogen as functions of time, when container volume is halved.

opment of nitrous oxide and molecular nitrogen: the maximum nitrous oxide concentration is about halved with respect to the simulation results in Fig. 2, the maxima of nitrous oxide and nitrite are reversed, and the nitrogen concentration has increased at the end of the simulation. Halving the container volume roughly doubles the increase of the gas pressure in the soil container, because the same amount of gas is produced in a smaller volume. The distribution coefficient of nitrous oxide between water and gas phases remains constant, and consequently its concentration in the water phase will be doubled. Then, the relative growth rate on this electron acceptor may increase when concentrations are of the order of the half-saturation values (Table 1, Eq. (5)), and the use of this electron acceptor for growth and maintenance will increase too (Table 1, Eq. (10)). Though the increase of the gas pressure of nitrous oxide relative to atmospheric pressure in the model soil container was low—a maximum of 1.2% only was recorded during the simulation, depicted in Fig. 2—the model suggests that it has an appreciable influence on the results. The fact that the maxima of nitrite and nitrous oxide are reversed in the results of Cooper and Smith (1963) with respect to the experiment presented in this paper, may, at least in part, be explained by differences in gas pressures, because the model indicated a gas pressure of nitrous oxide relative to atmospheric pressure of 3.1% in the case of Cooper and Smith. The importance of these theoretical observations is twofold. First, denitrification patterns reported by different workers cannot be compared directly; rather such results should be related to one another by model studies. Second, differences in local gas pressures of nitrous oxide in field soils will affect the ratio N_2O/N_2, and thus not only the biochemistry of the denitrification process is a determining factor for the numerical value of this ratio. The N_2O/N_2 ratio is the subject of research in connection with the possible contribution of nitrous oxide to destruction of the ozone layer in the stratosphere (Letey et al. 1980*b*, 1980*c*).

CONCLUDING REMARKS

The presented simulation model proved to give a reasonable description of the denitrification process measured in laboratory incubation vessels. A major difficulty, however, is that no coherent data sets exist to date to parameterize the model. Simulation results obtained with such data sets would enable one to put forward more definite conclusions about the quality of the model. Therefore, besides the introduction of minor improvements in the present model, e.g., the consumption of other electron acceptors than nitrate for growth and for maintenance processes after the depletion of nitrate, it seems appropriate neither to principally modify the model nor to develop more complicated models, e.g., that of Cho and Mills (1979), that are inherently more difficult to parameterize. Rather, attention should be given to gathering coherent data sets, including both determinations of the parameters needed in the present model and the time course of denitrification products. Such data should be be used to further test the model to be able to judge its predictive value in ecological studies concerning denitrification.

Sensitivity analysis of the presented model may help to design the experiments that are needed to determine these parameters.

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