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Model of bacterial growth influenced by substrate C:N ratio and concentration

Franck Touratier[1,*], Louis Legendre[1], Alain Vézina[2]

[1]Département de biologie, Université Laval, Québec, Québec G1K 7P4, Canada
[2]Institut Maurice-Lamontagne, Ministère des Pêches et Océans, CP 1000, Mont-Joli, Québec G5H 3Z4, Canada

ABSTRACT: The present study describes and explores a model to determine bacterial growth, respiration and net ammonium remineralization as a function of the average substrate C:N ratios and concentrations of organic substrates. Several experimental data sets were used to estimate critical parameters of the model. These data sets suggest that carbon and nitrogen growth efficiencies play a central role in the computation of bacterial growth, respiration, and net ammonium remineralization. The new parameterization of these processes, which takes into account the variability of carbon and nitrogen growth efficiencies with the C:N ratio of organic substrates, agrees with the observed experimental trends. The structure of the model and the results are compared to those of other models. According to the output of our model and to experimental data, which were used to validate parts of the structure, bacterial growth increases with organic substrate concentration and decreasing C:N ratios. As a consequence, a low carbon content relative to nitrogen in the substrates does not limit bacterial growth.

KEY WORDS: Bacterial growth · Excretion · Respiration · Model · Nitrogen · Carbon

INTRODUCTION

Bacterial growth and metabolic rates are key variables in modern biological oceanography. Bacteria are the central component of the microbial food web (e.g. Legendre & Rassoulzadegan 1995), and the quality and concentration of organic substrates may explain several aspects of the efficiency of that web (i.e. its ability to transfer organic carbon to higher trophic levels) and the competition for ammonium between phytoplankton and bacteria. Net bacterial ammonium remineralization becomes negative when the substrate C:N ratio reaches a certain threshold (Lancelot & Billen 1985). Above this threshold, there is a strong competition for ammonium between bacteria and phytoplankton in surface waters (Rivkin & Anderson 1997), in which case most ammonium is taken up by heterotrophic bacteria (Wheeler & Kirchman 1986, Hoch & Kirchman 1995, Rivkin & Anderson 1997). Several factors are involved in this: the relative biomasses, ammonium half-saturation constants for uptake as a function of cell size, and maximum growth rates of bacteria and phytoplankton, and the nitrogen growth efficiency of bacteria as a function of the substrate C:N ratio. It is still debated whether bacteria act as a link towards higher trophic levels or as a sink, because they respire much of the assimilated organic carbon. Many experimental studies were conducted and models proposed to explore the link-sink problem (e.g. Ducklow et al. 1986, Taylor & Joint 1990, Ducklow 1991), but most of these did not take into account the quality of assimilated substrates, which may be a critical factor to understand the role of bacteria in pelagic ecosystems.

The factors affecting bacterial growth are numerous and not yet well understood. In particular, it is not clear what limits the growth of bacteria in nature, but it is of general interest to know which chemical element limits bacterial growth, especially for ecological modeling. Most models consider only 1 biogeochemical unit (e.g. Andersen & Nival 1988, Fasham et al. 1990), which is often nitrogen or phosphorus because the availability of nitrate/ammonium or phosphate may limit primary production. In other words, to simplify the structure of

*E-mail: ftourat@botzoo.uct.ac.za

the models, the unit of bacterial biomass and processes is imposed by the element which limits phytoplankton growth. If the limiting element for bacterial and phytoplankton growth is the same, such models are likely to be useful. If, however, bacteria and phytoplankton are limited by different elements, the value of the models is questionable. The solution is to consider several units for the same model, in order to correctly represent the effects of several potentially limiting elements, keeping in mind that only 1 element actually limits the growth of an organism.

The limitation of growth can be looked at from 2 different viewpoints. The first is related to the quantity of substrates, i.e. it is the concentration of substrates which is limiting, irrespective of the element considered. The second is related to the quality of substrates, so that the influence of element ratios (e.g. C:N, C:P, N:P) on bacterial growth must be assessed to determine which element is limiting. The present study only considers carbon and nitrogen (see the second hypothesis in 'Description of the model'), so that 2 cases can occur: nitrogen will limit bacterial growth if the growth rate increases with a decreasing substrate C:N ratio, or carbon will be the limiting factor if the growth rate increases with an increasing substrate C:N ratio. Hence, the C:N ratio of the substrates will be used here as a surrogate for substrate quality. The influence of substrate quality and quantity on bacterial growth has been documented in anoxic sediments (Blackburn 1980, 1995, Blackburn & Henriksen 1983). In the water column, most studies have focused on the influence of substrate concentration on bacterial growth (e.g. Azam et al. 1983, Peduzzi & Herndl 1992), very few being devoted to bacterial responses to substrate quality. Results from the latter studies suggest that carbon and nitrogen growth efficiencies depend on substrate C:N ratios (Lancelot & Billen 1985, Goldman et al. 1987). Phosphorus and perhaps other elements may have comparable roles, but little is known about this. In spite of experimental difficulties in determining bacterial growth efficiencies, the literature reflects growing interest on the influence of substrate quality (Caron et al. 1988, Goldman & Dennett 1992, Legendre & Rassoulzadegan 1995, Fenchel et al. 1998). The quality of assimilated substrates also influences bacterial carbon and nitrogen growth efficiencies, which are functions of the substrate C:N ratio (e.g. Goldman et al. 1987). When these efficiencies are known, it is possible to estimate which percentages of assimilated organic carbon and nitrogen are respired and excreted, and which are used for the production of new biomass (e.g. Billen et al. 1990, Weaver & Hicks 1995). Carbon and nitrogen growth efficiencies are, however, not independent because stoichiometry for these elements must be maintained in bacterial biomass (Goldman et al. 1987).

The representation of bacterial growth and metabolism in ecosystem models falls broadly into 3 classes, each mathematical model offering specific advantages for the exploration of bacterial dynamics. The first class consists of models in which bacteria are implicit, i.e. the bacterial biomass (or abundance) is not described by a state variable. Examples are the Nutrient(s)-Phytoplankton-Zooplankton (NPZ) models of e.g. Andersen & Nival (1988), Radach & Moll (1993), and Carlotti & Radach (1996), where there is direct remineralization of dead organic matter (fecal pellets and dead organisms) into nutrients. In the second class of models, bacteria are a state variable. With this representation, an estimate of bacterial biomass is included in the structure of the model and remineralization processes, if considered, are often influenced by this biomass. Examples are the models of Fasham et al. (1990), Andersen & Rassoulzadegan (1991), and Thingstad et al. (1997). Most ecological models fall into the first 2 classes. Because these models describe only 1 biogeochemical cycle (often nitrogen or phosphorus) and, consequently, do not consider the quality of the substrate, their structure does not allow the assessment of competition between bacteria and phytoplankton, or the link-sink problem. In the third class of models, bacterial growth is influenced by substrate quality. In all classes of models, complexity may be quite different.

A few models only belong to the last class. These include the models proposed by Parnas (1975) and Anderson (1992), which are of limited use. These models only consider bacterial growth whereas other models, like those of Moloney & Field (1991), Moloney et al. (1991), and Moloney (1992), try to simulate the development of the main components of the planktonic network. There exists, in addition, biotechnological models, whose ability to study natural systems is limited as discussed below.

We present here a new model to assess the effects of substrate C:N ratio on growth, respiration, and excretion. The model combines experimental observations on the relationships between growth efficiencies and C:N ratio with simple mass balance concepts, to compute the fluxes (growth, respiration, excretion) required in ecosystem models and their changes as a function of substrate quantity and quality. We estimate the parameters required by the model using experimental data, and show how its predictions differ from those of alternative models.

The model proposed in the present study goes beyond that of Parnas (1975), which is too simple to extensively analyse experimental results. Moreover, the latter model does not consider the influence of substrate concentration. In addition, our model aims at greater structure simplicity than the model of Ander-

son (1992), whose simulated results do not reproduce observed trends in growth efficiencies in relation to substrate C:N ratio, as explained below.

In the following sections, the organic substrates for bacterial growth refer to the 'direct substrates' defined by Billen & Servais (1989). These are small organic molecules, which are produced by bacterial exoenzymatic hydrolysis of high molecular weight organic compounds (Billen et al. 1990).

The main objectives of the present study are: (1) To build up and assess the structure of a bacterial growth model, which takes into account the influence of quality and quantity of substrates on bacterial respiration and net ammonium remineralization. (2) To use available experimental results, obtained for aerobic bacteria, to determine the parameters of the model concerning the influence of substrate quality. (3) To compare the structure or output of the present model with those of other models.

DESCRIPTION OF THE MODEL

The model is presented in 2 steps, to simplify the description. In the first step, the influence of substrate concentration on bacterial growth is considered and, in the second, the influence of the C:N ratio of the assimilated substrate is taken into account. Three main hypotheses are used to limit the complexity of the model. The release of dissolved organic matter by bacteria is assumed to be insignificant. This assumption is probably valid, although some qualitative evidence suggests that bacteria may excrete small-sized organic material (Itturriaga & Zsolnay 1981, Novitsky & Keplay 1981). Hence, all assimilated material must be respired, excreted (ammonium), or used for production of new biomass. Second, only carbon and nitrogen have the potential to limit bacterial growth; other elements like phosphorus and vitamins are considered to never be limiting. Third, the bacterial C:N ratio is assumed to remain constant, which is consistent with the study of Goldman et al. (1987). These authors showed that the variability of the bacterial C:N ratio was small when bacteria were exposed to a wide range of growth conditions.

First step: influence of substrate concentration on bacterial growth

The description, values, and units of the parameters and variables used in the model are listed in Table 1. The assimilation of a substrate by bacteria is described by a Michaelis-Menten function, which is often used in model studies (e.g. Andersen & Rassoulzadegan 1991):

$$A_c = A_m \frac{C_o}{k_A + C_o} B_c \quad (1)$$

At low concentration of organic carbon (C_o), when C_o is much lower than k_A (half-saturation constant for organic substrates, Table 1), the assimilation of organic carbon (A_c) is directly proportional to the concentration of organic carbon (Fig. 1). At a high organic carbon concentration, when C_o is much higher than k_A, A_c is maximum and independent of C_o (therefore, the ratio $A_c:B_c \approx A_m$, where A_m is the maximum assimilation rate and B_c is bacterial carbon biomass; for this formulation, see Billen et al. 1988, and Billen & Servais 1989). The organic nitrogen contained in the substrates (N_o) which is assimilated by bacteria is:

$$A_n = \frac{A_c}{(C:N_s)} \quad (2)$$

where $C:N_s$ is the C:N atom ratio for organic substrate. Fluxes A_c and A_n specify what enters the bacteria, i.e. the quantity of carbon and nitrogen and the ratio of these elements. Depending on the concentrations of substrate, the model represents completely different physiological behavior of bacteria. Active bacteria, like other organisms, need a minimum substrate concentration to survive. Above this minimum value (the substrate concentration threshold for maintenance, C_b), bacteria are able to build up biomass and divide. When the substrate concentration (C_o) is lower than the concentration threshold for maintenance (C_b), growth is negative, i.e. carbon must be drawn from cellular reserves to satisfy the cost of maintenance. At substrate concentration C_b, the carbon obtained from substrates is equal to the carbon spent for maintenance, with a resultant steady state in bacterial biomass. At substrate concentration C_b, the respiration for maintenance (R_b: respiratory loss of all carbon contained in assimilated substrates) is computed using Eq. (1):

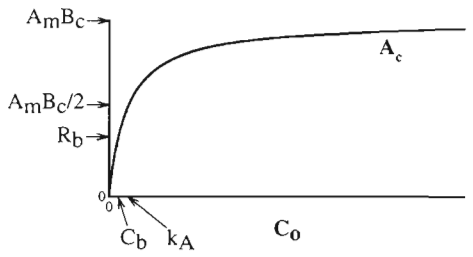


Fig. 1. Influence of the concentration of organic carbon (C_o) on the assimilation of organic carbon (A_c) by bacteria. A_m: maximum assimilation rate; B_c: bacterial carbon biomass; C_b: substrate concentration threshold for maintenance; k_A: half-saturation constant for substrate assimilation; R_b: respiration for maintenance

Table 1. Parameters and variables used in the model (Wd: without dimension). Parameters $C:N_b$, Ym, and α were derived (see text) from 2 experimental data sets of Goldman et al. (1987; Expts A and B) and of Lancelot & Billen (1985; Expt C)

	Description	Value			Unit
		Expt A	Expt B	Expt C	
Parameter					
A_m	Maximum assimilation rate		3		d^{-1}
B_c	Bacterial carbon biomass		1		mmol C m^{-3}
C_b	Substrate concentration threshold for maintenance		0.5		mmol C m^{-3}
k_A	Half-saturation constant for substrate assimilation		1		mmol C m^{-3}
k_{N_i}	Half-saturation constant for ammonium uptake		0.5		mmol N m^{-3}
k_Y	Half-saturation constant for curve Y		0.05		mmol C m^{-3} d^{-1}
N_i	Ammonium concentration		0.5		mmol N m^{-3}
$C:N_b$	C:N atom ratio for bacteria	4.7	5.97	4.66	mmol C (mmol N) $^{-1}$
Y_m	Growth efficiency when $C:N_s = C:N_b$ and when the substrate concentration is saturating	0.626	0.478	0.4	Wd
α	Factor for the slope A of Y_n	0.399	0.064	0	Wd
Variable					
A	Slope of Y_n				Wd
A_c	Assimilation of organic carbon				mmol C m^{-3} d^{-1}
A_n	Assimilation of organic nitrogen				mmol N m^{-3} d^{-1}
B_n	Bacterial nitrogen biomass				mmol N m^{-3}
C_i	Inorganic carbon concentration				mmol C m^{-3}
$C:N_s$	C:N atom ratio for organic substrate				mmol C (mmol N) $^{-1}$
C_o	Organic carbon concentration in the substrate				mmol C m^{-3}
E_n	Net ammonium remineralization				mmol N m^{-3} d^{-1}
I_{N_i}	Ammonium limitation				Wd
N_o	Organic nitrogen concentration in the substrate				mmol N m^{-3}
P_c	Net carbon production				mmol C m^{-3} d^{-1}
P_n	Net nitrogen production				mmol N m^{-3} d^{-1}
R_b	Respiration for maintenance				mmol C m^{-3} d^{-1}
R_c	Respiration				mmol C m^{-3} d^{-1}
S_1	Value of $C:N_s$ when $Y_c = 1$				mmol C (mmol N) $^{-1}$
S_2	Value of $C:N_s$ when $Y_n = 1$				mmol C (mmol N) $^{-1}$
Y	Growth efficiency when $C:N_s$=$C:N_b$ and when substrate concentration is not saturating				Wd
Y_c	Carbon growth efficiency ($Y_c = P_c : A_c$)				Wd
Y_n	Nitrogen growth efficiency ($Y_n = P_n : A_n$)				Wd

$$R_b = A_m \frac{C_b}{k_A + C_b} B_c \quad (3)$$

Since the substrate concentration threshold for maintenance (C_b) is constant, the respiration for maintenance (R_b) is also constant (Fig. 1) so that, depending on the value of A_c, the model predicts 4 different physiological states for the bacterial cells, as schematized in Fig. 2.

When the fluxes A_c and A_n are null (Fig. 2a), i.e. C_o = 0, the respiration for maintenance is completely sustained by the cellular carbon reserves, and the respiration (R_c) is equal to the respiration for maintenance ($R_c = R_b$). In terms of nitrogen, the bacteria must, in the first state, excrete ammonium (the net ammonium remineralization E_n is positive) in order to maintain the stoichiometric balance between carbon and nitrogen in the bacterial biomass.

When there is assimilation of organic carbon, at a rate lower than the respiration for maintenance ($0 < A_c < R_b$, Fig. 2b), all carbon from substrates is invested in respiration. This carbon is, however, not enough to meet all metabolic requirements of bacteria, so that the cell must still use carbon from its reserves. All the nitrogen in assimilated substrates must, in the same way, be excreted to keep the bacterial C:N ratio ($C:N_b$) constant. Because some carbon from the bacterial biomass is used for respiration, a stoichiometric quantity of nitrogen must, in the second state, be excreted from the biomass.

When the assimilation of organic carbon is equal to the respiration for maintenance ($A_c = R_b$, Fig. 2c), the carbon contained in substrates exactly meets the metabolic requirements of bacteria. All assimilated nitrogen must be excreted into the medium to maintain the stoichiometry. Consequently, in the third state, carbon and nitrogen cellular reserves are not used and the biomass remains constant.

In terms of production, a lower assimilation of organic carbon (A_c) causes the production to be more negative. The mathematical formulation of processes involved, i.e. net production in terms of carbon (P_c) and nitrogen (P_n), respiration (R_c), and net ammonium remineralization (E_n), are the same for states a, b, c in Fig. 2 (see Table 2, for the range $0 \leq A_c \leq R_b$).

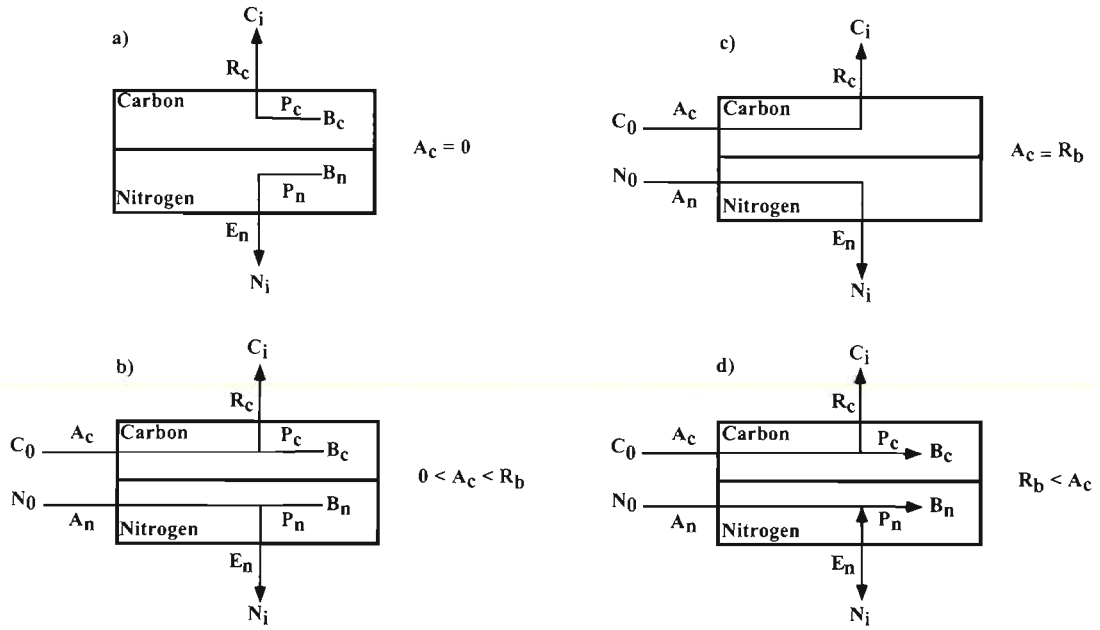


Fig. 2. Carbon and nitrogen fluxes in the model, for 4 physiological states of a bacterial cell: (a) $A_c = 0$; (b) $0 < A_c < R_b$; (c) $A_c = R_b$; (d) $R_b < A_c$. A_c: assimilation of organic carbon; A_n: assimilation of organic nitrogen; B_c: bacterial carbon biomass; B_n: bacterial nitrogen biomass; C_i: inorganic carbon concentration; C_o: organic carbon concentration in the substrates; E_n: net ammonium remineralization; N_i: ammonium concentration; N_o: organic nitrogen concentration in the substrates; P_c: net carbon production; P_n: net nitrogen production; R_b: respiration for maintenance; R_c: respiration

When the concentration of substrate is high enough to allow bacterial growth (state d, Fig. 2), the physiological behavior changes drastically. Because the assimilation of organic carbon exceeds the respiration for maintenance ($A_c > R_b$), the excess carbon can be used for building up biomass or division. Specific equations for that state are given in the $R_b < A_c$ column of Table 2. These equations are well known and were often used in the literature (e.g. Billen et al. 1990, Weaver & Hicks 1995). The carbon and nitrogen contained in the organic substrates (e.g. carbohydrate, amino acids) are used by bacteria (see Egli 1995) for multiple purposes (e.g. structural, energetic). It is not possible to consider all types of substrates, and all processes involved in the bacterial utilization and transformation of substrates containing carbon and nitrogen. A simple approach to the widely variable composition of organic substrates ($C:N_s$) is to consider that most of the regulation performed by bacteria to maintain their stoichiometry (constant $C:N_b$) is achieved through the catabolic processes of respiration and excretion. In the model, the variability of carbon and nitrogen growth efficiencies is used to simulate this regulation. In this fourth state (Fig. 2d), the quality of assimilated substrates becomes a predominant factor in determining carbon and nitrogen net production, respiration, and net ammonium remineralization, so that computing these processes requires the determination of carbon

Table 2. Equations of the model for net production in terms of carbon and nitrogen, respiration, and net ammonium remineralization, for the 4 different states of the model (Fig. 2a to d)

Variable	$0 \le A_c \le R_b$ States a, b and c in Fig. 2		$R_b < A_c$ State d in Fig. 2	
Net carbon production	$P_c = -R_b + A_c$	(2a)	$P_c = A_c Y_c$	(2e)
Net nitrogen production	$P_n = -\frac{(R_b - A_c)}{(C:N_b)}$	(2b)	$P_n = A_n Y_n$	(2f)
Respiration	$R_c = R_b$	(2c)	$R_c = A_c(1 - Y_c)$	(2g)
Net ammonium remineralization	$E_n = A_n + \frac{(R_b - A_c)}{(C:N_b)}$	(2d)	$E_n = A_n(1 - Y_n)$	(2h)

(Y_c) and nitrogen (Y_n) growth efficiencies (Eqs. 2e to 2h, Table 2). This is further discussed in the second step of the model description.

Second step: influence of C:N$_s$ ratio on bacterial growth

A simple situation is when the C:N ratio for organic substrates is identical to the bacterial C:N ratio (C:N$_s$ = C:N$_b$), because the carbon and nitrogen growth efficiencies, Y_c and Y_n, must then be equal in order to keep C:N$_b$ constant (Goldman et al. 1987). This particular value for growth efficiency is called Y (in the present case, $Y = Y_c = Y_n$). The growth efficiency (Y) is modelled as a function of organic carbon assimilation (Ac; Fig. 3):

$$Y = Y_m \frac{(A_c - R_b)}{k_Y + (A_c - R_b)} \quad (4)$$

When organic carbon assimilation (A_c) is lower or equal to the respiration for maintenance (R_b), growth efficiency Y is not defined (Fig. 3). That situation corresponds to states a, b, and c in Fig. 2, whose equations (Eqs. 2a to 2d, Table 2) are independent of the growth efficiency. When A_c is slightly higher than R_b, Y is directly proportional to A_c. At high organic carbon assimilation, Y is maximum ($Y \approx Y_m$, where Y_m is the maximum growth efficiency when C:N$_s$ = C:N$_b$) and independent of the assimilation of organic carbon. The half-saturation constant k_Y specifies the shape of the curve Y.

When C:N$_s$ ≠ C:N$_b$, the situation becomes quite complex because experimental results (Lancelot & Billen 1985, Goldman et al. 1987) suggest that bacteria adapt their carbon and nitrogen growth efficiencies ($Y_c \neq Y_n$) to keep their C:N$_b$ constant. The variability of Y_c and Y_n as a function of organic substrate quality (C:N$_s$) is poorly understood, but the experimental data show that the function $Y_n = f$(C:N$_s$) can be approximated by a straight line (see also the section 'Estimation from experimental data of parameters C:N$_b$, Y_m, and α of the model'). In the present model, Y_n is therefore linearly related to C:N$_s$ (Eq. 3a, Table 3), where the slope A is given by Eq. (3b) (Table 3). Y_n depends on: the value of C:N$_b$, which is considered to be constant; the variable C:N$_s$ value; and the variable growth efficiency (Y), defined for C:N$_s$ = C:N$_b$. In view of keeping the stoichiometric balance of carbon and nitrogen in bacterial biomass, the carbon growth efficiency (Y_c) must be a function of Y_n, C:N$_b$, and C:N$_s$ (Eq. 3c, Table 3).

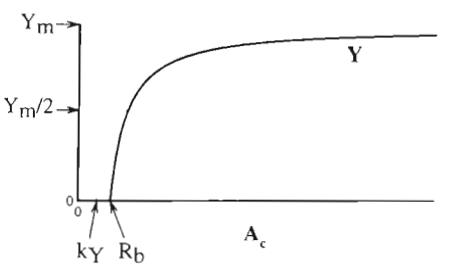


Fig. 3. Influence of the assimilation of organic carbon (A_c) on the growth efficiency (Y) when C:N$_s$ = C:N$_b$. k_Y: half-saturation constant for curve Y; R_b: respiration for maintenance; Y_m: maximum growth efficiency when C:N$_s$ = C:N$_b$

In the next section, we will see that the same experimental data suggest that the slope A of Y_n ranges between 2 values, which are, in the model (Eq. 3b, Table 3), 0 and Y(C:N$_b$)$^{-1}$. The boundaries of this range represent 2 extreme cases of the bacterial physiological behavior, to adapt the nitrogen and carbon growth efficiencies to variable C:N$_s$. In order to simplify the interpretation of the slope value, parameter α is introduced in Eq. (3b) (Table 3), where $\alpha = 1 - [A(\text{C:N}_b)\,Y^{-1}]$; when $A = Y(\text{C:N}_b)^{-1}$, α = 0 and, when $A = 0$, α = 1. The 2 extreme cases α = 0 and α = 1 are schematized in Fig. 4a,b, respectively. When α = 0 (Fig. 4a; do not consider yet the situation for C:N$_s$ > S_2), the bacteria maintain their C:N$_b$ by keeping their carbon growth efficiency (Y_c) constant. When α = 1 (Fig. 4b), the bacteria keep their nitrogen growth efficiency (Y_n) constant. For intermediate cases (0 < α < 1, Fig. 4c), neither Y_c or Y_n are kept constant.

The carbon growth efficiency Y_c cannot be ≥1 because part of the assimilated carbon is respired. The specific value of C:N$_s$ for which Y_c = 1 is threshold S_1, which is computed with Eq. (3d) (Table 3). Threshold S_1 determines the lower boundary of the C:N$_s$ values used in the model because there are no experimental data showing what happens when C:N$_s$ ≤ S$_1$. When α = 0 (Fig. 4a), S_1 = 0 (Eq. 3d, Table 3), so that any C:N$_s$ value may be used in the model since there is always carbon in the substrates (i.e. C:N$_s$ is never null). When α > 0 (Fig. 4b,c), S_1 > 0, so that C:N$_s$ must be chosen >S_1.

When Y_n < 1, net ammonium remineralization (E_n; Eq. 2h, Table 2) is positive, so that ammonium is excreted in the medium. When Y_n > 1, net ammonium remineralization becomes negative, so that ammonium is taken up by bacteria to maximize their production, in which case there is competition with phytoplankton (Lancelot & Billen 1985). It is therefore interesting to know the C:N$_s$ value for which Y_n = 1, because this value is a threshold between 2 very different physiological states. This threshold is called S$_2$, and it is computed with Eq. (3e) (Table 3). When α = 1 (Fig. 4b), threshold S_2 does not exist, and net ammonium remineralization is always positive, so that ammonium uptake never occurs. When α < 1 (Fig. 4a,c), ammonium uptake is possible when C:N$_s$ > S_2, but bacterial growth may become ammonium limited if the availability of

Table 3. Equations of the model used to describe the role of the C:N_s ratio in the various processes

Variable	Equations of the model when $S_1 < C:N_s \leq S_2$		Equations of the model when $S_2 < C:N_s$	
Nitrogen growth efficiency	$Y_n = A[(C:N_s) - (C:N_b)] + Y$	(3a)	$Y_n = A[(C:N_s) - S_2] + 1$	(3g)
Slope for Y_n	$A = (1-\alpha)\frac{Y}{(C:N_b)}$	(3b)	$A = (1-\alpha)\frac{Y}{(C:N_b)} \cdot I_{N_i}$	(3h)
Ammonium limitation	Ammonium is not limiting		$I_{N_i} = \frac{N_i}{k_{N_i} + N_i}$	(3i)
Carbon growth efficiency		$Y_c = \frac{Y_n (C:N_b)}{(C:N_s)}$	(3c)	
S_1 threshold		$S_1 = \frac{\alpha Y (C:N_b)}{1+(\alpha-1)Y}$	(3d)	
S_2 threshold		$S_2 = \frac{1-\alpha Y}{(1-\alpha)\frac{Y}{(C:N_b)}}$	(3e)	
E_n:A_c ratio	$\frac{E_n}{A_c} = \frac{1-\alpha Y}{(C:N_s)} - \frac{(1-\alpha)Y}{(C:N_b)}$	(3f)	$\frac{E_n}{A_c} = \left[\frac{1-\alpha Y}{(C:N_s)} - \frac{(1-\alpha)Y}{(C:N_b)}\right] I_{N_i}$	(3j)

this nutrient in the medium is low. To represent the effect of such a limitation on the nitrogen and carbon growth efficiencies, Eqs. (3g) to (3i) (Table 3) are used in the model for $C:N_s > S_2$. A straight line still describes the Y_n relationship (Eq. 3g, Table 3), but the slope A (Eq. 3h, Table 3) is influenced by the ammonium limitation, I_{N_i} (Eq. 3i, Table 3). When $I_{N_i} = 1$, then Eqs. (3g) and (3h) are the same as Eqs. (3a) and (3b). When $I_{N_i} < 1$, the nitrogen growth efficiency (Y_n) is lowered. Since the carbon growth efficiency (Y_c) is a function of Y_n (Eq. 3c, Table 3), it is also lowered. Fig. 4 shows 3 examples of the effect of I_{N_i} on Y_n and Y_c ($I_{N_i} = 1$, 0.5, and 0). The parameterization proposed in this study comes from the conclusions of Lancelot & Billen (1985), who thought that a limitation by ammonium would result in a decrease of Y_c and Y_n. This opinion is also supported by the results of Rivkin & Anderson (1997) for the Gulf Stream and the Sargasso Sea, where the addition of ammonium to the medium resulted in a significant increase of bacterial growth rates.

Since the nitrogen and carbon growth efficiencies are functions of Y (Eqs. 3a and 3c, Table 3), it is important to note that when Y tends towards 0 (Fig. 3), Y_n and Y_c also tend towards 0. This is not true for Y_n when $C:N_s > S_2$ (in this case, Y_n tends towards 1; Eq. 3g, Table 3), but the threshold S_2 becomes infinite (Eq. 3e, Table 3), so that the structure of the model remains consistent even when $Y = 0$.

When the organic carbon assimilation (A_c) is saturating for Y ($Y \approx Y_m$, Fig. 3), only 3 parameters ($C:N_b$, Y_m, and α) are required to determine the functions Y_n and Y_c for $C:N_s \leq S_2$. The derivation of the values of these parameters from experimental data is discussed in the following section.

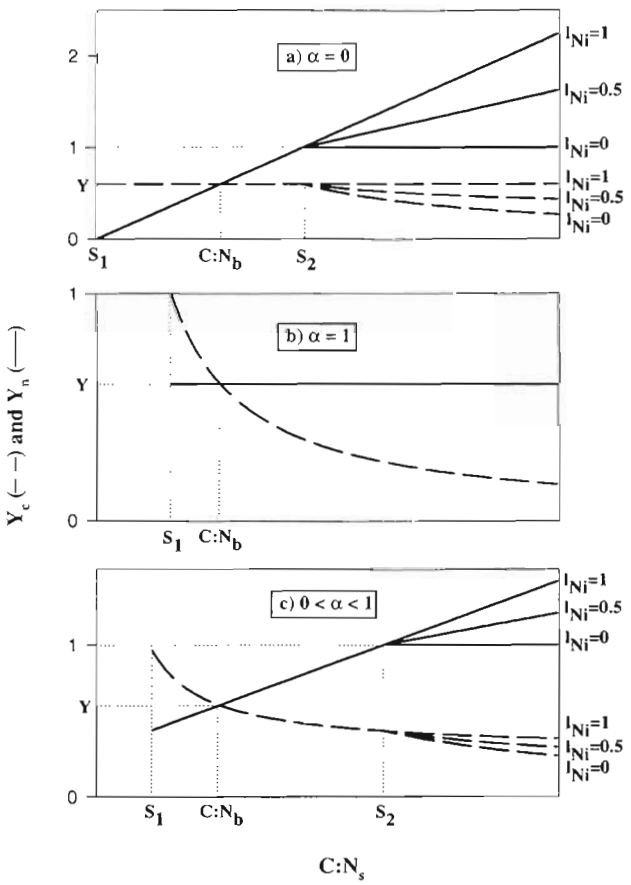
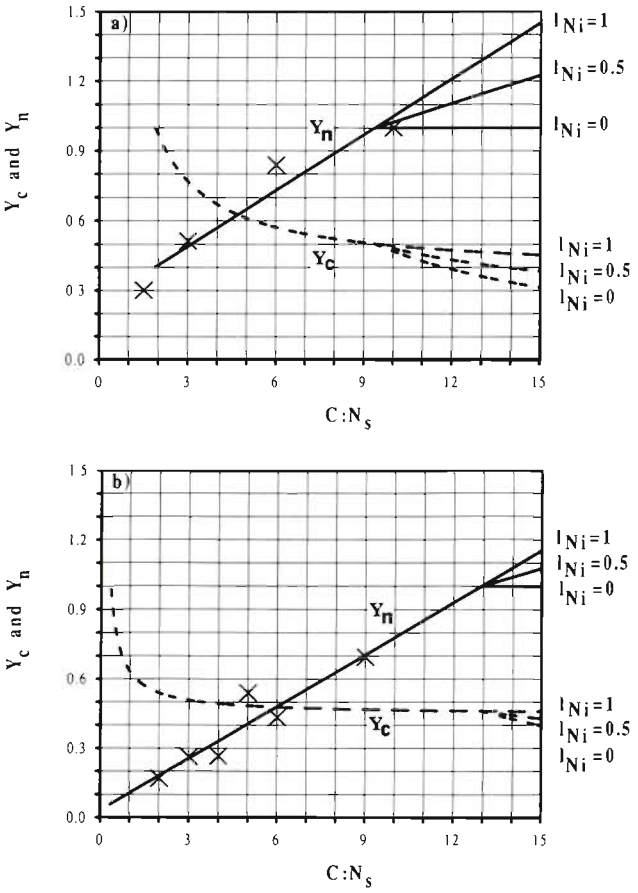


Fig. 4. Influence of the substrate C:N ratio (C:N_s) on the carbon (Y_c) and nitrogen (Y_n) growth efficiencies. When (a) $\alpha = 0$; (b) $\alpha = 1$; and (c) $0 < \alpha < 1$ (in this example, $\alpha = 0.5$). Three examples of the effect of the I_{N_i} limitation on Y_c and Y_n: $I_{N_i} = 1$, 0.5, and 0. $C:N_b$: C:N ratio for bacteria; I_{N_i}: ammonium limitation; S_1: value of $C:N_s$ when $Y_c = 1$; S_2: value of $C:N_s$ when $Y_n = 1$; Y: growth efficiency when $C:N_s = C:N_b$; α: factor for the slope A of Y_n



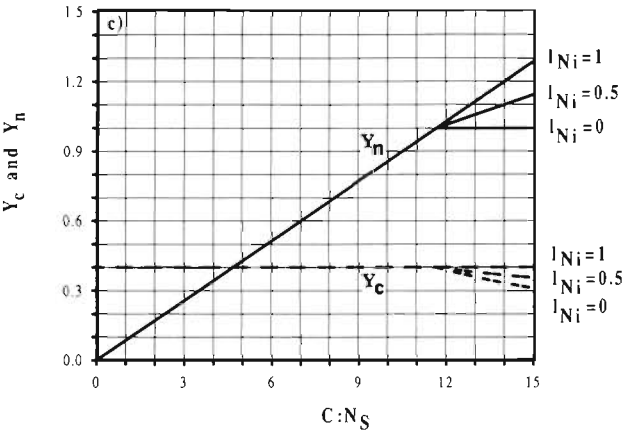


Fig. 5. Carbon (Y_c) and nitrogen (Y_n) growth efficiencies as a function of the C:N_s ratio. (a) Expt A: experimental data for Y_n (×) from Goldman et al. (1987), their Expt A (see Table 4), and model results for Y_c and Y_n. (b) Expt B: experimental data for Y_n (×) from Goldman et al. (1987), their Expt B (see Table 4), and model results for Y_c and Y_n. (c) Expt C: model results for Y_c and Y_n using Eq. (7) given by Lancelot & Billen (1985)

ESTIMATION FROM EXPERIMENTAL DATA OF PARAMETERS C:N_b, Y_m, AND α OF THE MODEL

Experimental data of Goldman et al. (1987)

Two experiments, called A and B (Table 4), were conducted by Goldman et al. (1987). Substrates of different quality were added to cultures of marine bacteria (see Goldman et al. 1987, for details of experimental conditions), for which nitrogen regeneration and carbon growth efficiencies were estimated. For Expts A and B, the range of C:N_s given by the authors was 1.5 to 10 mmol C (mmol N)$^{-1}$. The results of Goldman et al. (1987) for ammonium regeneration efficiency correspond to $(1-Y_n)100$ in the present model, from which it was easy to compute the values of nitrogen growth efficicency (Y_n, Table 4). Plotting these values against the corresponding C:N_s for Expts A and B (Fig. 5a,b, respectively) shows that the relationship $Y_n = f(C:N_s)$ can be approximated by a straight line:

$$Y_n = 0.08\ (C:N_s) + 0.25 \quad \text{(Expt A)} \quad (5)$$

$$Y_n = 0.075\ (C:N_s) + 0.031 \quad \text{(Expt B)} \quad (6)$$

For each experimental point, Goldman et al. (1987) determined the C:N_b ratio. The average ratios for Expts A and B were 4.7 and 5.97 mmol C (mmol N)$^{-1}$, respectively. Knowing the C:N_b value, the regression curve for Y_n in each experiment (Eqs. 5 and 6), and using Eqs. (3a) and (3b) of the model (Table 3), it was possible to compute parameters Y_m and α for each experiment (Table 1). For this computation, constant

Table 4. Experimental data sets (Expts A and B) from Goldman et al. (1987) used to estimate parameters Y_m and α of the model. In all cases, $N_o = 100$. The Y_n values were calculated as explained in the text. The composition and the carbon and nitrogen contents of substrates are not available for Expt C of Lancelot & Billen (1985)

C:N_s	N_o	C_o	Y_n
Expt A			
1.5	(Arginine)	150 (Arginine)	0.300
3	(Arginine)	150 (Arginine) 150 (Glucose)	0.512
6	(Arginine)	150 (Arginine) 450 (Glucose)	0.839
10	(Arginine)	150 (Arginine) 850 (Glucose)	0.999
Expt B			
2	(Glycine)	200 (Glycine)	0.168
3	(L-Alanine)	300 (L-Alanine)	0.262
4	(L-Aspartate)	400 (L-Aspartate)	0.265
5	(Glutamate)	500 (Glutamate)	0.539
6	(Isoleucine)	600 (Isoleucine)	0.433
9	(Phenylalanine)	900 (Phenylalanine)	0.695

Y_m was used instead of variable Y because the organic carbon content of substrates used by Goldman et al. (1987) was always saturating ($C_o \geq 150$ mmol C m^{-3}). When C_o is saturating, the A_c flux is also saturating and, given Eq. (4), $Y \approx Y_m$.

For Expts A and B, and using the appropriate parameters ($C:N_b$, Y_m, α; Table 1), the Y_c curves are drawn on Fig. 5a,b. Goldman et al. (1987) did not give their experimental data for Y_c, but the simulated Y_c curves (Fig. 5a,b) can be compared with the representation given by Goldman et al. (1987; their Fig. 4, not shown here). The agreement between the simulated and experimental Y_c curves is very good. From Eqs. (3d) and (3e) (Table 3), the values for S_1 and S_2 are 1.88 and 9.37, and 0.33 and 12.94 mmol C (mmol N)$^{-1}$ for Expts A and B, respectively.

In the context of Expts A and B, when $C:N_s > S_2$, 3 examples of Y_c and Y_n curves are given in Fig. 5a,b, corresponding to 3 values of I_{N_i}. Since data for $C:N_s > S_2$ are not available in Goldman et al. (1987), it is not possible to constrain this part of the model using their data.

Experimental data of Lancelot & Billen (1985)

Although Lancelot & Billen (1985) did not provide the composition and concentrations of organic substrates, it is possible to estimate the parameters of our model from their data (called Expt C). The authors provide the function $E_n:A_c = f(C:N_s)$, which expresses the trend in their experimental results (equation expressed in term of mass ratios; see their Fig. 14, not given here). After conversion to molar ratios, the original equation becomes:

$$\frac{E_n}{A_c} = \frac{1}{(C:N_s)} - 0.0857 \qquad (7)$$

A value $C:N_b = 4.66$ mmol C (mmol N)$^{-1}$ (see Table 1) was chosen because it is representative of the range given by Luria (1960) and Lucas et al. (1981), which is 3.85 to 5.6 mmol C (mmol N)$^{-1}$. Lancelot & Billen (1985) used the same range in their study. Ratio $E_n:A_c$ can be calculated with our model. For $S_1 \leq C:N_s \leq S_2$, the ratio can be obtained from Eqs. (2h) (Table 2) and (3a) (Table 3) and, for $S_2 < C:N_s$, from Eqs. (2h) (Table 2) and (3g) (Table 3). Corresponding expressions for $E_n:A_c$ are Eqs. (3f) and (3j) (Table 3). Combining Eqs. (7) and (3f) provides new estimates of Y_m and α (Table 1), which are representative of the experimental results of Lancelot & Billen (1985). From the Y_m, α, and $C:N_b$ values for Expt C, it is possible to draw the corresponding Y_c and Y_n curves (Fig. 5c). The values for thresholds S_1 and S_2 are then 0 and 11.66 mmol C (mmol N)$^{-1}$, respectively.

RESULTS AND DISCUSSION

Model predictions of growth, respiration and excretion

Results from the model are given below for the parameters ($C:N_b$, Y_m, and α) derived from Expts A and C. The model results for Expt B are not shown because the 3 parameters for this experiment have intermediate values between those of Expts A and C (except $C:N_b$, which is the highest, see Table 1). The other parameters in Table 1 (from A_m to N_i) are the same for Expts A and C, to facilitate the comparison of results. The values of these parameters were chosen arbitrarily, but they are realistic. The aim of the present study is not to model a specific ecosystem for which the parameters should be accurately known, but instead to explore the general behavior of the model. The values of parameters N_i and k_{N_i} (the ammonium concentration and the half-saturation constant for ammonium uptake, respectively) were chosen in order to obtain $I_{N_i} = 0.5$, which is an intermediate situation showing the effect of nitrogen limitation when $C:N_s > S_2$.

The results for Expts A and C (Figs. 6 & 7, respectively) illustrate the changes of growth, respiration, net ammonium remineralization, and carbon and nitrogen growth efficiencies as a function of C_o and $C:N_s$. All fluxes computed with the model were divided by the corresponding bacterial carbon or nitrogen biomass (B_c or B_n, respectively), to obtain specific rates. The range of variation of C_o is 0 to 20 mmol C m^{-3}, to cover rates with $C_o < C_b$ and at saturating C_o. Ratio $C:N_s$ ranges between threshold S_1 (when $Y \approx Y_m$) and 15 mmol C (mmol N)$^{-1}$.

In Expt A, bacterial growth, net positive ammonium remineralization rate, and carbon growth efficiency (Fig. 6a,c,d, respectively) all show the same general trend of increasing values with increasing C_o and decreasing $C:N_s$. As embedded in the model structure, bacterial growth is negative for $C_o < C_b$. When $C:N_s > S_2$, there is net ammonium uptake by bacteria (Fig. 6c). The isoline $E_n:B_n = 0$, in Fig. 6c, indicates the boundary between net excretion and net assimilation. Because threshold S_2 is a function of Y (Eq. 3e, Table 3), S_2 increases with decreasing concentrations of C_o. For $C_o < C_b$, the net ammonium remineralisation rate is always positive because the growth rate is negative. In the same range of C_o values ($C_o < C_b$), the carbon growth efficiency is not defined (Fig. 6d). At saturating concentrations of C_o, the variablity of Y_c as a function of $C:N_s$ is very close to that in Fig. 5a. The respiration rate and the nitrogen growth efficiency (Fig. 6b,e, respectively) increase with both C_o and $C:N_s$. As for Y_c, Y_n is not defined when $C_o < C_b$ and the change of Y_n for saturating values of C_o is similar to that in Fig. 5a.

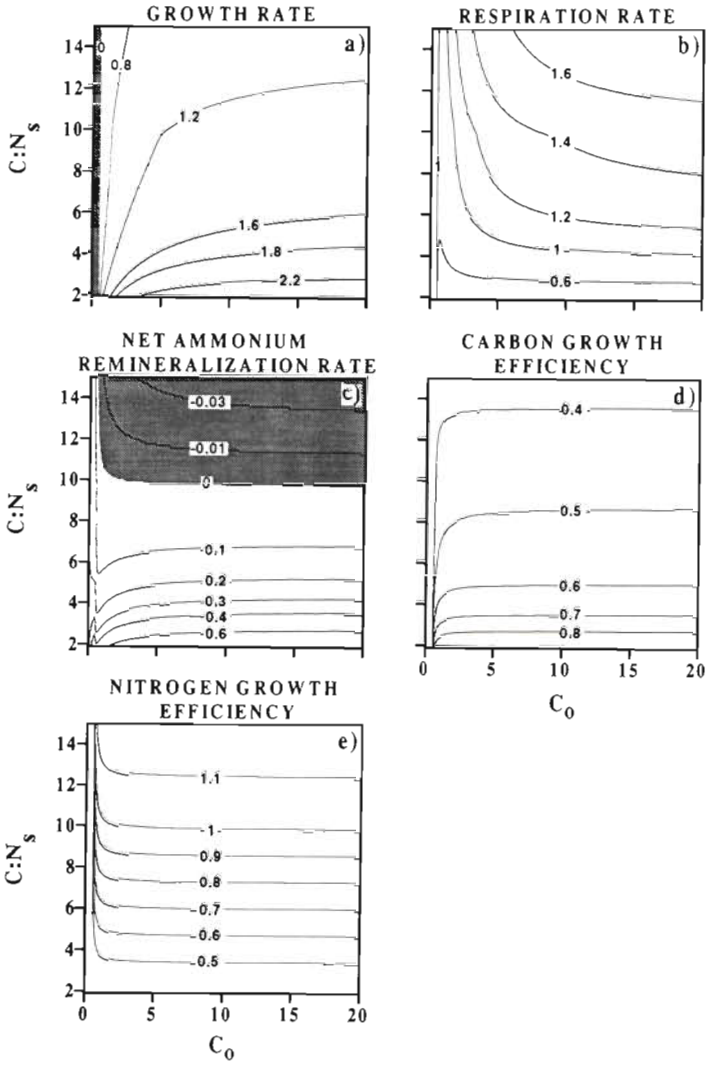


Fig. 6. Model results considering the influence of both the organic carbon concentration (C_o) and the C:N_s ratio, using the parameters from Expt A (Table 1). (a) Specific growth rate (P_c:B_c); (b) specific respiration rate (R_c:B_c); (c) specific net ammonium remineralization rate (E_n:B_n); (d) carbon growth efficiency (Y_c); (e) nitrogen growth efficiency (Y_n). Shaded areas correspond to negative values

In general, the trends for simulations based on Expt C (Fig. 7) are similar to those based on Expt A (Fig. 6). Bacterial growth, however, is lower because of higher respiration and excretion. In Fig. 7c, there is net ammonium uptake at higher C:N_s than in Fig. 6c, which is consistent with the respective S_2 thresholds at $Y \approx Y_m$, i.e. 9.37 and 11.66 mmol C (mmol N)$^{-1}$ in Expts A and C, respectively.

The model output suggests that the quality of the substrates assimilated by bacteria (represented by the C:N_s ratio) strongly influences their growth, respiration, and excretion. This is achieved by modifying the carbon and nitrogen growth efficiencies, in order to maintain the stoichiometry of these elements in the biomass. An important remark is that low C:N_s ratios never limit bacterial growth. The experimental results of Goldman et al. (1987) and of our model for Expts A and B show that, when the C:N_s ratio decreases, the growth rate increases. In the same way, the results of Lancelot & Billen (1985) and of the present model for Expt C lead to the conclusion that a low content of carbon relative to nitrogen in the substrate does not limit bacterial growth because, for C:N_s < S_2, the carbon growth efficiency and the growth rate remain constant.

Comparison with other models

Microbiologists are acquainted with the model of Parnas (1975), which is described by a single equation:

$$\frac{E_n}{A_c} = \frac{1}{(C:N_s)} = \frac{Y_c}{(C:N_b)} \quad (8)$$

The interest of that model lies in its simplicity, and its ability to reproduce and analyse experimental results on the influence of the C:N_s ratio on bacterial metabolism. The model was used by Billen (1984), after which it was experimentally verified by Lancelot & Billen (1985), and Goldman et al. (1987).

Eqs. (3f) and (3j) (Table 3) are similar to Eq. (8). This equation can be derived from other equations in the present model. Rearranging Eqs. (3a) to (3c) and (3f) (Table 3), when C:$N_s \leq S_2$, leads to Eq. (8). Combining Eqs. (3c), (3g), (3h) and (3j) (Table 3), when S_2 < C:N_s, one ends up with the same equation. Combining Eq. (8) with Eq. (2h) (Table 2) leads to Eq. (3c) (Table 3). This shows that a relationship exists between Y_c and Y_n in the model of Parnas (1975), where variable Y_n does not appear explicitly and in which the shapes of curves Y_n and Y_c are not specified. The main difference between the model of Parnas (1975) and ours is that, in the latter, Y_n is described by a straight line, which is consistent with experimental data (Eqs. 5 & 6). A main advance of the present model is that the shapes of the Y_n and Y_c curves (Fig. 5) and the range of variation of slopes A (through parameter α) are specified.

A much more complex model is that of Anderson (1992). Both the model of Anderson (1992) and ours aim at studying the influence of the quantity and quality of assimilated substrates on bacterial growth. Comparing the structures of the 2 models is not easy because of the many differences. It is interesting, however, to compare the model results. Fig. 7 of Anderson (1992, not shown

here) provides all the information needed for this comparison, i.e. specific growth rate of bacteria, carbon respired, net ammonium remineralized, and carbon and nitrogen growth efficiencies. Wide differences exist between Figs. 6 & 7 and the results of Anderson (1992). He found the growth rate to increase with $C:N_s$, whereas the present results suggest the opposite. The general trends of respiration, ammonium remineralization, and nitrogen growth efficiency are the same in the 2 models, but the results for carbon growth efficiency are drastically different. In Anderson (1992), the carbon growth efficiency increases with increasing $C:N_s$, whereas Y_c decreases here. This difference explains the difference noted above between the 2 models for the bacterial growth rate. The experimental results of Goldman et al. (1987) and Lancelot & Billen (1985) suggest that Y_c decreases or remains constant with increasing $C:N_s$ (Figs. 5, 6 & 7). Anderson (1992) concluded from his results that carbon limits bacterial growth for $C:N_s < S_2$. Again, the experimental results and the output of the present model suggest the opposite.

We tried to understand the above differences between models. Using our model, we changed the value of parameter α, which determines the slope of Y_n, so as to obtain results similar to those of Anderson (1992). Negative values of α reproduced the general trends of values obtained by Anderson (1992), where both Y_c and Y_n increase with increasing $C:N_s$, but such α values are outside the range of values in our model ($0 \leq \alpha \leq 1$). When $C:N_s$ is low, this means that bacteria increase both their respiration and their excretion to maintain their $C:N_b$. This situation may be plausible, but it does not agree with experimental results.

The purpose of Anderson (1992) was to explain the variation of growth efficiencies, whereas the aim of the present model is to represent as best as possible the processes involved in bacterial growth (respiration, excretion, etc.) In other words, the present model does not have any explanatory value as to the variability of parameters Y_m and α among Expts A, B, and C. The small number of experimental results does not allow one to understand the basic processes involved, but it could be that the different origins of bacterial populations used in the experiments largely contributed to the observed differences.

For low values of C_o, there are 2 other differences between our results and those of Anderson (1992). The first is that the growth rates in the present model are then negative, whereas those of Anderson (1992) are not. The structure of the latter model assumes bacterial respiration to be growth-specific only whereas, in the present model, biomass-specific respiration occurs when $C_o < C_b$. There is little information in the literature on this topic, so that there are no data supporting the possibility of negative bacterial growth. For large heterotrophic organisms, however, negative growth occurs when food is absent or in low concentration. The concept of 'individual threshold', introduced by Lampert (1977), specifies the food concentration required to exactly balance the metabolic losses. Such thresholds have been determined, for example, for rotifers (Stemberger & Gilbert 1985), copepods (Lam-

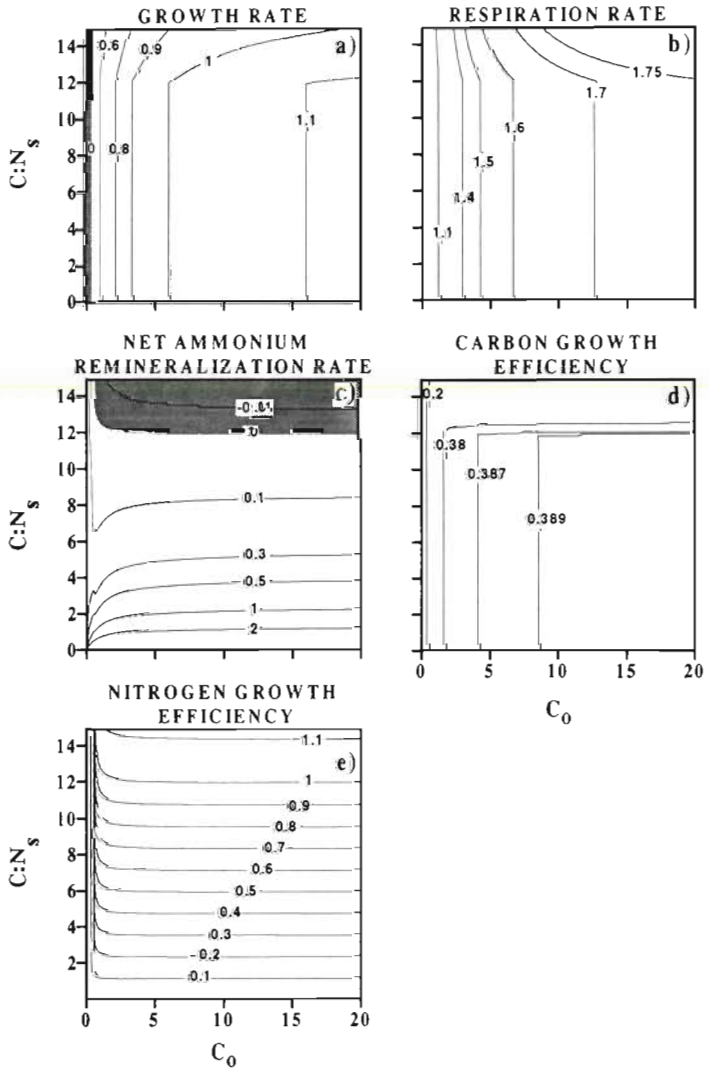


Fig. 7. Model results considering the influence of both the organic carbon concentration (C_o) and the $C:N_s$ ratio, using the parameters from Expt C (Table 1). (a) Specific growth rate ($P_c:B_c$); (b) specific respiration rate ($R_c:B_c$); (c) specific net ammonium remineralization rate ($E_n:B_n$); (d) carbon growth efficiency (Y_c); (e) nitrogen growth efficiency (Y_n). Shaded areas correspond to negative values

pert & Muck 1985), and cladocerans (Gliwicz 1990, Sterner & Robinson 1994). Since, most of the time, moderate to very high concentrations of substrate have been used in bacterial cultures (Egli 1995), it is not known if a threshold for maintenance (C_b) exists for bacteria. In any case, if this threshold does not exist, the structure of our model can handle the absence of a threshold (see below $C_b = 0$).

The second difference between the 2 models is the following: in the present model, the growth efficiency Y (and therefore Y_n and Y_c) increases with A_c (Eq. 4), whereas the carbon and nitrogen growth efficiencies remain constant in the model of Anderson (1992). To our knowledge, no relationship has yet been established between Y and bacterial growth or assimilation rates. Straile (1997), however, found such a relationship for large heterotrophic organisms, where he observed an initial increase of the gross growth efficiency with food concentration. If such a relationship exists for bacteria and a Michaelis-Menten function is used to approximate it, the half-saturation constant k_A used in the present model should be very low. As the value of k_A tends towards zero, Eq. (4) becomes $Y = Y_m$ and the growth efficiencies becomes independent of A_c, A_n, P_c, or P_n. Hence, the general results of the present model could be similar to those of Anderson (1992) when C_o concentrations are low, by simply using $C_b = 0$ and $k_A = 0$. These changes would mean no biomass-specific respiration and no relationship between growth efficiencies Y and A_c, respectively.

The original aspects of the present model relative to previous ones are: (1) compared to the model of Parnas (1975), the shape of the function which describes the variability of nitrogen growth efficiency (Y_n) as a function of the substrate C:N ratio ($C:N_s$) is imposed (a straight line), which is in accordance with the experimental results of Lancelot & Billen (1985) and Goldman et al. (1987); (2) our model takes into account the influence of substrate concentration on bacterial growth, whereas the model of Parnas (1975) does not; (3) compared to the model of Anderson (1992), the slope of the linear function $Y_n = f(C:N_s)$ is bound by 2 specific values, which have a physiological meaning; all the slopes derived from the experimental data sets used in the present study fall into that range, whereas the results obtained with the model of Anderson (1992) do not show such a trend. These advances have a direct effect on the computation of nitrogen and carbon growth efficiencies, so that they allow a better representation of processes which are influenced by the quality of substrates (i.e. bacterial production, respiration, and net ammonium remineralization). These processes have a major effect on the functioning of the microbial food web, so that the present model can be very useful for assessing both the competition between bacteria and phytoplankton for ammonium and the link-sink problem.

Several experimental (e.g. Cooney et al. 1976, Herbert 1976) and modeling (e.g. Bader 1978, Baltzis & Fredrickson 1988, Haas 1994) studies originating from the biotechnological sciences are of special interest to the present study because their aims are similar to ours. It must be noted, however, that the hypotheses used for the construction of these models largely differ from those used by oceanographers and limnologists (e.g. Parnas 1975, Anderson 1992, and the present study). For example, simple elements like carbon and nitrogen are used as units in the present study, whereas complex substrates like glucose and amino acids are the units of models in biotechnology. It follows that the approach developed in biotechnology cannot be easily applied to natural aquatic systems, because it would considerably increase the number of state variables needed to represent the pool of labile dissolved organic matter. Another difference is that the uptake kinetics of complex substrate are the main factors that control bacterial growth in biotechnological models, whereas growth efficiencies play a central role in the present model. In comparison with the models used in biotechnology, the main advances of our model are that: (1) Our simpler approach only considers the carbon and nitrogen contents of organic substrates, irrespective of their forms. Goldman et al. (1987) found that the major determinants of bacterial growth efficiencies were the relative magnitudes of $C:N_s$ and $C:N_b$, and not necessarily the forms of available carbon and nitrogen. Even if the biotechnological models of Bader (1978), Baltzis & Fredrickson (1988), and Haas (1994) only consider dual substrate limitation, their structures are quite complex because the 2 organic or inorganic substrates can be complementary, partially complementary and partially substitutable, entirely substitutable, or another intermediate case (Baltzis & Fredrickson 1988). The complexity of such models would rapidly increase with the number of substrates considered, which limits their use for natural waters. (2) Since the complexity of our model does not increase with the number of substrates available for bacterial growth, it can be used to analyse systems with several substrates. (3) The experimental data sets used in the present study show that growth efficiencies vary widely with $C:N_s$. Our model is able to represent this variability, whereas biotechnological models are not. (4) Our model can be integrated in ecosystem models with a minimum increase of their complexity.

The results of our model show that its structure is realistic, because simulated bacterial growth efficiencies, which are influenced by the quantity of substrates, behave as expected. For low concentration of substrate, however, doubts still exist about the struc-

ture (presence/absence of the threshold C_b, and the shape of curve Y), reflecting the lack of knowledge on that subject. For high concentration of substrate, the model reproduces fairly well the influence of the quality of assimilated substrates, i.e. the carbon and nitrogen growth efficiencies are very similar to those observed in experimental conditions. This meets Objective 1 stated in the 'Introduction'.

Although it was not possible to determine the reason(s) for the variability of parameters α and Y_m among Expts A, B, and C, we showed that such parameters, which are linked to the influence of the quality of substrates, can be derived from available data (Objective 2). Moreover, the values of parameter α fall between the 2 extreme cases ($\alpha = 0$ and $\alpha = 1$) discussed above.

Comparison of the structure of our model with that of Parnas (1975) showed that the former seems to be more precise for several reasons: (1) our model takes into account the influence of the quantity of substrate, whereas the model of Parnas does not; (2) the shapes of the Y_n and Y_c curves are specified in our approach (Y_n seems to be linearly related to $C:N_s$); (3) the slope of Y_n may vary among the experiments, but the value of parameter α must be between 0 and 1. Available experimental data confirm the last 2 points. Comparison of the output of our model with that of Anderson (1992) showed that the structure of the former is better because the simulated results are closer to the observations. We showed that negative values for parameter α are plausible, but not supported by observations. This achieved our Objective 3.

Since the quality of the substrate can strongly influence the main processes involved in bacterial growth (respiration, and net excretion), it seems essential to include this factor in ecosystem models. Because the quality of substrates potentially determines the competition for ammonium between phytoplankton and bacteria and the link-sink outcome, this factor may control the efficiency of the microbial food web. Hence, when the bacterial biomass is high in a given ecosystem, the predictive or analytical value of Class 1 and 2 models (see the 'Introduction') will be low.

The structure of the model proposed in this study can be seen as an improvement of the model of Parnas (1975), which only considers the relationship between the carbon and nitrogen growth efficiencies. As mentioned above, the present model is more precise, but the quality of the validation remains the same. Comparison with the results of the model of Anderson (1992) showed differences concerning the limitation by carbon. Our conclusion is that, according to experimental results, low $C:N_s$ increases the growth rate of bacteria, so that a low carbon content with respect to nitrogen does not limit the growth rate. This conclusion is well reproduced by the model.

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