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Model of interactions between dissolved organic carbon and bacteria in marine systems

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ABSTRACT: We propose a theoretical model describing the interactions between dissolved organic carbon (DOC) and bacteria and the mechanisms leading to DOC accumulation. The model assumes that DOC cycling time-scales may vary depending on the chemical characteristics of the dissolved organic matter (DOM) and describes the temporal variability of the bacterial growth efficiency (BGE) in response to changing availability of nutrients, semi-labile and semi-refractory DOC. The conceptual framework is tested in a zero-dimensional numerical model in 2 different contexts: a diatom–bacteria system, and a microbial loop system (with bacteria, pico-phytoplankton and heterotrophic nano-flagellates). Sensitivity analyses were performed on both systems by varying the initial conditions of nutrients. Model simulations highlight the link between DOC accumulation and nutrient availability and reproduce some of the observed bacterial and microbial loop features such as the competition between bacteria and phytoplankton for nutrients and the BGE decrease in the transition from eutrophic to oligotrophic conditions. In the microbial loop simulations the model reaches a steady state and the system sustains itself without invoking external sources of N and P.

KEY WORDS: Bacterial growth efficiency · DOC cycling · Microbial loop · Ecological modelling

INTRODUCTION

Dissolved organic carbon (DOC) in the ocean is one of the largest carbon pools on Earth. Consequently, understanding the processes governing DOC production, accumulation and consumption is a very important goal for the quantitative assessment of the global carbon cycle. The extracellular release of recently fixed photosynthate is the major DOC production process in the marine ecosystem (Maranon et al. 2004). This DOC source is particularly important in defining the structure of the marine planktonic trophic web. Since the released carbon is available for uptake by heterotrophic bacteria, there is a direct link between primary and bacterial production that is essential for the cycling of matter through the food web (Ducklow & Carlson 1992, Legendre & Rassoulzadegan 1996). DOC cycling in marine ecosystems is almost completely governed by bacteria over different timescales depending on its biochemical characteristics. A small fraction, about 2% of the dissolved organic matter (DOM) pool, constitutes the so-called 'labile' fraction and turns over rapidly (hours to days). The 'semi-labile' fraction is cycled by bacteria on timescales of weeks to months (Ogawa & Tanoue 2003), while the so called 'refractory' DOC pool is biologically cycled on time-scales ranging from centuries to millennia (Kirchman et al. 1993, Carlson & Ducklow 1995, Cherrier et al. 1996, Ogawa & Tanoue 2003).

The open ocean refractory DOC concentration is almost constant (~40 mmol m^{-3}) throughout the water column. This implies that the semi-labile DOC (related to the primary production processes) is given by the excess of surface DOC (60 to 80 mmol m^{-3}) over that in deep water (Ogawa & Tanoue 2003). Molecular structure and the carbon to nutrient ratios are very important for understanding the origin, processing, age and lability/refractivity characteristics of DOM. Previous research has shown that the C:N, C:P and N:P ratios of the bulk DOM are higher in deep waters, where the

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refractory component is dominant, than in the surface ocean (Clark et al. 1999, Ogawa et al. 1999, Carlson et al. 2000, Ogawa & Tanue 2003). This suggests that phosphorus and nitrogen in DOM are rapidly (with a preference for phosphorus over nitrogen) remineralized while the carbon is relatively preserved.

Bacterioplankton cycles the semi-labile DOC, but also contributes to this pool through the release of extracellular mucopolysaccharides that form mucilaginous protective capsules around the cell (capsular material) and/or slimes and fibrils (Stoderegger & Herndl 1998, del Giorgio & Cole 1998, Azam et al. 1999). The release rate of capsular material has been estimated to be about 25% of the bacterial respiration rate. The capsular material release is also considered as a pathway of energy dissipation that may contribute to the maintenance of intracellular stoichiometry (del Giorgio & Cole 1998).

Bacterially driven DOC cycling is particularly important in oligotrophic systems where heterotrophic bacteria constitute the major living carbon pool in the euphotic zone (Jürgens et al. 2000) and prokaryotes are governing the primary and secondary production (Whitman et al. 1998). In such systems there is significant competition between bacteria and phytoplankton for inorganic nutrients (Thingstad & Rassoulzadegan 1999, Hagström et al. 2001), and heterotrophic nanoflagellate excretion products (DOM and inorganic nutrients) are a significant source of nutrients for both bacteria and pico-phytoplankton (Hagström et al. 2001). This ecosystem structure is often referred to as the 'microbial loop' (Azam et al. 1983).

Despite the remarkably constant open ocean mean DOC concentration (Fajon et al. 1999), coastal areas exhibit processes of seasonal DOC accumulation (Ogawa & Tanoue 2003, Giani et al. 2005) that has been found to be caused both by sustained, bloom-related, phytoplankton DOC excretion and/or by a weakened bacterial efficiency in organic carbon cycling (Carlson et al. 1994, Williams 1995).

A bloom-related increase in DOC concentration could indicate that the efficiency of the flux of energy and organic carbon through the bacteria–protozoa–metazoa chain may vary in time. Low bacterial growth efficiency (BGE) boosts the bacterial respiration and hence causes a decrease in biomass. BGE tends to be high in eutrophic environments and decrease along the eutrophy to oligotrophy continuum (Eiler et al. 2003), apparently a consequence of a decoupling between anabolism and catabolism when bacterial growth is constrained by a lack of organic substrate or governed by inorganic nutrient uptake (del Giorgio & Cole 1998). Although it is well known that the carbon flux into bacteria (and BGE) may be limited by DOM quality, inorganic nutrients and temperature (Church et al. 2000), it is not yet clearly understood how external nutrient concentrations and the carbon to nutrient ratios of organic substrates affect the BGE and the degradability of DOC.

In the present study, we propose a theoretical model describing the DOC–bacteria interactions leading to DOC accumulation. The model assumes that DOC cycling time-scales may vary dependently of the chemical characteristics of the DOM and describes the temporal variability of the BGE. The conceptual framework is tested in a zero-dimensional (0D) numerical model. We use numerical simulations to try to answer to the following questions: Can a simplified microbial loop-like system sustain itself by recycling nitrogen and phosphorus without invoking external sources? Why do nutrient-limited bacteria not outcompete phytoplankton to the point where phytoplankton biomass is reduced to a level where the system production of organic carbon is so low that bacteria become carbon limited? (Thingstad & Rassoulzadegan 1999).

THE CONCEPTUAL MODEL

The basic assumption of the model, consistent with the observations summarised above, is that the lability/refractivity characteristics of DOM depend on 2 factors: the C:N and C:P ratios, and the structure of organic molecules constituting the DOM matrix. In order to account for these factors, DOM was assumed to be partitioned into 3 broad and distinct classes/state variables, each corresponding to different degrees of lability and having different production pathways.

Since we focus on DOC–bacteria interactions on a time-scale relevant to the observed processes of DOC accumulation, the 3 classes of DOC considered by the model cover only the labile and the semi-labile DOC fractions. However, we define the DOC fraction more difficultly remineralized by bacteria as 'semi-refractory', to avoid confusion with the truly refractory DOC (with a turnover time of 100s to 1000s of yr) that is not considered in our work. The phosphorus and nitrogen components of DOM are included only in the labile fraction ($R1$ state variable) because they are more rapidly remineralized by bacteria. Refractory DON is mainly derived from the bacterial cell walls not ingested by the grazers (mainly constituted by peptidoglycans fragments; McCarthy et al. 1998) and is supposed to have a much longer turnover time; therefore, it is not assumed to significantly affect the DOM–bacteria interactions on the time-scales investigated in this work. A schematic representation of our proposed DOM–bacteria interactions, including the assumed DOM partitioning, is given in Fig. 1.

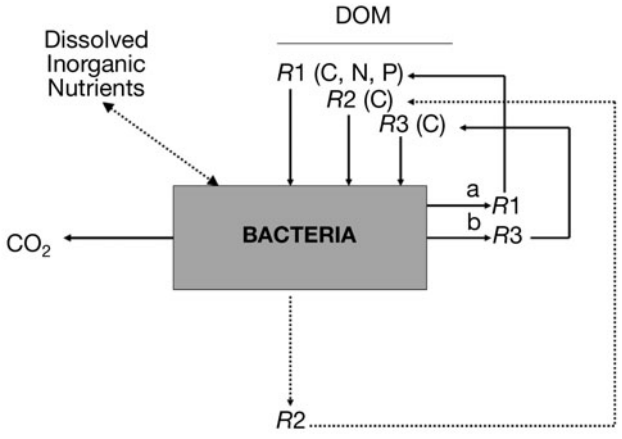

Fig. 1. Schematic representation of DOM–bacteria interactions. *R*1 = labile organic matter, *R*2 = semi-labile organic carbon, *R*3 = semi-refractory organic carbon; a = excretion due to mortality, b = fraction of DOM uptake released as capsular material. Dotted arrows indicate carbon to nutrient ratio dependent fluxes

The most labile fraction of the total DOM pool (*R*1 in Fig. 1) is produced by phytoplankton, zooplankton and bacteria via lysis, mortality and sloppy feeding (zooplankton only) processes. This DOM class/state variable is characterized by C:N and C:P ratios that reflect those of the producing functional groups. The characteristic turnover time-scale is assumed to be 1 d.

The semi-labile DOM fraction (*R*2 in Fig. 1) is produced by phytoplankton and bacteria excretion in order to achieve/maintain their internal 'optimal' stoichiometry. The production process of semi-labile DOM can be considered as a release of excess carbon and, therefore, negligible N and P pools are assumed. The characteristic turnover time-scale is assumed to be 10 d.

DOM released by bacteria as capsular material (*R*3 in Fig. 1) is the semi-refractory fraction. This component of the DOM pool is also assumed (as is the semi-labile fraction) to be DOC only and to be formed by high molecular weight substances such as polysaccharide fibrils (Heissenberger et al. 1996), which are fairly resistant to enzymatic attack (Stoderegger & Herndl 1998). Therefore, the characteristic turnover time-scale is assumed to be greater (100 d) as this material is degradable by bacteria on time-scales of 2 to 3 orders of magnitude higher with respect to the labile DOC (Stoderegger & Herndl 1998).

In the following section a mathematical description of the model is given. It has to be stressed that the model does not consider processes such as DOM exposure to UV radiation (McCallister et al. 2005) changes in bacterial community structure (Carlson et al. 2002) that are considered important for the overall DOC cycling processes.

Table 1. DOM components, producers and composition

Variable	Producers	Process	Composition
*R*1 (labile)	Phytoplankton Zooplankton Bacteria	Mortality, sloppy feeding	C, N, P
*R*2 (semi-labile)	Phytoplankton Bacteria	Exudation, release	C
*R*3 (semi refractory)	Bacteria	Capsular material release	C

Mathematical formulation

A schematic description of DOM classification and production is given in Table 1. Bacteria are described by the state variables B_c, B_n and B_p, which are the bacterial carbon, nitrogen and phosphorus biomasses, respectively. The time rate of change of B_c, B_n and B_p is due to different processes that are described in a general form by:

$$\frac{\partial B_c}{\partial t} = \left.\frac{\partial B_c}{\partial t}\right|_{R1_c,R2,R3}^{\text{uptake}} - \left.\frac{\partial B_c}{\partial t}\right|_{R2}^{\text{release}} - \left.\frac{\partial B_c}{\partial t}\right|_{R3}^{\text{release}} - \left.\frac{\partial B_c}{\partial t}\right|_{CO_2}^{\text{respiration}} - \left.\frac{\partial B_c}{\partial t}\right|_{R1_c}^{\text{mortality}} - \left.\frac{\partial B_c}{\partial t}\right|_{Z_c}^{\text{grazing}} \quad (1)$$

$$\frac{\partial B_n}{\partial t} = \left.\frac{\partial B_n}{\partial t}\right|_{R1_n}^{\text{uptake}} + \left.\frac{\partial B_n}{\partial t}\right|_{NH_4}^{\text{uptake/remin}} - \left.\frac{\partial B_n}{\partial t}\right|_{R1_n}^{\text{mortality}} - \left.\frac{\partial B_n}{\partial t}\right|_{Z_n}^{\text{grazing}} \quad (2)$$

$$\frac{\partial B_p}{\partial t} = \left.\frac{\partial B_p}{\partial t}\right|_{R1_p}^{\text{uptake}} + \left.\frac{\partial B_p}{\partial t}\right|_{PO_4}^{\text{uptake/remin}} - \left.\frac{\partial B_p}{\partial t}\right|_{R1_p}^{\text{mortality}} - \left.\frac{\partial B_p}{\partial t}\right|_{Z_p}^{\text{grazing}} \quad (3)$$

The equations are written in the form $\left.\frac{\partial A}{\partial t}\right|_Y^X$, where A is the state variable, X the process described and Y the other state variables involved in the process (Vichi et al. 2003): $R1_c$, $R1_n$ and $R1_p$ are the carbon, nitrogen and phosphorus content of the labile fraction of the DOM, respectively, $R2$ and $R3$ are the semi-labile and semi-refractory DOC, respectively, CO_2 is the carbon dioxide, PO_4 is the phosphate, NH_4 is the ammonium and Z_c, Z_n and Z_p are the carbon, nitrogen and phosphorus biomasses of the heterotrophic nano-flagellates, respectively.

Starting with the carbon uptake we have:

$$\left.\frac{\partial B_c}{\partial t}\right|_{R1_c,R2,R3}^{\text{uptake}} = \min(G_{\text{env}}, G_{\text{sub}}) \quad (4)$$

where G_{env} is the maximum potential uptake of DOC given by:

$$G_{env} = f^t f_O r_O B_c \quad (5)$$

and G_{sub} is the substrate availability given by:

$$G_{sub} = n_1 R1_c + n_2 R2 + n_3 R3 \quad (6)$$

where f^t is a temperature-dependent growth function (Blackford et al. 2004), r_O is the potential uptake, n_1, n_2 and n_3 are the characteristic uptake time-scale for each of the 3 DOM classes (see Table 2) and f_O is the oxygen-regulating factor parameterized with a Michaelis-Menten formulation using the half saturation constant h_b for oxygen:

$$f_O = \frac{O_{sat}}{O_{sat} + h_b} \quad (6.1)$$

where O_{stat} is the relative oxygen saturation.

The dissolved organic phosphorus and nitrogen uptake is based on the uptake of the labile DOC ($R1_c$) following:

$$\left.\frac{\partial B_{n,p}}{\partial t}\right|_{R1_{n,p}}^{uptake} = \left.\frac{\partial B_c}{\partial t}\right|_{R1_c}^{uptake} \frac{R1_{n,p}}{R1_c} \quad (7)$$

where $B_{n,p}$ is the nitrogen and phosphorus bacteria content and $R1_{n,p}$ is the dissolved organic nitrogen or phosphorus concentration.

Table 2. Model parameters used in the study

	Symbol	Value
Environmental effects		
Half O_2 saturation	h_b	0.3125
Uptake		
Max. spec uptake rate at 10°C (d^{-1})	r_O	4.0
Availability of $R1_c$ (d^{-1})	n_1	1
Availability of $R2$ (d^{-1})	n_2	0.1
Availability of $R3$ (d^{-1})	n_3	0.01
Loss terms		
Respired fraction of C uptake	η_b	0.6
Respired fraction of C uptake under low O_2 concentration	η_b^0	0.2
Fraction of C uptake released as capsular material	α	η_b 0.25
Mortality (d^{-1})	d_O	0.05
Rest respiration at 10°C (d^{-1})	b_O	0.01
Max. daily zooplankton ingestion rate (d^{-1})	r_{Oz}	2.0
Zooplankton half saturation constant for food (mg C m^{-3})	h_z	45.0
Nutrients dynamics		
Optimal N/C ratio (mmol N mg^{-1} C)	n^{opt}	0.0167
Optimal P/C ratio (mmol P mg^{-1} C)	p^{opt}	0.0019
Half saturation constant for N uptake (mmol m^{-3})	h_n	0.5
Half saturation constant for P uptake (mmol m^{-3})	h_p	0.1

It should be stressed that DOC uptake is not constrained by nitrogen and phosphorus availability in the DOM. The bacteria can balance their 'optimal' internal carbon to nutrient ratio level (following Goldman et al. 1987) by assimilating dissolved inorganic nitrogen and/or phosphorus, if available, or conversely, by releasing the excess DOC into the $R2$ pool (as detailed below).

The carbon release and the dissolved inorganic N and P uptake/remineralization are regulated by the Goldman et al. (1987) C:N (n^{opt}) and C:P (p^{opt}) ratio (C:N:P = 45:9:1) as follows (ammonium uptake and remineralization are governed by the same rules as in the case of phosphorus):

$$\left.\frac{\partial B_c}{\partial t}\right|_{R2}^{release} = \max\left[0, \max\left(1 - \frac{qpb}{p^{opt}}\right), \left(1 - \frac{qnb}{n^{opt}}\right)\right] B_c \nu \quad (8)$$

and

$$\left.\frac{\partial B_p}{\partial t}\right|_{PO_4}^{uptake/remin} = \nu\left(qpb - p^{opt}\right) B_c f_n \quad (9)$$

where

$$f_n = -1 \quad \text{if} \quad qpb - p^{opt} > 0 \quad (9.1)$$

$$f_n = \frac{PO_4}{PO_4 + h_p} \quad \text{if} \quad qpb - p^{opt} < 0 \quad (9.2)$$

PO_4 is the phosphate concentration, qpb and qnb are the dynamically varying N:C and P:C cellular ratios, ν is the characteristic time-scale of the process (supposed to be 1 d) and h_p is a Michaelis-Menten half saturation constant (see Table 3). A fixed quota of bacteria production is directed to the semi-refractory DOC pool in order to describe the capsular material release observed by Stoderegger & Herndl (1998):

$$\left.\frac{\partial B_c}{\partial t}\right|_{R7}^{release} = \left.\frac{\partial B_c}{\partial t}\right|_{R1c,R2,R3}^{uptake} (1 - \eta_b)\alpha \quad (10)$$

where η_b is the respired fraction of carbon uptake and α is the daily fraction of bacterial production released as capsular mucopolysaccharide material (see Table 3). The respiration term is calculated according to Blackford et al. (2004) and is the sum of the rest and activity respiration:

$$\left.\frac{\partial B_c}{\partial t}\right|_{CO_2}^{respiration} = \left.\frac{\partial B_c}{\partial t}\right|_{CO_2}^{activityresp} + \left.\frac{\partial B_c}{\partial t}\right|_{CO_2}^{restresp} \quad (11)$$

The activity respiration term in Eq. (11) is given by:

$$\left.\frac{\partial B_c}{\partial t}\right|_{CO_2}^{activityresp} = \left[\eta_b O_{sat} + \eta_b^0 (1 - O_{sat})\right] \left.\frac{\partial B_c}{\partial t}\right|_{R1_c,R2,R3}^{uptake} \quad (12)$$

where η_b^0 is the fraction of carbon uptake respired at low oxygen concentration. The rest respiration term is given by:

$$\left.\frac{\partial B_c}{\partial t}\right|_{CO_2}^{restresp} = b_O f^t B_c \quad (13)$$

where b_O is the daily rest respiration activity.

Table 3. Experimental configuration. The model was tested in 2 idealized systems: System 1: a bacteria–primary producer (diatoms) system; System 2: a simplified microbial loop consisting of bacteria, pico-phytoplankton and heterotrophic nano-flagellates (see Fig. 2)

Functional groups	Expt	SiO_2 (mmol Si m^{-3})	PO_4 (mmol P m^{-3})	NO_3 (mmol N m^{-3})	NH_4 (mmol N m^{-3})	DOC (R2) (mmol C m^{-3})
System 1						
Diatoms, Bacteria	1.1	3	0.15	2.5	0.5	0
	1.2	6	0.30	5.0	1.0	0
	1.3	12	0.60	10.0	2.0	0
	1.4	3	0.15	2.5	0.5	400
System 2						
Pico-phytoplankton	2.1		0.15	2.5	0.5	0
Heterotrophic nano-	2.2		0.30	5.0	1.0	0
flagellates, Bacteria	2.3		0.60	10.0	2.0	0

The background mortality is described by a simple first order equation (Blackford et al. 2004) in order to mimic viral lysis:

$$\left.\frac{\partial B_c}{\partial t}\right|_{R1_c}^{mortality} = f^t d_O B_c \quad (14)$$

$$\left.\frac{\partial B_{n,p}}{\partial t}\right|_{R1_{n,p}}^{mortality} = \left.\frac{\partial B_c}{\partial t}\right|_{R1_c}^{mortality} \frac{B_{n,p}}{B_c} \quad (15)$$

where d_O is the specific mortality rate.

The loss terms due to the grazing are also formulated according to Blackford at al. (2004):

$$\left.\frac{\partial B_c}{\partial t}\right|_{Z_c}^{grazing} = f^t r_{O_z} \frac{B_c}{B_c + h_z} Z_c \quad (16)$$

and

$$\left.\frac{\partial B_{n,p}}{\partial t}\right|_{Z_{n,p}}^{grazing} = \left.\frac{\partial B_c}{\partial t}\right|_{Z_{c,p}}^{grazing} \frac{B_{n,p}}{B_c} \quad (17)$$

where r_{O_z} is the maximum specific daily ingestion rate and h_z is the half saturation constant for the food.

DOM production by phytoplankton and zooplankton

DOM production by phytoplankton and zooplankton is formulated according to Blackford et al. (2004). The zooplankton is assumed to produce only labile DOM ($R1$ variable) while the DOM produced by the phytoplankton is divided into labile (derived from lysis) and semi-labile (derived from exudation) DOM.

Bacterial growth efficiency

The BGE is estimated as the ratio between the net bacterial carbon production (BCP) and the total carbon uptake:

$$BGE = BCP / \left.\frac{\partial B_c}{\partial t}\right|_{R1_c,R2,R3}^{uptake} \quad (18)$$

where BCP is given by:

$$BCP = \left.\frac{\partial B_c}{\partial t}\right|_{R1_c,R2,R3}^{uptake} - \left.\frac{\partial B_c}{\partial t}\right|_{CO_2}^{respiration} - \left.\frac{\partial B_c}{\partial t}\right|_{R2}^{release} - \left.\frac{\partial B_c}{\partial t}\right|_{R3}^{release} \quad (19)$$

SIMULATION SETUP

The model for DOM–bacteria interactions described above was cast into a simplified 0D version of the biomass and functional group based European Regional Sea Ecosystem Model (ERSEM, Baretta et al. 1995), fully described by Blackford et al. (2004).

The model was run in 2 idealized systems (Fig. 2) — System 1: a bacteria–primary producer (diatoms) system. The experiment allowed the competition between phyto- and bacterioplankton for dissolved

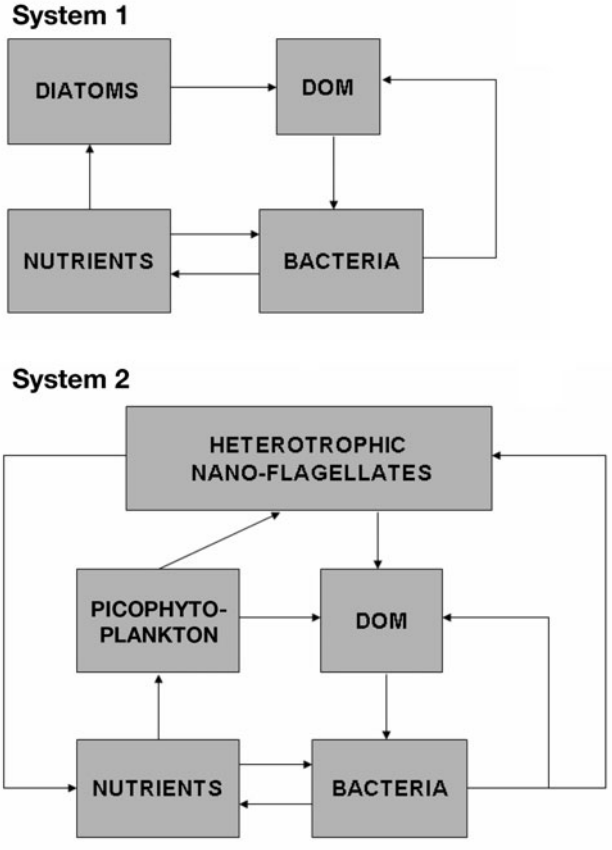

Fig. 2. Schematic representation of simulated systems. The model has 2 idealized systems; Systems 1 and 2 are defined in Table 3

nutrients and the processes leading to DOC accumulation to be investigated. System 2: a simplified microbial loop consisting of bacteria, pico-phytoplankton and heterotrophic nano-flagellates. Here the focus was on investigating whether a microbial loop structure can sustain a food web without invoking external sources of N and P.

The initial conditions were those used for the simulation of the ultra-oligotrophic Cretan Sea ecosystem (Allen et al. 2002). All experiments/simulations were run for an integration time corresponding to 1500 d. The central experiments for these 2 systems are labelled 1.1 (System 1) and 2.1 (System 2). Two sensitivity experiments were performed for each system by increasing the initial nutrient conditions as shown in Table 3. An additional and final sensitivity experiment for System 1 was designed by adding 400 mmol m^{-3} of semi-labile DOC after an integration time of 1260 d (Expt 1.4, Table 3). All the simulations were run in batch mode with a 12:12 h dark:light cycle and a constant temperature of 20°C. The bacterial parameters used are given in Table 2. All other phytoplankton and heterotrophic nano-flagellate parameters are taken from Blackford et al. (2004).

RESULTS

The analyses presented below focus on the biomass, BGE, carbon and phosphorus fluxes because the model has been run in a P-limited context. The same simulations carried out in a N-limited context gave analogous results and consequently are not discussed.

System 1

The System 1 simulations investigate the competition between phytoplankton and bacteria for dissolved nutrients and the accumulation of DOC in relation to nutrient availability. The simulations show a periodical behaviour for all the variables. An example of this behaviour is shown for bacterial and diatom biomass (Fig. 3) and for the labile, semi-labile and semi-refractory DOC (Fig. 4). The diatom bloom (Fig. 3) is periodically followed by a peak in bacteria. The 3 components of the model DOC pool also reach a repeating cycle. After the first 100 d, the labile DOC is in the 0.2 to 1 mmol m^{-3} range, the semi-labile DOC 0 to 30 mmol m^{-3}, and the semi-refractory DOC 5 to 7 mmol m^{-3}.

The results clearly indicate that the model, after a relatively short adjustment period, enters a stable repeating cycle, with oscillations of approximately 100 d. In order to investigate the functioning of the

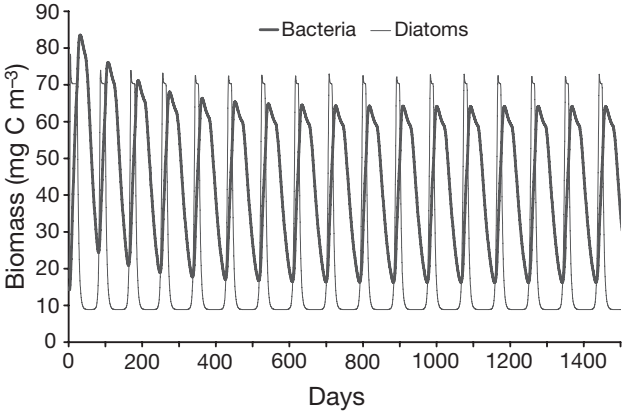

Fig. 3. Expt 1.1. Diatom and bacterial biomasses

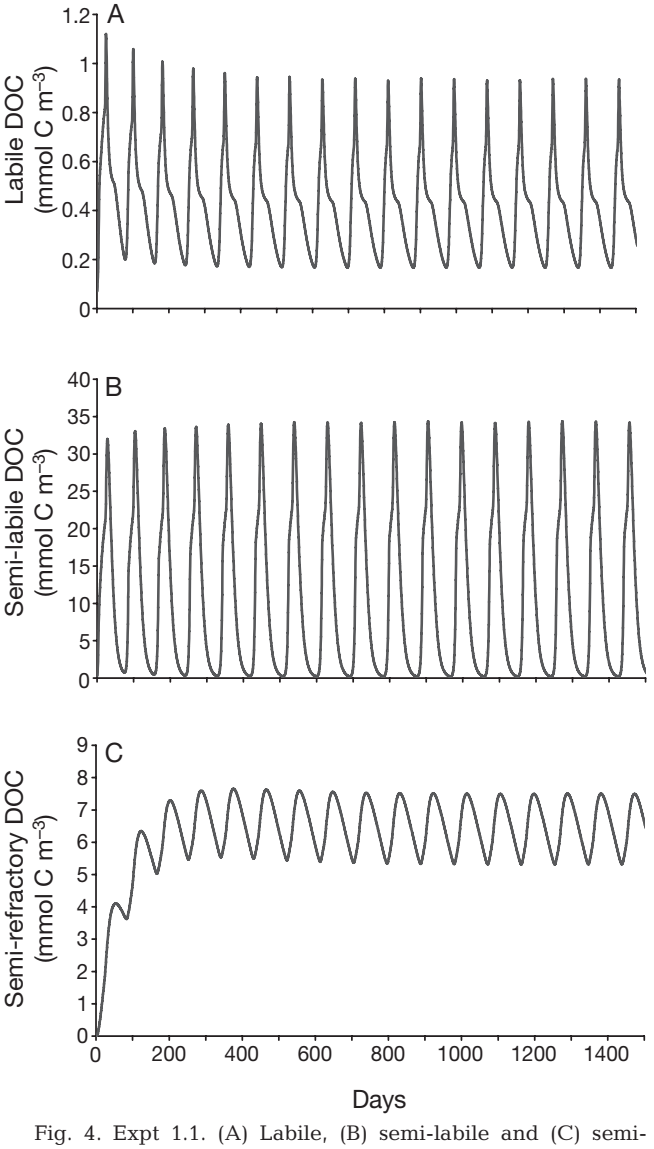

Fig. 4. Expt 1.1. (A) Labile, (B) semi-labile and (C) semi-refractory DOC concentrations

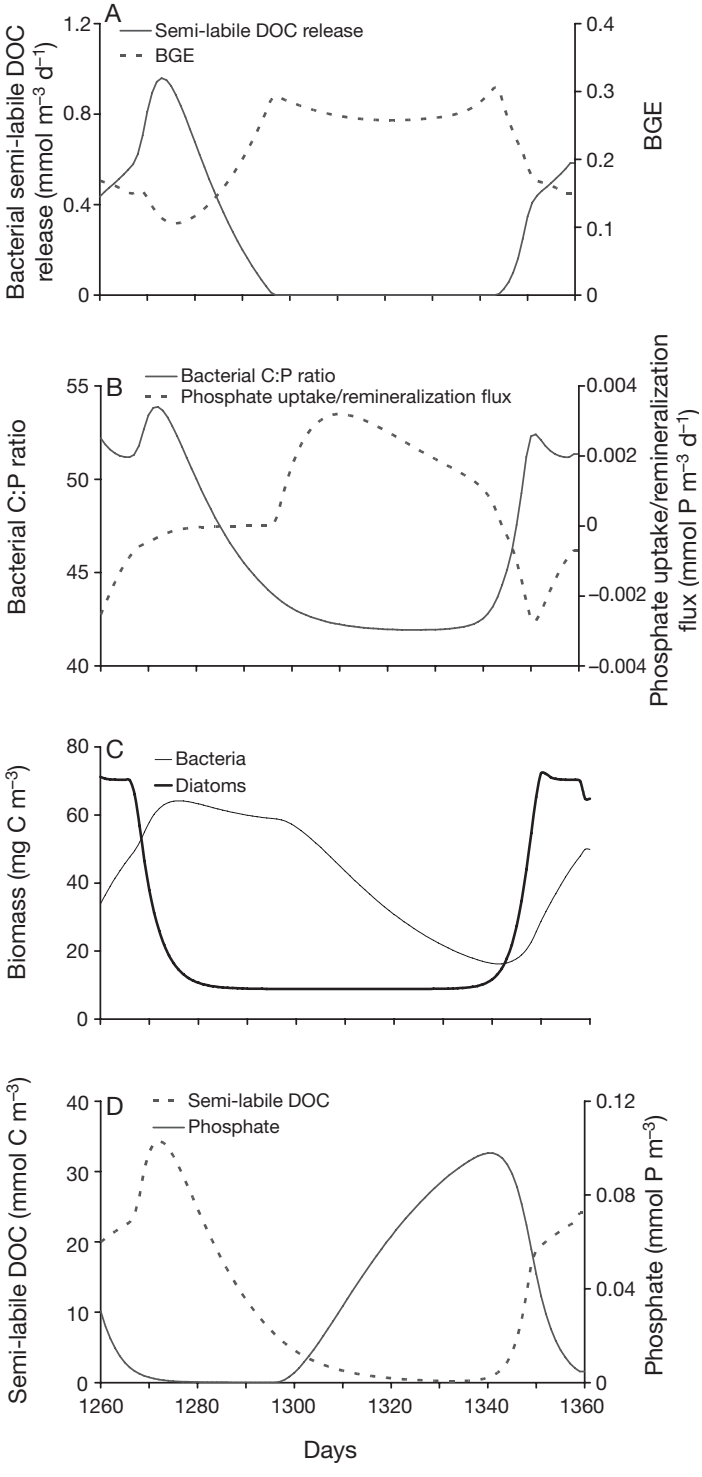

Fig. 5. Biomasses and fluxes over 100 d (from Day 1260 to Day 1360) during Expt 1.1. (A) Bacterial growth efficiency (BGE) and bacterial semi-labile DOC release flux. (B) Carbon to phosphorus bacterial internal molar ratio and phosphate bacterial uptake/remineralization flux (negative values indicate uptake, positive remineralization). (C) Diatom and bacterial biomass. (D) Semi-labile DOC concentration and phosphate concentration

system during a stable phase unaffected by the initial conditions, biomasses and fluxes over 100 d (between Day 1260 and Day 1360) are shown in detail (Fig. 5). Fig. 5A shows the BGE and the bacterial release of semi-labile DOC. When BGE is at minimum value (0.15 after 1290 d) the release of semi-labile DOC is maximum, indicating that bacteria are losing carbon in order to maintain their optimal stoichiometry. This is confirmed by the behaviour of the internal bacterial carbon to phosphorus ratio and the remineralization flux of phosphate (Fig. 5B). When the carbon to phosphorus ratio is higher than the reference ratio (Days 1260 to 1292), bacteria try to uptake phosphate and to lose carbon, implying nutrient limitation. When the bacterial carbon to phosphorus ratio is lower then the reference ratio (Days 1292 to 1345), bacteria remineralize phosphate as a consequence of carbon limitation. The remineralization of phosphate mediated by carbon-limited bacteria allows the diatoms to out-compete bacteria and to form a bloom (Figs. 3 & 5C). Once diatoms are strongly nutrient limited, the consequent release of DOC ends bacterial C-limitation and bacteria return to dominate the system. This pattern is illustrated in Fig. 5D, which shows the concentration of the semi-labile DOC and phosphate. Semi-labile DOC increases progressively from Days 1260 to 1310 when the pool of phosphate is depleted. Subsequently, DOC concentration decreases to reach negligible concentrations corresponding to maximum phosphate concentrations.

The temporal variability of the DOC/DOP ratio and the bacterial net production (Fig. 6) show a similar trend, indicating that bacterial activity and chemical composition of organic substrate are coupled (Azam et al. 1999). Sensitivity experiments performed using initial conditions with higher nutrient concentrations (Expts 1.2 and 1.3) do not show a different general

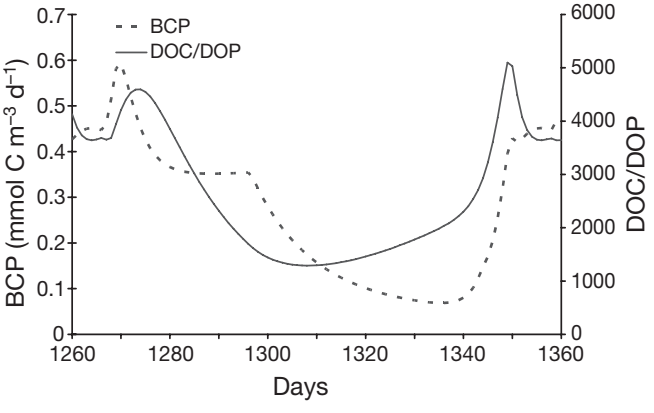

Fig. 6. Bacterial carbon production (BCP) and DOC/DOP ratio over 100 d (from Days 1260 to 1360) during Expt 1.1

behaviour of the system. Increasing the available chemical energy induces a faster periodicity and higher biomass (Fig. 7). The addition of 400 mmol m^{-3} of semi-labile DOC (Expt 1.4, Fig. 8) after 1260 integration days determines a temporal perturbation of the system: from Days 1260 to 1300 bacteria are strongly nutrient limited and the BGE decreases dramatically, reaching a value of 0.05. After this adjustment period the system re-achieves the behaviour observed in Expt 1.1.

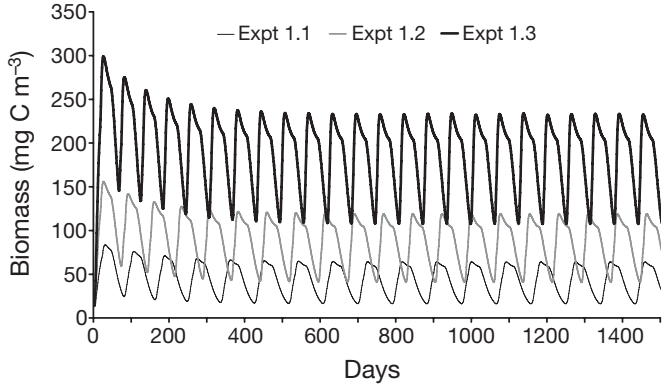

Fig. 7. Bacterial biomass in Expts 1.1, 1.2 and 1.3

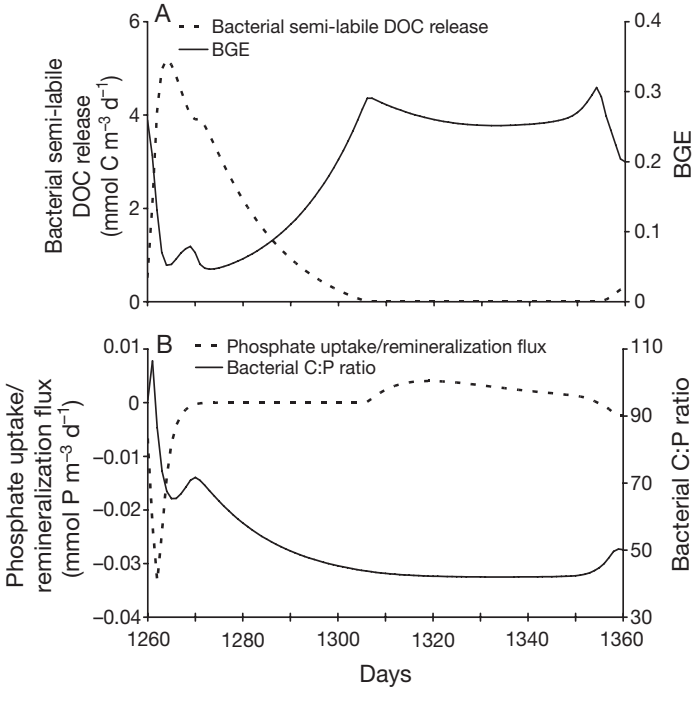

Fig. 8. Expt 1.4. Simulation from Days 1260 to 1360. (A) BGE and bacterial semi-labile DOC release. (B) Carbon to phosphorus bacterial internal molar ratio and bacterial phosphate uptake/remineralization flux (negative values indicate uptake, positive remineralization)

System 2

Fig. 9A shows the pico-phytoplankton, heterotrophic nano-flagellates and bacterial biomasses for Expt 2.1. The gross primary production and the community respiration for Expt 2.1 are shown in Fig. 9B. After an initial period of adjustment (~200 d) the system reaches a steady state. Fig. 10 shows the pico-phytoplankton, heterotrophic nano-flagellate and bacterial biomasses during the steady state for Expts 2.1, 2.2 and 2.3. Under the most oligotrophic conditions (Expt 2.1) the pico-phytoplankton, heterotrophic nano-flagellate and bacterial biomasses are very similar, while, in the higher nutrient simulations (Expts 2.2 and 2.3), pico-phytoplankton and heterotrophic nano-flagellate biomasses are greater than for bacteria. Fig. 11 illustrates the phosphate flux through bacteria, pico-phytoplankton and heterotrophic nano-flagellates. In all the implementations tested, heterotrophic nano-flagellate remineralization provides phosphate to both pico-phytoplankton and bacteria, which then compete with each other for the limiting nutrient. The competition between bacteria and pico-phytoplankton for phosphate is stronger at low nutrient concentrations and, in the most limited experiment (2.1), the bacterial uptake flux is higher than that of pico-phytoplankton.

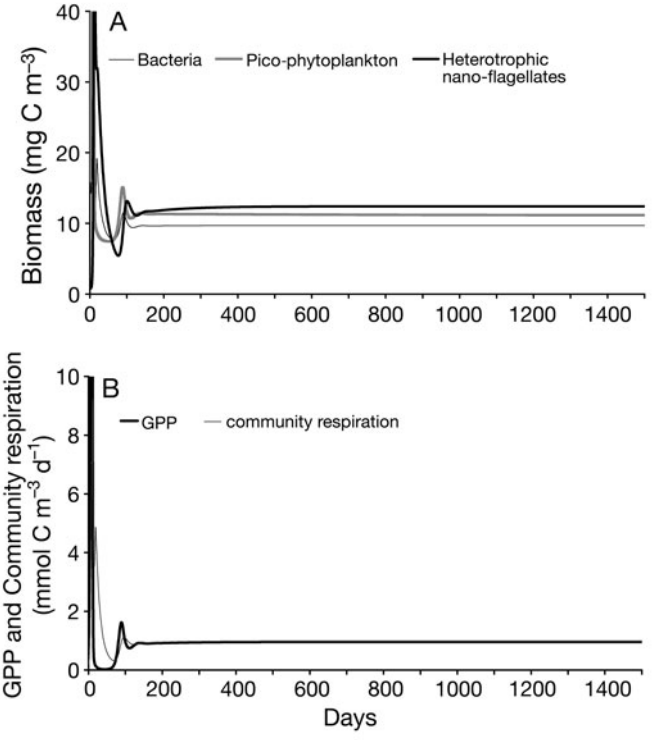

Fig. 9. Expt 2.1. (A) Pico-phytoplankton, heterotrophic nano-flagellates and bacterial biomasses. (B) Gross primary production (GPP) and community respiration

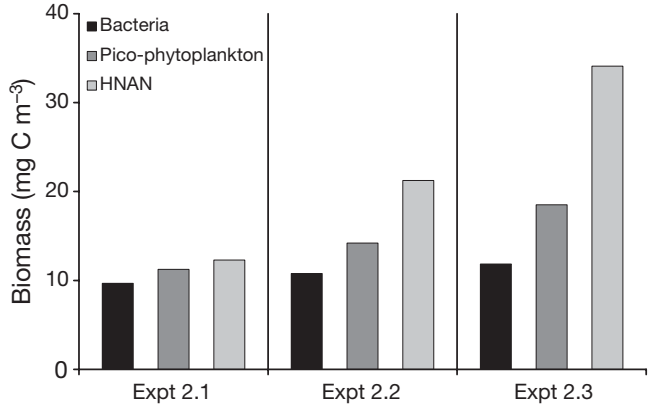

Fig. 10. Bacteria, pico-phytoplankton and heterotrophic nano-flagellates (HNAN) biomasses during the steady state of Expts 2.1 to 2.3

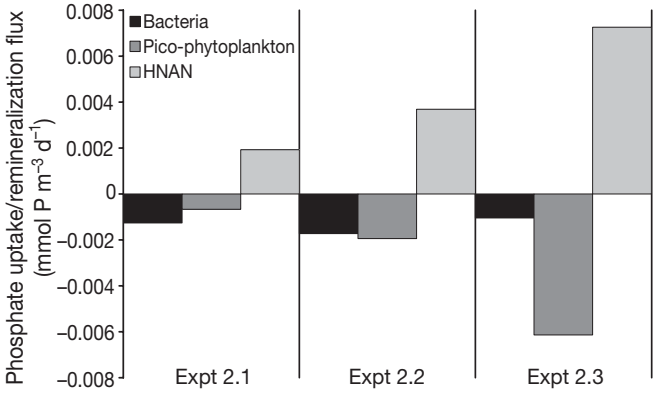

Fig. 11. Phosphate flux thhrough bacteria, pico-phytoplankton and heterotrophic nano-flagellates (HNAN) during the steady state of Expts 2.1 to 2.3, negative values indicate uptake, positive remineralization

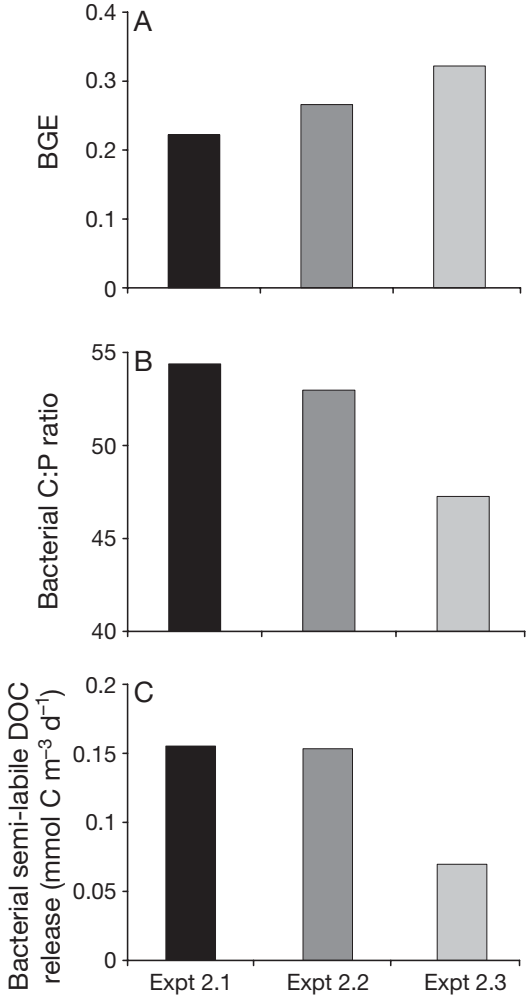

Fig. 12. (A) BGE, (B) carbon to phosphorus bacterial internal ratio and (C) bacterial semi-labile DOC release during the steady state of Expts 2.1 to 2.3

The BGE (Fig. 12A) increases with increasing nutrient availability, ranging from 0.22 to 0.33. The carbon to phosphorus ratio (Fig. 12B) and the flux of bacterial semi-labile carbon release (Fig. 12C) indicate that during the steady state bacteria are permanently nutrient limited in all 3 simulations. The release of semi-labile DOC increases as nutrient availability decreases as does the internal bacteria carbon to phosphorus ratio. The amount of semi-labile and semi-refractory DOC (Fig. 13) increases with increasing nutrient availability.

DISCUSSION

Several models describing the microbial loop and the DOC cycle have been proposed in the literature. The first version of ERSEM (Baretta et al. 1995), and the

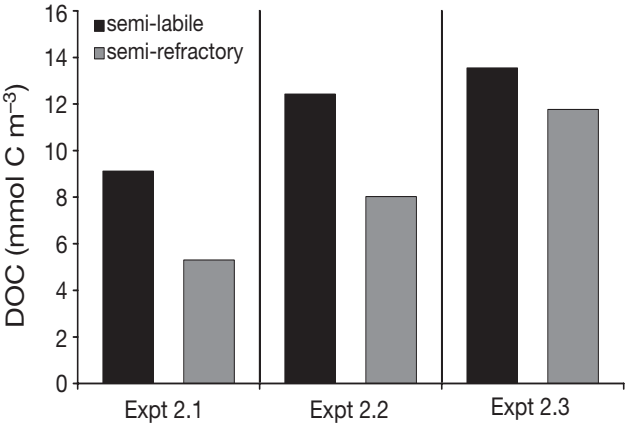

Fig. 13. Semi-labile and semi-refractory DOC concentrations during the steady state of Expts 2.1 to 2.3

model proposed by Fasham et al. (1990), have only a single DOM state variable. In general, DOM was rapidly consumed by bacteria and any DOM accumulation was prevented. The model proposed by Anderson & Williams (1998) and some ERSEM developments (Allen et al 2002, Vichi et al 2003, Blackford et al. 2004) proposed a more realistic DOC parameterization by partitioning the DOM pool into 2 different compartments, the labile and semi-labile pool.

The model proposed here is conceptually different from previous models in 3 important characteristics: (1) Bacteria can actively produce DOM not only by lysis or mortality but also by capsular material release and exudation. (2) DOC state variables (labile, semi-labile and semi-refractory) are defined on the basis of the processes governing its production. (3) The flux of DOC through bacteria and the carbon release into the semi-labile component allows the estimation of a time-dependent BGE, as a function of respiration and release of surplus carbon. Its variation can be thought of as an adaptive mechanism to different trophic conditions.

We also use the concept of 'optimal' carbon to nutrient ratios, but we allow the possibility of adaptation to different intracellular nutrient ratios. Consequently, the modelled bacteria act as a 'biological filter' of organic matter, their capacity to retain organic carbon (and convert it to biomass) being dependent on the availability of both organic and inorganic nutrients.

The diatom–bacteria system (Expts 1.1 to 1.4) shows how bacteria can compete with phytoplankton for external nutrients. This occurs when the DOC to nutrient (both organic and inorganic) ratio is higher with respect to the carbon to nutrient reference ratios of bacteria. At low primary production rates bacteria become C-limited and start to remineralize phosphate, allowing diatoms to grow again. This mechanism allows the system to sustain itself in a repeating cycle, but not in a steady state.

DOC accumulation is mainly due to the semi-labile DOC excreted by phytoplankton and bacteria. Semi-labile DOC flux from bacteria indicates the transition between a carbon-limited and a nutrient-limited condition. The magnitude of this flux can be strongly enhanced by the presence of allochthonous DOC (Expt 1.4, Fig. 8), which increases the competition between bacteria and phytoplankton for PO_4 and NH_4 and lowers the BGE. The contribution of the semi-refractory DOC (bacterial capsular material) to the overall DOC pool is limited, but it does not reach as low a concentration as the semi-labile DOC does. This allows the system to maintain a background value of DOC around 5.5 mmol m^{-3} (Fig. 4C). This kind of DOC is the most refractory in our model, and is cycled by bacteria on a time-scale of months. Following Ogawa et al. (2001), we argue that the slow utilization of this carbon by bacteria, implying ecto-enzyme activities, occasionally produces fragments from macromolecules (capsular material) which could form the truly refractory, but small sized, DOC.

In the microbial loop simulations, the presence of a top-down control (heterotrophic nano-flagellates) constrains the oscillations of the system, which after an initial unstable phase reaches a steady state where the gross primary production matches the loss due to community respiration. Bacteria, pico-phytoplankton and heterotrophic nano-flagellates manage to survive without external nutrient supply and primary production is sustained by the nutrients regenerated by the heterotrophic nano-flagellate activity (Hagström et al. 2001). In this context BGE is related to the availability of nutrients, and ranges from 0.22 to 0.33, which is in good agreement with the observations in terms of behaviour and values (del Giorgio et al. 1997, del Giorgio & Cole 1998). Bacterial biomass becomes increasingly dominant relative to phytoplankton with increasing oligotrophic conditions, reflecting a behaviour well described in the literature (Cho & Azam 1990, Thingstad & Rassoulzadegan 1999). Additionally, the slope of the relationship between primary production and bacterial production ($y = 1.272x + 1.483$ for Expt 2.1; $y = 0.560x + 3.675$ for Expt 2.2; $y = 0.194x + 7.388$ for Expt 2.3) increases as the available nutrients decrease, in agreement with the trend observed in the Mediterranean Sea (Turley et al. 2000).

The total amount of the modelled DOC (that represent the bulk semi-labile pool) ranges from 13 to 25 mmol m^{-3} and increases with increased initial nutrient concentrations. These values, albeit reasonable, are lower with respect to those reported in the literature for the semi-labile oceanic DOC (20 to 40 mmol m^{-3}). It is possible that DOC cycling processes not considered by the model (DOM exposure to UV radiation and changes in bacterial community structure) might account for this difference.

Model simulations allow us to support the 'theoretical solution' proposed by Thingstad & Rassoulzadegan (1999) to a variation of the classic Hutchinson (1961) paradox: Why do nutrient limited bacteria not outcompete phytoplankton until the point of phytoplankton biomass is reduced to a level where the system production of organic carbon is so low that bacteria become carbon limited?

Thingstad & Rassoulzadegan's (1999) solution implies that a mechanism such as predation could avoid carbon limitation for bacteria and our analysis of the model simulations leads us to the same conclusion. Simulated bacteria, in a diatom–bacteria system, periodically fall into a carbon-limited condition. In the

microbial loop simulations (with the presence of the heterotrophic nano-flagellates), bacteria are permanently nutrient limited, as the carbon to phosphorus cellular ratio is higher than the reference ratio. This implies that bacteria can survive under conditions of very low nutrient concentrations (starvation) because of the decoupling of nutrient assimilation and carbon metabolization, enabling the uptake of DOC without converting it to biomass but just to compensate for the loss due to respiration. Conversely, the ability of bacteria to survive under carbon limitation is a transitory condition because the process of respiration causes biomass loss.

CONCLUSIONS

The model proposed in this study describes some important features of the bacterial behaviour observed in marine systems. For example, the variation of BGE with the variation of external nutrient concentration. Model bacteria aim to reach their internal 'optimal' stoichiometry, taking up both organic and inorganic N and P, and releasing any excess of carbon as semi-labile carbohydrates. When the carbon to limiting nutrient ratio is higher than the optimal bacterial carbon to nutrient ratio, BGE decreases and DOC accumulation may occur. Bacteria contribute to the DOC accumulation in 2 ways: by decreasing their BGE (and hence the degradation activity), and consequently losing semi-labile DOC, and by actively producing semi-refractory DOC.

The model can reproduce a quasi steady state system with a simplified microbial loop structure without invoking external supplies of N and P. This could explain how a microbial food chain can sustain itself in natural ultra-oligotrophic systems such as the eastern Mediterranean and subtropical gyres.

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