

## **Toward the Development of Generally Applicable Models of the Microbial Loop in Aquatic Ecosystems**

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**Abstract.** Simulation modeling has been an integral, albeit ad hoc, component of the field of aquatic microbial ecology for the past two decades. One of the most critical steps in simulation modeling is the initial formulation of a clear set of questions and goals. It is doubtful that a single generic model could be constructed to address adequately all questions of interest concerning the microbial loop because of the tremendous range in time scales that define these questions. Progress in the field of aquatic microbial ecology will benefit from an integrated research program including experimental and modeling approaches. A submodel of bacterial utilization of various qualities of organic matter that we have under construction is presented. This submodel will be a component of a larger model to evaluate the effects of quality and quantity of organic matter and inorganic nutrient inputs on estuarine food web structure and efficiency. The overall model will be general enough in its structure that it should be applicable to a wide range of questions concerning the microbial loop, with time scales ranging from hours to days.

### **Introduction**

The field of aquatic microbial ecology has matured substantially since the publication of Pomeroy's 1974 *BioScience* article [9] that first conceptualized the importance of dissolved organic matter (DOM) and bacteria in pelagic food webs. Simulation modeling has been an integral, albeit ad hoc [11], component of the field. Whereas initial models were mostly descriptive, exploring mass balance relationships among food web components [8, 10, 12], later models specifically examined control mechanisms [1, 4, 7, 15]. Each of the simulation models has differed with respect to the question(s) being addressed, the level of complexity modeled, and the degree of mechanistic control included.

### **Defining the Question**

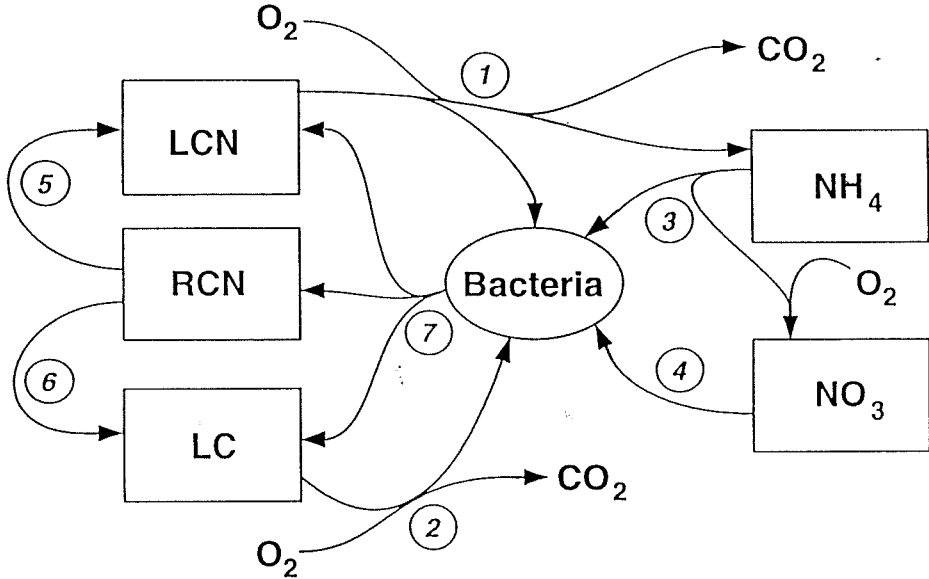
One of the most critical steps in simulation modeling is the initial formulation of a clear set of questions and goals. We would like to have a single generic model that could be used to address all questions of interest concerning the microbial loop.

However, because of the tremendous range in time scales between microbial processes such as bacterial growth (hours) and processes of global significance such as global warming (decades), no single generic model can be envisioned that will be useful for addressing all ecological questions in which microbes are involved. The choice of compartments to model, the degree of compartmental aggregation, and the requirements for precise mechanistic control are all dictated by the question to be addressed.

What exactly is the question being addressed? In the case of the Wright and Coffin models [13–15], it was to understand the controls of the cycles of bacterial, heterotrophic flagellate, and ciliate biomass in a salt marsh tidal river. Through modeling the system, the dynamics could be explained by a cycle of predator–prey interactions commencing with organic substrate supply tidally introduced from the salt marsh and concluding with ciliate grazing of flagellate biomass. The time scales of interest ranged from hours for bacterial growth rates to days for substrate resupply. There was no need in their models to include compartments representing the highest trophic levels in their ecosystem (time scale of months to years) or to include inorganic nitrogen dynamics. In the case of the Fasham models [4], the goal was to examine the effects of different food web and flow structures on system function and to look at the performance of food webs in different physical settings. As the Fasham models were to be linked with global oceanic circulation models, simplicity was a high priority. Consequently, linkage between microbial and meta-zoan food webs was not addressed; only two heterotrophic compartments were included, bacteria and bacteriovores. The modelers' inclusion of nitrogen as a parameter controlling primary and bacterial production reflected the results of physiological and culture studies showing that bacteria utilize inorganic N, compete with phytoplankton for N [2, 8], and that N availability controls bacterial growth efficiency [5, 6].

### **Modeling the Microbial Loop**

In all cases, progress in addressing important questions in microbial ecology will benefit from a combined and integrated research program including both experimental and modeling approaches. A variety of models will be required to address all questions concerning the microbial loop. Models that will address questions of global scale, such as the response of aquatic ecosystems to regional and global increases in atmospheric CO<sub>2</sub> concentration, temperature, cloudiness, and N inputs and how these changes will affect oceanic C storage, will differ greatly from models that will address questions at the community level, such as the effect of viral infection on the dynamics of microbial populations or the magnitude of dissolved organic carbon entering a system that is consumed by the microbial community. In the first case we can envision models that relegate microbes to a single heterotrophic compartment that degrades various organic carbon and nitrogen fractions in the sea. The time scale of interest would be decades. In the second set of models the microbial loop might be expanded to include various sources and qualities of organic matter and inorganic nutrients as well as specific compartments representing viruses, bacteria, phytoplankton, and higher trophic levels within the microbial loop. The time scale of interest would be hours.



**Fig. 1.** Flow diagram for bioenergetic model, bacteria only. Circled numbers correspond to flows  $F_i$ . The dissolved organic matter pool is divided into labile nitrogenous (LCN), labile nonnitrogenous (LC), and refractory nitrogenous (RCN) fractions.

**A Generic Model of the Microbial Community**

There is value in the construction of a generally-applicable model, operating at the time scale of hours to days, that could be used to examine interrelationships between autotrophic and heterotrophic organisms of the microbial loop. Of specific interest would be the inclusion of inorganic nutrient dynamics (N or P), various organic matter qualities, and links to metazoan food webs. We are interested in such a model and have been working toward its development. The overall question of interest that guides our model development is, how will variations in organic matter quantity and quality and nutrient inputs affect coastal food web structure, productivity, and efficiency? We envision the model being of sufficient generality that it could be used to examine controls on organic matter production and decomposition on continental shelves and to examine the influence of various land-use changes as they affect food web structure in estuaries.

In order to examine the effects of quality and quantity of organic matter inputs to coastal food webs, the model being developed specifically examines the coupling between C and N that results from stoichiometric growth requirements, and the bioenergetics and kinetics of organic versus inorganic nitrogen utilization. We are approaching this task through the creation of a series of submodels that ultimately will be combined. As a result of our emphasis on organic matter, our first submodel focuses on organic matter of various compositions and bacteria. It will be expanded to primary producers and higher trophic levels as development progresses. To illustrate the approach, the interactions of the bacterial compartment with the dissolved organic and inorganic nutrient compartments are described here (Fig. 1).

**Table 1.** Biochemical reactions ( $r_{ij}$ ) used to represent flows  $F_1$  to  $F_4$  in Fig. 1

Flow	Biochemical reactions	Free energy
$F_1$	$r_{11}$ : $C_{\alpha 1}H_{\beta 1}O_{\gamma 1}N \rightarrow$ bacteria	$\Delta G_{11}^{\circ}$
	$r_{12}$ : $C_{\alpha 1}H_{\beta 1}O_{\gamma 1}N + O_2 \rightarrow$ bacteria + $H_2O + \alpha 1CO_2$	$\Delta G_{12}^{\circ}$
	$r_{13}$ : $C_{\alpha 1}H_{\beta 1}O_{\gamma 1}N \rightarrow$ bacteria + $NH_3$	$\Delta G_{13}^{\circ}$
	$r_{14}$ : $C_{\alpha 1}H_{\beta 1}O_{\gamma 1}N + O_2 \rightarrow \alpha 1CO_2 + NH_3 + H_2O$	$\Delta G_{14}^{\circ}$
$F_2$	$r_{21}$ : $C_{\alpha 2}H_{\beta 2}O_{\gamma 2} \rightarrow$ bacteria	$\Delta G_{21}^{\circ}$
	$r_{22}$ : $C_{\alpha 2}H_{\beta 2}O_{\gamma 2} + O_2 \rightarrow \alpha 2CO_2 + H_2O$	$\Delta G_{22}^{\circ}$
$F_3$	$r_{31}$ : $NH_4^+ \rightarrow$ bacteria + $H^+$	$\Delta G_{31}^{\circ}$
	$r_{32}$ : $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$	$\Delta G_{32}^{\circ}$
$F_4$	$r_{41}$ : $NO_3^- \rightarrow$ bacteria + $OH^-$	$\Delta G_{41}^{\circ}$

**Table 2.** Constraints and maximization used in linear programming problem to determine reaction extents ( $r_{ij}$ )

Type	Constraint
Kinetic (CI)	$F_i = \sum_j r_{ij} \leq r_{i,max} = f(C)$
Bioenergetics (CII)	$\sum_{i,j} r_{ij} \Delta G_{ij}^{\circ} \leq \Delta G_{min}^{\circ}$
Composition (CIII)	$R_C - (CN_B + \delta) R_N \leq 0$
	$R_C - (CN_B - \delta) R_N \geq 0$
Maximize	$(R_C + R_N)$

Dissolved organic matter (DOM) is separated into three compartments consisting of labile nitrogenous (LCN), labile nonnitrogenous (LC), and refractory nitrogenous (RCN) pools, while dissolved inorganic nitrogen (DIN) is divided into ammonium and nitrate pools. The LCN and LC pools represent DOM that is readily utilized by bacteria, such as amino acids, DNA, and RNA (LCN pool), and polysaccharides and lipids (LC pool). Refractory DOM, such as humics, are lumped in the RCN pool. The compartments are interconnected by seven flows ( $F_i$ ), of which the first four ( $F_1$  to  $F_4$ ) can lead to bacterial biosynthesis.

So that proper utilization of the available resources occurs in a manner consistent with microbial metabolism, including remineralization and immobilization of nitrogen, the first four flows are represented as a sum of lumped biochemical reactions (Table 1). Labile organic material with low C:N ratio (LCN) can be utilized for C-, N-, or C,N-bacterial synthesis, or for energy production as presented in Table 1, where each reaction ( $r_{ij}$ ) is associated with a free energy change,  $\Delta G_{ij}^{\circ}$ . The LC, ammonium, and nitrate pools can be utilized similarly, as shown.

Although flows for the reactions given in Table 1 cannot be uniquely determined from typically measured quantities, a unique solution can be obtained by maximizing bacterial synthesis rate subject to constraints on uptake kinetics, bioenergetics, and bacterial composition, as described in Table 2. The uptake of nutrients from any of the four available pools is limited by a maximum rate due to kinetic constraints. Since flows  $F_1$  to  $F_4$  are given by  $\sum_j r_{ij}$ , the first type of constraint is of the form

$$\sum_j r_{ij} \leq r_{i,\max} \quad (\text{Constraint I})$$

where  $r_{i,\max}$  is the maximum rate for flow  $i$ , which is a function of substrate concentration ( $c$ ), such as in a Monod-type expression.

Some of the biochemical reactions mentioned above and listed in Table 1 liberate free energy (negative  $\Delta G^\circ$ ), such as the oxidation reactions, while others require energy to proceed, such as those that lead to bacterial biomass synthesis. Thermodynamic constraints dictate that the free energy summed over all the reactions must be less than or equal to zero in order for the overall reaction to proceed. As growth does not proceed in a reversible manner, some free energy is dissipated, so that the free energy for the overall reaction will be less than zero. Consequently, the second type of constraint takes the form

$$\sum_{ij} \Delta G^\circ_{ij} r_{ij} \leq \Delta G^\circ_{\min} \quad (\text{Constraint II})$$

where  $\Delta G^\circ_{\min}$  is the typically observed free energy change for an overall biosynthetic reaction.

The C:N ratio for bacteria represents a third type of constraint. The rates of bacterial carbon and nitrogen synthesis (denoted by  $R_C$  and  $R_N$ , respectively) are given by the stoichiometry of the DOM pools and the extents of the above reactions,  $r_{ij}$ . If the C:N ratio of the bacteria is given by  $CN_B \pm \delta$ , where  $\delta$  is the allowed variability in the C:N ratio, then the third type of constraint is given by  $CN_B - \delta \leq R_C/R_N \leq CN_B + \delta$ , or in linear form:

$$R_C - (CN_B + \delta)R_N \leq 0 \text{ and } R_C - (CN_B - \delta)R_N \geq 0. \quad (\text{Constraint III})$$

The flows for the reactions  $r_{ij}$ , hence  $F_1$  to  $F_4$ , are determined by solving the linear programming problem:

Maximize  $(R_C + R_N)$  Subject to constraints CI, CII, and CIII.

Flows  $F_5$  and  $F_6$  represent the degradation of refractory organic matter (OM) into the labile pools LCN and LC, respectively, and flow  $F_7$  represents bacterial lysis due to starvation or viral infection. These three flows are given by first-order decay expressions, so are not involved in the linear programming problem. To observe the dynamics of the system, a standard state model is constructed from atom balances for C and N around each of the six compartments, and integrated forward in time. At each time step, the linear programming problem is solved to determine reaction extents, hence flows  $F_1(t)$  to  $F_4(t)$ , from the current substrate concentrations at time  $t$ .

The advantage of this approach is the flexibility it allows in modeling the uptake and remineralization of nutrients in aerobic or anaerobic environments based on first principles. The disadvantages are the increased complexity and the difficulties in estimating free energies for substrates in poorly defined pools. However, literature exists [5, 6] regarding uptake and exudation of organic and inorganic matter, so that predicted substrate utilization will be testable.

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## References

1. Anderson TR (1992) Modelling the influence of food C:N ratio, and respiration on growth and nitrogen excretion in marine zooplankton and bacteria. *J Mar Res* 14:1645–1671
2. Bratbak G, Thingstad TF (1985) Phytoplankton–bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism. *Mar Ecol Prog Ser* 25:23–30
3. Caron DA, Goldman JC, Bennett MR (1988) Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles. *Hydrobiologia* 159:27–40
4. Fasham MJR, Ducklow HW, McKelvie SM (1990) A nitrogen-based model of plankton dynamics in the oceanic mixed layer. *J Mar Res* 48:591–639
5. Goldman J, Caron DA, Dennett MR (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnol Oceanogr* 32:1239–1252
6. Kirchman DL, Keil RG, Wheeler PA (1990) Ammonium recycling limits nitrate use in the oceanic subarctic Pacific. *Limnol Oceanogr* 35:1258–1266
7. Maloney CL, Field JG (1991) The size-based dynamics of plankton food webs. I. A simulation model of carbon and nitrogen flows. *J Plankton Res* 13:1003–1038
8. Pace ML, Glasser JE, Pomeroy LP (1984) A simulation analysis of continental shelf food webs. *Mar Biol* 82:47–63
9. Pomeroy LR (1974) The ocean's food web, a changing paradigm. *BioScience* 24:499–504
10. Steele JH (1974) *The structure of marine ecosystems*. Harvard University Press, Cambridge
11. Wetzel RL (1994) Modeling the microbial loop: an estuarine modeler's perspective. *Microbial Ecology* 28:331–334
12. Wetzel RL, Christian RR (1984) Simulation model studies on the interactions between carbon substrates, bacteria, and consumers in a salt marsh estuary: Duplin River, Sapelo Island, Georgia, USA. *Bull Mar Science* 35:601–614
13. Wright RT (1988) Methods for evaluating the interaction of substrate and grazing as factors controlling planktonic bacteria. *Arch Hydrobiol Beih* 32:229–242
14. Wright RT (1988) A model for short-term control of the bacterioplankton by substrate and grazing. *Hydrobiologia* 159:111–117
15. Wright RT, Coffin RB (1994) The microbial loop in a saltmarsh estuary: steady state vs. constant change. (Paper presented at Microbial Loop Symposium)