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# DYNAMIC ENERGY BUDGET REPRESENTATIONS OF STOICHIOMETRIC CONSTRAINTS ON POPULATION DYNAMICS

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*Abstract.* Metabolism, and thus population dynamics, can be limited by energy, carbon, nitrogen, and/or other nutrients. This is due to homeostasis, the relatively constant composition of biomass. Yet growth-rate-dependent changes in the composition of biomass do exist. The dynamic energy budget (DEB) theory provides the framework to deal with these simultaneous limitations and stoichiometric restrictions. We illustrate the application with three examples. First, we discuss simple single-species growth of a chemolithoautotroph to illustrate the interactions between nutrients and substrates in growth. We show how the macrochemical reaction equation with variable yield coefficients can be decomposed in a number of subprocesses with constant yield coefficients. We then discuss a simple predator–prey system, where nutrients are accumulated in the prey, which no longer have a constant composition of biomass. The implication is a varying conversion efficiency from prey to predator, with consequences for qualitative aspects of population dynamics. We illustrate the principles with a grazer (a heterotrophic consumer) feeding on algae (autotrophic producers). The algae frequently have an excess of energy in the form of carbohydrates, which are excreted and serve as food supplements for the heterotroph. This exchange of carbohydrates against nutrients is basic to a symbiosis, our third example of application of DEB theory for solving stoichiometric problems in species interactions. The algae are no longer grazed as long as the grazer is able to extract nutrients from other sources. Depending on parameter values of the system, the coexistence can be very stable and further integrate into a single entity with mixotrophic properties. This process is basic to the evolution of the eukaryotic cell and to the organizational structure of metabolism. Mixotrophs can specialize under particular environmental conditions into autotrophs or heterotrophs, which again can associate in symbiotic relationships. The chemical composition of membranes testifies to the frequent occurrence of this process, which can now also be understood quantitatively.

*Key words: autotrophs and heterotrophs; bifurcation theory; dynamic energy budget (DEB) theory; ecosystem dynamics; Liebig's minimum rule; macrochemical reaction equation; mixotrophy; nutrient limitation; producer–consumer dynamics; stoichiometry; symbiosis; syntrophy.*

## INTRODUCTION: NUTRIENTS AND ENERGY

If a zebra eats grass, one gets less grass and more zebra till the grass supplies are so poor that the zebras can hardly reproduce. Most population-dynamic models try to describe this process on the basis of simple assumptions about the growth of grass, in order to analyze the population dynamics of zebras. The problem with the application of those models in biological research is the fact that grass growth is usually nutrient limited, and that the zebra excretes most of the nutrients locked into grass biomass, and eventually returns almost all nutrients as legacy. This obviously stimulates grass growth, which is usually not modeled explicitly. The same applies to lions, which feed on zebras. The inefficient conversion of zebra into lion comes with a nutrient leak, which stimulates grass growth and so zebra production. The lions, therefore, not only eat zebras, but also feed them indirectly. The indirect quantitative support of the zebra by the lion received less attention than the direct qualitative support, which is well know since Darwin: lions prefer weak individuals (less effort, less health risks). Apart from genetic selection, and reduction of the probability of epidemics of contagious diseases, lions affect competition for grass between weak and strong zebras, to the benefit of the strong (reproductive) ones. The combination of quantitative and qualitative aspects is required for evolutionary insight. Factors such as the effects of weather and climate, and migrations in spatially heterogeneous environments, make this insight not easy to acquire.

This example illustrates that we should focus on the fate of nutrients to understand the quantitative relationships between grass, zebras, and lions, as further illustrated in Daufresne and Loreau (2001). A few different types of nutrient usually limit growth in their roles as building blocks, but energy is of importance as well for (thermodynamic) work. Organisms usually

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extract energy in the form of carbohydrates, lipids, and proteins. These polymers have a dual role as supplies of energy as well as building blocks; the part that functions as energy supply results in the production of nutrients (and carbon dioxide, water, and heat). Excretion of nutrients is, therefore, basic to metabolism. Some bacteria manage to extract energy from certain mineral compounds, but energy in a community usually originates from light that is fixed by plants, algae, or certain bacteria, and passed to other organisms via the food web.

Because autotrophs acquire energy and nutrients independently, they store them independently within the cells (body), while their structural composition is rather constant. The latter property is called ''homeostasis.'' Autotrophs' homeostasis implies that grazers acquire energy and nutrients in rather fixed ratios; predators then acquire energy and nutrient in almost perfectly fixed ratios.

We here discuss the stoichiometric restrictions on metabolism and interactions between organisms in the context of the dynamic energy budget (DEB) theory for the uptake and use of nutrients and substrates. Introductions to this theory can be found in (Kooijman 2000, 2001, Nisbet et al. 2000). This theory is based on energy and mass balances in an explicit way, and can handle contraints that are more complex than simple element ratios; conservation of elements is necessary, but frequently not sufficient (Anderson et al. 2004). It can also handle particular changes in biomass composition, which is essential to understand growth patterns (Vrede et al. 2004). To simplify the discussion we take the organism's surface area that is involved in assimilation proportional to its structural mass. This eliminates the distinction between the individual and population level. It is frequently a good numerical approximation for organisms that divide, i.e., organisms that reset their volume frequently. It is not appropriate for large-bodied multicellulars, where we need formulations in terms of individual-based population dynamics. Our approach can be implemented in such formulations in a straightforward way (Kooijman 1995, Kooijman et al. 1999, Kooijman and Nisbet 2000), but is obviously a bit more complex.

As an introduction to more complex transformations, we first discuss the decomposition of growth of chemolithoautotrophic bacteria, and show how this already-rather-complex chemical transformation can be decomposed into simpler ones. This serves as an exercise to deal with stoichiometric restrictions, while biomass has a variable composition. We then discuss a simple prey–predator system and show that the conversion of prey into predator is not constant, and depends on the composition of the preys' biomass. We finally study a symbiotic system that is based on the exchange of nutrients. This example illustrates a basic relationship between organisms.

The rates of transformations that are involved will be specified in terms of that of ''synthesizing units.'' A short section just introduces this concept first.

## SYNTHESIZING UNITS

A *synthesizing unit* (SU) (Kooijman 1998) can be conceived as a (generalized) enzyme that behaves according to the association–dissociation rules as prescribed by classic enzyme kinetics, with two modifications: the kinetics is not specified in terms of substrate concentrations, but in terms of arrival fluxes of substrate molecules to the enzyme, and the enzyme–substrate complex does not dissociate (enzymes are only released after transformation of substrate into product). If arrival is controlled by diffusion (or convection) in a well-mixed environment, the arrival rate is proportional to the concentration of substrate. The transformation $y_{AC}A + y_{BC}B \rightarrow C$, then amounts to

$$J_C = \frac{J_{Cm}}{1 + K_A/M_A + K_B/M_B - (M_A/K_A + M_B/K_B)^{-1}}$$

$$J_A = -y_{AC}J_C \qquad J_B = -y_{BC}J_C$$

where $M_A$ and $M_B$ stand for the amounts of $A$ and $B$ in a unit volume, respectively, $J_{Cm}$ for the maximum flux of Carbon $C$, which is achieved for $M_A$, $M_B \rightarrow \infty$. The half-saturation constants $K_A$ and $K_B$ include transport phenomena, binding probabilities, and stoichiometric requirements. The expression is an approximation only; stoichiometric details appear in the exact expression (Kooijman 1998, 2000). Arrival can also be the result of an intracellular allocation process, which is linked to metabolic transformations; we will meet such a situation in the discussion on predation. Arriving fluxes imply the existence of rejected fluxes that require a destination, which places allocation in a central position in metabolic organization; allocation is basically a partitioning of fluxes to specific destinations.

The numerical behavior of a SU closely follows Liebigs' minimum rule, except for a narrow window of concentrations where several substrates limit the transformation simultaneously. So, if $M_A/K_A \ll M_B/K_B$ or $M_A/K_A \gg M_B/K_B$ we have

$$J_C \simeq \frac{J_{Cm}M_A}{K_A + M_A} \quad \text{or} \quad J_C \simeq \frac{J_{Cm}M_B}{K_B + M_B}.$$

However, in the case of SUs, the transition from one limiting substrate to another at changing substrate concentrations in the environment is smooth, which is rather essential in combination with reserves; substrates can be absent in the environment, while not (yet) limiting growth, due to the presence of reserves. This implies complex algorithms for evaluation of the moment at which the switch occurs for each individual, which can be avoided with SUs because of the absence of switches.

The application of SU kinetics in the dynamic energy budget (DEB) theory is in the assimilation process, which transforms substrates in the environment into reserves, and in the growth process, which transforms reserves into structural mass. Each reserve, and each structure, has a single type of SU, thus the basic DEB model has two types of SUs. The flux of substrate that is rejected by the growth SU is returned to the reserves, or excreted into the environment with a fixed fraction. If all rejected substrate would be returned, no upper boundary for that reserve would exist. The return fraction quantifies the damming up of nonlimiting reserves, with the remarkable consequence that the growth rate *in*creases with limiting-reserve density, but *de*creases with the nonlimiting ones. Excretion can be treated implicitly in single-reserve–single-structure systems by inclusion into the overheads of assimilation, maintenance, and growth. It must be treated explicitly, however, in multivariate cases, because excretion of one reserve depends on the dynamics of the others.

In the above-mentioned transformation, substrate $A$ as well as $B$ are required to produce $C$ and are processed in a parallel way; such substrates are called ''complementary.'' If transformations $A \rightarrow C$ and $B \rightarrow C$ are possible, $A$ and $B$ are called ''substitutable substrates,'' and the SU expression for the product $C$ for serially processed substrates becomes

$$J_C = \frac{J_{Cm}}{1 + (M_A/K_A + M_B/K_B)^{-1}}$$

with

$$J_{Cm} = \alpha J_{Am} + (1 - \alpha)J_{Bm}$$

$$J_A = y_{AC}\alpha J_C \qquad J_B = y_{BC}(1 - \alpha)J_C$$

$$\alpha = \left(1 + \frac{M_B K_A}{M_A K_B}\right)^{-1}.$$

The coefficient $\alpha$ weighs the contribution of $A$ in the production of $C$. The arriving flux $A$, which is proportional to the concentration $M_A$, will not be processed completely, partly because the SU is not always in the binding stage when a particle arrives, partly because the binding probability can be $<1$. The processed fluxes of $A$ and $B$ follow from the production rate $J_C$. These reduce to $J_{Am}$ and $J_{Bm}$ for large production capacities, and the production flux becomes a simple addition: $J_C = J_{Cm}(M_A/K_A + M_B/K_B)$. The strength of the SU concept becomes clear in more complex situations, such as in gradual transitions from the parallel complementary to the serial substitutable cases and co-metabolism (Brandt 2002), in substrate inhibition and slow adaptation to available substrates (Brandt 2002), in simultaneous limitations by more than two substrates (see the next section, and Kooijman et al. [2002]).

## SINGLE-SPECIES POPULATION GROWTH

The chemical impact of an individual on its surroundings can be summarized in the macrochemical reaction equation. Contrary to ordinary chemical reaction equations, the yield coefficients are not constant, but depend on substrate availability. This is because maintenance has priority over growth and because the chemical composition of biomass depends on its growth rate. According to the DEB (dynamic energy budget) theory, the macrochemical reaction equation can be decomposed into several constituting processes with fixed yield coefficients; the relative importance of these subprocesses depends on environmental conditions. We illustrate the principle with autotrophs that grow on simple nutrients, methanotrophs,

$$\mathrm{CH_4} + Y_{CX}\mathrm{CO_2} + Y_{OX}\mathrm{O_2} + Y_{NX}\mathrm{NH_3} + Y_{HX}\mathrm{H_2O}$$
$$\rightarrow Y_{WX}\mathrm{CH}_{n_{HW}}\mathrm{O}_{n_{OW}}\mathrm{N}_{n_{NW}}$$

where the chemical indices of biomass, $n_{HW}$, $n_{OW}$, and $n_{NW}$, depend on nutrient availability. Biomass consists of structural mass $M_V$ and reserve mass $M_E$ (both measured in moles of carbon [C-moles]). The ''reserve density'' is defined as the ratio of the reserve and structure masses $m_E = M_E/M_V$. Then, the chemical indices of biomass can be written as $n_{iW} = (n_{iV} + m_E n_{iE})/(1 + m_E)$, where the chemical indices for structure $n_{iV}$, and for reserve $n_{iE}$ are fixed ($i$ is $H$, $O$, or $N$), and the reserve density $m_E$ depends on environmental conditions (the concentrations of methane, carbon dioxide, dioxygen, and ammonia, assuming abundant water).

The basic DEB model for metabolism assumes that substrates (and nutrients) are transformed into reserves (the transformation is called ''assimilation''), and reserves are transformed into structure (growth), while reserves are also used for maintenance purposes. All three transformations come with an overhead, which implies the production of ''minerals'' (carbon dioxide, water, ammonia) or other compounds. Both reserve and structure are taken to be generalized compounds: mixtures of chemical compounds that do not change in composition. The implication is that the transformations are subjected to stoichiometric constraints and that both substrates and reserve have a dual role: they are used to drive the transformation and they serve as building blocks (anabolic substrates).

Both the chemical indices $n_{iW}$, and the yield coefficients $Y_{iX}$ depend on the availability of nutrients, which makes the representation in terms of the macrochemical reaction equation less useful and calls for a decomposition of underlying simpler transformations with constant yield coefficients. The varying yield coefficients and chemical indices complicate the concept ''*the* reaction rate'' that is widely applied in chemical kinetics; each compound is appearing or disappearing at its own rate in a more complex transformation, while energy and mass balances provide constraints on these rates. Reactions of this type should be characterized by a *vector*-valued rate, rather than a scalar.

Classic physiology delineates *catabolism* (the collection of processes that decomposes compounds to release energy and building blocks for the synthesis of new compounds) and *anabolism* (the collection of processes that synthesizes new compounds that become part of new biomass). These concepts are more complex in the context of the DEB theory. Assimilation and growth both have catabolic and anabolic aspects because both reserve and structure are taken to be generalized compounds that involve synthesis from building blocks that are extracted from substrate and reserve, respectively.

These aspects of assimilation and growth can be written as generalized transformations. We here use classic notation for chemical transformations with yield coefficients $Y_{ij}$, which are negative if one of the compounds disappears and the other appears. This points to a notational problem that is hard to deal with in a consequent way, due to the various possible levels of organization that can be considered (population, organism, cell, organelle, molecule). Yield coefficients $Y$ are ratios of fluxes—but notice that yield coefficients $y_{ij}$ have almost the same interpretation, but they are treated as positive constant mass–mass couplers. Specific fluxes $j_i$ are here taken to be positive.

Methanotrophs use methane ($CH_4$) as energy source; methane is the only carbon source in Type I methanotrophs, such as *Methylomonas*, *Methylomicrobium*, *Methylobacter*, and *Methyloccus*, which use the monophosphate pathway to process formaldehyde ($CH_2O$), a metabolite of methane. Methane and carbon dioxide ($CO_2$) are carbon sources for Type II methanotrophs, such as *Methylosinus* and *Methylocystis*, which use the serine pathway to process formaldehyde. These organisms can also fix dinitrogen. We here selected type I methanotrophs to illustrate the stoichiometric principles because only very simple compounds are involved.

Table 1 gives the yield coefficients and the chemical indices of the seven compounds that are involved in five transformations for growing Type I methanotrophs. Phosphates and micronutrients are not included for simplicity's sake. The yield coefficients are on methane for assimilation, and on reserve for maintenance and growth. If we assemble a matrix of chemical indices $\boldsymbol{n}$, with four elements in the rows and seven compounds in the columns, and a matrix of yield coefficients $\boldsymbol{Y}$, with seven compounds in the rows and five transformations in the columns (Table 1 presents $\boldsymbol{Y}'$ rather than $\boldsymbol{Y}$, for typographic reasons), then the conservation law for elements implies that $\boldsymbol{nY}$ is a 4 × 5 matrix of zeros. This law does not tell anything about rates, but it determines all yield coefficients. In other words: the yield coefficients are such that $\boldsymbol{nY} = 0$; it takes only simple bookkeeping and some patience to solve for these yield coefficients. For the five transformations given in Table 1, this gives the following yield coefficients:

$$Y^A_{NX} = -n_{NE}$$

$$Y^A_{HX} = 2 - Y^A_{NX}3/2 - n_{HE}/2$$

$$Y^A_{OX} = -Y^A_{HX}/2 - n_{OE}/2$$

$$Y^M_{HE} = -n_{NE}3/2 + n_{HE}/2$$

$$Y^M_{OE} = -1 + n_{OE}/2 - Y^M_{HE}/2$$

$$Y^G_{NE} = n_{NE} - n_{NV}$$

$$Y^G_{HE} = n_{HE}/2 - n_{HV}/2 - Y^G_{NE}3/2$$

$$Y^G_{OE} = n_{OE}/2 - n_{OV}/2 - Y^G_{HE}/2.$$

It is thus possible to decompose the macrochemical reaction equation into five transformations in a unique way, given knowledge about the chemical composition

TABLE 1. The yield coefficients (upper panel) and the chemical indices (lower panel) for the seven compounds that are involved in the five transformations of a Type I methanogen with one reserve and one structure.

| | | Yield coefficients | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Symbol | Definition† | Methane, $X$ | Carbon dioxide, $C$ | Water, $H$ | Dioxygen, $O$ | Ammonia, $N$ | Reserve, $E$ | Structure, $V$ | Specific rates |
| Yield coefficients, $Y$ | | | | | | | | | |
| $A_C$ | assim. (cat.) | −1 | 1 | 2 | −2 | 0 | 0 | 0 | $(y_{XE} - 1)j_{EA}$ |
| $A_A$ | assim. (ana.) | −1 | 0 | $Y^A_{HX}$ | $Y^A_{OX}$ | $Y^A_{NX}$ | 1 | 0 | $j_{EA}$ |
| $M$ | maintenance | 0 | 1 | $Y^M_{HE}$ | $Y^M_{OE}$ | $N_{NE}$ | −1 | 0 | $j_{EM}$ |
| $G_C$ | growth (cat.) | 0 | 1 | $Y^M_{HE}$ | $Y^M_{OE}$ | $N_{NE}$ | −1 | 0 | $(1 - y_{VE})j_{EG}$ |
| $G_A$ | growth (ana.) | 0 | 0 | $Y^G_{HE}$ | $Y^G_{OE}$ | $Y^G_{NE}$ | −1 | 1 | $y_{VE}\, j_{EG}$ |
| Chemical indices | | | | | | | | | |
| $C$ | carbon | 1 | 1 | 0 | 0 | 0 | 1 | 1 | |
| $H$ | hydrogen | 4 | 0 | 2 | 0 | 3 | $n_{HE}$ | $n_{HV}$ | |
| $O$ | oxygen | 0 | 2 | 1 | 2 | 0 | $n_{OE}$ | $n_{OV}$ | |
| $N$ | nitrogen | 0 | 0 | 0 | 0 | 1 | $n_{NE}$ | $n_{NV}$ | |

*Notes:* Following microbiological tradition, the substrate is chosen as the reference in the yield coefficients, i.e., methane for assimilation, and reserve for maintenance and growth. Given the chemical indices $n$, the yield coefficients $Y$ follow from the conservation law for elements, and the specific rates $j_{EA}$, $j_{EM}$, and $j_{EG}$ are specified by the dynamic energy budget (DEB) theory; see *Single-species population growth.* The yield coefficients for the catabolic aspect of growth equal those for maintenance.

† Key to abbreviations: assim. = assimilation, cat. = catabolic, ana. = anabolic.

of reserve, structure, and nutrients. This knowledge can be acquired from a study of changes in the composition of biomass as a function of the growth rate. The specific rate of appearance of ammonia in association with maintenance, for instance, is $j_{NM} = n_{NE} j_{EM}$; that of dioxygen is $Y^{M}_{OE} j_{EM}$. If we assemble the rates in Table 1 in a five-vector $\boldsymbol{k}$, the seven-vector of specific rates of appearances or disappearances of compounds is given by $\boldsymbol{Yk}$, where each rate can be positive as well as negative.

The assimilation flux of reserves depends on the concentrations of the complementary compounds methane, dioxygen, and ammonia. The SU rule for the assimilation rate of reserves $j_{EA}$ for $x = X/K_X$, $o = O/K_O$, $n = N/K_N$ works out as follows:

$$j_{EA} = j_{EAm}/[1 + x^{-1} + o^{-1} + n^{-1} - (x + o)^{-1} - (x + n)^{-1} - (o + n)^{-1} + (x + o + n)^{-1}]$$

where $X$, $O$, and $N$ are the concentrations of methane, dioxygen and ammonia, and $K_X$, $K_O$, and $K_N$ are the half-saturation constants; $j_{EAm}$ is the maximum specific assimilation rate of reserves. The consumption of methane, dioxygen, and ammonia follow from the production of reserve via fixed coupling coefficients. A rather small range of concentrations of methane, dioxygen, and ammonia limit assimilation simultaneously. In many practical applications we have, at abundant dioxygen and $x \ll n$ or $x \gg n$,

$$j_{EA} \simeq \frac{j_{EAm}}{1 + x^{-1}} = \frac{j_{EAm} X}{K_X + X}$$

or

$$j_{EA} \simeq \frac{j_{EAm}}{1 + n^{-1}} = \frac{j_{EAm} N}{K_N + N}.$$

This is the familiar standard formulation for single-substrate limitation. The use of reserve for metabolism is a first-order process on the basis of densities, so density dynamics is $d/dt\ m_E = j_{EA} - k_E m_E$ where $k_E$ is the reserve turnover rate.

The specific maintenance flux of reserve is constant at rate

$$j_{EM} = y_{EV} k_M.$$

The flux of reserve associated with growth (including overheads) is

$$j_{EG} = y_{EV} r$$

with

$$r \equiv M_V^{-1} \frac{d}{dt} M_V = \frac{m_E k_E - j_{EM}}{m_E + y_{EV}}$$

where specific growth rate $r$ equals the ratio of two quantities: $m_E k_E - j_{EM}$, which is the reserve flux that is released from the reserves minus the losses through maintenance, and $m_E + y_{EV}$, which is the specific cost for new reserve plus structure. So the growth rate depends on the reserve density $m_E$, not on the nutrient concentrations directly. Growth ceases at reserve density $m_E = j_{EM}/k_E$, where all mobilized reserves are used for maintenance.

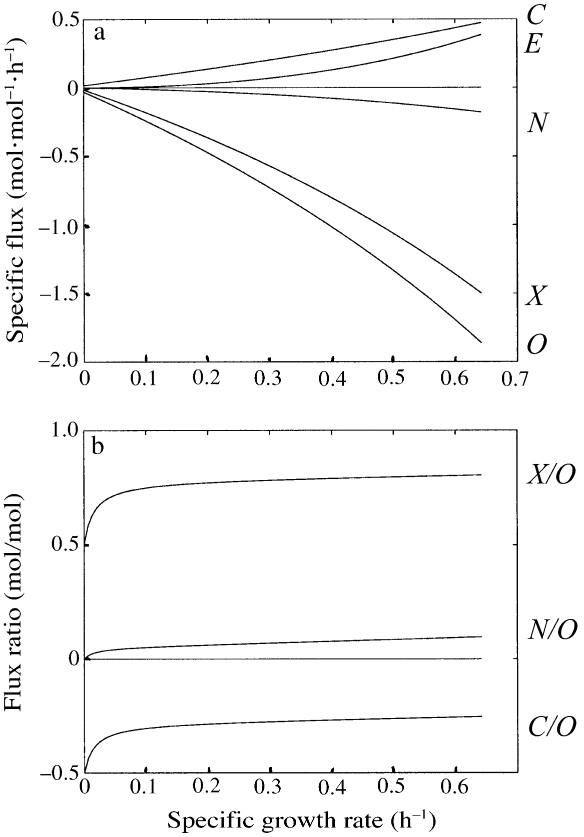

FIG. 1. Fluxes of compounds as functions of specific growth rate. (a) The specific fluxes of carbon dioxide ($C$), reserves ($E$), ammonia ($N$), methane ($X$), and dioxygen ($O$) as a function of the specific growth rate of a methanotroph. Those of water and structure are not shown. (b) The ratio of the fluxes of methane (top curve), carbon dioxide (bottom curve), and ammonia (middle curve), with that of dioxygen. Parameters: maximum specific assimilation rate (of $E$) $j_{EAm} = 1.2$ mol·mol$^{-1}$·h$^{-1}$); yield coefficients $y_{EX} = 0.8$ mol/mol and $y_{VE} = 0.8$ mol/mol; maintenance rate constant $k_M = 0.01$ h$^{-1}$; reserve turnover rate $k_E = 2.00$ h$^{-1}$; chemical indices of reserve and structure: $n_{HE} = 1.8$; $n_{OE} = 0.3$; $n_{NE} = 0.3$; $n_{HV} = 1.8$; $n_{OV} = 0.5$; $n_{NV} = 0.1$.

This completes the specification of the rates in Table 1 in terms of the state of the organism (amounts of reserve and structure) and of the environment (concentrations of nutrient and substrate).

Fig. 1 gives the specific fluxes of compounds as functions of the specific growth rate. It also gives the ratio of the carbon dioxide and dioxygen fluxes, and that of ammonia and dioxygen. Many text books deal with these ratios as being proportional to the specific growth rate. This obviously does not apply here.

The result we obtained is that we can relate the yield coefficients and chemical indices of biomass to (varying) concentrations of nutrients in the environment, and to a (varying) reserve density, which involves a number of constant energy-budget parameters. These constant parameters are the specific maintenance rate $k_M$, the reserve turnover $k_E$, the yield of structure on reserve $y_{VE}$, the chemical indices of reserve and structure, and the parameters of the assimilation process. Some text books mention that methanotrophs consume two methane molecules for each produced carbon dioxide molecule. Our analysis shows, however, that such a fixed relationship does not exist; it is very sensitive to environmental conditions.

Methane burning in assimilations' catabolic transformation should generate enough energy to drive assimilations' anabolic component. For the chemical potential $\mu_X$ of methane and $\mu_E$ of reserve, we require $\mu_X j^C_{XA} > (\mu_E - \mu_X) j^A_{XA}$ or $\mu_X(1 - y_{EX}) > (\mu_E - \mu_X) y_{EX}$ or $y_{EX} > \mu_E/\mu_X$.

Notice that ammonia is taken up as well as excreted—a phenomenon that only recently attracted attention in algal physiology. We know a priori that ammonium uptake always exceeds excretion at steady state.

The summary of this exercise is that, given the concentrations of the rate-limiting compounds (here methane, dioxygen, ammonia, and biomass), the DEB theory specifies the rates of the organizing transformations (assimilation, maintenance, and growth), and stoichiometric constraints specify the appearance and disappearance of all compounds that are involved. The rates can be used in the specification of a dynamical system (where the transformations affect the concentrations), such as a batch culture, a chemostat, or as part of a more complex system, that involves other chemical or biological species. It is important to realize that the macrochemical reaction equations are still far away from a detailed chemical description of metabolism. Compounds can be produced in one part of the pathway, and used in another part, and do not occur in the macrochemical reaction equation. The chemical variability of biomass can be increased by delineating more types of reserves. This extension is natural in the context of the DEB theory, but it obviously increases the number of parameters, which should be balanced by a sufficient increase of goodness of fit with experimental data. Such data are frequently not available.

## PREDATION

When one organism eats another one with a chemical composition that can vary, there is a need to deal with conversion efficiencies of prey into predator in a bit more detail than is usual in ecological theory. Things simplify considerably when, as the basic formulation of DEB (dynamic energy budget) theory assumes, biomass can be decomposed into a single reserve and a single structure that do not change in composition. The assimilation process of the predator then should specify how the two components of its prey, together with nutrients from the environment, transform into predator reserves. Think for instance of daphnids feeding on algae. Alga's main carbon component, cellulose, is of no nutritional value for the daphnid. It is the starch and lipids in alga's reserves that are daphnids' main energy sources, while it also needs ammonia and phosphate, for instance, as building blocks. Daphnids can obtain part of these nutrients from the intra-cellular reserves of the alga; sometimes they can also obtain them directly from the environment (Parker and Olsen 1966, Urabe et al. 1997). So the nutritional value of the alga for the daphnids is not a constant, but varies, and depends on environmental conditions.

The implications of a variable nutritional value of the producer $P$ (alga) for the consumer $C$ (daphnid) can be illustrated with the following simple dynamical system:

$$\frac{d}{dt}P = r_P P - j_{PA} C$$

$$r_P = \frac{k_N m_N}{y_{NP} + m_N} \qquad j_{PA} = \frac{j_{PAm} P}{K + P} \tag{1}$$

$$\frac{d}{dt}C = (r_C - h)C$$

$$r_C = \left[\frac{1/r_{CP} + 1/r_{CN} - 1}{(r_{CP} + r_{CN})}\right]^{-1}$$

$$r_{CP} = y_{CP} j_{PA} - k_{MP} \qquad r_{CN} = y_{CN} m_N j_{PA} - k_{MN} \tag{2}$$

where producers' reserve density is $m_N = N/P - n_{NC}C/P - n_{NP}$, for a total amount of nutrient $N$, i.e., all nutrient that is not in producers' or consumers' structure is in producers' reserve. The chemical indices $n_{NP}$ and $n_{NC}$ stand for producers' and consumers' moles of nutrient content per mole of carbon. The amount of nutrient in the environment is taken to be negligibly small. The consumer has a constant hazard rate $h$, and dead producers decompose instantaneously. The producers' reserve turnover rate is $k_N$, and producers' maintenance is neglected. This simplification of the DEB theory amounts to Droop's kinetics (Droop 1974) for the producer (with a very small half-saturation constant, and a very large specific maximum uptake rate). Consumers' reserves are not taken into account. This simplification of the DEB theory amounts to Marr-Pirt's kinetics (Pirt 1965) for the consumer. The Marr-Pirt's kinetics results from the DEB model as a limit for increasing reserve turnover rates. The expression for the growth rate of the consumer follows from the SU kinetics and the assumption that assimilates from producers' reserve and that from structure are complementary and parallelly processed with a large capacity. There is no need to set a maximum to the capacity here, because that is already set by the maximum specific assimilation rate $j_{PAm}$. Notice the SU (synthesizing unit) formalism here deals with rates, rather then concentrations, as is basic to its derivation.

We will also study a model where the consumer consumes the structural part of the producer only. Then the growth rate of the consumer follows a simple Holling type-II functional response, that is $r_C = r_{CP}$. In other words, the consumer is no longer limited by the reserves of the producer.

### *Model analysis*

The two ordinary differential equations (Eqs. 1 and 2) describe the dynamics of the two populations. This autonomous system can be analyzed using dynamical systems theory, see Guckenheimer and Holmes (1985) and Kuznetsov (1998) for an introduction into the general theory and Bazykin (1998) and Kooi and Hanegraaf (2001) for applications to simple food chains and food webs. This theory deals with the dependency of the qualitative long-term dynamical behavior on parameters.

Mathematically critical values where this behavior changes are fixed by the steady-state (or periodic) solutions and criteria that determine the stability properties. These criteria concern eigenvalues of the Jacobian matrix evaluated in the equilibrium (for limit cycles similar criteria hold). Since the producer–consumer system is two dimensional there are two eigenvalues. As a result only a few types of bifurcations are possible. Three ''standard'' types of bifurcations are important here. In a tangent bifurcation point ($T_e$) stable and unstable equilibrium branches coincide and disappear under parameter variation. This point marks a region where an interior equilibrium (i.e., where the equilibrium values of the two state variable $P$ and $C$ are positive) can exist. A transcritical bifurcation point (TC) marks a region where a predator can invade the producer system. In both bifurcation points one eigenvalue is zero. In a Hopf bifurcation point ($H^-$) the real parts of the two complex conjugated eigenvalues are zero. On one side of the critical parameter value these real parts are negative and the equilibrium is stable (saddle focus) and on the other side they are positive and the equilibrium is unstable (saddle source), and the solution converges to a (stable) limit cycle. These points are so-called ''local'' bifurcations since they can be deduced from local information at the point.

We will also deal with a so-called ''global'' bifurcation where the local informations is not sufficient, but orbits have to be followed through the state space. An orbit starting close to an unstable (saddle) equilibrium point that returns after a large excursion through the state space back to the point itself is called a ''homoclinic'' orbit. This can occur with saddle points where one eigenvalue is positive (repelling) and the other is negative (attracting). The critical parameter value marks a homoclinic bifurcation.

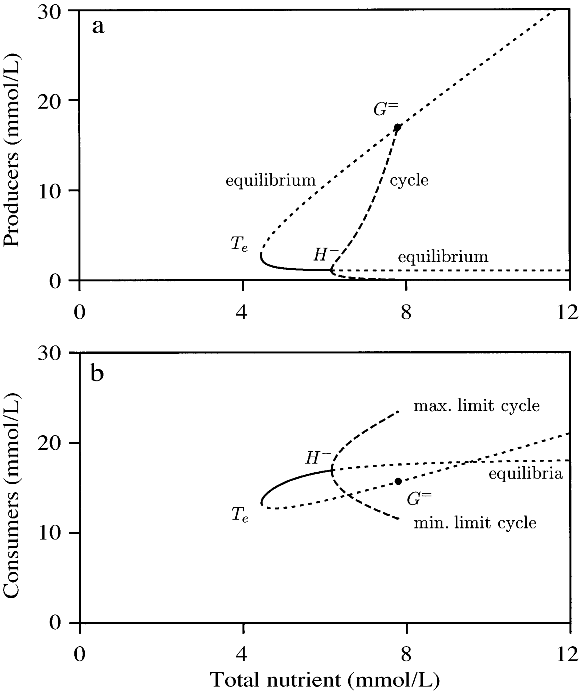

FIG. 2. Bifurcation diagrams of producer–consumer dynamics in a closed system, producers requiring reserves of consumer (i.e., without free nutrient in the environment). The total amount of nutrient is used as the bifurcation parameter. The producer follows Droop's kinetics (Droop 1974), the consumer follows Marr-Pirt's kinetics (Pirt 1965) and has a constant hazard rate. Producers' reserve and structure are complementary for consumers.

At very low nutrient levels, the system cannot exist. At intermediary nutrient levels, the system has a point attractor. A tangent ($T_e$) and a Hopf bifurcation point ($H^-$) mark the boundaries of these intermediary nutrient levels. At larger nutrient levels, the system oscillates with increasing amplitude. A homoclinic bifurcation point ($G^=$) marks the upper boundary of this interval; the system cannot exist at higher nutrient levels. Parameter values are: $h = 0.005$ h$^{-1}$, $n_{NP} = 0.15$ mol/mol, $n_{NC} = 0.25$ mol/mol, $y_{CN} = 0.8$ mol/mol, $y_{CP} = 0.5$ mol/mol, $y_{NP} = 0.15$ mol/mol, $K = 10$ mM, $j_{PAm} = 0.15$ mol·mol$^{-1}$·h$^{-1}$ $k_N = 0.25$ h$^{-1}$, $k_{MP} = 0.002$ h$^{-1}$, $k_{MN} = 0.001$ h$^{-1}$. (See *Predation* for parameter definitions and the system of differential equations.)

A critical parameter value where the long-term dynamic behavior changes under parameter variation is called a bifurcation point in the parameter space. When two parameters are varied simultaneously, these points form a curve in a two-parameter parameter space. They are the boundaries in the parameter space with different qualitative long-term behavior, such as an equilibrium or a limit cycle where the population densities vary in time periodically. Generally these bifurcation points and curves have to be approximated numerically. We used the computer package AUTO (Doedel et al. 1997) to calculate the bifurcation points and curves. The computer package HomCont (Champneys et al. 1996), implemented in AUTO has been used to study the homoclinic bifurcation to a saddle. Fig. 2 shows the asymptotic dynamics of the system (1, 2). We observe that it shows the typical paradox of enrichment (Rosenzweig 1971): the system starts oscillating above a certain nutrient level $H^-$. For higher nutrient levels where the system fluctuates, the larger the nutrient level, the larger the amplitude of the oscillations. At a

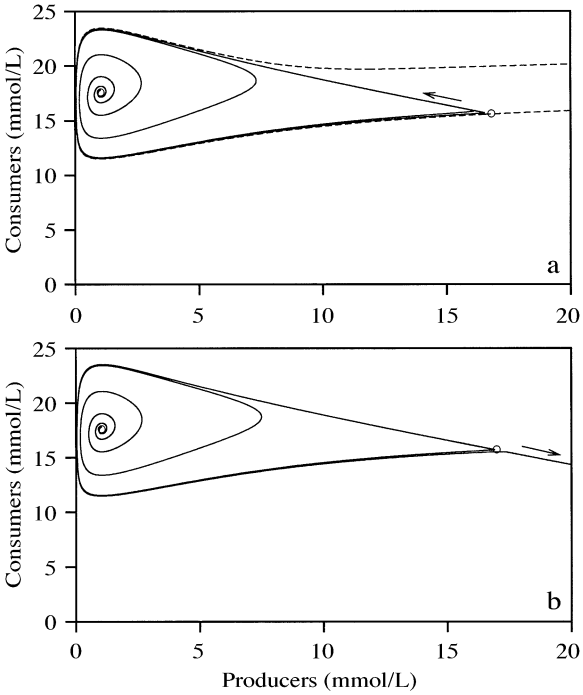

FIG. 3. Orbits of the producer–consumer system of Fig. 2 for nutrient levels (a) just below ($N = 7.775$ mM) and (b) above ($N = 7.825$ mmol/L) the homoclinic bifurcation point at $N = 7.795775$ mmol/L. Orbits that start within the dashed separatrix in (a) result in a stable oscillation (one such orbit is indicated), while other orbits lead to extinction of the consumer. This separatrix breaks open for higher nutrient levels (b), and all orbits lead to extinction of the consumer (one such orbit is indicated). The saddle point (one negative and one positive eigenvalue), and the spiral source (positive real part of the complex conjugate eigenvalues) are indicated. The stable equilibrium where the consumer is absent (point $P = N/n_{NP}$ and $C = 0$) is not shown.

certain nutrient level the peak producer's value approaches the unstable equilibrium value. The critical nutrient level marks a homoclinic bifurcation indicated by $G^{=}$. Fig. 3 gives orbits in the phase plane ($C$, $P$) for nutrient levels just below and above this value. These curves were calculated with the computer package DsTool (Back et al. 1992). Below $G^{=}$ there is a limit cycle with a very large period since the orbit stays for long episodes close to the saddle point. At the bifurcation point this cycle is called a ''homoclinic cycle.'' At this point the cycle breaks up and for nutrients levels above $G^{=}$ the orbit converges always to the stable solution $m_N = C = 0$ and $P = N/n_{NP}$, that is, the consumer goes extinct. Hence, the system has an upper boundary for nutrient, above which it cannot exist. Contrary to standard kinetics, the system has a lower bound for the nutrient level, $T_e$, due to the maintenance costs of the consumer. This is further illustrated by a bifurcation diagram in Fig. 4 for the system (1, 2) with $r_C = r_{CP}$. This diagram resembles that of classical predator–prey models cf. Gurney and Nisbet 1998) and Kooi and Hanegraaf (2001). There are two qualitative effects: both the tangent bifurcation point $T_e$, forming the lower limit, and the homoclinic point $G^{=}$, being the upper limit, disappear. Now the consumer goes extinct at very low nutrient levels by a gradual decrease of the consumer population via a transcritical bifurcation TC. For nutrient levels higher than $H^{-}$ where the system fluctuates, the larger the nutrient level the larger the amplitude of the oscillations. This continues in principle to unbounded nutrient level; however, the producer population in Fig. 4 reveals unrealistic low minima at high nutrient levels where extinction due to demographic stochasticity is likely (Fryxell and Lundberg 1998).

Notice that consumers cannot invade the producer population with a very small inoculum size in the case of co-limitation by producers' reserve, but it can in absence of this co-limitation (compare Fig. 2 and 4). We can conclude that the nutritional details of the producer–consumer interaction affect their kinetics in a qualitative way. Muller et al. (2001) discuss a very similar producer–consumer model, which deviates slightly in the specification of consumers' growth (implementation of maintenance and of maximum growth).

In Fig. 5 the two-parameter bifurcation diagrams for both models are depicted. We use the total amount of

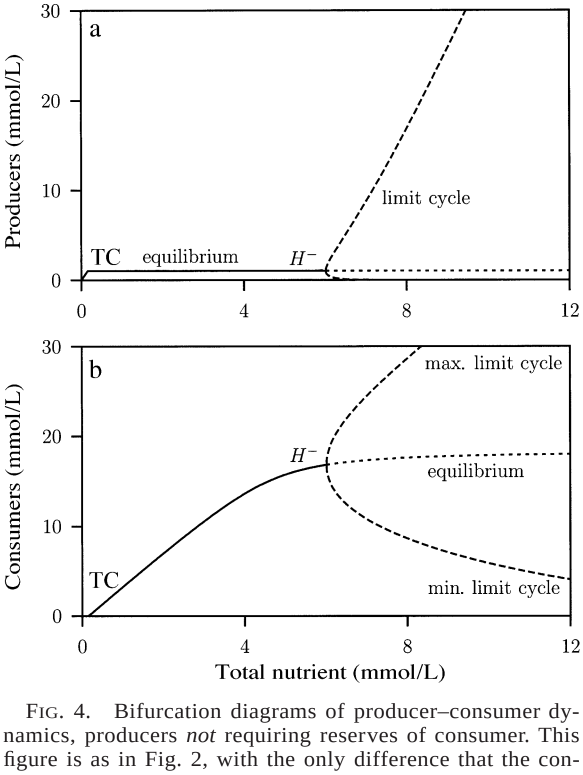

FIG. 4. Bifurcation diagrams of producer–consumer dynamics, producers *not* requiring reserves of consumer. This figure is as in Fig. 2, with the only difference that the consumer is not limited by producers' reserve, so $r_C = r_{CP}$. Notice the absence of the tangent and homoclinic bifurcation points. A transcritical bifurcation point (TC) marks the branching with the trivial equilibrium where producers and consumers are absent. The parameter values used in this bifurcation diagram are the same as in Fig. 2. Producers' minima become extremely small for increasing nutrient levels.

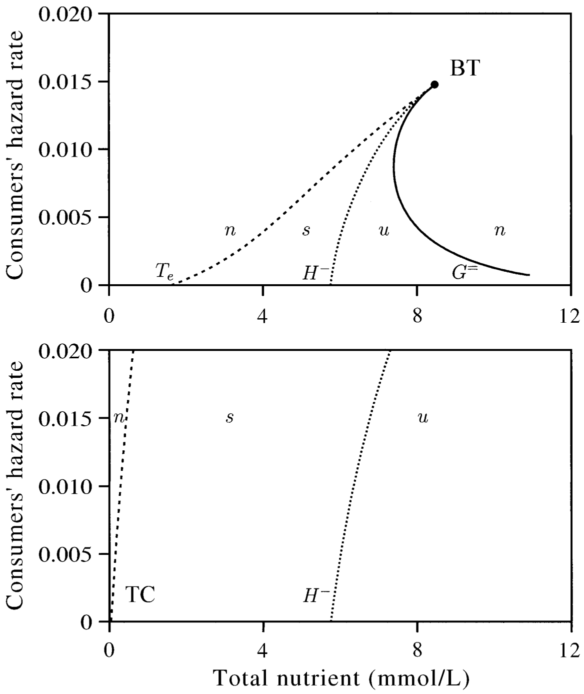

FIG. 5. The two-parameter bifurcation diagram for the producer–consumer system, as in Fig. 2a and in Fig. 4b, using the total nutrient level and consumers' hazard rate as bifurcation parameters. The consumer requires producers' structure *and* reserve (a) or producers' structure only (b). Three areas are indicated: *n*, no coexistence; *s*, stable coexistence; *u*, unstable coexistence (oscillations). (a) When the consumer needs both the structure and energy components of the producer, the tangent ($T_e$), Hopf bifurcation point ($H^-$), and homoclinic point ($G^=$) bifurcation curves meet in a Bogdanov-Takens point (BT). (b) When the consumer needs only the structural component of the producer, the transcritical (TC) and Hopf bifurcation curves diverge, and the region where coexistence is possible (*s*) is much larger.

nutrient $N$ and the consumer specific loss rate (hazard rate, $h$) as bifurcation parameters while all other species-specific parameters are fixed. When the consumer needs both components of the producer the tangent ($T_e$), Hopf bifurcation point ($H^-$), and homoclinic point ($G^=$) bifurcation curves meet in a Bogdanov-Takens point (BT; Kuznetsov 1990). Hence there is an upper limit for the loss rate of the consumers ($h$) where both populations can coexist. When on the other hand the consumer needs only the structural component of the producer, the transcritical (TC) and Hopf ($H^-$) bifurcation curves, diverge and hence region where coexistence is possible is much larger than in the previous case. The curve $G^=$ terminates for a $h$ value where the minimum of the producer population during the homoclinic orbit becomes unrealistically low.

## SYMBIOSIS

Almost all transformations from food into feces plus a little bit of biomass and from maintenance processes release nutrients that can be used by autotrophs for growth. Autotrophs are frequently limited in their growth by nutrients, and have energy (carbohydrates) in excess, which they partly excrete. These excreted carbohydrates can be used by heterotrophs as supplement to their autotroph diet (Muscatine et al. 1983, 1984, 1989). Heterotrophs reuse part of the nitrogen waste, and combine it with these carbohydrates to produce biomass. This reduces their waste production, and so autotrophic growth is reduced as is the subsequent excretion of carbohydrates, a strongly stabilizing feedback mechanism. This reciprocal syntrophy forms the basis of most symbiotic relationships between organisms and easily leads to biomass ratios of the partners that vary only in a narrow range.

Quantitative knowledge of the exchange of compounds is essential to the understanding of production and the maintenance of biodiversity, and of the metabolic organization of cellular physiology; eukaryotes evolved from an increasingly integrated symbiotic consortium of symbiotic organisms. The exchange is generally rather open in a community, where the compounds are excreted into the environment, and their concentrations can vary. The exchange can gradually become more direct and some species seek each other's company, and exchange compounds on a flux basis, with hardly any accumulation in the environment. This can eventually lead to endo-symbiosis (Kooijman et al. 2003).

Let us sketch the quantitative aspects, assuming that a population of pelagic unicellular chlorophytes lives together with a population of ciliates in a spatially homogeneous environment.

### *Autotrophs*

Suppose that for simplicity's sake, photosynthesis $CO_2 + H_2O + \text{light} \rightarrow CH_2O + O_2$ is only limited by carbon dioxide, $CO_2$. Carbohydrate $CH_2O$ is both energy and carbon source for the autotroph. We delineate two reserves, carbon reserves $E_C$ and nitrogen reserves $E_N$, which are taken to be generalized compounds. The assimilation rate of both types of reserves $E_C$ and $E_N$, depends hyperbolically on the concentrations of carbon dioxide and ammonia, respectively.

The use of the reserves, the catabolic rates, are first-order processes on the basis of reserves densities, i.e., ratios of the reserves and the structure. After subtraction of the maintenance costs, which are proportional to the amount of structure, both catabolic fluxes arrive at the synthesizing units (SU) for the synthesis of structure, $V$. We are, therefore, dealing with the transformation

$$y_{E_CV}E_C + y_{E_NV}E_N \rightarrow V + \text{minerals}.$$

As will be explained below, the application of SU rules for the synthesis of structure from the two reserve fluxes leads to the specific growth rate:

$$r = \left\{ \sum_{i\in\{C,N\}} \left[\frac{m_{E_i}(k_E - r) - j_{E_iM}}{y_{E_iV}}\right]^{-1} - \left[\sum_{i\in\{C,N\}} \frac{m_{E_i}(k_E - r) - j_{E_iM}}{y_{E_iV}}\right]^{-1}\right\}^{-1}$$

where $m_{E_i} = M_{E_i}/M_V$ is the reserve density $i$, $k_E$ is the turnover rate of the reserves (which are taken to be equal here), and $j_{E_iM}$ is the structure-specific maintenance costs for reserve $i$. This equation for the specific growth rate is implicit; the growth rate also occurs at the right-hand side because of the ''dilution by growth'' the reserve density not only decreases by the use of the reserves, but also by the increase of the structure. Mathematically, as well as numerically, implicit equations do not pose extra problems, relative to explicit ones.

The flux $m_{E_i}(k_E - r)$ represents the catabolic flux; the flux $m_{E_i}(k_E - r) - j_{E_iM}$ arrives at the SUs for the synthesis of structure, and the flux $(m_{E_i}(k_E - r) - j_{E_iM})/y_{E_iV}$ has the role of $j_i'$ in the general expression for the production rate of the SU. We assume that the binding probabilities equal 1. Since the reserve dynamics implies an upper bound for the reserve density, and so an upper bound for the catabolic flux, we assume that the maximum production capacity, $J_m$, for structure is large enough to avoid co-limitation of the growth of structure.

The upper bound for reserve densities depends, however, on the fate of the reserves that are rejected by the SUs for growth. If all rejected reserves are fed back to the reserves, the upper bound can disappear for the nonlimiting reserve. To avoid such a situation, excretion of (some of the) rejected reserves is essential. We assume that a fixed fraction, $1 - \kappa_{E_i}$, of rejected reserves is excreted, the remaining fraction is fed back to the reserve from where the flux originated. If this excreted fraction $1 - \kappa_{E_i}$ is close to 0, nonlimiting reserves can accumulate to high levels; so high levels for the nonlimiting reserve can be expected for low growth rates, and thus low levels of the limiting reserve. The technical process of biological phosphate removal makes use of this phenomenon, for instance. If the fraction is close to 1, we have the opposite effect: nonlimiting reserve increases with the growth rate.

The dynamics of the reserve density $i$, for $i \in \{C, N\}$, now amounts to

$$\frac{d}{dt}m_{E_i} = j_{E_iA} - (1 - \kappa_{E_i})(k_E - r)m_{E_i} + \kappa_{E_i}(j_{E_iM} + y_{E_iV}r) - rm_{E_i}$$

where $j_{E_iA} = j_{E_iAm}(1 + K_i/X_i)^{-1}$ is the specific assimilation for reserve $E_i$, with half-saturation constant $K_i$ and nutrient concentration $X_i$ in the environment. The derivation uses the specific catabolic flux $(k_E - r)m_{E_i}$, which follows for the first-order kinetics, while a fraction $\kappa_{E_i}$ of the rejected specific catabolic flux $(k_E - r)m_{E_i} - j_{E_iM} - y_{E_iV}r$ is fed back to the reserve. The last term, $rm_{E_i}$, represents the dilution by growth. The excretion, therefore, amounts to

$$j_{E_iE} = (1 - \kappa_{E_i})[(k_E - r)m_{E_i} - j_{E_iM} - y_{E_iV}r].$$

This is the full excretion flux for carbohydrates, but for ammonia (and other 'minerals') we also have excretion fluxes that are associated with maintenance and growth (in the overhead costs for growth). The fluxes are fully determined by the conservation laws for mass, and do not require any extra assumptions to specify their size. The total specific ammonia excretion is given by

$$j_{NE_+} = (1 - \kappa_{E_N})(k_E - r)m_{E_N} + \kappa_{E_N}(j_{E_N,M} + y_{E_NV}r) - n_{NV}r.$$

This result follows from the expression for $-d/dt\, m_{E_N}$ where we take $j_{E_NA} = 0$, ignore the term for dilution by growth, $rm_{E_N}$, and then subtract the flux that is fixed in new structure, $n_{NV}r$.

This mechanism allows growth, for example, in an ocean where light and carbon, but no nutrients, are available at the surface, and nutrients, but no light, at the bottom of the mixing layer, while the cells move up and down in wind-driven circulations. At each location, growth conditions are far from optimal, but, thanks to the wind, growth can be considerable.

### *Heterotrophs*

Apart from assimilation, the metabolic organization of heterotrophs is in fact the same as that for autotrophs, but we usually need not delineate more than one reserve (again taken to be a generalized compound), due to the limited variation of its chemical composition. When a heterotroph feeds on an autotroph, it eats its structure, but also its reserves. There is usually no reason to believe that excreted autotrophic reserves cannot be taken up as well by the heterotroph. The nutritional value of autotrophic reserves for the heterotroph usually exceeds that of autotrophic structure, which frequently contains a lot of cellulose. This is the reason why total organic carbon is a poor measure for the amount of food for the heterotroph.

Heterotrophs' specific growth is given by

$$r = \frac{m_E(k_E - r) - j_{EM}}{y_{EV}} \Rightarrow r = \frac{m_E k_E - j_{EM}}{m_E + y_{EV}}$$

and the dynamics of its reserve density is

$$\frac{d}{dt}m_E = j_{EA} - k_E m_E.$$

These dynamics are the same as for the autotroph for $\kappa_{E_i} = 0$, and no colimitation by two reserves. The catabolic fluxes are obtained for full excretion of rejected reserves, $\kappa_E = 0$, although hardly any reserve will be rejected because of absence of co-limitation and a large growth capacity. There is no need to model excretion in this case, because we can and should allow for overhead costs of growth and maintenance; explicit modeling of excretion is only necessary to deal with the dynamics of more types of (independent) reserves.

Heterotrophs' assimilation is more difficult to model. We will assume that, apart from the autotroph, another organic substrate $X$ is available that contains nitrogen, which can serve as an alternative food source for the heterotroph. This organically bound nitrogen is not available for the autotroph, and forms the basis of a symbiotic relationship between the autotrophs and the heterotrophs.

The specific assimilation flux can be found from the application of the rules for the behavior of SUs, and written in terms of contributions by the organic substrate and autotrophic structure and reserves, and excreted autotrophic reserves in the environment:

$$j_{EA} = \left( \frac{\sum_i j'_{EA_i}}{\sum_i j'_{EA_i} j_{EA_i m}} + \frac{1}{\sum_i j'_{EA_i}} \right)^{-1} \quad \text{for } i \in \{X, V, E\}$$

$$j'_{EA_X} = y_{EX} b_X X \qquad j'_{EA_V} = y_{EV_A} b_{V_A} X_{V_A}$$

$$j'_{EA_E} = [j_{EA_C}^{-1} + j_{EA_N}^{-1} - (j_{EA_C} + j_{EA_N})^{-1}]^{-1}$$

$$j_{EA_i} = y_{EE_i} \left( m_{E_i} \frac{j'_{EA_V}}{y_{EV_A}} + b_{E_i} X_{E_i} \right) \quad \text{for } i \in \{C, N\}$$

where $V_A$ refers to the structure of the autotroph, and $E$ (without index) to the reserve of the heterotroph. The parameters $b_X$, $b_{V_A}$, $b_{E_C}$, and $b_{E_N}$ combine a meeting and a binding probability. Although the expressions already look complex enough, they are perhaps too simplistic from a metabolic point of view, because autotrophs' structure and reserves might be partially complementary for the heterotroph. Theory for such mixtures are discussed in Brandt (2002).

The assimilation efficiencies $y_{EV_A}$ and $y_{EX}$ imply the presence of products (e.g., feces). For simplicity's sake, we will assume that these products are mineral. The heterotroph excretes minerals, just like the autotroph, in association with assimilation, maintenance, and growth. The excretion products represent an important feedback to the autotroph. Careful bookkeeping shows that the total specific excretion of ammonia amounts to

$$j_{NE_+} = j_{V_A A}(n_{NV_A} + m_{E_N}) + \alpha_N \alpha_E j_{EA} n_{NE} + j_{XA} n_{NX} + (k_E - r) m_E n_{NE} - j_{EA} n_{NE} - r n_{NV_H}$$

where

$$\alpha_E = \frac{j_{EA_E}}{j_{EA_X} + j_{EA_V} + j_{EA_E}}$$

is the fraction of the assimilation of heterotrophic reserve from autotrophic C reserve, and

$$\alpha_N = \frac{b_{E_N} X_N}{m_{E_N} j_{EA_V} / y_{EV_A} + b_{E_N} X_N}$$

is the fraction of ammonia relative to ammonia plus consumed autotrophic N reserve. The first three terms represent the processed fluxes of nitrogen (structure plus N reserve), ammonia, and substrate. The next three terms represent the catabolized nitrogen from the reserves, and the fixed nitrogen in the reserve and in the structure.

### *Syntrophic interactions*

Before we set up the dynamics of the autotroph–heterotroph system, we note that all ingredients for the symbiosis are already present; the autotroph excretes carbohydrates, which can be used for energy purposes by the heterotroph, while the heterotroph excretes ammonia, which can be used by the autotroph. Part of this ammonia originates from the organic substrate, which was not accessible for the autotroph, without interference by the heterotroph.

The uptake and use of substrates by the autotroph and the heterotroph being determined, we now are ready for chemostat dynamics, given a throughput rate $h$ and concentrations of ammonia $X_N$ and substrate $X$ in the feed. The specific growth rates equal the throughput rate at steady state (if it exists). Assuming that carbon dioxide and light are not limiting, the specific growth rate of the autotroph at steady state reduces to

$$r = \frac{m_{E_N} k_E - j_{E_N M}}{m_{E_N} + y_{E_N V}}$$

which implies that the N-reserve density amounts to $m^*_{E_N} = (y_{E_N V} h + j_{E_N M})/(k_E - h)$. This is only positive if $h < k_E$. Similar results apply to the heterotroph. It is not sure at all that a steady state exists; however, it can oscillate, behave chaotically, or one or both partners might wash out. Fig. 6 shows that the ratio of the steady-state amounts of auto- and heterotroph structures hardly changes for ranges of ammonia and substrate concentrations and a given set of parameter values. It is only a small step to a constant ratio, which allows the structures to be taken together in a single new one (mixotroph). The amount of variation of the ratio depends on parameter values; to change parameters and study the effect on steady states, one can use the software package DEBtool.[4]

The transition from herbivory to symbiosis can easily be made by the herbivore (i.e., heterotroph) if the yield coefficients $y_{EV}$ are small (which means that the heterotroph hardly gains from consuming autotrophs' structure), and the half-saturation constants $K_{E_C}$ and $K_{E_N}$ are small (which means that the heterotroph can push autotrophs' excreted reserve concentrations to very low levels). A direct physical contact between the partners facilitates the exchange of material.

The transition from symbiosis to mixotrophy is automatic, if the ratio of the amounts of structural masses of both partners no longer change as functions of the nutrient and substrate concentrations in the environ-

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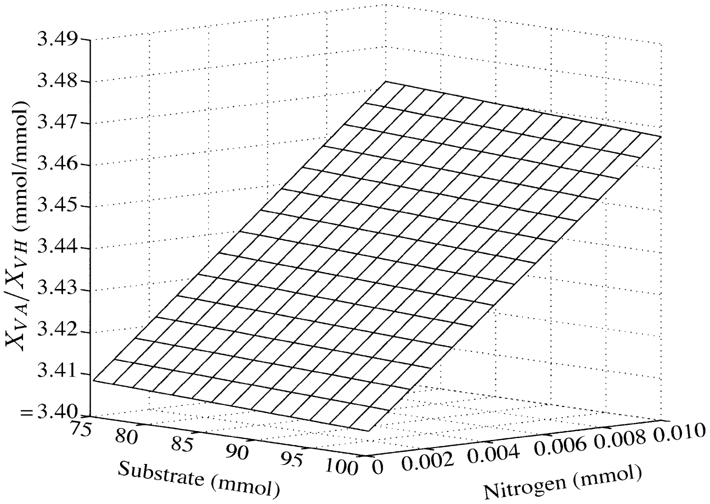
[4] URL: ⟨http://www.bio.vu.nl/thb/deb/⟩

FIG. 6. The ratio of the steady-state amounts of the structure ($V$) of symbiotic auto- (A) and heterotrophs (H) in a chemostat as function of substrate and nitrogen concentrations in the feed at a fixed throughput rate (of 0.05 $h^{-1}$). Notice that this ratio varies only within the range 3.41–3.47, while the steady-state amount of the heterotroph varies in the range 19–26 mmol/L (not shown).



ment. The quantitative details of this transition is at this moment under study by us within the context of the dynamic energy budget (DEB) theory, and is fundamental to evolutionary consistency. The interaction of mixotrophs with their environment is discussed in Kooijman et al. (2002).

Part of the specific costs for growth relate to the synthesis of the assimilatory machinery, so the specific growth costs for the mixotroph are expected to exceed the mean specific growth costs of both partners. This points to the evolutionary ''advantages'' of specialization, a process that can in principle be captured within the theory of adaptive dynamics (Metz et al. 1996, Geritz et al. 1997, 1998, 2002). The understanding of environmental conditions that select for specialization and those that select for mixotrophy is of importance to understand the evolution of prokaryotes. It might well be possible that mixotrophs can co-exist with auto- and heterotrophs, and that mixotrophs can invade in an autotroph–heterotroph community and vice versa.

## DISCUSSION AND CONCLUSIONS

The present approach can be extended to include more realistic situations. One example is an individual–structure in population dynamics, which results from particular surface-area-to-volume relationships, where assimilation is linked to individuals' surface area, and maintenance to volume. This also affects reserve dynamics, via a size-dependent turnover rate. Other examples include a spatial structure, and the process of aging, and decomposition of biomass.

Several authors have shown that stoichiometric constraints on the consumer level introduce qualitatively new dynamics in simple autotroph–herbivore models (e.g., (Andersen 1997, Loladze et al. 2000, Grover 2002)). Stoichiometric constraints imply a hump-shaped herbivore null cline, which again allows for additional stationary states, including the possibility of deterministic extinction of the herbivore population through a homoclinic bifurcation similar to that in Fig. 3. The models of Andersen (Andersen 1997) and Loladze et al. (Loladze et al. 2000) involved a piece-wise linear ad hoc formulation on the stoichiometric effect of autotroph nutrient content on herbivore growth. The first-order discontinuities implied by piece-wise linearity will obviously limit the domain of local stability analysis. In contrast, the present model is based on mechanistically well-founded principles (synthesizing units), with the desirable property of being analytical everywhere.

The exchange of carbohydrates against nutrients, as has been discussed earlier, also applies to plants, where the root plays the role of the heterotroph, and the shoots that of the autotroph. This results in a ratio of the masses of shoots and roots that respond dynamically to changes in the environment in a ''natural'' way, using a relatively small number of parameters. The model plant compensates for suboptimal growth conditions by enhancing relative investments in roots or shoots, without using any optimality argument; light reductions can lead to larger shoot: root ratios, for instance, whereas nutrient or water reductions can have the opposite effect.

Nutrient recycling dominates the fate of an ecosystem, and especially that of rather closed ones. Systematic theoretical work on the relationships between ecosystem structure and function is still in its childhood. In closed, spatially homogeneous systems, canonical communities (Kooijman and Nisbet 2000), i.e., simple three-species communities of consumers, producers, and decomposers, and single-species mixotroph communities (Kooijman et al. 2002) seem to share a gross feature of the steady-state distribution of nitrogen as a function of the total carbon in the system: dissolved inorganic N decreases proportional to total C (see Fig. 7). Single-species mixotroph communities are obviously less plastic than canonical communities, and the steady-state distribution of C as function of the total

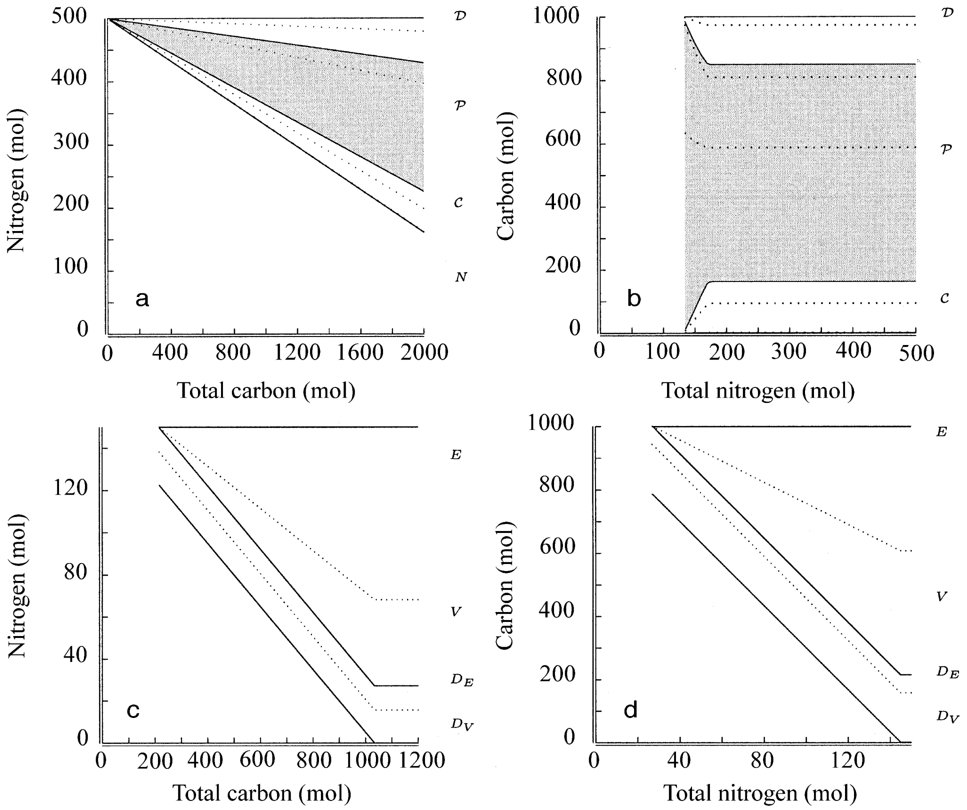


FIG. 7. The steady-state distribution of carbon and nitrogen in a three-species canonical community (panels a and b) and a single-species mixotroph community (panels c and d) while increasing the total amount of carbon (a and c) or nitrogen (b and d). Both communities are spatially homogeneous, open for energy, closed for mass, and have constant light. The amounts of carbon and nitrogen are plotted cumulatively, from bottom to top.

The top panels show minerals [(a) dissolved inorganic nitrogen, N or (b) dissolved inorganic carbon, C (very small, not labeled], detritus (very small, not labeled), consumers $\mathcal{C}$, (structure and reserve), producers $\mathcal{P}$ (structure, C- and N,C-reserves, gray shaded), and decomposers $\mathcal{D}$ (structure and reserve). The producers have three carbon components and two for nitrogen, because one reserve lacks nitrogen.

The bottom panels show minerals [(c) dissolved inorganic nitrogen or (d) carbon], detritus (dead structure $D_V$ and reserve $D_E$), and biomass (living structure $V$ and reserve $E$). Modified from Kooijman and Nisbet (2000) and Kooijman et al. (2002).

N in the system is rather different. A lot of work still has to be done to determine the sensitivity of results like these for choices of parameter values.

These analyses show that homeostasis has far-reaching implications for the coexistence of organisms and for transformation efficiencies. These effects can only be understood with the help of models of sufficient resolution. These models should minimally include the (changes in) the chemical composition of biomass. Although it is essential to keep the models as simple as possible, models that are too simple miss the point.

More information about the DEB research program and its results can be found at the home page of the Department of Theoretical Biology of the Free University in Amsterdam, The Netherlands. You can download the software package DEBtool and use it to analyze the symbiosis dynamics numerically (see footnote 4).

## ACKNOWLEDGMENTS

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