Limnol. Oceanogr., 53(4), 2008, 1685–1696

Excess carbon in aquatic organisms and ecosystems: Physiological, ecological, and evolutionary implications

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Abstract

Cells and organisms, both autotrophs and heterotrophs, commonly face imbalanced access to and uptake of elements relative to their requirements. C is often in excess relative to key nutrient elements like N or P in photoautotrophs. Likewise, one of the lessons from ecological stoichiometry is that the growth of consumers, especially herbivores and detritivores, is commonly limited by N or P such that they also experience C in excess in relative terms. "Excess" implies wastage, yet this definition, which is consistent with purely stoichiometric arguments, is by no means straightforward. In fact, many organisms put this apparently surplus C to good use for fitness-promoting purposes like storage, structure, and defense or mutualistic goals like symbiosis. Nevertheless, genuine excesses do occur, in which case the remaining "leftover C" must be disposed of, either in organic or inorganic form via increased metabolic activity and respiration. These fluxes of C in various forms have major effects on the C balance of organisms, as well as governing the energy flux and C pathways at the ecosystem level. We here discuss evolutionary and ecological implications of "excess C" both at the organism and ecosystem level.

Carbon, the basic building block of organic matter itself, is often nonlimiting for the growth of aquatic autotrophs, although in some instances limitation is by light, which leads to restricted supply of fixed C. It should also be noted that enhanced supply of inorganic C in the form of CO_2 can lead to increases in photosynthesis (Riebesell et al. 2007). Primary production is usually controlled by nutrient elements such as N, P, Si, or Fe. Whereas the growth of consumers is believed to be commonly limited by their net rate of intake of food (generally quantified in units of C), limitation may in fact be by various aspects of food quality. Zooplankton production can, for example, be stimulated by dietary addition of nutrient elements (Sterner and Elser 2002) or specific biochemicals such as essential fatty acids (Müller-Navarra et al. 2004). C must then be in excess. The advance of stoichiometric theory has bolstered these findings, high P : C and N : C ratios in consumers imposing nutritional constraints on growth (Hessen 1992; Elser et al. 2000). P and N are the essential building blocks for RNA and protein, respectively. Hence the rate of protein synthesis—and thus growth rate—is mutually regulated by these elements. Liebig-style, stoichiometric theory can be used to calculate which dietary substrates are in shortest supply relative to demand, showing the potential for limitation by nutrient elements (Anderson and Hessen 1995, 2005). The whole concept of limitation is, however, fraught with difficulty given the complex metabolism of organisms, the multitude of factors contributing to their nutrition at different stages of their life cycles, the combined effects of quantity and quality in limiting growth, and the variability in the nutritional environment.

At a simpler level, one may think instead of nutrition in terms of obtaining a balanced diet that contains the right mix of C-rich substrates for energy and protein-rich material for growth (Kleppel 1993). In humans, the adverse effects of consuming too many C-rich foods, inducing conditions such as obesity, are all too familiar.

But what of aquatic organisms, many of which have a limited storage capacity for C? When faced with unbalanced supply relative to somatic demands, e.g., in the case of phytoplankton undertaking photosynthesis under high light but low mineral P, or grazers consuming C-rich prey, excess C must be returned to the environment. For metazoans, this C may be ingested but subject to enzymatic discrimination in the gut, leading to high defecation of C-rich compounds (DeMott et al. 1998). If assimilated, it may be either released in dissolved organic form (Darchambeau et al. 2003) or as respiration decoupled from other metabolism (Trier and Mattson 2003).

Stoichiometric theory is based on the Liebig minimum principle, where there is a single limiting nutrient, with everything else in excess. The concept of optimum nutrition is little different, with deviations away from a balanced diet being regarded as unfavorable for organisms. In reality, matters are not so clear cut. Even when C is in excess relative to P, a consumer may also suffer deficiency of energy or C, as is seen in growth rate responses in *Daphnia* fed algae along a gradient of C : P and at different levels of food quantity (Fig. 1). With decreasing food quantity, growth is reduced as a consequence of less substrate being available either to provide energy or as building blocks for growth. This reduction of growth with increasing C : P for

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Acknowledgments

This review has benefited from collaborative projects and discussions within an international group of colleagues financed by the Centre for Advanced Study at the Norwegian Academy of Science and Letters. This manuscript has been improved by comments from two anonymous reviewers.

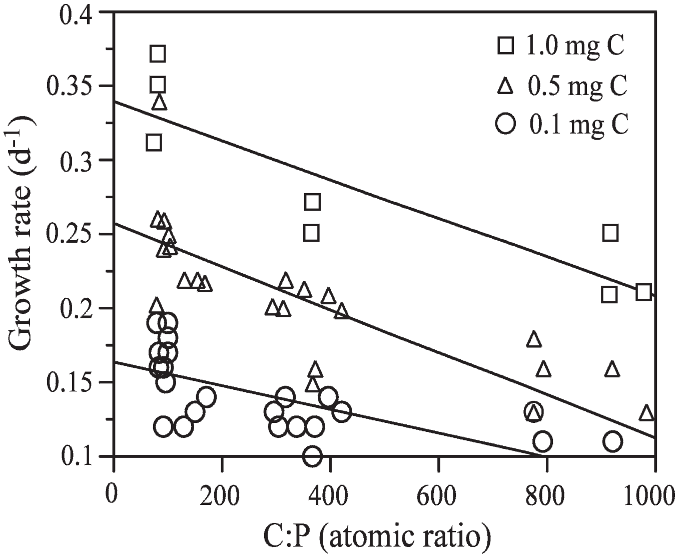

Fig. 1. Growth rate of *Daphnia magna* as related to food quantity (mg L^{-1} C of *Selenastrum capricornutum*) and C : P ratio of food (data from Hessen et al. 2002).

any given food concentration reflects that an increasing fraction of ingested C must be disposed of and goes to waste as access to P becomes the limiting factor. P deficiency at high C : P in food thus causes an extra toll on growth due to reduced growth efficiency—adding to the effects of suboptimal food quantities.

Plants and animals do of course require nutrients and C for basic somatic growth. Carbon also serves many other functions in organisms, providing an outlet for C that would otherwise be in excess. In the case of phytoplankton, for example, C may be used for the production of mucilage, spines, or toxins that act as deterrents to grazers (Tollrian and Harvell 1999). Indeed, one might argue that strict wastage of C might be expected to be a rare phenomenon from an evolutionary point of view. In the event that C-rich compounds are utilized for various fitness-promoting purposes, they are not then excess in a formal sense. Thus, a precise definition of "excess C," and thereby the concept of balanced nutrition, needs to consider not only the requirements of somatic growth but also the other potential fitness-improving benefits offered by C-rich compounds.

The way in which organisms process C has far-reaching implications for food-web function and the fate and sequestration of C in ecosystems (Hessen et al. 2004). For example, Cebrian (1999) made the intriguing discovery that autotroph stoichiometry governs the accumulation and burial of refractory detritus in both aquatic and terrestrial ecosystems. In communities with palatable plants (low C : P or C : N) subject to high turnover rates, a high share of production is channeled into herbivores, rather than being sequestered in detritus. Aquatic autotrophs, particularly planktonic algae, have comparatively high turnover, low C : N and C : P ratios, and high C transfer efficiency to herbivores relative to terrestrial ecosystems, a consequence of the greater need for C-rich structural matter in the latter (Elser et al. 2000). Heterotrophs living in aquatic ecosystems nevertheless commonly face excess C, especially when judged from the imbalance in C : P between resources and cellular composition (Fig. 2). The fate of this excess C, in autotrophs as well as heterotrophs, has major effects on structure and function both within and between trophic levels.

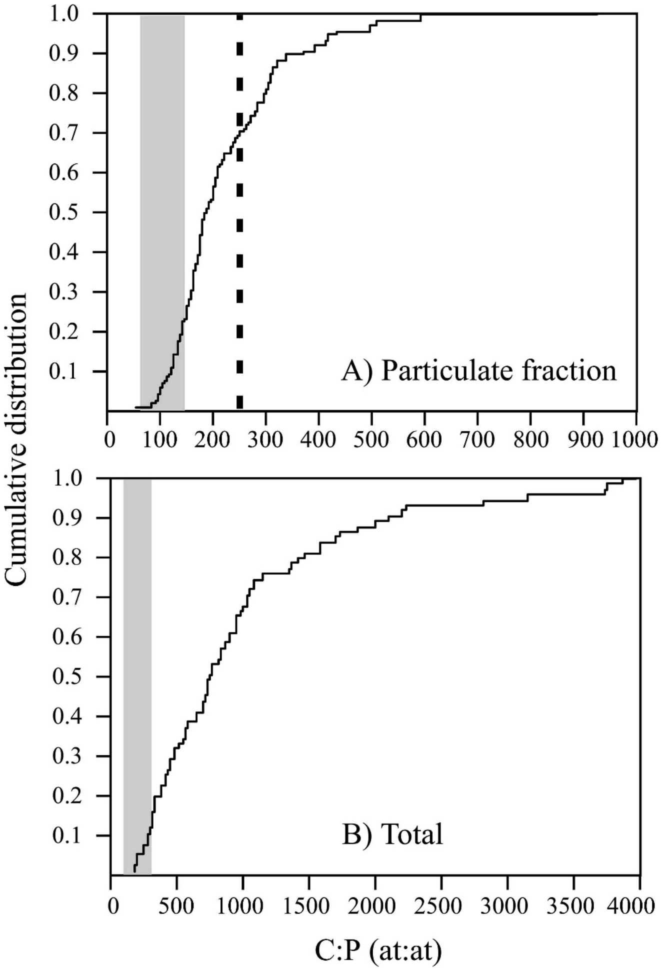

Fig. 2. (A) Cumulative frequency of sestonic C : P and (B) total organic carbon (TOC) : total P in Norwegian lakes (average of 4 samples in 125 lakes). Shaded bars represent span of reported C : P in zooplankton (Andersen and Hessen 1991) and heterotrophic bacteria (Vadstein 2000), and broken line indicates suggested threshold for P limitation in *Daphnia* (Anderson and Hessen 2005).

In this review, we examine the flux and fate of excess C in aquatic phytoplankton, heterotrophic bacteria, and metazoans, with emphasis on the latter group. The very concept of excess C will be subject to scrutiny, given both inherent problems in the concept of limitation itself, and also the evolutionary adaptations of organisms to utilize all substrates at their disposal for fitness-promoting purposes. Resulting implications for ecosystem processes and overall ecosystem C balance will be highlighted.

The concept of optimal nutrition

In nature, consumers are commonly limited by their access to food. Setting this aspect of nutrition aside and focusing on food quality, an optimal, or balanced, diet is, in general terms, one that maximizes the growth or fitness of organisms. In the aquaculture and farming industries, for example, considerable effort has been put into identifying ideal mixes of growth supplements to improve yields. Only rarely, however, were limiting factors invoked when interpreting experimental results (e.g., Fox et al. 1995), particularly in aquatic organisms. Yet in the pure sciences stoichiometry, underpinned by the concept of individual components of diet being limiting, rose to the fore. Given the assumption of homeostasis, then a single substrate (usually C, N, or P) is, at least theoretically, limiting production at any one time, excesses in others having to be dealt with. Limiting elements should consequently be used with high efficiency and thus with minimal release. Experimental studies involving bacteria and zooplankton did indeed show that as the concentration of nutrient elements was decreased relative to C in food substrates, regeneration of nutrients decreased to near-zero rates (Olsen et al. 1986; Goldman et al. 1987), indicative of nutrient element limitation.

The identification of limiting factors is thus central to stoichiometric theory. Threshold elemental ratios (TERs) can be determined that define the crossover from one limiting substrate to another (e.g., Anderson and Hessen 1995, 2005). In this context, perhaps the best definition of optimal nutrition (the optimum occurring at the TER) is a diet that gives rise to minimal disposal of elements such as C, N, and P as waste products. Is, then, C in excess when food offers high C-to-nutrient ratios? The evidence, both observational and theoretical, points to this being so in many instances. Phytoplankton, for example, are exposed to high-light, low-nutrient conditions throughout the vast oligotrophic gyres of the open ocean, often excreting dissolved organic carbon (DOC) into the surrounding environment in a manner that could be interpreted as an excess of photosynthesis over what can be utilized for growth (Berman-Frank and Dubinsky 1999). Similarly, experimental studies showing growth responses to high-nutrient prey (e.g., DeMott et al. 1998), theoretical calculation of TERs (e.g., Anderson and Hessen 2005), and more advanced models that explicitly consider the maintenance requirements of organisms (e.g., Kuijper et al. 2004; Anderson et al. 2005) have shown the potential for C to be in excess in aquatic zooplankton. Heterotrophic bacteria also release surplus elements in a stoichiometrically predictable manner, acting as mineralizers or consumers of nutrients depending on the C-to-nutrient ratios in culture media (Goldman et al. 1987; Tezuka 1990).

The case for nutrient limitation, and hence carbon excess, in zooplankton and bacteria appears to be self evident given that these organisms are often approximately homeostatic with relatively (with respect to their food) high N and P compared with C in their biomass (Andersen and Hessen 1991; Chrzanowski et al. 1996). Indeed, by definition the old adage "you are what you eat" does not hold under homeostasis since any excess intake of C or other elements will necessarily be disposed of. In reality, however, an optimal diet does not necessarily equate to the composition of body tissues. How many people, for example, would prefer a meal consisting of a solitary T-bone steak as compared with a mix of meat and vegetables, perhaps followed by a sticky (C-rich) dessert? Body composition is thus not the only factor that determines optimal nutrition—the demands of metabolism also need to be taken into account. For example, given the low C gross growth efficiencies of marine bacteria (del Giorgio and Cole 1998) and zooplankton (Straile 1997), one could instead argue that limitation by C is a likely outcome, with nutrient elements rather than C being in excess (Anderson and Hessen 1995).

In many ways this is a "chicken-or-egg" discussion since in fact the low C growth efficiencies seen in consumers may often be a consequence, rather than cause, of nutrient limitation, in which case they can only be used in stoichiometric calculations with care. Recent stoichiometric models have moved away from using empirical growth efficiency parameters, replacing them with process-based descriptions of maintenance and addressing terms such as protein turnover and basal metabolism directly (Kuijper et al. 2004; Anderson et al. 2005). With this progress has come a realization of the importance of nutrient elements in maintenance and the theoretical case for nutrients acting as limiting factors has been re-emphasized (Kuijper et al. 2004; Anderson et al. 2005).

It is also important to note that the concept of excess C generally relates to growth. Different ontogenetic stages may impose different associated nutritional demands in organisms. Juveniles tend to have higher demands for P because of their high growth rates (and thus high demands for P-rich RNA) and are thus more likely to experience excess C than adults or senescent individuals. As a consequence, although population growth rate may be constrained by high C : P in food (i.e., food quality), maintenance of population density depends more on food quantity. Chemostat experiments in which phytoplankton quantity and quality were regulated by light intensity (high light yields high biomass and high C : P and vice versa for low light) demonstrated that whereas low-light treatments result in fast population growth of *Daphnia* due to high food quality (i.e., low C : P), highest standing stocks of consumers are established in high light, even if these treatments had algal C : P far above the consumers' demands for balanced growth (Andersen et al. 2007). This finding illustrates that the although high growth rate and reproduction require a diet that is stoichiometrically balanced, a population of individuals requiring substrates only for maintenance can be adequately sustained on a plentiful diet of nutritionally deficient food.

Taking advantage of excess C

A simple dichotomy is seen in traditional stoichiometry whereby C is either used for balanced growth or is in excess. In reality, however, much of the C that exceeds the demands for somatic growth may be put to various fitness-promoting uses by organisms. Principally, the benefits of excess C may be either as structures and structural defenses, storage, virus repellents, protective metabolites (toxins), energy storage and energy use, heat gain, mutualism, and symbiosis, or more subtle mechanisms leading to effects at the ecosystem

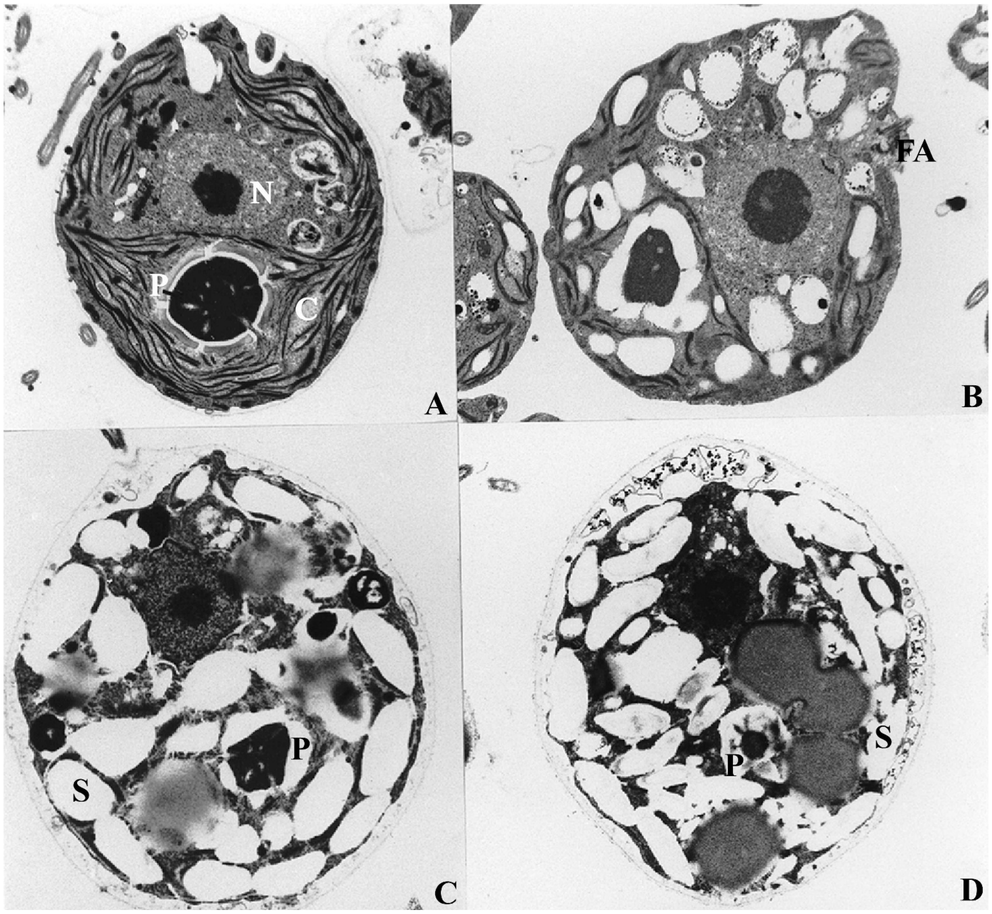

Fig. 3. Transmission electron microscope (TEM) photos of different strains of *Chlamydomonas reinhardtii*. (A) Nutrient-saturated cell at magnification of ×8,800. (B) Nutrient-saturated, cell-wall-deficient mutant at ×8,480. (C) P-limited cell at ×7,000. (D) N-limited cell at ×8,500. Abbreviations: C = chloroplast, FA = flagellar apparatus, N = nucleus, P = pyrenoid, S = starch granulae (from Van Donk et al. 1997).

level. In this context, we define "excess C" as that C that is surplus to the demands of somatic growth, which could be allocated to other fitness-promoting uses or released as waste (evolution will of course act on both somatic growth and other fitness-promoting uses of C simultaneously, in which case the notion that somatic growth takes priority is a supposition purely for the theoretical analysis of C budgets). Thus, surplus C in the food relative to the needs for balanced growth is not necessarily a burden on organisms. If, however, it cannot be profitably used by organisms, then we deem this C to be leftover C (or futile C), i.e., true wastage, which must be returned to the environment to maintain consumer homeostasis.

The growth of organisms is a function of their internal nutrient status because they can only operate on the basis of nutrients that they have acquired (Ågren 1988). Storage thus provides a means of ensuring an ongoing balance of substrates for growth and metabolism, buffering against nutritional imbalances in a changing environment. Whereas storage (obesity) is usually seen as being unwelcome in humans, it is generally fitness-promoting in the natural world (as it once was for humans, too). It must not therefore be seen as an evolutionary malfunction, but, to the contrary, as a means of usefully sequestering C that, at least according to simple stoichiometric calculations, is temporarily in excess.

Storage of carbon is widespread in phytoplankton, serving multiple roles in terms of energy supply and buoyancy control. Actively photosynthesizing algal cells can store carbon under conditions where fixation rates exceed the intake of essential nutrients required for growth (Myklestad 1974; Guerrini et al. 2000). Under mineral nutrient deficiency, excess C is commonly stored as lipids, starch, and storage glucans (Fig. 3). Although the elemental makeup of marine phytoplankton has all too often been equated with the Redfield ratio of 138 : 106 : 16 : 1 for $-O_2 : C : N : P$ (Redfield et al. 1963), a panacea for modelers, C : nutrient ratios are variable and often considerably exceed this ratio (Daly et al. 1999; Mei et al. 2005).

Aquatic vertebrates frequently store C as adipose tissue that may serve as insulation (in homeotherms) and energy reserves. Invertebrates like zooplankton show less variability in their elemental ratios (Andersen and Hessen 1991; Koski 1999), suggesting a limited capacity for storage of carbon. Some zooplankton species, however, notably high-latitude copepods such as *Calanus finmarchicus*, have significant lipid stores that are used both in gonad formation and metabolism during the ascent from deep water after diapause (Jónasdóttir 1999). As a result, spawning occurs in advance of the spring phytoplankton bloom (Niehoff et al. 1999) and the juvenile stages developing as food become plentiful. Lipids, triacylglycerols, and carbohydrates also vary considerably in freshwater zooplankton such as *Daphnia* (Lemcke and Lampert 1975), although much of this variability is due to starvation and associated depletion of somatic tissue rather than regulation of excess C. Most species, including *Daphnia*, store some lipids under high food conditions, the resulting "lipid score" being commonly used as a sign of nutritional condition (Goulden et al. 1982).

Drawing a parallel with terrestrial systems, structural defenses are an obvious outlet for C that might otherwise be left over, and are widespread in prey species. In phytoplankton, C may be allocated to spines, increased cell wall thickness, and C-rich extracellular polymers that serve as grazing repellents. Nutrient-stressed chlorophytes, for example, allocate C to cell-wall structures, impairing access to digestive enzymes and increasing the likelihood of viable gut passage (Van Donk et al. 1997). Release of carbon in the form of gelatinous, extracellular compounds may also serve as an antigrazing mechanism by blocking digestive enzymes (Porter 1976). Small osmotrophs use C simply to increase cell size as an antipredator strategy (Thingstad et al. 2005).

Zooplankton, too, may invest carbon into structural defenses (Tollrian and Harvell 1999). Some metazoans such as salps and jellyfish utilize carbohydrates to make extremely C-rich body tissue that makes them less attractive to predators. The freshwater cladoceran class *Holopedidae* (represented by two species only) is surrounded by a quite remarkable gelatinous mucopolysaccharide mantle that serves both as a buoyancy control and as an antipredator defense. Toxins represent a further fitness-promoting outlet for carbon, a suite of grazer-repelling compounds having been identified across various taxa (Turner and Tester 1997). Although C-rich secondary metabolites and toxic compounds in terrestrial plants are commonly utilized as toxic predator repellents by invertebrate grazers (generally associated with "warning" color patterns), there are fewer examples of this phenomenon among aquatic invertebrates.

Release of C-rich compounds that screen off ultraviolet (UV) radiation serves as another example of the fitness-promoting use of C that at a first glance may seem in surplus. Polysaccharide matrices at cell surfaces, mucosporine-like amino acids, and C-rich carotenoids may all act as UV-screening agents, playing a major role in the well-being of many aquatic organisms. The release of UV-absorbing DOC into surface waters by zooplankton could also be a UV protection mechanism (Williamson et al. 2007). Bacteria and phytoplankton may also benefit from the production of extracellular layers of organic C, "bacteria films" consisting of C-rich polymers offering a multitude of fitness-promoting purposes ranging from signaling to protection against viruses and desiccation (Costerton et al. 1987).

An intriguing use of organic C is seen in metabolic symbiosis. Symbiosis in integrated organisms is simply a trade of nutrients for energy, serving as a good example of coevolutionary adaptations that mutually benefit from release of substances that would otherwise have contributed to leftover C. In general, the autotrophic partner in the relationship releases excess carbohydrates from photosynthesis, whereas the heterotroph in return releases nutrients. This exchange may eventually evolve from mutual benefits in free-living organisms toward a true symbiosis by full cellular integration. The classical aquatic example is corals (Dubinsky and Berman-Frank 2001), where this trade of energy and elements has been crucial both for permanent symbiotic partnerships at the organismal level as well as in the evolution of organelles (Kooijman et al. 2003). Other benefits of the coral–zooxanthellae symbiosis include protection from predators and staying close to light, while the coral's ability to sequester calcium carbonate is enhanced. Aquatic protists also commonly regulate their host–symbiont associations relative to the prevailing light and nutrient regimes (Stabell et al. 2002).

Although not exhaustive, the examples of evolutionary adaptations that make use of C described above nevertheless show that C in excess relative to somatic needs for balanced growth may be used for a multitude of fitness-promoting purposes. In many instances, however, organisms will have to get rid of the leftover C that cannot be stored or otherwise used.

Dealing with leftover C

Despite the possible uses for excess C, genuine surpluses do nevertheless occur, plants and animals being left to deal with them. Phytoplankton may, for example, experience periodic high irradiance as they mix in the water column, leading to uncoupling between C fixation and population growth (Dubinsky and Berman-Frank 2001). Photosynthetic organelles are damaged when exposed to strong light if the energy is not dissipated (Powles 1984). Photoprotection is one means to prevent damage by intense or UV light (Columbo-Pallotta et al. 2006), but decreases quantum yield (which is disadvantageous when light intensities are lower) and has associated production and maintenance costs. An alternative is for algae to proceed with photosynthesis and then dispose of the surplus fixed C, one possible means being the Mehler reaction (Radner and Kok 1976). Jensen and Hessen (unpubl.) found that chlorophytes strongly increased their dark respiration rates when cultured under P deficiency (raising cellular C : P ratios from 80 to 600) (Fig. 4), although other studies have found respiration to decrease in response to P limitation (Theodorou et al. 1991). It is not clear whether organisms may incur fitness-reducing costs through increased respiration. If cells are saturated with C for metabolic purposes and storage, respiring it does not incur net costs in terms of C balance and energy expenditure. Overall fitness could, however, be decreased if there are associated requirements for other minerals, e.g., in terms of increased needs for enzymes involved in the tricarboxylic acid cycle. Perhaps the most effective way of disposing of the leftover C

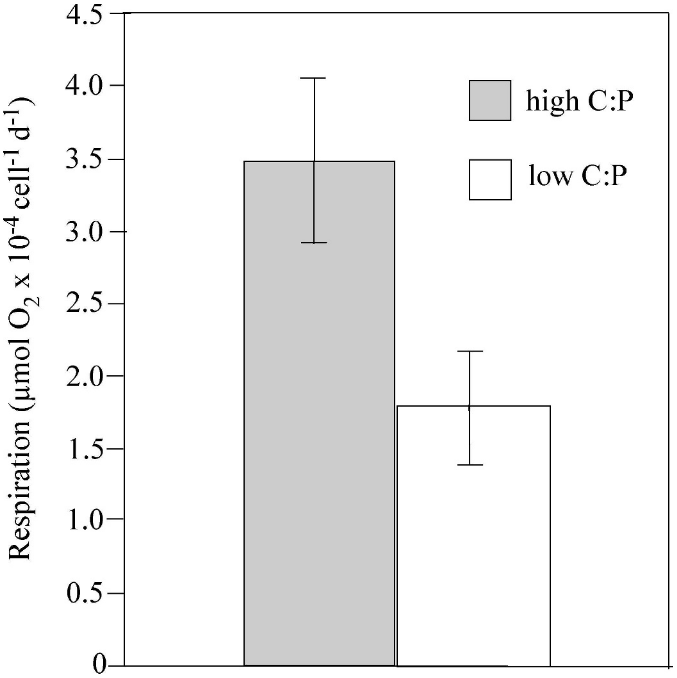

Fig. 4. Cell-specific dark respiration in *Selenastrum capricornutum* grown under P-saturated medium (cellular C : P: 80 by atoms) and P-depleted medium (C : P: 700 by atoms). (Jensen and Hessen unpubl.)

resulting from ongoing photosynthesis under conditions where nutrients are insufficient to permit growth is simply to release it as DOC. High release rates of DOC by phytoplankton are generally associated with oligotrophy (Magaletti et al. 2004), frequently in the form of transparent exopolymer particles (Passow 2002), although note that Maranón et al. (2004) found that the percentage extracellular release in the Rio de Vigo was highest under suboptimal irradiances. Despite Bjørnsen (1988) labeling DOC loss a "property tax" on phytoplankton, there appears to be no net penalty to the algal cells given that any energetic costs can be met from the leftover C itself.

Limitation of heterotrophic aquatic bacteria by nutrients may be commonplace both in both freshwater (Vadstein 2000) and marine systems such as the Mediterranean Sea (Zohary and Robarts 1998). Nutrient-limited bacteria may dispose of excess C via increased respiration or release of C-rich polymers, notably extracellular polysaccharides (Decho 1990). Although, as described above, this release may often serve fitness-promoting purposes, several studies have shown a decoupling between the uptake of glucose (or other precursors of pyruvate) and growth rate under nutrient limitation, indicating that surplus energy in such cases is simply "overflow metabolism," "wastage," or "futile cycles" (Russel and Cook 1995); depletion of adenosine triphosphate (ATP) energy by heat generation is then without any net substrate-to-product conversion. Nutrient limitation of bacteria may be manifested as high respiratory rates, especially in DOC-rich lakes (Smith and Prairie 2004). In a series of experiments with *Klebsiella aerogenes*, Tempest and Neijssel (1975) found that when C was in excess the production of pyruvate, gluconate, succinate, and other products explained less than 50% of metabolic activity, the remainder being excreted as polysaccharides, some lost as proteins or by respiration. In all cases, respiration (as O_2 consumption) was shown to increase with dilution rate, but the lower respiration seen in C-limited cells relative to those limited by P and N is indicative of respiration acting as a means of disposing of leftover C (Fig. 5). The interaction between nutrients and C in microbial systems is, however, complex, with, for example, nutrient addition experiments showing various responses to addition of different substrates (Thingstad et al. 1998), rather than simple Liebig-style limitation. Furthermore, bacteria may be able, to some extent, to selectively strip out and utilize those nutrient-rich compounds that they require from the bulk organic matter pool (Hollibaugh and Wong 1999; Danovaro et al. 2000), thus avoiding the problem of leftover C at source and leaving C to accumulate in the environment.

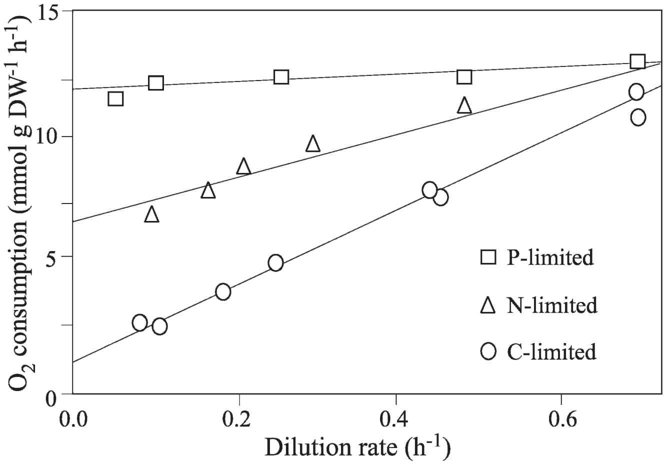

Fig. 5. Respiration rates (as O_2 consumption) for P-, N-, and C-limited cells of *Klebsiella aerogenes* (modified from Tempest and Neijssel 1975).

In moving from unicellular organisms to metazoans, things become more complicated, there still being a surprising lack of knowledge on how invertebrates handle leftover C. It should be stressed that both quality and quantity of food are important in nutrition, the effect of the former being superimposed on any shortfall in quantity by imposing an extra toll on C-use efficiency. In addition, zooplankton growth rate will in many cases be reduced simply by decreased assimilation efficiency caused by limited access to C in their diet due to structural properties of their food (Anderson et al. 2004).

Regarding diet quality in metazoans, there has been a long controversy with regard to identifying the compounds that limit growth in zooplankton, various studies demonstrating decreased growth rate due to mineral nutrient deficiency (Sterner and Elser 2002) and lack of polyunsaturated fatty acids (Müller-Navarra et al. 2004), vitamins, or sterols (von Elert et al. 2003). Assuming homeostasis, food quality constraints necessarily require that consumers dispose of leftover C, yet the metabolic routes and fate of this C may differ between species. There are three principal routes for consumers to dispose of leftover C (Fig. 6): first,

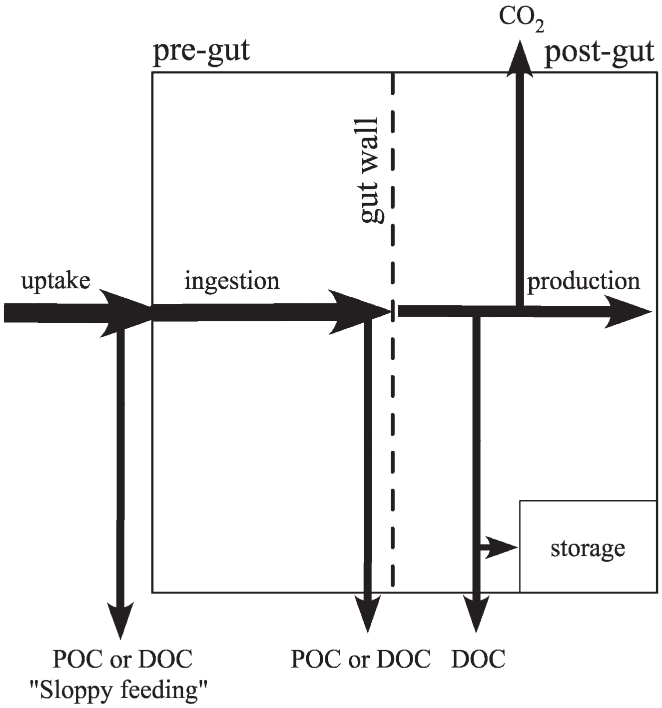

Fig. 6. Conceptual illustration of potential pre- and post-assimilatory fates of excess C in a metazoan. Width of arrows indicates relative size of fluxes (after Hessen et al. 2004).

enzymatic adjustment that downregulates the uptake of C-rich compounds across the gut wall, maximizing the assimilation of nutritionally deficient minerals but not C; second, release of organic C; or third, increased metabolic activity and respiration to CO_2.

The regulation of extracellular enzyme activity both in photoautotrophs and heterotrophic bacteria in response to the availability of specific nutrients and C compounds demonstrates that there is a "preassimilitaory" screening and enzymatic adjustment to match intracellular elemental demands. On the other hand, the strong stoichiometric variability and commonly recognized storage or release of substrates in excess also demonstrates "postassimilatory" responses in these groups. For metazoans, there is surprisingly little exact knowledge on the mechanisms for stoichiometric regulation of leftover C. Regarding pre-assimilatory regulation, the study of DeMott et al. (1998) showed that C assimilation efficiency (AE) in *Daphnia* declined, whereas AE for P remained constant at high food C : P, thereby reducing the C : P net assimilation ratio. Similarly, Darchambeau (2005) argued in favor of enzymatic regulation over the gut wall as a prime mechanism for regulating acquisition of elements, combined with regulation of gut residence time. The benefits of preassimilatory regulation are that there would be no need for involvement of the metabolic machinery (with associated costs), and that production of certain enzymes (like lipases and carbyhydrases) could be relaxed when C is surplus to requirements.

Conversely, one could argue that food has to be assimilated for animals to monitor its composition and make metabolic adjustments (Anderson et al. 2005). In this case, leftover C could then either be disposed of as DOC or oxidized and released as CO_2 (Jensen et al. 2006; Jensen and Hessen 2007). Several studies demonstrated release of DOC by zooplankton (Vadstein et al. 2003), yet it is difficult to establish whether this occurrs pre- or post-absorption by the gut. The experiments of Darchambeau et al. (2003) suggested an extensive release of assimilated DOC for zooplankton consuming P-deficient diets. Release of organic C requires little in the way of metabolic investment. An alternative is respiration, the importance of this means of homeostatic regulation being stressed by Sterner et al. (1997), who advocated the need for estimates of specific dynamic action (the increase in respiration associated with feeding) and how it relates to food quality. Disposal by respiration involves biochemical oxidation that could imply metabolic advantages, but also cellular disadvantages such as the production of free radicals.

Distinguishing between the different pathways for C utilization in organisms is necessary to assess the potential for respiration as a means of dealing with leftover C. Recent theoretical stoichiometric approaches have accordingly separated basal metabolism and the costs of assimilation and growth (Anderson and Hessen 2005; Anderson et al. 2005). Elevated rates of metabolism seem to play a role in the disposal of leftover C in some animals (Fu and Xie 2004). The study of Jeyasingh (2007) is particularly intriguing in this respect since, by comparing respiratory rates measured as O_2 consumption in five different species of *Daphnia* offered P-sufficient (molar C : P = 150) and P-deficient (molar C : P = 800) food, it demonstrated not only that respiration rates were consistently higher on P-deficient diets, but also how this apparent respiration of leftover C caused deviations from the quarter-power metabolic scaling (Gillooly et al. 2002). That ingestion rates were similar for high- and low-P diets, yet increased appendage beat rate and heartbeat rate were observed under P-deficient diets (despite ingestion being similar for both high- and low-P diets), indicates that the animals were using both behavioral and physiological mechanisms to increase respiration and thereby dispose of leftover C.

Plath and Boersma (2001) also observed an increase in appendage beat rate in *Daphnia magna* fed P-limited algae and argued that it may act as a mechanistic explanation behind increased disposal of C. Other studies have, however, found that clearance rate is unaffected by food quality (Darchambeau et al. 2003; Van Donk et al. 1997; Jeyasingh 2007). Philippova and Postnov (1988) showed that filtering costs in *D. magna* constituted only a relatively small fraction of total metabolic costs at high food concentrations, in which case it seems unlikely that increased filtration could be a major factor in regulating stoichiometric balance.

Disposal of excess C via increased "work" or "wastage respiration" has been demonstrated for terrestrial invertebrates as an analogue to what is frequently described in homeotherms. Zanotto et al. (1997) measured how an imbalance in digestible carbohydrate relative to dietary protein in locusts induced elevated CO_2 outputs. Trier and Mattson (2003) also suggested that the decrease in growth efficiency in spruce budworm (*Choristoneura fumiferana*) when feeding on a diet with a high energy : protein ratio and increased net intake of food could be credited to such "waste" of excess C by diet-induced thermogenesis. In other words, increased oxidation of C-rich substrate would yield ATP in excess, which then subsequently should generate heat without catabolism. If such mechanisms exist in terrestrial insects that face diets with fluctuating and commonly high dietary C, there is every reason to believe that the same should be true for aquatic invertebrates. The rapid heat exchange between tiny aquatic animals and their ambient medium should be substantial, however. Hence for aquatic invertebrates the metabolic gain of excess ATP is dubious.

Stoichiometric theory assumes that there are no costs per se of disposing of leftover C. In reality, however, there are likely to be metabolic costs of disposing of unwanted substrates, these costs having been highlighted by the geometric approach to nutrition of Raubenheimer and Simpson (1999). They showed that the fitness of various terrestrial organisms, such as locusts, is maximized at intake targets (rather than threshold elemental ratios) that are optimal for nutrition, performance being reduced as surpluses or deficits occur across the range of substrates in ingested food (rather than in response to a single limiting substrate). In the aquatic realm, Boersma and Elser (2006) argued that disposing of excess P incurs a cost such that growth decreases for food with high P content in a variety of animals, including cladocerans and fish. Indeed, Plath and Boersma (2001) noted that growth in *D. magna* was depressed when fed algae with more than ~1% P. Similarly, Boersma and Elser (2006) concluded that high P content in food incurs real costs to consumers that decrease growth, reproduction, and survival, and therefore the usual stoichiometric assumption that such costs are insignificant is unjustified. As yet, however, the exact nature of these costs is far from fully understood.

Excess C and food-web interactions

The ratio of N or P to C in organism biomass has important consequences for the cycling and fate of C in ecosystems. The C : N and C : P ratios in organisms in aquatic systems are generally less pronounced than the stoichiometric mismatch between elemental ratios in plants and grazers in the terrestrial biosphere (Elser et al. 2000). A tight correlation is found across ecosystems between C : N or C : P in plant biomass and biomass turnover, grazing efficiency, and temporary C burial as detritus (Cebrian 1999). Forest ecosystems, for example, represent one extreme of this scale, characterized by exceptionally high C : nutrient ratios in plant tissue, largely a result of the woody and C-rich supportive structures such as cellulose and lignin that reduce grazing and turnover. Consequently, terrestrial ecosystems are often characterized by high standing biomass of nutrient-poor plant biomass, with relatively little fixed C entering the food web and high C sequestration. Pelagic systems represent the other end of the scale, characterized by small and nutritious autotrophs with comparatively low C : nutrient ratios in plant biomass and consequently with high turnover rates and a major share of fixed C diverted up the food chain (Hessen et al. 2004).

Although excess C may be more widespread in terrestrial than in aquatic food webs (Cebrian 1999; Elser et al. 2000), it is nevertheless an important modulator of C sequestration and food-web dynamics in the latter. Unlike aboveground terrestrial ecosystems, the flux and fate of various forms of autocthonous C play essential roles in aquatic ecosystem metabolism, community organization, and competitive outcomes between and among functional groups and taxa. Competition, commensalism, and mutualism may be tightly interwoven and thus hard to disentangle when it comes to ecosystem responses to excess C. For example, phytoplankton and heterotrophic bacteria will mutually exchange pools of C, the release of organic C (exudation) serving as a substratum for bacterial growth. The very concept of the microbial loop is built on this process. This commensalism (Gurung et al. 1999) may turn into mutualism if the phytoplankton benefit from inorganic C provided by bacterial respiration, although this source of CO_2 will probably only rarely be a main determinant of phytoplankton C fixation (Danger et al. 2007). A mutualistic relationship may also occur if bacteria act as true remineralizers in the sense that they provide dissolved nutrients for autotrophs.

Under conditions of P limitation of aquatic heterotrophic bacteria (Vadstein 2000), the relationship between heterotrophic bacteria and photoautotrophs could, however, be seen as competition rather than mutualism. If an increasing share of the P pool is allocated into bacterial biomass, primary production will decline. This reduction typically occurs under scenarios with high availability of allochthonous DOC that reduces the osmotroph dependency of autotroph exudates (Hessen et al. 1990; Jansson et al. 2000). A high load of allochthonous DOC may occur in coastal areas influenced by riverine inputs, as well as in many lakes in boreal regions. Allocthonously influenced systems of this kind are typically characterized by a high ratio of heterotrophic : phototrophic biomass and production, resulting in net heterotrophy and a vigorous export of CO_2 to the atmosphere (Hessen et al. 1990; Prairie et al. 2002).

Processes leading to imbalanced uptake of C over other nutrients in autotrophs (e.g., high pCO_2, high light : nutrient ratio) would a priori stimulate the release of DOC, which in turn stimulates bacterial growth, intensifying competition with phytoplankton for nutrients and thus skewing autotrophs toward nutrient limitation (Fig. 7). The resulting changes in the elemental balance of autotrophs, e.g., elevated C : P or C : N ratio, may lead to increased primary production and carbon export, for example via transparent exopolymer particles (TEP) (Riebesell et al. 2007).

From an organism's point of view, the route by which by C in stoichiometric excess is lost might seem irrelevant. As long as it is in excess in the strict sense, i.e., it is leftover C, it will not contribute directly to increased fitness and simply give rise to reduced C growth efficiency. In actual fact, the form in which C is released is not trivial, the resulting indirect food web effects depending on whether release is in organic or inorganic form. Release in organic form promotes heterotrophic processes, whereas respiration of

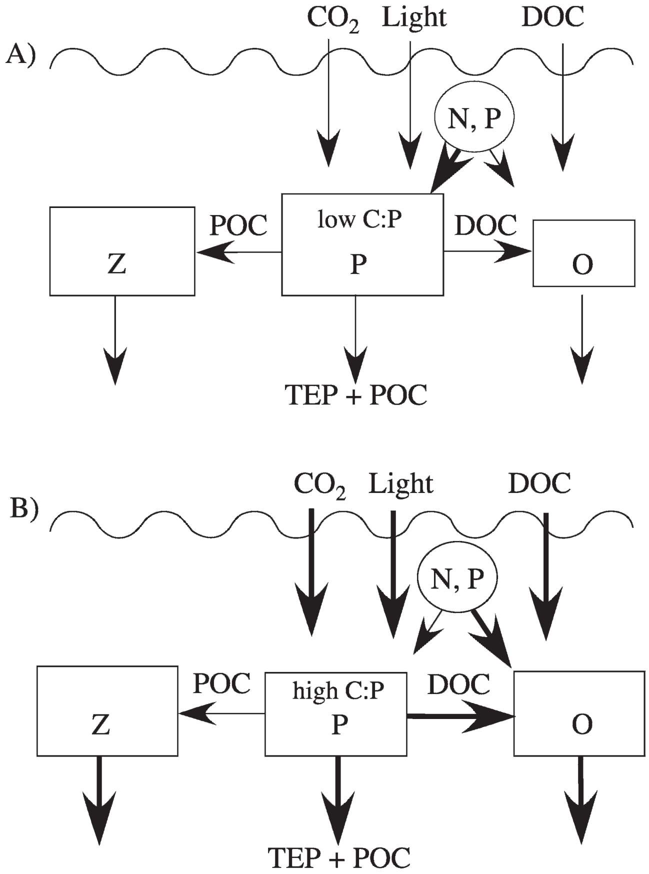

Fig. 7. Conceptual illustration of stoichiometrically driven C flux in lakes or oceans under two different scenarios: (A) the "background" scenario; (B) increased levels of CO_2, light, or allochthonous DOC. Elevated levels of CO_2 and light enhance C allocation to the photoautotrophs (P), which could cause elevated C : P, or increased release of DOC to the osmotrophs (O) (or both) that in turn increase their competitive uptake of N and P. Export of particulate organic carbon, and possibly TEP, may be expected to increase because of cycling of carbon by the autotrophs, the osmotrophs, and the zooplankton (Z). See text for further elaboration of these arguments.

excess C to CO_2 may promote algal growth (Riebesell et al. 2007), the two pathways offering different potential for C sequestration. Excretion of DOC by phytoplankton would appear somewhat enigmatic—why should autotrophs apparently fuel the growth of their competitors for nutrients, the heterotrophic bacteria? One reason might simply be that they are not evolutionarily adapted to increasing their respiratory outputs sufficiently. Increased metabolic rate to burn off excess C could generate reactive oxygen species, causing mutations and cellular damage, which requires investment in protective mechanisms. Alternatively it could be, as we have seen, that DOC serves an important function as a virus or grazer repellent. If DOC is the cheapest way of getting rid of leftover C, more metabolically expensive solutions would invoke group selection arguments. This is not an evolutionarily stable strategy if this sacrifice was for the good for the common populations (i.e., the autotrophs in general) but to the disadvantage of individual cells.

Zooplankton may also be a significant autochthonous source of DOC (Vadstein et al. 2003), with major implications for the balance of auto- and heterotrophic processes in lakes, since grazing often removes phytoplankton with higher efficiency than bacteria, and much of the released DOC serves as a C source for bacteria. Hence, to the extent that DOC release shifts the competition between autotrophs and heterotrophic bacteria in favor of the latter, it would seem beneficial to respire away excess C rather than releasing it in organic form. Again, there may be physiological constraints on elevated respiratory activity in metazoans.

We have seen that individual processes within or between organisms will cumulatively determine C sequestration at the community, ecosystem, and consequently even the global level. Hence the flux, fate, and organismal trade of various forms of C are in essence the major drivers for all ecosystem processes as well as the global C cycle. For aquatic ecosystems, one could expect feedback scenarios toward more C-rich systems, which again would promote less efficient energy transfer. Specifically, low nutrient quotas in phytoplankton would mean more nutrients reclaimed by the consumers and less N and P recycled. This, along with increased releases of organic C, could intensify the competition for mineral nutrients between phytoplankton and heterotrophic osmotrophs, induce even more nutrient-replete autotrophs, etc. In systems where autotrophs are enriched in C (e.g., due to high light : nutrient ratio or high CO_2 : nutrient ratio), zooplankton will release less P but more C, further reinforcing the poor seston quality.

Balanced growth in organisms implies a net uptake of C, N, and P (and a multitude of other elements) in certain proportions. Relative deficiency in one element would a priori imply excess of others, yet only when there is a continued gross intake of excess elements can one really speak about being in excess in a metabolic context. Aquatic autotrophs do frequently fix more C than is generally needed for balanced growth, and this is most pronounced under situations where the photosynthetic machinery is stimulated by high levels or light and CO_2 and nutrient concentrations are kept low. This decoupling of C fixation and nutrient (particularly P) uptake gives rise to both a strong departure from Redfield proportions in cells due to temporary storage, and also release of leftover C in organic or inorganic form. In similar fashion, heterotrophs, both bacteria and metazoans, also frequently face food quality limitation in terms of low proportions of nutrient elements and hence face an excess intake of C that is released in organic or inorganic form. The concept of excess C is not, however, straightforward, because this C can be used for a suite of fitness-promoting purposes in organisms. The flux and fate of "excess" C in organisms and ecosystems is thus a major driver both in an evolutionary and ecological context.

Future work and models in this field should go beyond the simple "black-box" and mass-balance concept in organisms, and include costs and benefits associated with both excesses and deficits. We need a much better understanding of these costs and penalties. Although the disposal routes for unwanted, excess C have been identified, further research is required to understand why particular routes are favored over others. Not least, future work needs to consider that excess C from a balanced-growth point of view may promote fitness for the organism in subtle ways. Such improvements will guide insight into energy and C cycling not only within organisms and food webs, but may also provide a framework for the understanding of C cycling at the ecosystem level.

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Received: 18 October 2007
Accepted: 4 February 2008
Amended: 12 February 2008