Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world

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The relative supply of energy and elements available to organisms in the environment has strong effects on their physiology, which, in turn, can alter important ecological processes. Here we consider how resource imbalances affect three basic physiological processes common to all organisms: elemental uptake, incorporation, and release. We review recent research that addresses these core issues (uptake, incorporation, and release) as they relate to elemental homeostasis in autotrophs and heterotrophs. Our review shows the importance that organism elemental homeostasis plays in determining the types of physiological processes used to acquire, assemble, store, and release biogenic elements, which are found in widely varying ratios in the environment. Future research should examine the degree to which organisms assess their internal nutritional composition and that of their food sources within a multiple elemental and biochemical context. Also, scientists should explore if and how the stoichiometry of cellular and molecular responses underlying nutrient (elemental and biochemical) acquisition, incorporation, and release depends on the nutritional composition of food resources. These types of queries will further improve our understanding of the physiological processing of primary elements involved in growth, reproduction, and maintenance of organisms.

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One challenge that all organisms face is the acquisition of sufficient quantities of energy and elements needed for growth, reproduction, and maintenance. Accumulating evidence indicates organisms (plants, bacteria, fungi, and animals) are faced with and sometimes acquire an imbalanced mixture of energy and elements (Sterner and Elser 2002), which can place strong constraints on their growth and reproduction (Bruning 1991, Sterner and Schulz 1998, Aerts and Chapin 2000, Smith 2002). Elemental constraints on organisms can, in turn, alter the dynamics of populations (Elser et al. 1998, Chen et al. 2004), inter-specific interactions in food webs (DeMott and Gulati 1999, Denno and Fagan 2003)

and key processes in ecosystems (Sterner et al. 1997, Cebrian 1999). One key to understanding the stoichiometric linkages between organisms and ecological processes is knowledge of the physiology that contributes to the acquisition, incorporation, and release of energy and elements.

Organisms vary considerably in the degree to which they are stoichiometrically homeostatic; that is, the extent to which they regulate their internal elemental contents in relation to the available elemental supply. Evidence suggests that there are fundamental differences in the flexibility in the elemental composition of autotrophs and heterotrophs (Sterner and Elser 2002).

Organismal homeostasis of elemental content (or lack thereof) is largely controlled by a set of key physiological processes that regulate element uptake, incorporation, and release. The sum of these physiological processes, some of which enforce homeostasis (e.g. increasing nitrate uptake affinity by plants in nitrogen (N)-limited environments) and some of which do not (e.g. storage of excess nitrate by plants in N-replete environments), proximally control the elemental composition and the metabolism of organisms.

Physiology is a nexus at which imbalances of energy and element supplies are translated into ecological phenomena such as allelopathy, resource competition, herbivore defense, and selective feeding. Thus, a stoichiometric perspective on physiology should explicitly link the metabolism of organisms (e.g. protein synthesis) with the elemental constraints imposed by the environment. Such a stoichiometric framework may provide a more complete understanding of nutritional physiology because it allows one to compare elemental regulation among diverse taxa (e.g. vascular plants vs mammals) and to track the movement and balance of specific elements as they are used by individual organisms. For example, one could ask what physiological processes

dictate the movement of elements through a biogeochemical cycle (e.g. soil–plant–herbivore–predator–detritivore-soil) or within organisms (e.g. food/free elements to cells to excreted elements). A multiple element approach also provides a complementary perspective (to energetics) to examine the regulatory processes underlying key life-history traits of organisms and their role in ecosystems (Reiners 1986, Elser et al. 1996).

We have organized our thinking about physiological stoichiometry with a conceptual framework that highlights many of the important connections between resource supply, organism physiology, and environmental factors (Fig. 1). This framework considers how physiological processes affect, and are affected by, the movement of carbon (C) and other elements into and through an organism. For example, increasing C:x ratios (where x is any element) in food leads to less ingestion of x relative to C (Fig. 2). This change in the ratio of acquired elements should lead to lower efficiencies of C accumulation and higher C:x release ratios by a homeostatic consumer (Fig. 2). This approach can also be extended to essential biochemicals and in places throughout the manuscript, we refer to nutrients to include both elements and biochemicals needed for
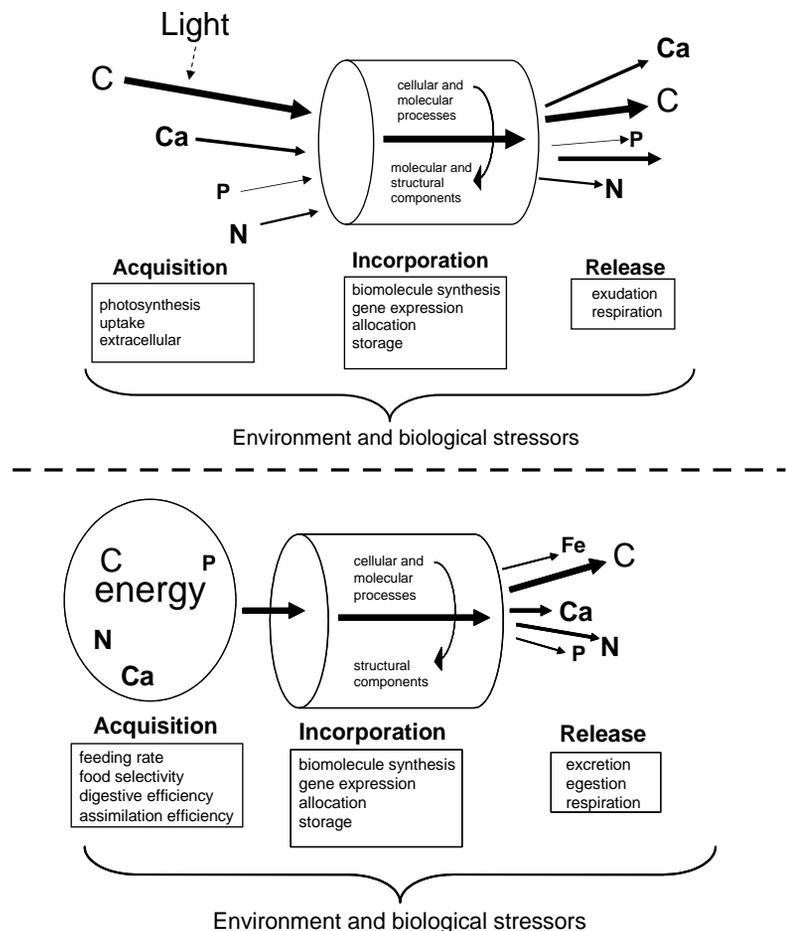

Fig. 1. Conceptual diagram illustrating primary processes regulating the acquisition, incorporation, and release of chemical elements for autotrophs and heterotrophs. Central to these processes are cellular and molecular responses that control the demand for elements in new structural material. These processes feed back into acquisition processes by affecting the production of nutrient uptake and digestive proteins. Disposal is also likely affected by these key cellular processes, as elements acquired in excess are preferentially released. Finally, environmental factors and biological stressors may alter organism physiological stoichiometry by affecting metabolic demands for particular elements.
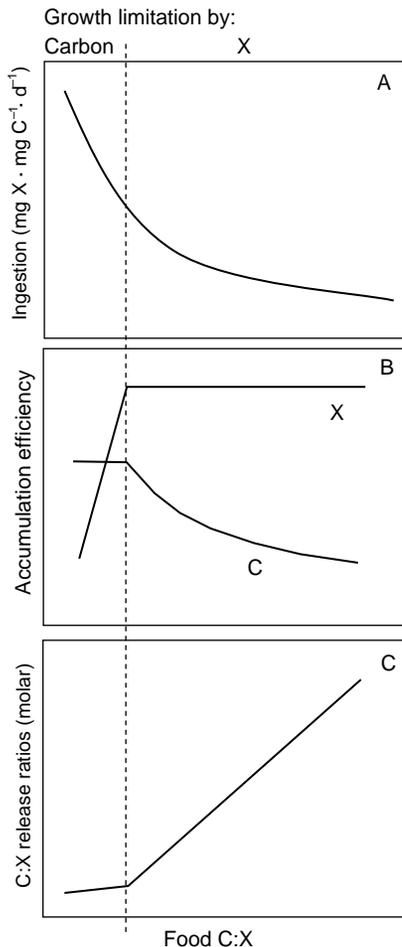
Fig. 2. Predicted trends in ingestion (A), accumulation efficiency (B), and C:X release ratios from (C) a homeostatic consumer eating food of differing C:X ratio where X is any non-C element. Here accumulation efficiency represents the fraction of element incorporated into growth relative to the amount ingested. The shapes of these predictions are based on modified equations from the consumer growth model presented in Sterner (1997) and under the condition of high food quantity.

organismal metabolism. In addition, these organismal physiological processes (and as we argue here, elemental demands) should be largely considered as context dependent, as environmental factors (e.g. light and temperature) can alter many processes (e.g. plant nutrient uptake, animal digestion, respiratory rates) that regulate the supply of and demand for multiple chemical elements.

We consider recent progress and possible future directions in understanding the constraints placed by stoichiometry on physiological processes relating to the uptake, incorporation, and release of multiple chemical elements by organisms as outlined by our conceptual framework (Fig. 1). Throughout, we integrate consideration of both autotrophs and heterotrophs in aquatic and terrestrial environments. While heterotrophs are generally considered to be more homeostatic with their elemental composition (Sterner and Elser 2002), we show evidence for a considerable degree of stoichiometric regulation of physiological processes in autotrophs as well. We draw examples from across habitats and trophic levels to illustrate the potential value of a stoichiometric perspective to understanding the physiology of a wide variety of organisms. Our hope is that examination of the connections between elemental supply ratios and organismal processes will advance our basic understanding of the interactions between organismal physiology and the environment.

Acquisition of resources

A variety of physiological processes are used by organisms to acquire nutrients. Autotrophs primarily use independently acquired inorganic elements, while heterotrophs mostly obtain elements bound together in the biochemical constituents of food. Autotrophs therefore often confront greater variability in the relative supply of elements available compared to heterotrophs. Nevertheless, both autotrophs and heterotrophs show some degree of selectivity during elemental acquisition in order to obtain the mixture of elements needed for growth and maintenance.

Ingestion is a key process that animals may modify to maintain elemental or biochemical homeostasis when faced with imbalanced resources. Animals can change their ingestion rate and degree of food selectivity to alter the balance of elements or biochemicals available for growth (Bairlein 1996, Witmer 1998, Plath and Boersma 2001, Raubenheimer and Simpson 2003, Darchambeau and Thys in press). Omnivorous feeding habits or dietary mixing of heterogeneous food sources may also increase the acquisition of the limiting nutrient, as has been observed for vertebrates (Izhaki and Safrel 1989, Rode and Robbins 2000), crustaceans (DeMott 1998, Acharya et al. 2004), and insects (Denno and Fagan 2003, Raubenheimer and Simpson 2003). Organisms using such physiological mechanisms (e.g. ingestion rate and feeding selectivity) to regulate nutrient uptake must be able to assess the nutritional state of itself and its food source (Simpson et al. 1995). Insects may assess their nutritional state based on hemolymph biochemical composition, which has been directly linked to feeding behaviour (Simpson and Raubenheimer 1993, Simpson et al. 1995). Insects can also determine the nutritional nature of their food using chemoreceptors specific for particular inorganic salts or biochemicals (i.e. sugars and amino acids) on various body parts (Chapman 1995) and potentially even in the gut (Champagne and Bernays 1991, Timmins and Reynolds 1992). Future endeavours examining ingestion as a mechanism of elemental homeostasis should consider not only the elemental

composition but also the biochemical characteristics of the food resources involved. It would also be worthwhile to examine the lowest component (e.g. N, amino acid or protein) recognized by chemoreceptors across a variety of organisms and elements important for growth.

Animals may also use post-ingestive processes to regulate the relative acquisition of biochemicals and elements (Fig. 2). Digestive enzymes are specific to particular molecules or types of molecules and both the suite and the activity of gut enzymes can depend on the biochemical composition of the food resource (Zhalka and Bdolah 1987, Lehane et al. 1995, Ivanovic et al. 2002). For example, proteolytic enzyme activity was highest in individuals fed a higher protein diet in starved cerambycid beetles (Ivanovic et al. 2002). The nature of the relationships between food quality, digestive enzyme quantity and quality, and the ratios of elements acquired is only beginning to be studied (Darchambeau in press). Differential acquisition of elements post-ingestion could be accomplished by adjusting the assimilation efficiencies of each element (Frost et al. 2004, Logan et al. 2004). Though element-specific assimilation efficiencies are rarely reported for animals, it does appear that crustacean zooplankton and insects can differentially assimilate C relative to other elements (Brodbeck et al. 1996, DeMott et al. 1998, Tang and Dam 1999). Future experiments should explore the effect of both food biochemical and elemental composition on the ratios and kinds of digestive enzymes produced in the gut and on the relative assimilation efficiency of different elements. Based on previous physiological studies, we would predict that particular suites of digestive enzymes would be activated across gradients of food quality. More fundamentally, cellular and molecular mechanisms underlying the expression of digestive enzymes could be explicitly examined relative to biochemical and elemental composition of the ingested food.

The preceding discussion involved metazoan heterotrophs. Here we briefly consider how unicellular heterotrophic organisms, such as bacteria, might fit within such an analysis. Analagous to release of digestive enzymes in animal guts, it is well known that microbes use several global regulatory systems, particularly two-component signal transduction systems (e.g. Pho and Ntr systems), that control the release of extracellular enzymes in response to limiting nutrient conditions (Atlas and Bartha 1998). However, studies are needed to explictly examine the stoichiometry of these enzymes and the microbial flexibility (at the level of species or community) in their production. Interestingly, it seems that nutrient limitation not only affects the levels and types of assimilatory enzymes produced by microbes, it also affects the elemental composition of those enzymes themselves (Baudouin-Cornu et al. 2004, Bragg and Hyder 2004). For example, assimilatory proteins in some

prokaryotes are relatively deficient in the elements (i.e. S, C and N) that they target for assimilation (Baudouin-Cornu et al. 2001). It is unknown if microbes can adjust protein stoichiometry on short time scales, that is, in response to shortterm fluctuations in nutrient availability. It would be interesting to know if microbes can substitute lower C:N amino acids in enzymes when C-limited, or if N-degrading enzymes in general have higher C:N ratios than phosphatases. Future work should measure the production and elemental composition of these extracellular products as they relate to the relative and absolute supplies of elements underlying microbial growth and metabolism.

Autotrophs take up nutrients by means of ion-specific transport proteins, typically consisting of constitutively expressed, low-affinity transporters and high-affinity transporters that are inducible under conditions of nutrient limitation. The uptake rate of particular elements is regulated, in part, by the internal store of that element and by the abundance of other elements as well. For example, uptake of NO_3^- in vascular plants is reduced when cellular levels of C and N metabolites are high (Grossman and Takahashi 2001) and when P is limiting (Chapin 1991). In this case, feedback from high N and low P content maintains some degree of elemental homeostasis. The feedback from high C does not, but it may be adaptive for the plant to reduce nitrate uptake when C accumulation indicates sufficient functioning of photosynthetic systems that themselves are N-intensive. Micronutrient content in autotrophs also affects acquisition of macronutrients. For example, urea uptake is associated with a requirement for nickel (Ni; Price and Morel 1991) and the uptake of NH_4^+ can be accompanied with an increased requirement for Fe (Muggli and Harrison 1996, Armstrong 1999). Autotrophs also consume nutrients beyond metabolic demands by decoupling their acquisition from the immediate demands for growth. This occurs even on very short time scales, as with surge uptake in phytoplankton (Stolte and Riegman 1995).

Elemental uptake by aquatic autotrophs is controlled, in part, by growth rate (demand) and external nutrient availability (supply). The ratio of acquired elements can vary considerably because some element-specific uptake mechanisms are not directly coupled. For example, net P-uptake was less sensitive than net photosynthesis (C-fixation) during exposure of lake phytoplankton to ambient levels of UVR (Frost and Xenopoulos 2002). More information on relative uptake rates and their regulation in phytoplankton would likely improve our understanding of autotroph growth dynamics as it relates to the supply of multiple elements. In particular, work should examine how the relative abundance and turnover of uptake proteins for specific elements change in relation to external nutrient supply ratios and internal growth demands.

In terrestrial plants, transport proteins that are selective for inorganic element uptake have been fairly well characterized for N species, but somewhat less so for P (Grossman and Takahashi 2001). More work is needed to help understand the contribution of elements resorbed from senescing leaves to the nutrient economy of terrestrial plants. In a recent study of global forest leaf stoichiometry, litter C:N ratios were nearly constant worldwide, despite significant biome-level differences in foliar C:N ratios (McGroddy et al., in press). This indicates that some leaf N is relatively immobile, perhaps because of strong links to C as structural proteins in cell walls. For any plant species, there must be a biochemical or physiological limit to the amount of N and P that can be resorbed, but most studies report only resorption efficiency in a single year, which is inadequate to estimate this limit (Killingbeck 1996). Additional biochemical studies of leaves over the course of senescence and of the resulting litter would indicate which biomolecules are more or less easily degraded during resorption, and how much nutrient is allocated to them.

Incorporation/assimilation of resources

The assembly of new biomass in the form of metabolic components, body structure, and reproductive tissues is a key process underlying organismal fitness. However, tradeoffs are almost inevitably involved with the allocation of limiting elements to different organismal traits (Zera and Harshman 2001). For example, increased allocation of N-based resources to immune responses likely constrains growth and reproduction in some vertebrates (Lochmiller and Deerenberg 2000). One approach to assess the extent of these tradeoffs and their stoichiometric basis is to decompose organismal structures into their biomolecular components (e.g. protein, fats, nucleic acids; Elser et al. 1996). Physiological processes underlying growth and maintenance of cells/bodies would thus each have a particular set of elemental demands, which if not met, could constrain the competitive ability of an organism.

One good example of these linkages is the growth-rate hypothesis (GRH), which predicts that organisms differ in body P content due to higher requirements of P-rich ribosomal RNA that support rapid rates of protein synthesis and faster growth (Sterner and Elser 2002). Numerous taxa, including bacteria, insects, algae and crustaceans, show strong connections between growth rate, biomass P and RNA content (Elser et al. 2000), although such connections may be broken under conditions where P is not limiting the growth of heterotrophs (Elser et al. 2003, Makino and Cotner 2004). One might also expect that inter-specific variation in N:P ratios would relate, in part, to differences in organism size. In general, rapidly growing, small organisms should have a lower N:P ratio than slower growing, larger organisms (Elser et al. 1996, 2000).

There is little evidence supporting the GRH from plants, for which a number of recent studies characterized N:P ratios (but not RNA concentration) and growth rate. A recent model by Ågren (2004) contradicts the GRH's prediction of a monotonic decrease in N:P ratio with increasing growth rate. The model conceptualizes autotroph C fixation as dependent on the protein synthesis rate (i.e. N), which is itself dependent on the amount of ribosomes (i.e. P). If so, autotroph C:N ratios should decrease linearly and C:P ratios quadratically with relative growth rate. The predicted result is a hump-shaped trend of N:P ratios versus growth rate, where N:P ratio initially increases, then hits a maximum and declines with increasing growth rate, as in the GRH. Element ratios from a freshwater alga and a tree seedling conformed to the model (Ågren 2004). For terrestrial plants in natural ecosystems, empirical evidence shows average C:N:P ratios can be fairly constant, almost Redfield-like, despite variation due to growth rate and nutrient supply (Knecht and Göransson 2004) or biome type (McGroddy et al. 2004). On the other hand, analysis of a global dataset of plant traits produced some indirect support for the GRH, suggesting that leaf N:P ratios decline in concert with increasing metabolic rates and faster leaf turnover times (Wright et al. 2004). Additional work on these questions is needed, especially in which plants are grown under more controlled nutrient conditions. This work should determine if, as suggested the model of Ågren (2004), the GRH needs to be modified to accommodate the possibility that the N-intensive requirements of energy acquisition dominate at low absolute growth rates while the P-intensive requirements of protein synthesis machinery dominate at high growth rate.

Plants can alter the stoichiometry of proteins and other compounds used in key cellular processes in response to light and nutrient limitation. For example, high light conditions stimulate particular pathways of N assimilation that result in high C:N amino acids such as glutamine and glutamate, while light or C-limited conditions inhibit these pathways and favor production of asparagine, a low C:N amino acid (Lam et al. 1996). Under P-limitation, plants synthesize alternative, low-P compounds to carry out structural and functional activities (Grossman and Takahashi 2001). For example, *Arabidopsis* plants starved for P, or defective in xylem loading of P, show decreased levels of phospholipids and increased levels of sulfolipids; substitution of sulfolipids may preserve the anionic character of thylakoid membranes under conditions of low P availability (Poirier 1991, Essigmann et al. 1998). P-limited plants can also use alternative pathways during glycolysis to reduce the need for phosphorylated adenylates (Duff et al. 1989).

While there is growing evidence of stoichiometric constraints on plant physiological processes at the cellular level, much more work is needed to elucidate the biochemical basis for these constraints. The problem is simply lack of data; our stoichiometric models for autotroph growth are becoming more biochemically based (Klausmeier et al. 2004, Ågren 2004), but empirical data relating N and P content to complex macromolecules lags behind. In addition to reporting elemental content, researchers should endeavor to characterize the allocation of elements to important classes of biomolecules in autotrophs (e.g. RNA vs protein content, structural vs photosynthetic protein, lipids vs water-soluble C etc.). This information may provide important information about biochemical and elemental food quality for herbivores (Anderson et al. 2004). In addition, such findings as the surprising correspondence between global foliar and fine root C:N:P ratios (Jackson et al. 1997, McGroddy et al. 2004) would be elucidated by a more detailed biochemical approach. Efforts are underway to link up disparate global databases of plant traits to shed light on the basic physiological tradeoffs that constrain plant morphology and function (Diaz et al. 2004, Wright et al. 2004); the next step will be to expand this analysis to the biomolecules underlying these different traits.

One abiotic factor that could have strong stoichiometric effects on the growth physiology of plants across the Earth is atmospheric CO_2. Increasing anthropogenic emissions of CO_2 will have strong effects on the growth and stoichiometry of autotrophs. Increased C:N ratios of terrestrial plant leaves under conditions of elevated CO_2 are one of the most robust and frequently observed trends from studies where CO_2 has been experimentally elevated (Körner 2000). This effect may result from the dilution of N by increases in total nonstructural carbohydrates and/or the down-regulation of the protein-rich photosynthetic system because of increased availability of CO_2 (Körner 2000). Crop plants also increase C:element ratios, which may have important consequences for public health given the key role these elements play in the human diet (Loladze 2002). Similarly, C:P ratios of a P-limited freshwater alga increased with elevated CO_2 concentrations (Urabe et al. 2003) but such effects were absent in rapidly growing marine phytoplankton (Burkhardt et al. 1999). More studies need to examine the effects of elevated atmospheric CO_2 (particularly under low nutrient supply) on autotroph stoichiometry, its cellular basis, and its subsequent effects on their primary consumers.

In heterotrophic consumers, understanding the stoichiometric controls on growth should be placed more explicitly in the context of the requirements of essential biochemicals (Anderson et al. 2004, Raubenheimer and Simpson 2004). Nutritional physiologists have shown the importance of protein and carbohydrate composition,

essential amino acids (EAAs), and fatty acids (EFAs) to growing consumers (van Tets and Hulbert 1999, Müller-Navarra et al. 2000, Boersma et al. 2001, Raubenheimer and Simpson 2004). Future research should strive to couple this knowledge of food biochemistry with its elemental composition. A recent analysis by Anderson et al. (2004) used a stoichiometric framework to analyze the importance of essential biochemicals to consumer growth. Within that framework, they estimated the EAA:total amino acid ratio in food relative to that needed by consumers. They found that EAA imbalances likely exist across a wide variety of animals including mollusks, fishes, birds, and mammals feeding on diverse arrays of food sources and inhabiting differing environments. The essential biochemical content and the elemental composition of food sources often co-vary (Weers and Gulati 1997, Jensen and Verschoor 2004) and this increases the difficulty of identifying causal effects of individual food components on consumer growth rates. One approach to resolve this problem could be the application of a geometrical framework approach (Raubenheimer and Simpson 2004), which would examine both elements and essential biomolecules in concert.

The elemental requirements of other life-history traits and their physiological basis should also be considered. In particular, immune responses likely require significant quantities of N, given the high protein demands of this key defense against infection (Lochmiller et al. 1993, Birkhead et al. 1999). It would be especially useful to determine if shortages of particular elements (P for ribosomes or N for immune proteins) mute immune function and increase an organism's likelihood of sustained infection. In addition, the stoichiometric demands for immune function need to be considered relative to other organismal activities (i.e. growth and reproduction). This work would provide additional insight in life history tradeoffs such as those that apparently exist between immune function and reproduction in some birds (Lochmiller and Deerenberg 2000). Similarly, work should examine the physiological repertoire available to host organisms to limit the availability of elements to disease organisms (Smith 1993, Smith and Holt 1996). For example, it appears humans constrain growth of Fe-demanding bacteria with a number of Fe-binding processes (Smith 1993). Such work would provide potentially important and useful information about how hosts combat infectious disease by manipulating the relative availability of internal elements.

Finally, we need more information on the complementary role of elements other than C, N and P in organismal physiology. Expanding the framework of physiological stoichiometry to additional elements may yield the same benefits that came from expanding single-element (C-based) models to two- or three-element

models. Elements other than C, N and P can limit organism growth, alter competitive interactions, and even exert control over biogeochemical cycling of the major elements. For example, Fe availability can alter C:Si ratios in diatoms (De La Rocha et al. 2000), limit primary production, and N-fixation by cyanobacteria, which can have cascading effects on phytoplankton community composition, marine food web structure and, ultimately, on the stoichiometry of upwelled nutrients (Falkowski 1997). Several other elements, including Mo, Cd and Zn, have the potential to limit primary production, constrain N acquisition, or slow P uptake (Howarth and Cole 1985, Morel et al. 1994, Cullen et al. 1999). For terrestrial plants, K and Ca are the most likely candidates to be limiting after N and P, especially where N pollution and acid rain strongly affect the relative availability of cations. Future work is needed to further elucidate the dynamics of these other elements within organisms under different supply ratios of nutrients and energy in a comprehensive set of taxa under different environmental conditions.

Release and storage of resources

The accumulation of excess C within organisms may have few benefits when other elements place strong constraints on growth and reproduction (Hessen et al. 2004). Consequently, organisms have a wide variety of mechanisms to release this excess C back into the environment. For example, aphids eliminate excess C by egesting large quantities of C-rich "honeydew" (Rhodes et al. 1996). Similarly, experimental work shows that *Daphnia* can increase the excretion of endogenous DOC, particularly when fed food having high C:P ratios (Darchambeau et al. 2003). The capability to dispose of surplus C through excretion may be affected by consumer food selectivity (Anderson et al. 2005). In particular, generalist feeders are hypothesized to better cope with elemental deficiencies in food (using post-absorption mechanisms) than do specialist feeders (Anderson et al. 2005).

Autotrophs, whose C:nutrient ratios tend to be more variable than those of heterotrophs, also obtain C in excess of metabolic demand, which they can use to acquire or protect scarce nutrient elements. As such, there may also be a positive fitness value from homeostatic maintenance when processes release C from the plant. For example, some plants feed photosynthate to mycorrhizal or N-fixing symbionts to increase uptake of P or N, respectively. Plant roots exude C-rich compounds, including organic acids and phosphatases (to increase P availability), sugars (to increase microbial activity), and ligands (to chelate metals) near the root zone (Uren 2000, Bertin et al. 2003). Plants synthesize

high C defensive compounds like lignin and tannins to defend tissue nutrients from herbivory. Anthocyanins, non-green pigments that contain no N, are produced under relatively high C, low N conditions, possibly to protect photosystems from oxidative damage and to allow nutrients to be efficiently resorbed during senescence (Martin et al. 2002, Schaberg et al. 2003). Terrestrial plants spend as much as 10% of their fixed C producing volatile organic compounds (VOCs) associated with a number of different possible functions, including herbivory defense and protection of leaf proteins from thermal stress (Penuelas and Llusia 2004). One of these VOCs, isoprene, is a breakdown product of dimethylallyl diphosphate (DMAPP), which serves in leaf pigment synthesis. It has recently been hypothesized that isoprene production may be a sort of metabolic "safety valve" to prevent scarce phosphate from being unnecessarily sequestered in DMAPP (Rosenstiel et al. 2004). Plants also have a non-phosphorylating respiratory pathway that does not produce energy in the form of ATP; use of this "wasteful" alternative oxidase may protect the photosystem from damage by free radicals (Vanlerberghe and McIntosh 1997, Maxwell et al. 1999).

The disposal of excess C may be achieved by a variety of other physiological processes in a range of organisms. Excess dietary C can be removed, in part, by increased heat production, diet-induced thermogenesis (DIT). DIT is found in animals as different as spruce budworms, *Daphnia*, locusts, and grizzly bears (Zanotto et al. 1997, Darchambeau et al. 2003, Felicetti et al. 2003, Trier and Mattson 2003). Physiological (i.e. metabolic pathways) and behavioural mechanisms may account for the increased heat production and respiratory C loss created by DIT (Zanotto et al. 1997, Darchambeau et al. 2003, Trier and Mattson 2003). Aquatic bacteria and autotrophs also can increase respiration when faced with high quantities of DOC or light (respectively) relative to nutrients (Robinson 1996, Alcoverro et al. 2000, Smith and Prairie 2004). Significant quantities of excess C can also be exuded by algae growing under high light, low nutrient conditions (Berman-Frank and Dubinsky 1999, Goto et al. 1999).

The storage of elements is likely an important driver of C:N:P ratios in many autotrophs and some heterotrophs. While storage reflects excessive supply of elements in an organism's elemental budget, the costs (energy or space consumption) and benefits (future growth) associated with the storage of multiple elements should be further explored. Storage of P is relatively well-studied in some organisms. For example, it is well known that heterotrophic bacteria store P as polyphosphate (Lötter and Murphy 1985) both as future sources of P and energy (Kornberg et al. 1999). Less is known about elemental storage in many metazoans.

Some freshwater zooplankton may acquire or assimilate at least modest amounts of P in excess of immediate growth demands over short time scales (Sterner and Schwalbach 2001). Similarly, P storage has been found in the heamolymph of an insect (Woods et al. 2002). Work is needed to explore how the cellular and molecular mechansims that animals use to create these stores of unused elements.

Autotrophs are capable of storing relatively large quantities of elements (compared to many heterotrophs) in their vacuoles. Some evidence suggests the N:P ratio of this storage may generally be low in plants. For example, fertilized plants accumulate relatively more excess P than excess N, expressed as a percentage of nutrients in unfertilized leaves (Judd et al. 1996, Cordell et al. 2001). This could be due to the high energy cost of nitrate reduction or because C fixation is more tightly coupled to N content (via Rubisco) than to P content. Similarly, phytoplankton also accumulate large stores of P in polyphosphate granules but more modest quantities of vacuolar N (Raven 1997). More work could also examine the molecular (e.g. up and down regulation of gene expression) and cellular (e.g. allocation of in specific cell bodies) controls on the relative storage of different elements. In addition, elemental storage increases the difficulty of interpreting the relationship between organismal elemental ratios and specific physiological functions (e.g. growth). X-ray microanalysis is a tool that may be especially useful for this question as it can identify specific storage bodies within cells (Heldal et al. 1985, 2003).

Elemental release ratios by consumers are predicted by ecological stoichiometry to reflect both the elemental composition of the food and the animal (Elser and Urabe 1999). Experimental studies should work to better establish the physiological bases of elemental release in a wide range of animal species. For example, the mode of physiological regulation employed by the animal to maintain a homeostatic elemental composition will likely affect the form and fate of the waste products. Animals can eliminate excess ingested elements by reducing digestive efficiencies of excess elements and releasing them in feces. Alternatively, the removal of assimilated elements can occur by post-absorptive processes resulting in the release of dissolved organic C and CO_2 (Darchambeau et al. 2003). Theoretic considerations of stoichiometric regulation indicate that post-absorption processes may be more effective than pre-absorptive processes for generalist feeders to dispose of surplus C and other elements in response to varying food quality (Anderson et al. 2005). Future research should determine the relative costs and benefits of pre- and post-absorption processes in the release of excess elements and their effects on biogeochemical cycling and trophic flows in ecosystems.

Concluding remarks

Ecological stoichiometry provides a useful perspective to examine food web processes and ecosystem function, because it links energy, elements, organisms, and ecological processes in ecosystems. Organismal physiology is a centerpiece of this approach because it largely constrains the ratios with which elements are acquired, incorporated, and released by the organism. Here, we explored the basis for the stoichiometry of key physiological processes in diverse organisms and reviewed recent progress in this field. It is clear based on this recent work that stoichiometric imbalances have strong effects on many physiological processes underlying growth, reproduction, and maintenance in autotrophs and heterotrophs. Future work should build on these foundations by specifically focusing on the connections between the relative supplies of energy and elements in the environment to the physiology and biochemistry of organisms and, subsequently, to their inter-specific interactions and biogeochemistry in diverse ecosystems.

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