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IDEA AND PERSPECTIVE

Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling

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Abstract

Ecologists have long recognized that species are sustained by the flux, storage and turnover of two biological currencies: energy, which fuels biological metabolism and materials (i.e. chemical elements), which are used to construct biomass. Ecological theories often describe the dynamics of populations, communities and ecosystems in terms of either energy (e.g. population-dynamics theory) or materials (e.g. resource-competition theory). These two classes of theory have been formulated using different assumptions, and yield distinct, but often complementary predictions for the same or similar phenomena. For example, the energy-based equation of von Bertalanffy and the nutrient-based equation of Droop both describe growth. Yet, there is relatively little theoretical understanding of how these two distinct classes of theory, and the currencies they use, are interrelated. Here, we begin to address this issue by integrating models and concepts from two rapidly developing theories, the metabolic theory of ecology and ecological stoichiometry theory. We show how combining these theories, using recently published theory and data along with new theoretical formulations, leads to novel predictions on the flux, storage and turnover of energy and materials that apply to animals, plants and unicells. The theory and results presented here highlight the potential for developing a more general ecological theory that explicitly relates the energetics and stoichiometry of individuals, communities and ecosystems to subcellular structures and processes. We conclude by discussing the basic and applied implications of such a theory, and the prospects and challenges for further development.

Keywords

Allometry, growth rate hypothesis, metabolic rate, nutrient limitation, organelle.

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INTRODUCTION

Since the early days of ecology, mathematical theory has contributed substantially to our understanding of the factors that control ecological communities (e.g. Verhulst 1838; Lotka 1925; Volterra 1926). Theories now exist to describe a variety of ecological phenomena including individual growth, population regulation, food-web structure and ecosystem dynamics. However, these theories are often derived using different assumptions, pertain to different levels of biological organization and yield predictions in different currencies (e.g. numbers of individuals, species or trophic levels in communities; fluxes of nutrients, energy or biomass in ecosystems). In the decades ahead, our ability to address the challenges brought on by environmental change will depend in part on developing a more predictive body of ecological theory that elucidates inter-relationships among biological currencies.

Two biological currencies in particular have been the focus of much theoretical work in ecology: energy, which is required to fuel the metabolic processes of survival, growth and reproduction in organisms; and materials (i.e. chemical elements), which are required to construct biomass (Lotka 1925; Lindeman 1942; Redfield 1958; Reiners 1986; Brown *et al.* 2004). Cellular and molecular biologists have long recognized that energy and materials are fundamentally linked through the biochemical reactions of metabolism (Morowitz 1968). However, understanding and quantifying

interrelationships between energy and materials – particularly for elements other than carbon (C) – has proven to be a far greater challenge for ecological phenomena (Elser & Hamilton 2007).

At the individual level, growth is among the most thoroughly investigated biological processes in terms of energy and nutrient requirements. A number of nutrient-based models have been developed that quantify how growth rates are affected by the availability of elements including nitrogen (N) and phosphorus (P) (e.g. Michaelis & Menten 1913; Monod 1950; Droop 1973). These nutrient-based growth models serve as the basis for resource-competition theories in ecology (e.g. Tilman *et al.* 1981). A number of energy-based models have also been developed that quantify growth rates in terms of metabolic rate (Brody 1945; von Bertalanffy 1957), and/or primary determinants of metabolic rate, namely body size and temperature (Arrhenius 1889; Kleiber 1961). These models also serve as the basis for ecological theories in areas such as food-web dynamics (e.g. Yodzis & Innes 1992; Brown *et al.* 2004; Reuman *et al.* 2008). However, energy- and nutrient-based models of growth, and ecological theories based on these models, have largely been developed independently.

Here, we aim to integrate common principles and assumptions that underlie these two classes of models to better understand how energy and nutrient availability *combine* to constrain individual-level processes, and thereby affect the flux, storage, and turnover of energy and elements in ecosystems. We do so by integrating key concepts from two emerging theoretical frameworks: the metabolic theory of ecology (MTE, Brown *et al.* 2004), which focuses on the importance of individual energetics to ecology, and ecological stoichiometry theory (EST, Sterner & Elser 2002), which focuses on the importance of element availability to ecology. MTE and EST have both placed special emphasis on understanding the mechanistic basis of individual growth down to the level of cellular organelles (e.g. Elser *et al.* 1996; Gillooly *et al.* 2002; Sterner & Elser 2002; West *et al.* 2002). Results of several recent studies suggest that integrating models and concepts of MTE and EST may lead to a new, more general theory that encompasses plant and animal physiology, and community and ecosystem ecology (e.g. Brown *et al.* 2004; Gillooly *et al.* 2005a; Kerkhoff *et al.* 2005, 2006; Niklas *et al.* 2005; Kerkhoff & Enquist 2006).

Towards this end, we begin here by reviewing some key aspects of MTE and EST that are important for linking these theories. In doing so, we define MTE and EST broadly to encompass the current work and the foundations upon which this work is based. Then, we show using recently published theory and data, along with some new theoretical formulations, how key features of these two theories may be combined mathematically based on four basic principles that apply to both unicellular organisms and multicellular animals and plants. We focus mainly on leaf- and individual-level rates of energy and material flux as they pertain to growth and nutrient dynamics. We conclude with a more general discussion of prospects and challenges for fully integrating MTE and EST.

CONTRIBUTIONS AND LIMITATIONS OF MTE AND EST

MTE and the energetic-invariance concept

The MTE focuses on understanding the interplay between physiology, ecology and evolution based on variation in metabolic rate among organisms (Brown *et al.* 2004). An underlying premise of MTE is that individual metabolism is fundamental to ecology because it is through metabolism that organisms interact with their environments. Over the last 10 years, MTE has yielded two general classes of models. The first predicts how two variables – body size and temperature – affect the metabolic rates of organisms, and how these differences in turn affect and are affected by differences in life history (West *et al.* 1997, 1999, 2002; Gillooly *et al.* 2001; Allen & Gillooly 2007). MTE's focus on size and temperature is based on a rich literature in comparative physiology, which has demonstrated that these variables are primary determinants of metabolic rate across the diversity of life (Arrhenius 1889; Krogh 1916; Kleiber 1961; Allen & Gillooly 2007). The second class of models explores the consequences of metabolic rate at different levels of biological organization, from the structure and function of cellular components (e.g. the respiratory complex, West *et al.* 2002) and genomes (Gillooly *et al.* 2005b; Allen *et al.* 2006), to the structure and function of cells (Savage *et al.* 2007), populations (Savage *et al.* 2004; O'Connor *et al.* 2007), communities (Reuman *et al.* 2008) and ecosystems (Enquist *et al.* 2003; Allen *et al.* 2005; Lopez-Urrutia *et al.* 2006).

One contribution of MTE – the energetic-invariance concept – is particularly relevant when considering integration with EST. This concept was put forward as an assumption in the model of West *et al.* (1997) (hereafter WBE), which was proposed to account for 'quarter-power scaling' of metabolic rate with body size. Quarter-power scaling has been documented for diverse taxa including mammals (Kleiber 1961), ectotherms (Peters 1983), plants (Niklas 1994) and unicells (Hemmingsen 1950). In this relationship, the mass-specific metabolic rate, B/M (W g^{-1}), decreases with increasing body size, M (in mass units of g), according to a power function of the form

$$B/M = B_o M^{-1/4}, \quad (1)$$

where B_o is a 'normalization constant' independent of size (W $g^{-3/4}$). The model derives the quarter-power scaling

relationship by invoking three simplifying assumptions: (i) biological distribution networks that deliver energy and materials to cells are fractal-like in their geometries; (ii) the energy required to distribute resources through these networks is minimized; and (iii) of particular relevance here, the terminal metabolic units (MUs) of these networks are energetically invariant, meaning that the energy flux per MU is independent of body size. At the most fundamental level, MUs can be defined as the protein complexes responsible for the transformation of energy and materials in metabolic organelles (West *et al.* 2002). Examples include respiratory complexes in the cristae of animal mitochondria and photosystems in the thylakoid membranes of plant chloroplasts.

The energetic-invariance concept [assumption (iii) of WBE], when combined with eqn (1), implies that the energy flux per MU, v_{MU}, is size-invariant, and therefore that the mass-specific metabolic rate increases proportionally with the density per unit mass of MUs, ρ_{MU} (g^{-1}):

$$B/M \propto \rho_{MU} v_{MU} \tag{2}$$

If assumptions (i) and (ii) are also upheld, WBE further predicts that the density of MUs should exhibit quarter-power scaling

$$\rho_{MU} = \rho_o^{MU} M^{-1/4}, \tag{3}$$

where ρ_o^{MU} is a normalization constant independent of size ($g^{-3/4}$). Thus, WBE predicts that declines in the mass-specific metabolic rate with increasing body size are directly related to declines in the densities of MUs, which comprise 'metabolic' biomass. Note also that combining eqns 2 and 3 demonstrates that $B_o = \rho_o^{MU} v_{MU}$ in eqn 1, meaning that the normalization constant is in part determined by the flux per MU.

Two aspects of eqns 2 and 3 are noteworthy. First, they apply not only at the molecular level, but also at higher levels of biological organization. Specifically, the definition of MU can be expanded to encompass cellular organelles responsible for energy and material transformation (e.g. mitochondria, chloroplasts, ribosomes), and the multicellular structures responsible for energy and material exchange between an organism and its environment (e.g. capillaries and alveoli in animals, fine roots and leaves in plants). For example, over a broad body-size range, densities of chlorophyll, chloroplasts and leaves are all proportional to rates of photosynthesis, and show approximately quarter-power scaling with plant mass (Niklas & Enquist 2001), suggesting that the energetic-invariance concept applies at all of these levels. Second, eqn 2, which embodies energetic invariance, is more general than eqn 3 in that it applies even if the other two assumptions of the WBE model are violated. For example, respiration rates of woody-plant seedlings and saplings do not exhibit quarter-power scaling (Reich *et al.* 2006), as is expected given that the space-filling assumption of WBE is violated (Enquist *et al.* 2007). Nevertheless, these data are consistent with the energetic-invariance concept (eqn 2) because respiration rates are proportional to total leaf mass (Reich *et al.* 2006; Enquist *et al.* 2007).

A second contribution of MTE has been to extend this concept of energetic invariance to encompass the kinetic effects of temperature on MU- and individual-level energy fluxes. In the case of mitochondria, the exponential effect of temperature on energy flux, v_{mito} (W), can be characterized by approximately the same Boltzmann–Arrhenius relationship, $e^{-E_r/kT}$, for animals, plants and unicells (Gillooly *et al.* 2001; Allen *et al.* 2005):

$$v_{mito} = v_o^{mito} e^{-E_r/kT} \tag{4}$$

Here k is Boltzmann's constant (=8.62×10^{-5} eV K^{-1}), T is absolute temperature in degrees Kelvin, and E_r is the average activation energy (≈0.65 eV, which corresponds to a 15-fold increase in respiration rates from 0–30 °C). Equations 3–4), in turn, can be combined to characterize the combined effects of body size and temperature on the respiration rate of an organism (Gillooly *et al.* 2001; Allen *et al.* 2005):

$$B/M = \rho_{mito} v_{mito} = b_o M^{-1/4} e^{-E_r/kT}, \tag{5}$$

where

$$b_o = \rho_o^{mito} v_o^{mito} \tag{6}$$

and ρ_{mito} is the density of mitochondria per unit mass (g^{-1}). Together eqns 4–6 demonstrate how individual metabolic rate can be expressed in terms of subcellular-level processes based on the densities and energy fluxes of MUs (in this case mitochondria).

Limitations of MTE models

There are many strengths and limitations of MTE and EST, but we will consider only those that are most pertinent to developing a more integrative theory. In this context, there are two limitations of MTE worth mentioning. First, the proposed mechanistic bases of MTE models require further theoretical investigation and experimental tests, especially at the subcellular level (see, for example, Clarke 2004). In

particular, the WBE model, which itself builds upon a long history of work (e.g. Thompson 1942), requires further evaluation before it can be viewed as the explanation for the ubiquity of quarter-power scaling in biology (Allen & Gillooly 2007). Nevertheless, it has proven useful for understanding biological scaling in general, and for understanding how MTE and EST can be integrated in particular. Second, MTE models generally do not account for the demonstrably important effects of factors other than size and temperature on metabolic rate, such as resource limitation (Monod 1950), which may induce changes in the metabolic normalization constant, b_o (Brown *et al.* 2004), or even the scaling exponent in some circumstances (e.g. Finkel 2001). Consequently, the variance left unexplained by MTE models can be substantial, as has been noted in some critiques (e.g. Tilman *et al.* 2004). Still, the approach of MTE has proven useful for generating first-order predictions for many ecological and evolutionary processes.

EST and the stoichiometric-invariance concept

Ecological stoichiometry theory focuses on understanding how differences in the balance of biologically important elements both affect and are affected by organisms and the environments in which they live (Sterner & Elser 2002). The foundations of this work are based on first principles of physics, chemistry and biology, including Lavoisier's law of conservation of mass and Proust's law of constant proportions. Since the early days of ecology, EST has applied these principles to better understand the structure and function of ecological systems at multiple levels. For example, at the individual level, Lotka (1925) noted that the stoichiometry of biomass differs substantially from that of the lithosphere due to the biological processing of materials. At the community level, Lindeman (1942) recognized that biota obey the laws of thermodynamics and mass-balance when energy and biomass are transferred between trophic levels. And at the ecosystem level, Redfield (1958) observed that the C : N : P stoichiometry of the world's oceans is maintained within relatively narrow bounds because phytoplankton biomass tends to obey the law of constant proportions. Anticipating the approach advocated here, Reiners (1986) recognized that predicting ecosystem dynamics from individual- and community-level processes would require explicitly linking element fluxes to energetics. Today, EST continues to successfully apply these principles to aid in understanding many aspects of the structure and function of ecological systems.

One particularly relevant contribution of EST, which we shall refer to as the stoichiometric-invariance concept, is the observation that the constituents of 'metabolic' and 'structural' biomass fractions comprising organisms are often relatively fixed with respect to elemental composition. Specifically, the elemental compositions of subcellular constituents (e.g. ribosomes), and of structure (e.g. skeleton, fat, wood), are often highly conserved across taxa (Williams & Frausto da Silva 1996; Sterner & Elser 2002), reflecting the common biochemical heritage of life (Morowitz 1968; Williams & Frausto da Silva 1996). This concept is useful for understanding the nutritional requirements of organisms for survival, growth and reproduction, and for understanding how these processes, in turn, affect the flux, storage and turnover of elements in ecosystems. For example, by invoking the concept of stoichiometric invariance, the 'growth rate hypothesis' of Elser *et al.* (1996) predicts that variation in whole-body P concentrations is partially attributable to increases in the densities of P-rich ribosomal RNA, ρ_{ribo} (g^{-1}), required to sustain higher growth rates. Given that the mass fraction of RNA in P, f_P^{RNA}, is *c.* 0.09 g P g^{-1} RNA for all organisms, and that ribosomes comprise most of the RNA ($f_{ribo}^{RNA} \approx 0.85$), the contribution of relatively P-rich RNA to whole-body P concentration, P (g P g^{-1}), can be expressed as

$$P = f_P^{RNA}\rho_{ribo}M_{ribo}/f_{ribo}^{RNA} + P_O = f_P^{RNA}[RNA] + P_O \qquad (7)$$

Here M_{ribo} is the mass of a ribosome, $[RNA] \equiv \rho_{ribo}M_{ribo}/f_{ribo}^{RNA}$ is the total RNA concentration, $f_P^{RNA}[RNA]$ is the concentration of RNA-associated P, and P_O is the concentration of other P 'pools' such as skeleton (see Table 1 for parameter definitions and estimates). So, eqn 7, which is our mathematical formulation of Elser *et al.*'s (1996) growth rate hypothesis, uses stoichiometric invariance and the law of conservation of mass to relate the elemental composition of the organism to that of subcellular constituents, in this case ribosomes.

A second contribution of EST has been the development of theory for understanding the effects of nutrient limitation on species. For example, this theory has helped ecologists to understand why biological activity is often constrained by a single limiting resource at a given point in time. This observation, which is referred to as Liebig's (1840) law, is in fact a consequence of stoichiometric invariance: if biomass has a fixed elemental composition, then whichever necessary element is most depleted in the environment tends to constrain the amount of biomass produced. For unicells, the effects of resource availability on biota are often quantified by the equations of Michaelis & Menten (1913),

$$V = V_{max}R/(K + R) \qquad (8)$$

or Droop (1973)

$$\mu = \mu_\infty(1 - Q_{min}/Q) \qquad (9)$$

Table 1 Kinetic and stoichiometric parameters used in models, along with estimates and sources

Parameter	Estimate	Source
Ribosome parameters		
Ribosome flux (v_o^{ribo})	1×10^{11} bonds ribosome^{-1} s^{-1}	Gillooly *et al.* (2005a)
Ribosome kinetics (E_s)	0.65 eV	Gillooly *et al.* (2005a)
Fraction of RNA in ribosomes (f_{ribo}^{RNA})	0.85 g rRNA g^{-1} RNA	Sterner & Elser (2002)
RNA fraction in P (f_P^{RNA})	0.09 g P g^{-1} RNA	Sterner & Elser (2002)
Ribosome mass (M_{ribo})	4.2×10^{-18} g rRNA	Gillooly *et al.* (2005a)
Amino-acid mass (M_{aa})	1.8×10^{-22} g	Gillooly *et al.* (2005a)
Animal parameters		
Endotherm flux (b_o)	3.9×10^{8} W g$^{-3/4}$	Gillooly *et al.* (2005a)
Ectotherm flux (b_o^s)	9.9×10^{7} W g$^{-3/4}$	Gillooly *et al.* (2005a)
Unicell flux (b_o)	2.8×10^{7} W g$^{-3/4}$	Gillooly *et al.* (2005a)
Respiratory kinetics (E_r)	0.65 eV	Gillooly *et al.* (2001)
Energy content of ATP (E_{ATP})	5×10^{-20} J	Gillooly *et al.* (2005a)
Fraction of metabolic energy allocated to protein synthesis (α)	0.2	Gillooly *et al.* (2005a)
Energy to produce biomass (E_M)	1140 J g^{-1}	Gillooly *et al.* (2008)
Ontogenetic growth parameter (ω)	3.13	Gillooly *et al.* (2002)
P assimilation efficiency (A_P)	0.5–0.9	Frost *et al.* (2006)
Gross growth efficiency (GGE_C)	0.09–0.77	Frost *et al.* (2006)
Energy per unit C flux (ε)	2×10^{4} J g^{-1} C	Nelson & Cox (2004)
Plant parameters		
N scaling (β_o^N)	0.02	Niklas *et al.* (2005)
P scaling (β_o^P)	0.002 g$^{-1/3}$	Niklas *et al.* (2005)
Fraction of leaf N in protein ($f_{protein}^{leaf-N}$)	0.55 g protein g^{-1} N	Niklas *et al.* (2005)
Protein retention efficiency (r_e)	0.6	Niklas *et al.* (2005)

depending on whether resource limitation is considered in terms of external or internal nutrient concentrations. In these expressions, V is the rate of resource uptake per cell (μmol nutrient cell^{-1} day^{-1}) at a given environmental resource concentration, R (μmol nutrient L^{-1}), K is the half-saturation constant, V_{max} is the rate of resource uptake when resources are not limiting, μ is the specific growth rate of a population (day^{-1}) at a given cell-nutrient quota, Q (μmol nutrient cell^{-1}), μ_∞ is the growth rate at infinite quota and Q_{min} is the minimum quota required for growth. Equations 8 and 9 serve as the basis for the influential R^* theory of resource competition in ecology (Tilman *et al.* 1981).

Another important consequence of stoichiometric invariance is that element ratios can be as important as absolute resource availability in constraining biological activity. In particular, stoichiometric invariance, when combined with conservation of mass for element fluxes between trophic levels, following Lindeman (1942), yields the prediction that there exists a threshold element ratio (TER) for food (prey) at which the nutrient limiting consumer growth shifts from one element to the other (Sterner & Hessen 1994). TER is a useful concept for linking MTE with EST because it encompasses the elemental composition of biomass, and the energetics of growth and respiration (Schindler & Eby 1997; Frost *et al.* 2006). For example, Frost *et al.* (2006) demonstrated that if the concentrations of P and C in consumer biomass are fixed (represented by P and C in the expression below), and food is readily available, the threshold C : P ratio of food, $TER_{C:P}$, is partly determined by the consumer's respiration rate, B, and its rate of C consumption, I_C (g C s^{-1}):

$$TER_{C:P} = \frac{A_P}{A_C - B/\varepsilon I_C} \times \frac{C}{P} = \frac{A_P}{GGE_C} \times \frac{C}{P} \qquad (10)$$

In this expression, the gross growth efficiency, $GGE_C \equiv A_C - B/\varepsilon I_C$, is the fraction of ingested C allocated to growth, A_C is the C assimilation efficiency, A_P is the P assimilation efficiency and ε is a conversion factor used to express C consumption in units of energy flux (J g^{-1} C, Table 1).

Limitations of EST models

Two limitations of EST models are pertinent to our discussion. First, EST models do not predict the partitioning of biomass between 'metabolic' and 'structural' fractions, and therefore do not yield *a priori* predictions on the elemental composition of an entire organism. For example,

while the growth rate hypothesis identifies an important link between whole-body P concentration and the concentration of ribosomal RNA (eqn 7), a substantial fraction of P is found in constituents other than ribosomes, particularly for multicellular organisms (Gillooly *et al.* 2005a; also see Fig. 3). Second, many variables in nutrient limitation models – including V_{max}, K, μ_∞, A_P, A_C and GGE_C in eqns 8–10 – are not predicted by EST and must therefore be empirically estimated. This limits the predictive power of EST models across taxa and environments.

PRINCIPLES FOR INTEGRATING MTE AND EST

The models of MTE and EST both utilize assumptions that are grounded in energetic principles and energy balance (e.g. Boltzmann–Arrhenius temperature kinetics), and principles of nutrient availability and mass balance (e.g. Leibig's law). Indeed, some of the patterns and relationships described by these two bodies of theory could be described equivalently in fluxes of energy or elements. Nevertheless, MTE and EST could both benefit from explicitly quantifying interrelationships between currencies. And, as we will demonstrate in this section, some limitations of these two theories can be addressed using four key principles that bridge MTE and EST. To be sure, none of these principles is new, but considered together in light of MTE and EST models, they may provide a roadmap for linking the biological currencies of energy and materials in an integrative theoretical framework. Specifically, these four principles integrate MTE and EST models by linking individual-level energetics and stoichiometry to higher-order processes based on two complementary forms of invariance – energetic and stoichiometric – at the level of cellular organelles. To demonstrate the utility and generality of these principles, we discuss recent models and analyses that apply these principles to both animals and plants (unicellular and multicellular).

Principle I: Fluxes of energy and materials are linked based on the kinetics and elemental compositions of subcellular structures and processes

Principle (I) is important for linking the structure and function of subcellular components to the structure and function of organisms, particularly with respect to growth. While this principle has long been recognized as a truism by biochemists and cell biologists (Morowitz 1968), it is less often considered by ecologists working at higher levels of biological organization. In part, this is because it has not been clear until recently how higher-level processes such as growth are related to the structure and function of organelles, either qualitatively or quantitatively. The energetic and stoichiometric forms of invariance, in fact, yield Principle (I) because together they imply that adding an additional MU to a unit of biomass induces a predictable change in both the energy flux and elemental composition of that biomass.

Two recently published models, the Gillooly *et al.* (2005a) model of RNA concentration in animals, and the Niklas *et al.* (2005) model of leaf growth in plants (see Table 1 for a list of model parameters) point to the importance of Principle (I). On the surface, these models appear quite different. But upon closer examination, one can see that both models assume energetic and stoichiometric invariance to derive unique predictions. Each model assumes that the masses and elemental compositions of ribosomes and amino-acids are fixed (stoichiometric invariance), and that ribosomes have a fixed capacity to synthesize proteins (energetic invariance). In addition, the model of Gillooly *et al.* (2005a) incorporates the well-established exponential effect of temperature on the rate of protein synthesis per ribosome:

$$v_{ribo} = v_o^{ribo} e^{-E_s/kT} \tag{11}$$

In this expression, v_o^{ribo} is a normalization constant independent of temperature (peptide bonds s^{-1}) and $E_s \approx E_r \approx 0.65$ eV.

The model of Gillooly *et al.* (2005a) builds on Elser *et al.*'s (1996) growth rate hypothesis (defined above) to yield predictions on whole-organism RNA concentration based on the size- and temperature-dependence of metabolic rate (eqn 5), and the observation that protein synthesis consumes a relatively constant fraction of metabolic energy in adult animals ($\alpha \approx 0.2$). The model predicts both the slope and intercept of the log–log relationship between the standardized RNA concentration, $\log\left[[\mathrm{RNA}]\left(b_o^s/b_o\right)\right]$, and body size, $\log[M]$, irrespective of temperature:

$$\log\left[[\mathrm{RNA}]\left(b_o^s/b_o\right)\right] = -\frac{1}{4}\log[M] + \log\left[\frac{\alpha M_{ribo} b_o^s}{4 v_o^{ribo} E_{ATP} f_{ribo}^{RNA}}\right] \tag{12}$$

This expression establishes the quantitative relationships of metabolic rate to the rates of ATP production and protein synthesis, and to the concentrations of RNA and RNA-associated P. It therefore links energy and materials at the cellular level to yield predictions at the level of the organism. Empirical data showing the relationship between RNA and body mass for diverse taxa support model predictions (Fig. 1).

Using a similar approach, Niklas *et al.* (2005) extended the model of Dobberfuhl (1999) to predict how the rate of accumulation of leaves in the canopy of a plant, $\mu_{leaf} = (1/M_{leaf})(dM_{leaf}/dt)$, varies with the total mass of

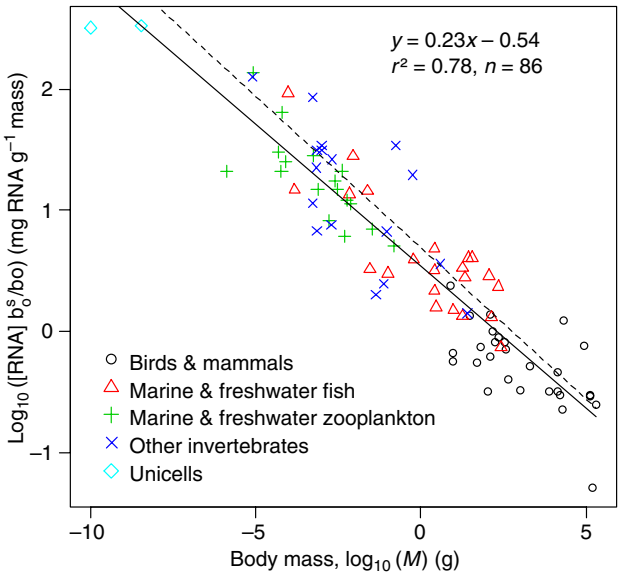

Figure 1 Whole-body RNA content corrected for taxon-specific metabolic normalization constants, b_o, plotted as a function of dry body mass for unicellular eukaryotes, multicellular ectotherms and endotherms (data compiled in Gillooly *et al.* 2005a). The normalization constant, b_o^s, used to control for differences among taxa in b_o (eqn 6) is arbitrary (see Table 1). The OLS-fitted solid line is close to the predicted dashed line of $y = -0.25x + 0.69$ mg RNA g^{-1} dry mass (eqn 12), which was calculated based on the parameter estimates in Table 1.

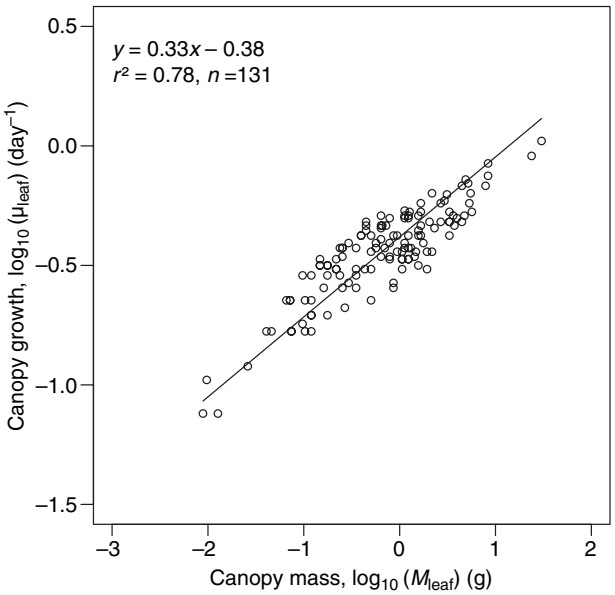

Figure 2 Relationship of mass-specific rate of leaf accumulation to total leaf-canopy size for 131 species (data from Niklas *et al.* 2005). The model (eqn 13) was fitted to the data by finding the value of f_{RNA}^{leaf-P} that minimized the sum of the squared deviations. Independent estimates for the other parameters of eqn 13 are listed in Table 1.

leaves, M_{leaf}. In the absence of direct measures of RNA concentration, Niklas *et al.* (2005) parameterized their model by combining previous estimates of leaf-level parameters with allometric expressions characterizing the size-dependencies of total canopy N ($N_{leaf} = \beta_o^N M_{leaf}$) and P ($P_{leaf} = \beta_o^P M_{leaf}^{4/3}$) (Table 1). Using these parameters, Niklas *et al.* (2005) proposed a model similar in form to the following (see Appendix S1 in Supporting Information):

$$\log[\mu_{leaf}] = \frac{1}{3}\log[M_{leaf}] + \log\left[\frac{f_{ribo}^{RNA} f_{RNA}^{leaf-P} \beta_o^P v_{ribo} M_{aa} r_e}{f_P^{RNA} M_{ribo} f_{protein}^{leaf-N} \beta_o^N}\right] \quad (13)$$

Equation 13 yields predictions for the slope and intercept of the log–log linear relationship between the rate of leaf growth and canopy size. However, as Niklas *et al.* (2005) pointed out, it was necessary to estimate the fraction of leaf P in RNA, f_{RNA}^{leaf-P}, from their data due to an absence of independent measurements. Given this caveat, Niklas *et al.* (2005) concluded that data for 131 plant species (depicted in Fig. 2) were broadly supportive of their model. Here, the fitted intercept in Fig. 2 yields an estimate of 9% for the fraction of P in RNA, after accounting for the temperature dependence of ribosome kinetics (eqn 11, Table 1), if we assume a growth temperature of 25 °C. This estimate is plausible given the range of estimates reported for ectothermic animals (see, for example, Gillooly *et al.* 2005a).

The studies of Gillooly *et al.* (2005a) and Niklas *et al.* (2005) both demonstrate how relationships between energy flux and elemental composition at higher levels of organization (leaf, individual) can be predicted based on subcellular structures and processes by combining the energetic- and stoichiometric-invariance concepts of MTE and EST respectively. These two models yield predictions on distinct phenomena for diverse organisms based on common principles and parameters. For example, the animal model of Gillooly *et al.* (2005a) and the plant model of Niklas *et al.* (2005) both assume essentially the same protein-synthesis rate at the level of the ribosome.

Principle II: Biomass is comprised of 'metabolic' and 'structural' pools, which can exhibit distinct allometries and elemental compositions

Principle (I) must be combined with Principle (II) to predict element dynamics at the level of the organism, and at the level of the ecosystem, as we discuss below. This is because, by itself, Principle (I) is only useful for relating fluxes (e.g.

growth rate in the models above) to MU-associated nutrients (e.g. ribosomal P), and not to total nutrient concentrations in biomass, which include metabolic and structural contributions. This need to distinguish between metabolic biomass (comprised of MUs) and the remaining structural biomass has been central to the development of MTE and EST. For example, in the WBE model of MTE, the size-dependence of metabolic rate is proximately related to changes in the densities of MUs (eqn 3). Thus, MTE yields predictions on the partitioning of biomass between metabolic and structural fractions. Similarly, in the Droop equation of EST, structure contributes to $Q_{\min}$ (eqn 9), which is the minimum internal nutrient content necessary for growth.

We illustrate the importance of Principle (II) using two examples that pertain to the flux, storage and turnover of P and N. First, we consider the formulation of Gillooly *et al.* (2005a) for the body-size dependence of whole-body P. As P associated with RNA, $f_{\mathrm{P}}^{\mathrm{RNA}}[\mathrm{RNA}]$, exhibits a predictable size dependence (Fig. 1), whole-body P concentration, P, is predicted to decline with increasing adult size, M, following eqns 7 and 12:

$$P = f_{\mathrm{P}}^{\mathrm{RNA}}[\mathrm{RNA}]/f_{\mathrm{ribo}}^{\mathrm{RNA}} + P_{\mathrm{O}} = \frac{f_{\mathrm{P}}^{\mathrm{RNA}}\alpha M_{\mathrm{ribo}} b_{\mathrm{o}}}{4 v_{\mathrm{o}}^{\mathrm{ribo}} E_{\mathrm{ATP}} f_{\mathrm{ribo}}^{\mathrm{RNA}}} M^{-1/4} + P_{\mathrm{O}} \tag{14}$$

Equation 14 assumes that P content in pools other than RNA, P_{O}, shows negligible change with size, and varies little among species. The model (solid line in Fig. 3), which was independently parameterized based on the biochemical parameters in Table 1 and an overall estimate of P_{O} = 0.006 g P g^{-1} for invertebrates (Elser *et al.* 2003), does reasonably well at predicting nonlinear declines in whole-body P concentration for insects collected from the Sonoran desert. This nonlinear relationship arises because the animal P in RNA systematically declines with increasing size (dashed line in Fig. 3).

Second, we consider the well-established positive relationship between leaf-level N and the leaf-level rate of photosynthesis (Field & Mooney 1986; Wright *et al.* 2004), which is a primary determinant of plant growth. Based only on Principle (I), one might conclude that this relationship indicates that all leaf-level N is contained in chloroplasts. However, using Principles (I) and (II), we can formulate a hypothetical model for this relationship to quantify the leaf-level N fractions in metabolic and structural constituents. Based on Principle (I), we first note that the rate of photosynthesis per unit leaf tissue, $B_{\mathrm{leaf}}/M_{\mathrm{leaf}}$, is equal to

$$B_{\mathrm{leaf}}/M_{\mathrm{leaf}} = \rho_{\mathrm{chlor}} v_{\mathrm{chlor}} \tag{15}$$

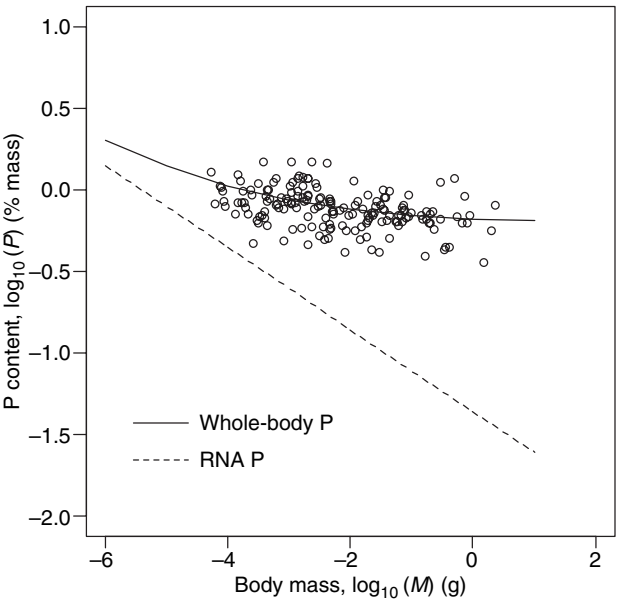

Figure 3 Relationships of whole-body P concentration and RNA concentration to adult size for 169 insect species from the Sonoran desert (Woods *et al.* 2004). The solid lines were predicted using eqns 12 and 14 based on the parameter estimates listed in Table 1. See Gillooly *et al.* (2005a) for further details on how these parameter estimates were obtained.

following eqn 2, where B_{leaf} is the total metabolic rate of the leaf canopy (W), ρ_{chlor} is the number of chloroplasts per unit mass of leaf (g^{-1}) and v_{chlor} is the photosynthetic flux per chloroplast (W). Then, based on Principle (II), we express the overall N concentration of a leaf, $N_{\mathrm{leaf}}/M_{\mathrm{leaf}}$, as the sum of the N pools associated with chloroplasts, $N_{\mathrm{chlor}}\rho_{\mathrm{chlor}}$, and other leaf constituents, $N_{\mathrm{O}}^{\mathrm{leaf}}$ (e.g. cell wall)

$$N_{\mathrm{leaf}}/M_{\mathrm{leaf}} = N_{\mathrm{chlor}}\rho_{\mathrm{chlor}} + N_{\mathrm{O}}^{\mathrm{leaf}}, \tag{16}$$

where N_{chlor} is the N content of a chloroplast (g N). Together, eqns 15 and 16 yield the following linear function:

$$\rho_{\mathrm{chlor}} v_{\mathrm{chlor}} = \frac{v_{\mathrm{chlor}}}{N_{\mathrm{chlor}}}\left(N_{\mathrm{leaf}}/M_{\mathrm{leaf}}\right) - \frac{v_{\mathrm{chlor}}}{N_{\mathrm{chlor}}} N_{\mathrm{O}}^{\mathrm{leaf}} \tag{17}$$

Provided that the invariance assumptions are upheld for chloroplasts, and that $N_{\mathrm{O}}^{\mathrm{leaf}}$ is independent of chloroplast density, eqn 17 predicts a linear relationship between the photosynthetic rate, $\rho_{\mathrm{chlor}} v_{\mathrm{chlor}}$ and leaf-level N concentration, $N_{\mathrm{leaf}}/M_{\mathrm{leaf}}$. A plot of leaf-level flux against leaf-level N concentration for 107 plant species supports this prediction by showing a linear function that accounts for 69% of the variation, consistent with Principle (I) (Fig. 4). And with respect to Principle (II), the fitted slope and intercept of this relationship yield an estimate for the average concentration of structural leaf N ($N_{\mathrm{O}}^{\mathrm{leaf}} = 0.006$).

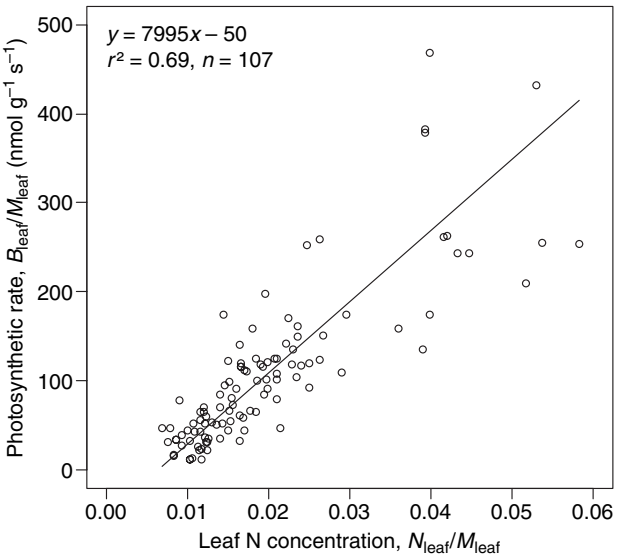

Figure 4 Relationship of photosynthetic rate per unit leaf mass to leaf N concentration for measurements taken from species around the world (Reich *et al.* 1999). The model (eqn 17) was fitted to the data using reduced major-axis regression.

Both examples above demonstrate the need to distinguish between metabolic and structural biomass when relating fluxes of energy and materials to the elemental composition of biomass. This issue is particularly relevant because the sizes of the metabolic and structural biomass fractions may exhibit distinct allometric scaling relationships, leading to more complicated relationships between overall nutrient concentration and size, as demonstrated by eqn 14 and the data depicted in Fig. 3. Moreover, the elemental compositions of these fractions may differ considerably, as demonstrated by the low estimate of N_O^{leaf} relative to leaf N for many of the taxa depicted in Fig. 4.

Principle III: At the level of the organism, fluxes of energy and elements are governed by metabolic rate and its determinants

Principle (III) is important for understanding how differences among species with respect to body size, temperature and resource availability influence the flux, storage and turnover of energy and elements in ecosystems. This principle, which is a central tenet of MTE, reflects the fact that the uptake and transformation of energy and materials by an organism requires metabolic energy (Brown *et al.* 2004). It has two important consequences. First, as body size and temperature have predictable effects on energy flux (eqn 1), these variables should also have predictable effects on material fluxes in nutrient-limitation models such as the Michaelis–Menten equation (eqn 8) and the Droop equation (eqn 9). Second, as the elemental composition of biomass is also inextricably linked to flux, following Principles (I)–(II), it should be possible to derive novel predictions on how body size, temperature and elemental composition combine to influence fluxes of energy and matter.

Three examples, one for unicellular plants and two for animals, illustrate the importance of combining Principles (I)–(III) to better understand nutrient cycling. First, we consider the study of Litchman *et al.* (2007), which uses a nutrient-uptake model for phytoplankton (Aksnes & Egge 1991) to derive predictions for the body-size-scaling of parameters in the Michaelis–Menten equation (Michaelis & Menten 1913; Briggs & Haldane 1925) and the equation of Droop (1973). With regard to the Michaelis–Menten equation, Litchman *et al.* (2007) predict a 2/3-power scaling exponent for maximum rate of N uptake, V_{max}, in relation to cell volume, V_{cell}:

$$V_{max} \propto V_{cell}^{2/3} \tag{18}$$

This predicted exponent differs from the value of 3/4 predicted by WBE because Litchman *et al.* (2007) invoke the assumption of Aksnes & Egge (1991) that the rate of nutrient uptake, and hence the cellular metabolic rate, is controlled by the surface area of the cell. As shown in Fig. 5, there is a tight relationship between V_{max} and cell volume for phytoplankton. Moreover, the slope of this log–log relationship is closer to 2/3 than 3/4 ($\bar{x} = 0.61$, 95% CI: 0.45–0.77). These findings are consistent with the surface-

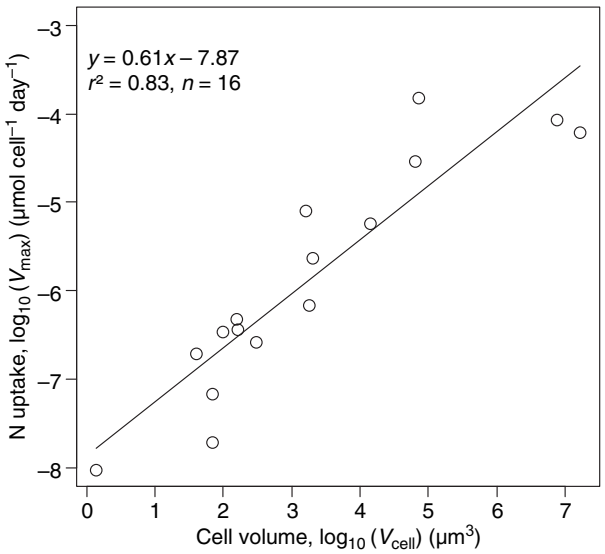

Figure 5 Relationship of maximum uptake rate of nitrate to cell volume for a compilation of data for diatoms, coccolithophores, dinoflagellates and green algae (Litchman *et al.* 2007). The 95% CI for the OLS-fitted regression slope is significantly less than unity (0.45–0.77), as predicted by eqn 18.

to-volume-scaling arguments of Litchman *et al.* (2007), although a 3/4-power slope is also consistent with their data. More generally, these findings are consistent with a large body of literature demonstrating that body size is a primary determinant of nutrient dynamics in unicellular and multicellular organisms (e.g. Wen *et al.* 1997; Vanni *et al.* 2002; Hall *et al.* 2007).

For the second example, we combine the RNA model of Gillooly *et al.* (2005a) (eqns 12 and 14) with the ontogenetic growth model of West *et al.* (2001) to characterize how body size, temperature and whole-body P influence the temperature-corrected rate of individual growth, $G\mathrm{e}^{Er/kT}$, where $1/G$ is the time from the juvenile stage to the size at adulthood, M:

$$G\mathrm{e}^{Er/kT} = \frac{b_{\mathrm{o}}M^{-1/4}}{4\omega E_{\mathrm{M}}} = \frac{f_{\mathrm{ribo}}^{\mathrm{RNA}} v_{\mathrm{o}}^{\mathrm{ribo}} E_{\mathrm{ATP}}}{\omega E_{\mathrm{M}} \alpha M_{\mathrm{ribo}} f_{\mathrm{P}}^{\mathrm{RNA}}}(P - P_{\mathrm{O}}) \qquad (19)$$

This expression includes all of the parameters in the RNA model along with E_{M}, which characterizes the energy to produce biomass (West *et al.* 2001; Gillooly *et al.* 2008; Moses *et al.* 2008), and ω, which characterizes taxon-specific attributes of the ontogenetic growth curve (Table 1, Appendix S1). This equation demonstrates that growth (a flux) can be expressed in terms of size and temperature, or in terms of stoichiometry and temperature, without reference to size, reflecting linkages between body size, temperature, metabolic rate, and the energy and density of P-rich ribosomes required for growth. The correspondence of model (eqn 19) to data is reasonable (Fig. 6), given that the cluster of four points exhibiting the largest negative deviations corresponds to only one of the eight species analysed. Moreover, the slope of the line, $(f_{\mathrm{ribo}}^{\mathrm{RNA}} v_{\mathrm{o}}^{\mathrm{ribo}} E_{\mathrm{ATP}})/(\omega E_{\mathrm{M}} \alpha M_{\mathrm{ribo}} f_{\mathrm{P}}^{\mathrm{RNA}})$, is independently calculated based on the parameter estimates listed in Table 1 rather than being fitted to the data. We note, however, that the confidence interval of the one fitted parameter, P_{O}, is substantial ($\bar{x} = 0.0006$, 95% CI: 0.00001–0.04), perhaps indicating significant variation in structural P among taxa.

For the third example, we extend the model above to predict the TER for food at which consumer growth shifts from nutrient (P) to energy (C) limitation, $\mathrm{TER_{C:P}}$. Frost *et al.* (2006) reported a significant negative relationship between $\mathrm{TER_{C:P}}$ and growth, and proposed that the growth rate hypothesis of EST could account for this relationship. By integrating EST with MTE using eqns 10, 12, 14 and 19, we can explicitly quantify this hypothesis:

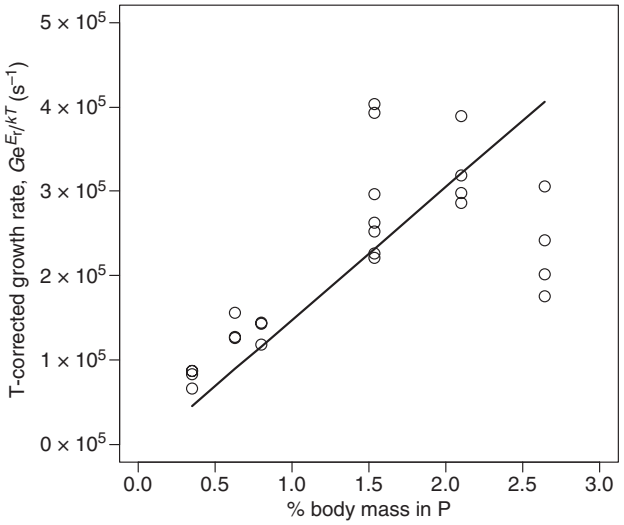

Figure 6 Relationship of temperature-corrected growth rate to whole-body P concentration for eight species of zooplankton (data compiled in Gillooly *et al.* 2002). The model (solid line, eqn 19) was fitted to the data by finding the value of P_{O} that minimized the sum-of-squared deviations. Independent estimates for all of the other parameters in eqn 19 are listed in Table 1.

Holding other variables constant, eqn 20 predicts a negative relationship between $\mathrm{TER_{C:P}}$ and G because maintaining a higher growth rate requires more P-rich ribosomes which, in turn, requires eating foods richer in P. In addition to providing the first theoretical support for the hypothesis of Frost *et al.* (2006), eqn 20 highlights that the extent to which growth is P-limited is influenced not only by the nutrient-use efficiencies ($\mathrm{GGE_C}$, A_{P}, A_{C}), but also by the kinetics and stoichiometry of ribosomes ($f_{\mathrm{ribo}}^{\mathrm{RNA}}$, $v_{\mathrm{o}}^{\mathrm{ribo}}$, M_{ribo}, $f_{\mathrm{P}}^{\mathrm{RNA}}$), following Principle (I), the amount of P stored in biomass pools other than RNA, P_{O}, following Principle (II), and the energetics of individual metabolism (B, I_{C}, E_{r}, ω, E_{M}, α), following Principle (III).

As a first step towards empirically evaluating this new model, we fit eqn 20 to the invertebrate data of Frost *et al.* (2006) using nonlinear least-squares regression. The fitted model yields an estimate for P_{O} (=0.0068; 95% CI: 0.0020–0.030) similar to previous estimates (Elser *et al.* 2003), and an estimate for $\mathrm{GGE_C}$ (=0.29; 95% CI: 0.09–0.65) within the range of values reported by Frost *et al.* (2006) (0.09–0.77) (see Fig. 7 legend for details on these calculations). Thus, predictions of the model appear broadly consistent

$$\mathrm{TER_{C:P}} = \left(\frac{A_{\mathrm{P}}C}{\mathrm{GGE_C}}\right)\left(\frac{f_{\mathrm{ribo}}^{\mathrm{RNA}} v_{\mathrm{o}}^{\mathrm{ribo}} E_{\mathrm{ATP}}}{\omega E_{\mathrm{M}} \alpha M_{\mathrm{ribo}} f_{\mathrm{P}}^{\mathrm{RNA}}}\right) \Big/ \left(G\mathrm{e}^{E_{\mathrm{r}}/kT} + \frac{f_{\mathrm{ribo}}^{\mathrm{RNA}} v_{\mathrm{o}}^{\mathrm{ribo}} E_{\mathrm{ATP}}}{\omega E_{\mathrm{M}} \alpha M_{\mathrm{ribo}} f_{\mathrm{P}}^{\mathrm{RNA}}} P_{\mathrm{O}}\right) \qquad (20)$$

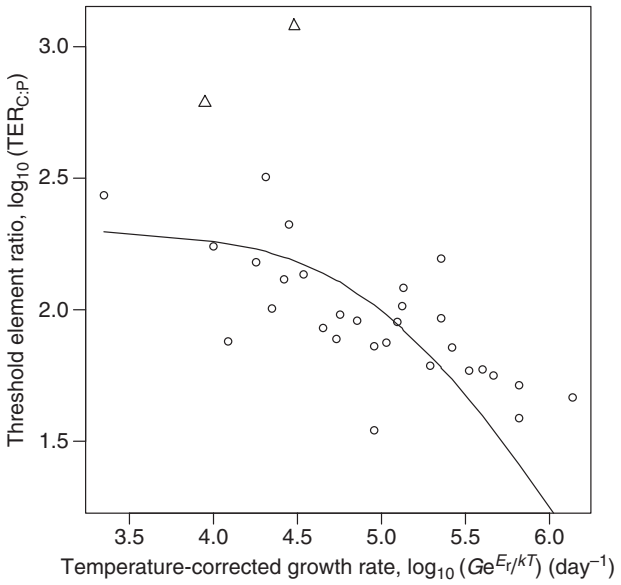

Figure 7 Relationship of threshold C-to-P ratio (mass basis) to temperature-corrected growth rate for invertebrate data compiled by Frost *et al.* (2006). Equation 20 was fitted to the data (solid line) by estimating two parameters using nonlinear least-squares regression, assuming a growth temperature of 25 °C. Excluding two points deemed to be outliers based on their leverage in the model fit (represented by triangles), this procedure yields an estimate of 0.0068 for P in pools other than RNA, P_O. The second parameter, which corresponds to the product $A_P C/\mathrm{GGE_C}$, yields an estimate of 0.29 for the gross growth efficiency, $\mathrm{GGE_C}$, given typical values of C (=0.48) and A_P (=0.8) reported by Frost *et al.* (2006), and estimates in Table 1 for other parameters. Results are similar if the two outliers are included in the analysis, although 95% CIs for P_O and $\mathrm{GGE_C}$ are substantially wider.

with the data of Frost *et al.* (2006). While these estimates capture the overall relationship of growth rate to $\mathrm{TER_{C:P}}$ (Fig. 7), it is important to recognize that there exist substantial differences among taxa with respect to other variables predicted by eqn 20 to affect $\mathrm{TER_{C:P}}$, including the energy required to produce biomass, E_M (see Moses *et al.* 2008), and the C:P stoichiometry of biomass (see Frost *et al.* 2006). For example, the datum exhibiting the greatest deviation from the fitted line (depicted by a triangular point in Fig. 7) had a *total* P concentration lower than our overall estimate for the nonmetabolic P fraction, P_O. Thus, more detailed analyses are needed to assess how multiple variables influence $\mathrm{TER_{C:P}}$, and thereby contribute to deviations about the fitted line in Fig. 7. Equation 20 provides a framework to perform such analyses.

Overall, the reasonable fits of models to empirical data in Figs 6 and 7 suggests that, by using Principles (I)–(III), it is possible to predict fluxes of energy and matter using models, assumptions, and principles that encompass the energetics-based approach of MTE and the mass-balance approach of EST.

Principle IV: The storage, flux and turnover of energy and materials in biological communities and ecosystems can be estimated by summing across individuals in that community

Principle (IV) is important when considering the total flux, storage and turnover of nutrients among communities of organisms. While conceptually straightforward, Principle (IV) yields novel predictions when combined with the other three principles above. For example, the total biomass per unit area, M_T, for a community comprised of J individuals in an area of size A is readily obtained by summing the biomass contributions of individuals comprising that community:

$$M_T = A^{-1}\sum_{i=1}^{J} M_i \tag{21}$$

Equation 21 is generic in form, and is therefore applicable not only to total biomass, but also to storage of elements such as N and P in biota. However, due to the size dependence of element ratios (following Principle II), storage is influenced not only by total biomass, but also by the size distribution of individuals. For example, Kerkhoff & Enquist (2006) presented theory and data indicating that the total quantities of N and P stored in phytomass, N_T and P_T, increase nonlinearly with M_T, when comparing ecosystems ranging from arctic tundra to tropical forest (i.e. $N_T \propto P_T \propto M_T^{3/4}$), because smaller-bodied plants contain higher concentrations of MUs and associated nutrients (as illustrated above for animals in eqn 14 and Fig. 3).

Principle (IV), when combined with Principle (III), can also be used to derive predictions on ecosystem flux by linking individual metabolic rate to population- and community-level processes. For example, if population abundance per unit area, J/A, is at equilibrium with the supply rate of a limiting resource in the environment, L, and if the flux by individual i, F_i, is proportional to metabolic rate (i.e. $F_i \propto B_i$), following Principle (III), then total flux per unit area for a population, F_T (flux $\mathrm{g^{-1}\ ha^{-1}}$), is equal to:

$$\begin{aligned} F_T &= A^{-1}\sum_{i=1}^{J} F_i \propto A^{-1}\sum_{i=1}^{J} B_i \\ &= b_o M_T \left\langle M_i^{-1/4}\right\rangle e^{-E_r/kT} \propto L, \end{aligned} \tag{22}$$

where $\left\langle M_i^{-1/4}\right\rangle$ is a quarter-power average for body size $[= (\mathrm{AM_T})^{-1}\sum_{i=1}^{J} M_i^{3/4}]$ (Enquist *et al.* 2003; Allen *et al.* 2005).

Equation 22 provides a general expression for the total flux of a given element in a community comprised of different-sized individuals, and at different body temperatures. Importantly, however, while total ecosystem flux is, by definition, equal to the sum of the individual fluxes, as specified in eqn 22, predicting F_T will be challenging for communities comprised of species that compete for multiple limiting resources rather than a single resource, L.

By combining Principles (III) and (IV), Habeck & Meehan (2008) recently presented evidence that total N flux is about the same for populations of herbivorous mammals that range in size from a mouse to a moose. They obtained this result by combining Damuth's (1981) rule, which describes the size-dependence of population abundance for mammals ($J/A \propto M_i^{-3/4}$), with the observation that N excretion rate is proportional to metabolic rate (i.e. $F_i \propto B_i \propto M_i^{3/4}$). Irrespective of Damuth's rule, Habeck & Meehan's (2008) data indicate that N excretion per unit biomass declines with increasing body size in the same way as mass-specific metabolic rate (i.e. $F_T/M_T \propto M_i^{-1/4}$, following eqn 22), consistent with the data depicted in Fig. 8.

The studies of Kerkhoff & Enquist (2006) and Habeck & Meehan (2008) highlight the potential of combining energetic and stoichiometric principles to derive predictions on the storage and flux of elements in entire ecosystems. While not explicitly discussed, the individual-level relationships used to derive population- and ecosystem-level predictions in these studies are ultimately related to the fluxes, densities and stoichiometries of MUs comprising biomass, following eqns 1–7 and Principle (I). These examples highlight the potential to explicitly link element flux, storage and turnover in ecosystems to the energetics and stoichiometry of cellular organelles.

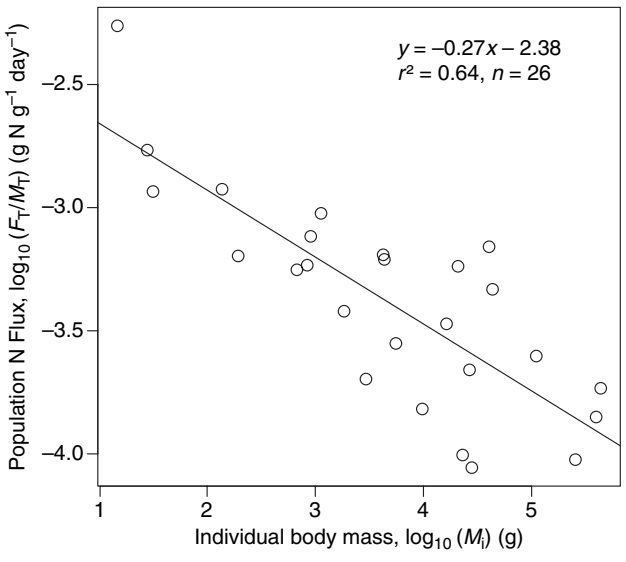

Figure 8 Size-dependence of average N flux per unit biomass for herbivorous mammal populations ranging in size from a mouse to a moose (species-level logarithmic averages of data compiled in Habeck & Meehan 2008). The fitted OLS regression slope is statistically indistinguishable ($P > 0.05$) from the predicted value of −0.25 (following eqn 22).

DISCUSSION

The models discussed above suggest that the four principles highlighted in this paper, when combined with models and concepts of MTE and EST, offer a useful framework for linking the flux, storage and turnover of energy and materials at different levels of biological organization. On the one hand, by combining the energetic-invariance concept of MTE with energy balance, it is possible, for example, to relate energy and material fluxes from leaves (eqn 15), individuals (eqns 2, 3, 5, 6) and ecosystems (eqn 22) to densities of metabolically active organelles including chloroplasts, mitochondria and ribosomes. On the other hand, by combining the stoichiometric-invariance concept of EST with mass balance, it is possible to relate organelle densities to the storage of essential elements like N and P in biomass (eqns 7, 14, 16). Together these complementary forms of invariance link the dynamics of energy and matter in individuals and ecosystems (eqns 12, 13, 17, 19, 22) to the kinetic and stoichiometric properties of organelles (eqns 4, 11; Table 1). These linkages are characterized using summation rules (e.g. eqns 21, 22) because energy and materials are fluxed, stored and turned over at all levels of biological organization.

Integration of these two theories may help address some of the limitations of MTE and EST. For example, with regard to EST models, mass-balance is insufficient to predict parameters of nutrient-limitation models such as those of Michaelis–Menten and Droop (eqns 8, 9). However, as demonstrated by the study of Litchman *et al.* (2007), it is possible to predict the size-dependence of nutrient uptake by incorporating energetics. And, with regard to MTE models, even after accounting for body size and temperature, the elemental composition of biomass has independent effects on biological rates, as demonstrated by eqn 19 and Gillooly *et al.* (2002, 2005a). More generally, the models and results presented here indicate that individual energetics and biomass stoichiometry are inextricably linked with each other, and with nutrient availability in the environment. For example, our expression for the TER (eqn 20) predicts that the extent to which growth is nutrient-limited is partly determined by biomass stoichiometry and individual energetics. Thus, models that integrate MTE and EST to predict the combined effects of all three classes of variables – biomass stoichiometry, energetics, resource

availability in the environment – should have greater predictive power.

We do not mean to imply that this proposed theory synthesis can account for all ecological phenomena. Rather, we argue that efforts along these lines offer the potential to provide a firmer foundation for understanding how energetic and stoichiometric constraints, including those imposed by attributes of organelles, combine to influence individual-level fluxes and stores of energy and matter. Such constraints can then be integrated into models aimed at predicting higher-order ecological phenomena, including competitive interactions among species, food-web structure and ecosystem dynamics.

Many important theoretical and empirical challenges remain to achieve full integration of MTE and EST. Here we highlight four more specific challenges. First, at the subcellular level, further work is needed to evaluate the energetic- and stoichiometric-invariance concepts or assumptions. Efforts along these lines are currently underway. For example, with regard to stoichiometric invariance, Elser *et al.* (2006a) demonstrated that the N contents of amino-acid residues are higher for proteomes of animals than of plants, which are more likely to experience N limitation. And with regard to energetic invariance, Tcherkez *et al.* (2006) showed that Rubisco kinetics vary among plant taxa in different CO_2 environments, and argued that this pattern reflects evolutionary optimization of protein structure to maximize photosynthesis. These examples illustrate that evolutionary mechanisms may influence nutrient cycling through their effects on the structure, function and stoichiometric composition of biological macromolecules. Such mechanisms would lead to deviations from the energetic- and stoichiometric-invariance assumptions used to construct the models discussed here, and may thereby induce variation about the predicted relationships in Figures 1–8. Evaluation of these two assumptions is therefore important for refining models, and more generally for assessing the extent to which evolutionary processes mediate linkages between energetics and stoichiometry.

Second, at the levels of tissue and individual, more experiments are needed that assess relationships between flux, stoichiometry and densities of subcellular constituents, and how these variables are affected by ecological conditions. With the exception of efforts to evaluate the growth rate hypothesis, surprisingly few studies have simultaneously measured these variables for the same individuals or tissues. Such research could benefit many areas of basic and applied ecology. For example, it may deepen our understanding of the biochemical basis of trait differences among species (e.g. leaf-level N and photosynthesis, Fig. 4), which contribute to fitness differences among species in particular environments and species turnover across environmental gradients (McGill *et al.* 2006). It may also aid in understanding of how species adapt to nutrient limitation and changing environmental conditions. For example, it is well established that the normalization constant of plants respiration, b_o (eqn 5), declines with long-term temperature increases. The biochemical basis of this phenomenon is still poorly understood despite its importance to C balance in ecosystems (Atkin *et al.* 2005). A straightforward extension of the theory presented here yields a novel hypothesis amenable to empirical testing. Given that $b_o = \rho_o^{mito} v_o^{mito}$ (eqn 6), and that plant respiration is ultimately constrained by photosynthesis, energetic invariance yields the prediction that respiratory acclimation occurs because plants grown at higher temperatures maintain lower mitochondrial densities, ρ_o^{mito}, owing to the stronger temperature dependence of respiration by mitochondria vs. photosynthesis by chloroplasts (Allen *et al.* 2005). The study of Armstrong *et al.* (2006) provides qualitative support for this prediction, but far more research is needed in this area.

Third, further work is needed to understand what factors control the sizes of structural-element pools, which can vary considerably among taxa, as demonstrated by the examples above. Knowing the sizes of these pools is important for predicting some phenomena, such as the relationship of growth rate to whole-body P concentration (eqn 19). Furthermore, when resources are abundant, some organisms consume, assimilate and store elements in excess of metabolic and structural requirements (Sterner and Elser 2002; Klausmeier et al. 2004). Explicitly modelling the contribution of this 'luxury consumption' to biomass stoichiometry, and how it varies with energetics and resource availability, seems critical for understanding why, for example, eqn 19 predicts that individual growth rate increases linearly with internal P content, whereas the Droop equation (eqn 9) models the rate of population increase as a saturating function of nutrient concentration.

A fourth challenge entails extending models to encompass the effects of non-steady-state dynamics on energetics and stoichiometry. For example, at the individual level, Elser *et al.* (2006b) reported declines in P content over ontogeny for five species of *Drosophila*. These declines may be related to decreased energy allocation to growth vs. maintenance as organisms approach adulthood. This hypothesis is qualitatively consistent with the MTE model of ontogenetic growth (West *et al.* 2001), but has yet to be quantitatively formulated and tested. Similarly, at the level of populations, MTE and EST models both indicate that resource allocation to the production of offspring should vary depending on whether populations are growing exponentially or are near carrying capacity (Klausmeier *et al.* 2004; Kuang *et al.* 2004; Savage *et al.* 2004). Yet, here again we lack a comprehensive understanding of non-steady-state dynamics. Such models may be important for understanding how populations are affected by energy and nutrient limitation, and more

generally for understanding how species respond to environmental change.

While these challenges point to the importance of future work at the intersection of MTE and EST, the examples presented here show how the energetics approach of MTE can be combined with the mass-balance approach of EST to yield novel, first-order predictions on the flux, storage and turnover of elements across species and environments. From the perspective of basic science, integration of these theories could help link different levels of biological organization, different biological currencies and different scientific disciplines. From an applied perspective, such integration could help address environmental issues. After all, current and future challenges of environmental change often involve simultaneous changes in the size structure, temperature and resource availability of ecological communities. And ultimately, environmental change affects ecological communities through its effects on the metabolism of the individuals and populations comprising those communities.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Equation derivations.

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