Opinion

The Ecology and Evolution of Stoichiometric Phenotypes

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Ecological stoichiometry has generated new insights into how the balance of elements affects ecological interactions and ecosystem processes, but little is known about the ecological and evolutionary dynamics of stoichiometric traits. Understanding the origins and drivers of stoichiometric trait variation between and within species will improve our understanding about the ecological responses of communities to environmental change and the ecosystem effects of organisms. In addition, studying the plasticity, heritability, and genetic basis of stoichiometric traits might improve predictions about how organisms adapt to changing environmental conditions, and help to identify interactions and feedbacks between phenotypic evolution and ecosystem processes.

**Ecological Stoichiometry: A Functional Trait Perspective**

There is a growing interest in understanding intraspecific functional trait variability so as to make better predictions about population dynamics, species interactions, and ecosystem functioning [1,2]. Although *stoichiometric traits* (see Glossary) are useful for connecting organisms to ecosystem processes [3–5], little is known about how intraspecific variation in these traits mediates ecological and evolutionary dynamics. Stoichiometric traits are an integral part of ecological stoichiometry (ES) theory, which focuses on the balance of multiple elements in ecological interactions and ecosystem processes [6]. Stoichiometric traits can vary among species [5–7], populations, and individuals of the same species [8–10], can influence how organisms affect energy flow and nutrient cycling in ecosystems (i.e., as functional effect traits) [6,11], and can mediate evolutionary responses to environmental variation and change (i.e., as functional response traits) [4,5]. Recent studies have also begun to document the occurrence [8,12,13] and ecological consequences [3,14] of rapid evolution of stoichiometric traits. Overall, such stoichiometric traits are a useful focal point for studying both interactions and feedbacks between phenotypic evolution and ecosystem processes.

ES theory posits that performance (e.g., growth rate) is partly determined by the match between the stoichiometry of resources and organism elemental phenotype (EP), which comprises multiple stoichiometric traits. However, what is the relationship between stoichiometric traits and fitness variation, and, more generally, how do organisms adapt to elemental imbalances in their diet or their resource uptake? Increasing nutrient supply can impact on the evolution of life-history traits by changing allocation trade-offs, particularly those associated with nutrient imbalances that negatively influence performance [15,16]. For example, we might expect either directional selection on stoichiometric traits to reduce elemental imbalances between a population and its resources [15,17], or fluctuating selection to maintain stoichiometric trait variation within populations when there is variation in resource quality through time. It is also possible that agents of selection on stoichiometric traits vary in their importance over the lifetime of organisms [15]. Juveniles and adults often vary in their nutritional needs because the nutritional demands for rapid growth (e.g., high dietary phosphorus, P) early in life might differ from those for

**Trends**

There is growing evidence for intraspecific variation in stoichiometric traits of animals, for a genetic basis of this variation, and for rapid evolution of these traits. In light of such observations, ecological stoichiometry has the potential to address evolutionary processes and identify mechanisms by which environmental heterogeneity drives the origins of biological diversity.

We suggest that addressing the evolution of stoichiometric reaction-norms could help in understanding when, and to what extent, variability in elemental phenotype in natural populations is due to plasticity of stoichiometric traits or genetic variation. We also propose strengthening the link between stoichiometric trait variability and fitness by building on nutritional geometry theory and function-valued methods. In addition, and because stoichiometric traits may not only respond to environmental variation but also influence ecosystem dynamics, studying the elemental phenotypes can contribute to our understanding of the nature of ecological evolutionary feedbacks.

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reproduction later in life (e.g., dietary lipids). Overall, the fitness consequences of maintaining (or not maintaining) stoichiometric homeostasis remain poorly understood.

We discuss here how stoichiometric traits can be an important intersection between ecological interactions, evolutionary dynamics, and ecosystem processes. To illustrate this, we review the primary drivers of intraspecific variation in the EP and the evidence that stoichiometric traits can evolve, potentially via multiple agents of natural selection. We advocate a better understanding of how stoichiometric trait variability [e.g., %P, %nitrogen (N), and the N:P ratio] relates to fitness variation, and suggest that studying the evolution of stoichiometric reaction norms might help to disentangle the role of phenotypic plasticity and genetic variation in determining the EP. We highlight how approaches from nutritional geometry (e.g., [18,19]) and function-valued methods (e.g., [20,21]) can help strengthening the link between fitness variation and elemental imbalances between consumers and their resources. We end by discussing how stoichiometric traits can improve our understanding of eco-evolutionary dynamics and feedbacks between ecology and evolution.

**EP Variability: Causes and Consequences**

The EP is made up of multiple stoichiometric traits associated with the composition, acquisition, assimilation, allocation, and excretion of key elements of an individual organism [22] (Box 1). Autotrophs are more stoichiometrically variable than heterotrophs owing to their rapid response to nutrient availability and ability to store nutrients [6]. For heterotrophs, there is growing evidence of variation in homeostasis among species, among individuals within populations [4,9], and within individuals throughout their ontogeny [10,17].

Variation in EP between and within populations is often attributed either to spatial heterogeneity in resource quantity and quality [9,23], or to non-spatial sources of intraspecific variation in EP, including body size, diet, morphology, and sex [7,10,24]. In addition, variation in EP among individuals has been attributed to variation in the cellular concentration (e.g., by weight) of nucleic acids, such as RNA and DNA, particularly when there is variation in ploidy or in elemental allocation associated with growth [12,25–27]. Such genomic variation at the cellular level can affect organismal stoichiometry, and thereby shape the nutritional quality of organisms (either as prey or detritus), and modify energy fluxes through the food web [11].

### Box 1. Agents and Targets of Selection

Phenotypic traits are defined as quantifiable measurements of an organism and can evolve when there is covariance between phenotype and fitness. The stoichiometric traits that define the elemental phenotype (EP) of an organism can both evolve in response to environmental selection and modify the environmental conditions that might determine selection pressures.

The EP is made up of several traits, such as organismal elemental composition (e.g., % of dry mass or the ratio of elements), which determines how acquired nutrients are used and recycled. These stoichiometric traits might affect the survival, growth, and reproduction of individuals, and consequently be targets of selection. Potential selection agents such as nutrient availability (i.e., food quantity and/or quality) and predation pressure are likely to affect fitness through changes in element allocation from routine metabolic processes to demanding physiological traits occurring during growth and reproduction, which can also reroute energy allocation away from homeostatic processes. Food quality affects the growth rate of consumers and therefore can be considered a selection agent. The selection targets would be the physiological mechanisms driving nutritional demand that are affected by food quality. Another target of selection might be genome size because it is composed of nucleotides and amino acids that require key elements, such as N and P, which are reallocated from DNA to RNA to sustain rapid growth [26,58]. Consequently, the N and P content of food have been suggested to act as selection agents in nutrient-limited environments because they can mediate the relative success of individuals differing in genome size, particularly between diploid and polyploid organisms [11,25]. Parasitism might also be a selection agent driving the evolution of EPs through nutrient-driven within-host infection dynamics [59].
Variation in EP can also occur within individuals throughout their ontogeny (Figure 1) as a result of different nutritional needs at early and adult life stages [28]. For example, maternal nutrition can influence offspring performance over a range of food quantities and qualities [29], and nutrition during early life stages can affect survival, growth, and adult performance [30]. Several pre- and post-ingestive homeostatic mechanisms have evolved to solve stoichiometric imbalance, including selective ingestion, assimilation, and excretion [7,31]. Such mechanisms are a dynamic property of consumers that vary ontogenetically so as to decrease the susceptibility of an organism to both deficiencies and excesses of nutrients for a given diet.

Intrapopulation variation in EP can originate from phenotypic plasticity, genetic variation, and genetic variation in plasticity. From an ecological perspective, previous work suggests that stoichiometric variation among species and populations can help in predicting community responses (e.g., species sorting) to global environmental change [4,5]. For example, latitudinal gradients in the N:P ratio of terrestrial plants [23] might explain variation in community composition of insects that differ in their propensity for P (e.g., Lepidoptera) or N limitation (Hemiptera) [32]. An evolutionary perspective on stoichiometric trait variation within populations would be useful for similar types of analyses because the amount and origin of trait variation might help in predicting how populations respond to environmental change. Specifically, we propose to focus on stoichiometric reaction norms as a way to study the evolution of EP in response to environmental change (Box 2). Comparing the slopes and intercepts of reaction norms might help in understanding phenotypic variation in response to genetic and environmental differences [33]. For example, flat reaction norms imply a lack of plasticity, but differences in mean values imply a genetic component of stoichiometric trait variability (Figure 1 in Box 2). Alternatively, non-flat reaction norms could indicate plastic effects on the trait, and one could test if variation in the reaction norm can be explained by genetic variation. Stoichiometric reaction norms that consider ontogenetic development could also be used to quantify variation in the sensitivity of organisms

![Figure 1. Ontogeny of Imbalances.](image)

The magnitude of the imbalance between consumer demand, defined by the elemental phenotype (EP) and food quality, might change ontogenetically. In this example, the stoichiometric traits of interest are the mean and the slope of the genotype lines, and suggest an interaction between genotype, ontogeny, and different homeostatic capacity (represented by the vertical arrows).
to elemental imbalance throughout ontogeny. In practice it is difficult to have continuous access to the complete ontogenetic EP through time, but breeding and rearing different genotypes in common-garden experiments could be a powerful way to identify how much phenotypic variation throughout ontogeny is explained by genetic differences.

**Link Stoichiometric Traits to Fitness**

One fundamental challenge for understanding the evolution of the EP is to establish a better link between EP and fitness. To start, it is important to recognize that ES studies typically use carbon (C) as a proxy for biomass to track energy flows between organisms and their environment. However, in most cases components of the body that are considered nutrients are more likely to be determinants of fitness variation than solely the elements and their ratios (e.g., C:P, N:P). In

**Box 2. Stoichiometric Reaction Norms**

Reaction norms describe how the phenotype expressed by a single genotype varies across a range of environments. Figure I shows a theoretical example of stoichiometric reaction norms across two different food qualities – with high and low C:P ratios for different genotypes (represented by three colors). Flat curves suggest no plasticity for a given genotype, in other words the phenotype is independent of food quality (Figure IA). Average reaction norms with a slope different from zero and parallel slope among genotypes (Figure IB) suggest that genotypes exhibit similar patterns of plasticity. Different slopes among reaction norms (Figure IC) suggest an interaction between genotype and environment. Connecting stoichiometric reaction norms with fitness responses might help to demonstrate whether phenotypically plastic responses are adaptive, but the link between stoichiometric trait variation and fitness is still poorly developed.

Stoichiometric reaction norms could be analyzed as function-valued traits so as to link them to selection gradients [21]. For example, if we assume that stoichiometric imbalance is negatively related to fitness, then we can assess the relationship between stoichiometric traits and fitness variation. As food quality changes over time, the imbalance between nutritional demand and supply, in other words organismal and environmental stoichiometry, respectively, might also change [17]. Such patterns could be compared among genotypes, as displayed in the hypothetical example displayed in Figure II, which exemplifies different patterns of stoichiometric trait variation across genotypes (represented by two different colors) through time (e.g., ontogeny) and the fitness consequences. Figure IIA shows variation of a stoichiometric trait between genotypes over time under constant food quality, for instance variation of %P fish body content throughout ontogeny [10]. Figure IIC shows genotypes with different homeostatic capacity that are provided with variable food quality over time.

![Figure I](image.png)

**Figure I. Effect of Food Quality on Elemental Phenotype (EP) of Different Genotypes (A–C).** EP can be affected exclusively by genotype (A) or by environment and genotype (B) in a predictable way. Reaction lines cross (C) when genotype interacts with environment.
fact, adaptive stoichiometric trait variation will probably involve elements that are either nutrients themselves (e.g., P) or close proxies of nutritionally-significant molecules (e.g., proteins are N-rich) [18,34]. By comparison, elements such as C that occur in a broader range of molecules might only be correlated with fitness in specific cases (e.g., in dietary lipids; see below).

Phosphorus is a key constituent of many biomolecules, including phospholipids, ATP, and nucleic acids [6], and its content in organisms has been shown to vary as a result of investment in P-rich RNA needed for growth [35]. Building on this idea, the growth rate hypothesis (GRH) posits that variations in organismal C:P and N:P ratios are associated with variations in growth rate as a result of variable investment in P-rich ribosomal RNA. In this view, the P content of food is considered to be an agent of selection on physiological processes associated with rapid growth (Box 1) [33,36]. For instance, contrasting responses to P availability by different Daphnia genotypes sampled from the same environment suggest that stoichiometric traits such as P use efficiency can evolve rapidly via clonal selection [37]. Interestingly, coexisting Daphnia genotypes
can differ strongly in their reproductive output over a broad range of dietary C:P ratios (e.g., 100 to 800) [38], suggesting that nutrient availability could be an important dimension of life-history evolution [15]. Although there is strong support for the GRH for invertebrates [35,39], less is known about the relationship between EP and fitness for organisms that store P in tissues with slow turnover, for example vertebrates that store P in bone [6].

Nitrogen is one of the main constituents in biological structural molecules, and tissue N content is often used as a proxy for proteins [6]. Several abiotic and biotic factors have been shown to affect N-based stoichiometric traits for a variety of organisms, ranging from primary producers to aquatic and terrestrial consumers [8]. N limitation can have important fitness consequences, particularly for herbivores because the N content of herbivores like that of all heterotrophs is notably higher than that of autotrophs [32,40]. This stoichiometric imbalance in herbivores has likely led to different adaptations to N-poor food, such as producing biomass using low-N materials (e.g., chitin instead of protein), or modifying elemental allocation to different body parts that differ in N content [41]. N limitation might also affect performance across life-history stages [42] or lead to the evolution of novel trophic interactions, such as omnivory or cannibalism, as alternative ways to meet nutrient demand and increase reproductive output [43,44].

Carbon occurs in multiple nutrients, such as lipids and carbohydrates, and therefore might co-vary with C-rich macro-compounds, such as dietary fatty acids [45]. For instance, variation in the ratio of essential fatty acids to C content of seston can strongly affect growth rate and egg production in Daphnia [46]. This is often observed in lakes where the dominance of cyanobacteria in phytoplankton communities, which lack important sterols and long-chain polyunsaturated fatty acids, directly affects Daphnia consumers and has driven specific adaptations [47]. However, establishing a direct link between organismal C-based stoichiometric traits and fitness is likely more difficult for other lipid molecules because different lipids can vary in their biological function but have similar C content.

A useful way to link stoichiometric traits with fitness variation is to build on approaches from nutritional geometry (Box 3). This approach uses a multidimensional nutrient space where each dimension represents a different nutrient [18,19,48–51]. A standard approach in nutritional geometry is to rear animals on a range of food qualities (e.g., different protein and carbohydrate contents are represented in each axis) which delimit an area in nutritional space. The location of the intake target within the available nutrient space represents the optimal combination of nutrients that fulfill the requirements of the animal. With this approach, consumer performance over a range of different nutritional scenarios can be measured as development rates or reproductive output. By doing so, one can assess the relationship between specific nutrients, and their elemental composition, and variation in fitness components [48,49]. Such approaches could also be extended by assessing how different genotypes vary in their performance within a multidimensional space determined by stoichiometric trait axes. This can be done for different life-history stages by measuring different fitness components, such as growth for early life stages and reproductive output for adults (Figure I in Box 3).

Establishing a link between stoichiometric traits and variation in fitness components throughout ontogeny is usually performed separately for different life stages because proxies of fitness are often quantified differently for juveniles and adults. This could be improved by using homeostasis as a continuous proxy of one fitness component through the life of an organism (Figure II in Box 2). This approach would assume that stoichiometric imbalance between consumers and resources is negatively correlated with performance, and account for ontogenetic changes in homeostatic mechanisms (Figure 1) as well as variability in resource quality and quantity. Such complex functions could be analyzed using a function-valued trait approach [20,21].
Stoichiometric Eco-Evolutionary Dynamics

Multiple agents of selection can drive the evolution of stoichiometric traits [8], but it is still unknown when this phenotypic evolution might affect biotic and abiotic conditions, and thereby influence prevailing environmental sources of natural selection [3]. Variability in resource demand by heterotrophs can affect nutrient recycling in natural ecosystems, as has been observed for fish with high P content that display low excretion rates [52,53]. In such cases, stoichiometric trait variation in the community [54], or at the population level [55], can change the balance of freely-available nutrients in the environment. Such changes to nutrient availability might subsequently affect the ecological and evolutionary dynamics of both autotroph and heterotroph populations that vary in their nutrient use physiology and elemental composition [11,26,36].

The dual role of stoichiometric traits as both functional effect and response traits makes them especially likely to be involved in feedbacks between phenotypic evolution and ecosystem processes. Models integrating ES with eco-evolutionary dynamics have begun to illustrate how the evolution of EPs can influence different levels of biological organization, from individuals to populations, communities, and ecosystems [3]. Although there are clear ES examples that the EP of animals can modify the environment [8], and that the evolution of EPs can be driven by environmental change [37], a better understanding of how stoichiometric traits evolve in response to natural selection will be useful to identify potential feedback between ecological, evolutionary, and ecosystem processes. One completely open question, for instance, concerns

Box 3. Application of a Geometric Approach to ES

In general, ES still lacks an approach to link stoichiometric traits to fitness and selection mechanisms that fit a broad range of organisms. Mechanisms such as selective feeding, differential assimilation of ingested nutrients, selective excretion of nutrients in excess, and nutrient storage have likely evolved in response to nutrient availability [60]. However, the evolution of stoichiometric-related traits and the mechanisms to obtain, retain, and concentrate resources throughout life remain unclear. ES has made progress toward establishing the link between elements and growth rate as a key life-history trait that is associated with fitness (e.g., GRH). However, ES could benefit from approaches such as nutritional geometry [18], where elemental ratios can be used in multidimensional geometric models to assess fitness across elemental phenotypes (Figure I), and from the analysis of function-valued traits (Box 2) to study adaptive plasticity. Variation in food quality requirements through life (e.g., early stages require more P for growth, and late stages require more C for energy storage), together with variability in available resources (Figure II), might change the strength and directionality of selection.

Figure I. Hypothetical 3D Model Showing Organismal Fitness Response to Food Quality Represented by N and P Content (Following a Nutritional Geometry Approach [18]). Note that in this example stoichiometric traits (%N and %P) are used instead of macromolecules (e.g., carbohydrates, proteins). Different optimal scenarios may be observed depending on nutritional strategies and experience throughout life history. In the hypothetical scenario shown here, early stages notably benefit (i.e., grow faster) from increased food quality, whereas adults do not benefit.
the genetic architecture of EP variation, and therefore how much EP variation can actually evolve within a single panmictic population. This leads to the question of how speciation and species richness within ecosystems affects EP variation, a potential theme for future speciation and biodiversity research.

Two recent studies have addressed the rapid evolution of stoichiometric traits that are involved in eco-evolutionary feedbacks. In a theoretical study, Yamamichi et al. [8] used a modeling approach to show that the rapid evolution of EP affects the nutrients available in the environment, and that this affected evolutionary processes. Consumers that rapidly evolved to new nutrient conditions caused a feedback that ultimately affected both adaptive evolution and population stability. In an elegant and unique experimental study, Declerck et al. [14] showed that phenotypic variation in growth rates and nutrient demand of a herbivorous rotifer affected nutrient recycling, particularly when P-limited food was provided. The subsequent changes in resource availability favored the phenotypes with a selection history of P limitation, thereby driving evolutionary processes and consequent feedback on standing stocks of primary production. This ultimately caused changes in population structure, grazing pressure, and the biomass ratio of consumers and resources. Both studies show the potential involvement of stoichiometric traits in eco-evolutionary dynamics and feedbacks. Moving forward, we can gain a better mechanistic understanding of the reciprocal interactions between EP variation and ecosystem dynamics by experimentally testing (i) how much individual organisms within a single genetic population can vary in their stoichiometry, (ii) how organisms with different stoichiometries affect ecosystem dynamics, (iii) how nutrient limitation and organismal elemental imbalance relate to fitness variation, and (iv) how organism-mediated modifications to the environment, particularly those governed by stoichiometric traits, affect fitness variation in subsequent generations [56].
Concluding Remarks and Future Directions

There is growing evidence that the EP can be highly variable across species, populations, and individuals, and can evolve in response to natural selection, but the effect of EP variation on ecosystems and its reciprocal effect on phenotypic evolution are still poorly understood (see Outstanding Questions). A necessary future step for ES to address this gap will be to strengthen the link between stoichiometric traits and heritable fitness using approaches other than the already established link between P and growth rate [35]. The recent integration between ES and nutritional geometry [48,49] can be extended to study how stoichiometric trait variation is related to fitness. The link between homeostasis and fitness can also be integrated with other frameworks encompassing homeostatic regulation in animals. For instance, Dynamic Energy Budget theory (DEB), which provides simple mechanistic rules describing the uptake and use of energy and nutrients [57], could be better integrated with ES [48] and across different organizational levels. Such an approach might provide a better mechanistic basis for understanding and predicting population and ecosystem responses to stoichiometric constraints of individuals, and uncover new mechanisms underlying eco-evolutionary feedbacks. Further progress integrating ES with other frameworks that address evolutionary dynamics will improve our understanding of how environmental heterogeneity might drive the origins of biological diversity, and how biological diversity can be affected by the accelerating environmental change that ecosystems are experiencing.

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Outstanding Questions

How variable are stoichiometric traits across ontogeny, and what are the fitness consequences of homeostatic imbalance resulting from variation in food quality?

What are the primary agents of selection that drive stoichiometric trait evolution?

How does selection driven by nutrient limitation act at the genomic, proteomic, and metabolomic levels, and what are the consequences for individuals, populations, and ecosystems?

How and when do ecosystem changes mediated by stoichiometric traits of organisms lead to eco-evolutionary feedbacks?

References
