Woodstoich III: integrating tools of nutritional geometry and ecological stoichiometry to advance nutrient budgeting and the prediction of consumer-driven nutrient recycling

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Within the last two decades, ecological stoichiometry (ES) and nutritional geometry (NG, also known as geometric framework for nutrition) have delivered novel insights into core questions of nutritional ecology. These two nutritionally explicit frameworks differ in the ‘nutrient currency’ used and the focus of their past research; behavioural feeding strategies in NG, mainly investigating terrestrial organisms, and trophic ecology in ES, mainly in aquatic settings. However, both NG and ES have developed in explaining patterns across various scales of biological organization. Integrating specific tools of these frameworks could advance the field of nutritional ecology by unifying theoretical and empirical approaches from the organismal to ecosystem level processes. Toward this integration, we identified 1) nutrient/element budgets as a shared concept of both frameworks that encompass nutrient intake, retention, and release, 2) response surface plots of NG as powerful tools to illustrate processes at the organismal level and 3) the concept of consumer-driven nutrient recycling (CNR) of ES as a useful tool bridging organism and ecosystem scales. We applied response surface plots to element budget data from an ES study to show how this approach can deliver new insights at the organismal level, e.g. by showing the interplay between egestion and excretion depending simultaneously on the consumed amount of carbon and phosphorus based on variation across individuals. By integrating concepts of ES and NG to model microbial uptake and mineralization of nitrogenous wastes reported in a NG study, we also demonstrate that considering biochemically explicit mineralization rates of organic wastes can improve predictions of CNR by reducing over- or underestimation of mineralization depending on the quality of the consumer’s diet. Our presented tools and approaches can help to bridge the organismal and ecosystem level, advancing the predictive power of studies in nutritional ecology at multiple ecological scales.
INTRODUCTION

Studies in nutritional ecology link animals to their environment through nutritional interactions (Parker 2003), thus linking animal biology with demographic responses (e.g. population and movement ecology) via foraging behaviour and physiology (Simpson and Raubenheimer 2012). The field of nutritional ecology has evolved from numerous disciplines that work at different levels of biological organization, from animal physiology, plant defence theory, plant and animal population and community ecology, ecosystem ecology and biogeochemistry, and foraging ecology. Early studies in nutritional ecology focused on single nutrients or currencies (e.g. energy in optimal foraging theory) at a time without considering the simultaneous, interactive effects of multiple nutrients on organism fitness (Raubenheimer et al. 2009). However, incorporating multiple nutrients has been shown to be valuable in predicting interspecific competitive outcomes, e.g. mechanistic resource competition (Tilman 1982), or animal behaviour, e.g. mechanisms underlying the mass movement of Mormon crickets (Simpson et al. 2006). Consequently it has been argued that nutritional ecology would benefit from a theoretical and quantitative framework that is (i) nutritionally explicit about multiple nutrients rather than single food components, (ii) organismally explicit about behavioural and physiological homeostasis and consequences of nutritional decisions at the organismal level, and (iii) ecologically explicit about the multiple interactions at different levels of biological organization (Raubenheimer et al. 2009, Simpson et al. 2010).

Within the last two decades, the two most active multi-nutrient frameworks, Nutritional Geometry (henceforth NG) (Simpson and Raubenheimer 2012) and Ecological Stoichiometry (henceforth ES) (Sterner and Elser 2002), have developed in parallel as promising candidates to meet the requirements of an integrative, overarching framework in nutritional ecology (Raubenheimer et al. 2009). Both frameworks
originating from questions focusing on different levels of biological organization, with organism feeding behaviour in NG and ecosystem biogeochemical cycles in ES. This differing initial focus has its origin mainly in different study systems, with NG predominantly investigating terrestrial, phytophagous insects and ES predominantly investigating aquatic, filter-feeding zooplankton. Despite these differences, the two frameworks share some key concepts (Raubenheimer and Simpson 2004). Notably, both frameworks have identified that organisms often face imbalanced diets and that an individual’s fitness can be maximized by satisfying nutritional demands through both pre- and post-ingestive regulatory mechanisms (e.g. food selection, digestion, and excretion), ultimately influencing the amount of nutrients retained or released by the individual (Fig. 1) (Sterner and Elser 2002, Simpson and Raubenheimer 2012, Raubenheimer et al. 2012). Further, organismal level processes, such as food choice or the selective release of nutrients, can drive higher level dynamics, such as social interactions (Lihoreau et al. 2015), ecosystem elemental cycling (Schade et al. 2011), or nutrient pollution (Cease et al. 2015).

The two frameworks differ primarily in their currencies used to define food, which in turn differ in their utility at different levels of biological organization. NG uses macro- and micro-nutrients, most often carbohydrates, lipids, proteins, and some specific elements (Simpson and Raubenheimer 2012) to describe food composition, whereas ES uses quantities and ratios of selected elements, notably C (carbon), N (nitrogen), and P (phosphorus) as proxies for more complex biomolecules (Sterner and Elser 2002). NG studies identify foods or nutrients that are selected by organisms and emphasize associated fitness consequences, thereby providing a broader understanding of organism level physiology and behaviour compared to studies in ES (Raubenheimer 2011, Raubenheimer et al. 2012). In contrast, the elemental currency of ES simplifies organism nutritional budgeting, addresses elemental constraints at multiple trophic levels including autotrophs and diverse heterotrophs, and uses concepts such as

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consumer-driven nutrient recycling (CNR) to bridge organism- to ecosystem-level processes, thereby providing a broader understanding of interactions at the organism-environment interface compared to NG (Sterner and Elser 2002, Schade et al. 2005).

Given the differing utility of ES and NG across levels of organization, integration of tools from each framework may provide greater insight at all levels of organization to ultimately better link organism and ecosystem-focused studies in nutritional ecology, as notably demonstrated in a few recent studies (Raubenheimer and Simpson 2004, Morehouse et al. 2010, Hawlena and Schmitz 2010, Harrison et al. 2014, Cease et al. 2015, 2016, Meunier et al. 2016). For instance, NG is more capable of capturing regulatory mechanisms of organism feeding behaviour, such as selective foraging for carbohydrates versus lipids that are equally carbon-rich, than the elemental currency approach of ES (Raubenheimer and Simpson 2004, Raubenheimer et al. 2009). Integrating methodological approaches of NG, such as state-space plots of nutrition (i.e. geometric models in which nutritional and growth states of an organism are presented within a multidimensional nutrient space), may resolve such issues (Raubenheimer and Simpson 2004, Simpson et al. 2004). Such state-space plots are usually used within NG to identify nutritional homeostatic regulation of organisms by investigating how feeding behaviour contributes to meeting specific nutritional targets or by their ability to over- or under-eat specific nutrients when confined to an imbalanced diet (Raubenheimer et al. 2012). State-space plots may also be adapted to analyse organism element budget components (Raubenheimer and Simpson 2004), thereby improving organism-level resolution of ES studies that use element currencies to bridge from organisms to ecosystems. The focus on the organism level, in turn, makes NG less suited for explaining higher-level phenomena (Morehouse et al. 2010), especially nutrient feedbacks between consumers and their resources that are ubiquitous in ecosystems and a key development from ES theory (Elser and Urabe 1999, Sterner and Elser 2002).

However, the biochemical resolution of NG may be useful in resolving the complexity
of consumer nutrient wastes, improving upon predictions of ES regarding CNR in ecosystems. Such integrative approaches could make nutritional ecology more explicit about nutrition, organisms, and ecology than achievable by either framework alone.

Here we propose novel approaches in nutritional ecology using specific complementary tools and concepts from ES and NG. For the first time, we utilize the response surface plot, a key tool in NG, to investigate organism elemental budgets derived from an ES study. This approach accounts for both ratios and quantities of ingested elements in two-dimensional nutrient space and better incorporates intraspecific variation in organism stoichiometric regulation. Next we illustrate how to advance the concept of CNR by incorporating different biochemical forms of waste products in a response surface context, as varying diets can alter both pre- and post-ingestive regulatory processes of consumers, thus affecting consumer-resource nutrient feedbacks. In an example using data derived from a NG study, we use the relationship between diet macronutrient and locust waste composition to better predict the amount of nitrogen mineralized from wastes over time. These data-driven examples provide two promising synergies between ES and NG toward an integrative nutritional ecology.

**NUTRIENT BUDGETS – A CONCEPTUAL CORNERSTONE OF ES AND NG**

Both ES and NG employ conservation of mass approaches to understand organism nutrition, i.e. all nutrients or elements ingested by an organism must be processed by the organism, with elements exhibiting strict mass balance while macronutrients are transformed into other compounds during regulation. Homeostatic regulation of the internal nutrient state can be a main driver influencing feeding behaviour or post-intake processing of nutrients (Sterner and Elser 2002, Raubenheimer et al. 2012). Intake can be regulated qualitatively by selective feeding behaviour or quantitatively by increasing or decreasing food consumption (Cohen et al. 1987, Simpson and Simpson 1990, Cruz-Rivera and Hay 2000). An animal can also regulate
the balance of nutrients after food ingestion by (1) changing digestion and absorption efficiencies of specific nutrients (Mayntz et al. 2005, He and Wang 2008, Clissold et al. 2010), which influences egestion, i.e. nutrients that are not absorbed after ingestion and released (Fig. 1) or by (2) excreting excessive nutrients or elements (Zanotto et al. 1993, Hessen and Anderson 2008), and enhancing respiration (Zanotto et al. 1997, Darchambeau et al. 2003, Jensen and Hessen 2007) (Fig. 1). These key mechanisms of homeostatic regulation can be summarized using nutrient or element budgets.

Nutrient budgets in NG are distinguished according to (1) whether they contribute to fitness (functional criteria) and (2) whether nutrients are retained or dissociated from the body in a given time period (methodological criteria) (Raubenheimer and Simpson 1995). The different components of nutrient budgets (e.g. ingestion, egestion, growth) can be quantified and visualized using a state-space ‘geometric’ approach (Fig. 2a) (Raubenheimer et al. 2009). The state-space model indicates the location of ingested nutrients within the two-dimensional nutrient space, as well as achieved absorption and growth. For each nutrient, growth, metabolic loss (e.g. excretion, respiration), and egestion sum to the amount the animal had ingested according to the mass balance rule (Fig. 2a). The use of the state-space model in NG incorporates the amounts of nutrients (horizontal or vertical arrows, Fig. 2a) as well as the ratios (diagonal lines, Fig. 2a). This is an important strength as it accommodates how the quantity as well as the ratio of ingested food influence the nutrient budget (Raubenheimer and Simpson 2004, Raubenheimer 2011). Such models allow the fate of ingesta to be mapped (i.e. absorption and retention in the tissues) as well as having the advantage of avoiding ratio-based indices that may involve some statistical and interpretive problems (Raubenheimer 1995, see below).

In ES, elements are budgeted individually, but budget compartments are commonly expressed as integrated nutritional indices, such as *assimilation efficiency* (AE = absorption/ingestion) and *gross growth efficiency* (GGE = growth/ingestion)
(Frost et al. 2006). While ES studies can include the quantity of elements (Urabe and Watanabe 1992, Sterner 1997), diets and the composition of consumer biomass are most often considered as distinct elemental ratios (e.g. C:N, C:P, or N:P ratios). By neglecting absolute amounts of element intake and using ratio-based indices (e.g. AE, GGE), these studies may misinterpret the effect on fitness and nutrient regulation (e.g. digestion efficiency) for any given diet composition (Lee et al. 2008, Clissold et al. 2010, 2013, Le Gall and Behmer 2014). For example, nutritional ratio indices such as AE impose isometry (i.e. linearity) on the relationship between numerator and denominator when such relationships (e.g. effects of consumption on assimilation) are often allometric (i.e. non-linear), which may cause erroneous interpretations of such ratio indices (Beaupre and Dunham 1995, Raubenheimer 1995). Therefore, ES would benefit from an extended state-space approach that accommodates allometry and uses both ratio and quantities of elemental budget components to gain a greater understanding of nutrient regulation (Raubenheimer and Simpson 2004).

**Applications of state-space plots to elemental budgets**

Here we show that the state-space approach can be readily applied to ES-related questions by presenting an element budgeting plot for larvae of the caddisfly *Pycnopsyche lepida* that were fed oak leaves of two different carbon:phosphorus (C:P) ratios for 33 days (Halvorson et al. 2015b) (Fig. 2b). On average, individual *P. lepida* larvae consumed less carbon and phosphorus on the higher dietary (C:P=2630) ratio compared to individuals on the lower (C:P=1590) ratio (Fig. 2b), suggesting no compensatory feeding for limiting P at high dietary C:P. Carbon growth of this detritivore is expectedly low, and the budgets of egested elements are very high. P egestion is high under P-sufficient conditions (C:P=1590) and also relatively high under P-limiting conditions (C:P=2630), probably because the majority of ingested food was not digestible. Consistent with ES theory, P excretion (i.e. P metabolic loss) is very low.
in the P-limiting diet treatment (~1% of ingestion), whereas it is considerably higher on
the P-sufficient diet (~15% of ingestion, Fig. 2b), indicating that excretion can be
regulated in P. lepida to retain this limiting element. In this example we show elemental
budgets based on average values of multiple individuals and for only two diet
treatments. However, such state-space plots might be easily crowded and difficult to
interpret when presenting budget components for multiple diet treatments
simultaneously together with multiple individuals instead of their averages. In the
following we will illustrate how to overcome such problems of complexity.

Here we show how to advance the current type of budget plot by employing a set
of response surface plots that present multiple diet treatments as well as multiple
individual animals (Fig. 3). Response surfaces, also called performance landscapes,
have been increasingly used in NG studies to illustrate various experimentally derived
fitness-related response variables across multidimensional nutrient space (Simpson et al.
2004, Lee et al. 2008, Fanson et al. 2009). They can be used to identify the nutrient
ranges over which particular nutrients limit growth due to deficiency or excess
(Simpson et al. 2004, Jensen et al. 2012, Sperfeld et al. 2012, 2016), but also for
comparing different fitness-related response variables and potential deviations in their
optima (Lee et al. 2008, Le Gall and Behmer 2014). Response surfaces can be
visualized using non-parametric thin-plate splines (Green and Silverman 1994), which
can be readily computed using the ‘fields’ package in the software R (R Core Team
2012). A notable strength of response surfaces is the potential to interpolate data by
predicting response outcomes for resource combinations that were not directly
measured, thereby allowing the investigation of patterns that would not be easy
otherwise (Lee et al. 2008, Sperfeld et al. 2012). Non-parametric thin-plate splines also
allow a less constrained visualization of the response shape across the resource space
compared to more static, parametric models, thereby facilitating the investigation of
data-driven response patterns (Blows et al. 2003, Sperfeld et al. 2016). Here we adopt

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this NG tool to create novel response surfaces, investigating response patterns of elemental budget components across the nutrient space (Fig. 3). Variation in the location of individual responses within the nutrient intake space is based on diet treatments that define the slope of nutritional rails across the nutrient space, as well as the different intake of individuals within each diet treatment reflected in the distribution of points along nutritional rails.

The response surfaces provide new insights into stoichiometric regulation by *P. lepida* that would not have been possible in a commonly used ES approach with averaged values of treatments and ratio-use indices (see Halvorson et al. 2015b). Carbon egestion by *P. lepida* larvae is of much greater magnitude than C metabolic loss across the nutrient intake space (cf. Fig. 3a&c), with a similar pattern for P egestion and P metabolic loss (Fig. 3b&d). Egestion is probably so substantial, because these organisms were eating recalcitrant leaf litter. Surprisingly, P metabolic loss decreases on very high P intake (Fig. 3d), suggesting that the animals switch to greater proportional release of excess P as egesta instead of excreta (cf. Fig. 3d with Fig. 3b, where P egestion increases steeply on high P intake). Growth, especially for C, plays a minor role in the overall budget for these organisms, but shows some variation across the intake space with highest values of P and C growth at high intake quantity of both elements (Fig. 3e&f).

Mass-specific growth rates as the best available fitness surrogate show a stoichiometric knife edge response (Fig. 4a), which is predicted to show highest growth rates at an intermediate diet C:P (Elser et al. 2005). At intermediate C and P intake quantity, growth rates decline from an optimum at medium dietary C:P=1590 towards lower and higher dietary C:P ratios, suggesting negative effects of high (2630) and low (1230) diet C:P on growth, i.e. a stoichiometric knife edge. There are also moderately high growth rates at high C and P intake quantities, which suggests that intake quantity may influence the strength of the knife edge, a response pattern that would be difficult
to identify when only relating to dietary C:P ratios. However, a potential knife edge at high intake quantities cannot be fully assessed, as there are no data available for high intake on the C:P=2630 rail (Fig. 4a).

Response surfaces can also present elemental ratios of key measures, such as consumer waste or body stoichiometry, that are usually in focus of ES studies. The C:P ratio of egestion resembles the C:P ratio of the intake and does not vary much along the consumption gradient (Fig. 4b), suggesting that most of the food is indigestible and thus the composition of this primary waste budget component can be predicted by what the animal eats (i.e. diet C:P ratio). However, total waste production strongly depends on C and P intake (see Fig. 3a&b), suggesting that the potential strength of detritivore-driven nutrient recycling may depend on the consumer’s feeding rates. Tissue stoichiometry of the whole larvae body at the end of the growth period (final body C:P) and the newly built biomass (growth C:P) show a qualitatively similar pattern (Fig. 4c&d). However, growth C:P is higher than body C:P, which adds to the growing evidence that these variables have different power to predict stoichiometric processes (Hood and Sterner 2014). Both tissue stoichiometry measures change with the C:P of intake (Fig. 4c&d), which corroborates the increasingly reported observation that animal consumers do not always show strict stoichiometric homeostasis (e.g. DeMott and Pape 2005, Lukas et al. 2011, Meunier et al. 2014). Together, the response surface plots provide enhanced resolution of the element budget of P. lepida larvae by addressing variation across individuals as well as effects of intake quantity and balance on distinct budget components. In this way, the response surface tool of NG – typically used for analysing organism fitness responses depending on macronutrient intake – can provide increased organismal resolution of ES studies by keeping the element currency. In the following we will expand upon these and further advantages of integrating response surface plots and element-based budgeting plots.

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Advantages of using element-based budgeting plots or response surface plots

One challenge with constructing organism nutrient budgets in NG is that macronutrients can be inter-converted (Zanotto et al. 1993) and are thus not conserved throughout the entire process of regulation. For example, carbohydrates can be absorbed, but converted into lipids for storage (Blaxter 1989), while proteins (amino acids) can be deaminated and used in energy metabolism (Wu 2013) and thus partly substitute carbohydrates and lipids as energy currency. Thus using NG to construct nutrient budgets may require measurements of multiple biomolecules over all regulatory pathways (Zanotto et al. 1993, but see Raubenheimer et al. 2009). This is in contrast to ES, where the budget of elements is constrained over the entire regulation pathway, because elements cannot be interconverted, unlike macronutrients. Therefore, we suggest that nutrient budgeting plots and response surface plots based on budget compartments may be used to analyse elemental budgets. In these state-space plots, new insights into organism homeostasis may be gained to better predict how organism physiology affects ecosystem processes.

The state-space approach of NG, which is useful for examining organism feeding behaviour, may also be applied to elemental currencies to incorporate pre-ingestive regulation (e.g. selective feeding) into the ES framework. Selective feeding is a means for animals to regulate nutritional homeostasis in the face of multiple diet options (Raubenheimer and Jones 2006, Hawlena and Schmitz 2010), but only very few studies in ES have investigated the role of diet selection in regulating stoichiometric homeostasis (e.g. Meunier et al. 2016, Cease et al. 2016). Indeed, homeostasis in ES is usually assessed on monospecific diets varying in elemental content, which may neglect the role of animal feeding decisions in regulating homeostasis. The lack of diet choice studies in ES originates from using rather unselective, filter-feeding cladocerans (e.g.
Daphnia) as early model organisms in ES theory development, which did not require consideration of food selection (Mitra and Flynn 2005). Also, diet choice experiments in aquatic environments may be difficult to conduct and impose logistical challenges that can be overcome as recently demonstrated by studies on more selective copepods (Boersma et al. 2016, Meunier et al. 2016), fish (Raubenheimer et al. 2005, Ruohonen et al. 2007, Andrews et al. 2008), and food quality-mediated habitat selection experiments on Daphnia (Schatz and McCauley 2007, Reichwaldt and Abrusan 2007). Isotope analyses, by labelling different food components of the diet (e.g. He and Wang 2008) or using stable isotopes (e.g. Boersma et al. 2016), offer another way of investigating food selection and/or discrimination in aquatic systems. Integrating NG state-space models will allow ES to advance beyond specific (aquatic filter-feeding) model organisms that do not show strong diet selection. Our example using data of a published ES study shall serve as motivation to consider tools and concepts of NG in reanalyses of ES datasets, or even more desirable, in the design of future ES experiments that can be analysed in a NG manner (e.g. Meunier et al. 2016, Cease et al. 2016).

Animals may also undertake complementary feeding to achieve the threshold elemental ratio (TERCP or TERCN) much as they regulate macronutrient intake to achieve the intake target, a target point within the nutrient space that reflects the amount and balance of nutrients the animal has to eat to achieve maximal fitness (Simpson et al. 2004, Raubenheimer and Jones 2006, Jensen et al. 2012). Although animals may more actively forage for specific macronutrients than for certain elements (Raubenheimer et al. 2009, Harrison et al. 2014), recent evidence suggests that some consumers forage actively for nutritionally important elements, such as P (Nie et al. 2015, Cease et al. 2016). Therefore, element-based budget and response surface plots are promising tools to examine feeding decisions based on elemental stoichiometry and such approaches
could help to compare the importance of pre- versus post-ingestive regulatory mechanisms in maintaining homeostasis.

The use of response surfaces can strengthen conclusions about how the quantity of ingested nutrients will affect nutrient allocation, as well as eventual fitness. ES theory predicts that food quantity affects organism nutrient requirements, namely that the TER_{C:P} and TER_{C:N} increase under lower food supply due to increased relative demand for C in energy metabolism (Sterner 1997, Frost and Elser 2002, Sterner and Elser 2002), but the quantity versus quality effects on stoichiometric regulation are poorly studied for most taxa (but see Frost and Elser 2002, He and Wang 2007). State-space plots employing response surfaces will arguably provide a powerful way to investigate how the amounts of consumed elements affect organism elemental budgets (Raubenheimer and Simpson 1994, 2004, Raubenheimer et al. 2009). Response surface plots could also be used to simultaneously examine organism nutrient budgets and fitness in response to intake of multiple nutrients. For example, response surface plots can be overlaid or directly compared to infer how different regulatory pathways (e.g. excretion or egestion) may relate to fitness surrogates (e.g. growth), as we illustrated for *P. lepida*. Such plots can be used to link selection pressures, such as intake targets associated with maximizing lifespan versus reproductive rates (Lee et al. 2008) and patterns of waste production, to understand broad effects of evolutionary processes on ecosystem nutrient cycling (Elser et al. 2000).

We suggest that the integrative approach of considering organism elemental budgets in a state-space response surface context will help resolve contemporary questions in ES theory. In particular, these plots may help explain what components of organism regulation drive the strength and occurrence of the stoichiometric knife edge, such as discerning roles of increased excretion versus reduced consumption on high element diets (Plath and Boersma 2001, Boersma and Elser 2006, Elser et al. 2016). This approach may also help ecologists understand whether consumer body
stoichiometry or consumer feedbacks on elemental cycling depend on the amount of food intake that can vary with food availability in time and space. Because response surface plots resolve data on the level of single individuals, they also better address the significant role individual variation plays in feeding behaviour and diet choice (e.g. Estes et al. 2003, Toscano and Griffen 2014). Such approaches may be especially helpful for future studies to address intraspecific variation in body stoichiometry or nutrient recycling (El-Sabaawi et al. 2014) compared to interspecific variation of stoichiometric traits as common ES foci (Vanni et al. 2002, Frost et al. 2006). Finally, response surface plots may help reveal whether the TER differs when animals are allowed to self-select diets or whether different fitness metrics are optimized on diets differing in elemental ratios, thereby improving predictions of organism fitness in ES studies.

**IMPROVING PREDICTIONS OF CONSUMER-DRIVEN NUTRIENT RECYCLING**

ES theory predicts feedbacks between consumers and their resources through the concept of consumer-driven nutrient recycling (CNR) (Elser and Urabe 1999, Sterner and Elser 2002). In ES, CNR is the process by which consumers release nutrients such as N and P back into the environment, thereby replenishing nutrient stocks for autotrophs, which will be taken up again by the consumers (Fig. 1) to drive elemental cycling in ecosystems (Vanni 2002, McIntyre et al. 2008). NG has yet to address consumer-resource feedbacks, partly because its macronutrient currency is most applicable for animal consumers and cannot directly link animal nutrient wastes to autotroph nutrient uptake as cohesively as the elemental currency of ES. Elements instead are easily interconverted between both organic and inorganic forms of animal wastes to predict mineralization and uptake by plants and microbes in the environment. However, the focus of ES on element stoichiometry and not the biochemistry or breakdown properties of released compounds may constrain predictions of organic
element remineralization and ultimately the strength of CNR in ecosystems (Liess and Haglund 2007, Hawlena and Schmitz 2010, Hawlena et al. 2012). We suggest that the focus of NG on macromolecule-based nutrients and pre- and post-ingestive regulatory processes (e.g. selective feeding, excretion) and ES on feedbacks between elements released by consumers and the elemental composition of their diet (i.e. CNR) may complement one another in discerning the functional role of consumers in element cycling within ecosystems. This should be especially important in systems where organic wastes play a significant role in mineralization, such as terrestrial systems (Jan et al. 2009, Hawlena et al. 2012, Metcalfe et al. 2014) or benthic systems (Liess and Haglund 2007, Halvorson et al. 2015a) compared to pelagic systems where ammonium and phosphate excreted from consumers are immediately available for autotrophs (Elser and Urabe 1999, Vanni 2002, Vanni et al. 2013).

Animals release waste products in a diversity of forms, leading to complex feedbacks between animal homeostatic regulation and the nutrient composition of their dietary resources (Liess and Haglund 2007). Egesta, i.e. released nutrients that were ingested but not absorbed by the gut (Fig. 1), might be biochemically similar to the food resource, but can differ in nutrient composition due to selective absorption by the consumer (Clissold et al. 2010). In contrast, excreta are produced after absorption as metabolic wastes and may differ biochemically from both the food resource and egesta. Nitrogenous wastes from insects, for example, may be composed of a mixture of protein (egesta) and amino and uric acids (excreta) in proportions that depend on carbohydrate and protein intake (Fig. 5) (Zanotto et al. 1993). Because each of these forms of N waste must be broken down in the environment in a different manner, the eventual effects on food resources and thus diet macronutrient composition may depend on the specific biochemical form of N waste. Here, we illustrate this concept using a dataset of compound-specific N waste production by *Locusta migratoria* fed diets varying in protein:carbohydrate ratios (Zanotto et al. 1993). We modelled N mineralization from...
these wastes in soil over time using compound-specific breakdown rates reported in the literature and subsequent mineralization by soil microbiota (Fig. 6, Fig. 7). We then compared the outcome to bulk N mineralization, ignoring the biochemical forms of organic N waste, to assess whether adding complexity by using compound-specific mineralization rates increases the predictability of CNR (Fig 8). This example highlights how the explicit foci of NG on organism physiology and ES on ecosystem dynamics are complementary in resolving nutrient feedbacks between animals and their resources.

In the example study, four distinct diets that differed in three protein:carbohydrate ratios resulted in variable N waste produced as frass (egesta + excreta) by fifth-stadium *L. migratoria* over an eight day period in the laboratory (Zanotto et al. 1993). As evident in the response surface plots, locusts released more N with increasing protein intake, suggesting locusts released excess N to regulate homeostasis (Fig. 5). Amino acid and uric acid release in particular is highest on the highest-protein intake (Fig. 5b,c), indicating high levels of protein absorption in these diets. Therefore, protein release is more evenly distributed across the nutrient space (Fig. 5a) compared to the other two waste components. This is reflected in a protein N release that increased 1.8-fold across the intake gradient, whereas amino acid N release increased 8-fold and uric acid N release increased 26-fold (Fig. 5). We modelled the temporal breakdown of N in these waste compartments using reported half-life times of protein and amino acids in soil (59 and 3.1 d, respectively) (Jan et al. 2009) and assumed uric acid decay rates were proportional to degradation of dry chicken litter (8% per day), which is composed predominantly of uric acid (Nahm 2003). We assume that microbes take up N according to the reported breakdown rates, which leads to the fastest N uptake rates for amino acids, followed by uric acid, and a comparatively very slow uptake of protein-derived N (Fig. 6a). Although the exact rates will vary among systems depending on factors such as soil moisture, temperature, and the microbial community (Nahm 2003, Jan et al. 'This article is protected by copyright. All rights reserved.')
2009), and some autotrophs may directly take up amino acids to bypass microbial pathways (Schimel and Chapin 1996), these compound-specific rates should reflect the relative breakdown feature of each major form of N waste and thus better predict mineralization rates over time compared to bulk frass N content. Indeed, the models indicate strong differences in the microbial total and percent N uptake from locust frass over time, driven by contrasting frass composition across the four diet scenarios (Fig. 6b,c).

We estimated the mineralization of N \( (M_N) \) and N retained in microbial biomass (growth, \( G_N \)) after N uptake \( (U_N) \) using the concept of N use efficiency (NUE) (Mooshammer et al. 2014), where NUE is defined as follows:

\[
NUE = \frac{U_N - M_N}{U_N} = \frac{G_N}{U_N}
\]  
(1)

We calculated \( M_N \) and \( G_N \) assuming a microbial mean NUE of 0.83 found in the organic soil horizon (Mooshammer et al. 2014) using the following equations:

\[
M_N = U_N - NUE \times U_N \quad (2)
\]

\[
G_N = NUE \times U_N \quad (3)
\]

A NUE of 0.83 indicates 83% of each N waste component that is taken up is incorporated into microbial biomass for growth. The remaining 17% of N uptake is returned by microbes to the soil as usable inorganic forms, such as ammonium that would be directly available to plants (Mooshammer et al. 2014). We consider the latter fraction as the mineralized part of the locusts’ wastes.

We also constructed a “null model”, which ignores the distinct uptake rates of each form of N waste and assumes all biochemical forms of organic N are mineralized equally, for comparison with the model considering compound-specific mineralization rates (integrative “ES/NG model”). The null model is based on average locust waste composition across diets (12.7% protein, 83.5% uric acid, and 3.8% amino acid; Zanotto et al. 1993), and predicts the proportion of total waste N taken up by microbes

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over time (Fig 6a). This way, the null model assumes a constant proportion of waste N taken up at any given time and thus, predicts uptake of bulk N waste regardless of waste biochemistry that actually varies with diet macronutrient composition (Fig. 5).

The divergent breakdown rates of each form of N waste (cf. Fig. 6) resulted in contrasting N growth of microbial biomass, as well as N mineralization from frass across the macronutrient intake plot (Fig. 7). Both the absolute amount of microbial N growth (=GN, Fig. 7a) or N mineralized (=MN, Fig. 7b) and the proportion of locust N waste that microbes took up and retained in biomass (=GN/total N waste, Fig. 7c) or mineralized over time (=MN/total N waste, Fig. 7d) were affected by the varying breakdown rates of the different N waste compartments. Even though the majority of N waste was retained in microbial biomass, both total microbial N growth and N mineralized were greater for frass from animals that fed on the highest protein:carbohydrate diet (Fig. 7a&b), simply because greater amounts of N waste were produced with greater protein intake (Fig. 5). These findings are according to expectations and could be predicted as well by only considering bulk N composition of wastes. However, our integrative ES/NG model improves on null model predictions by also showing how the proportion of N waste that is used for microbial growth or mineralized at a given time may depend on the biochemical form of N in frass (Fig. 7c&d). An ES approach treating all forms of N waste in the same way would predict uniform proportions of N mineralization or microbial growth across the nutrient space. This is evidently not the case when resolving the biochemical form of N waste as indicated by the varying proportions of both microbial N growth and N mineralized across the nutrient space in our integrative model (Fig. 7c&d). Nitrogen retained in microbial biomass or frass mineralization over time may thus be difficult to predict from bulk N waste alone.

In a comparison of mineralization based on the null model (Fig. 6a) to N mineralization predicted by the ES/NG model, we found that the former overestimated...
N mineralization by as much as 67% at low N mineralization, where wastes had a proportionately high protein content, but underestimated N mineralization by as much as 7% under higher predictions of N mineralization, where wastes were proportionately rich in uric and amino acids (Fig. 8). This is because the null model ‘averages out’ compound-specific uptake rates, reducing model sensitivity to slow uptake/mineralization rates of N as protein and fast uptake/mineralization rates as amino and uric acids. Such deviations could be ecologically significant, especially in N-limited systems, such as certain subhabitats in terrestrial and marine systems (Elser et al. 2007), where small differences in the rate of N mineralization could strongly affect primary production. Considering mineralization based on only bulk N waste would drastically overestimate the contribution of animals to nutrient recycling in such systems as our example demonstrates (i.e. locusts would actually release relatively more protein as a slower-breakdown form of N waste). Our proposed ES/NG model elaborates beyond conventional approaches considering bulk element compositions to show that microbe-mediated consumer-autotroph nutrient feedbacks depend on strategies of consumer homeostatic regulation that vary with diet macronutrient composition (Fig. 1).

Advantages of using response surface plots and biochemical explicitness to improve predictions of consumer-driven nutrient recycling

Our approach integrates organism-level knowledge, made possible by response surface tools of NG, into the crucial concept of ES theory that wastes from consumers influence ecosystem nutrient budgets and also play a feedback role that affects diet nutrient composition. The role of consumer selective feeding in nutrient recycling remains poorly understood (but see Hood et al. 2014) and response surfaces could provide a method to assess effects of intake amount and composition on nutrient release

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by consumers and recycling within the food web (Figs. 5, 7). By incorporating nutritional information of multiple individuals, response surfaces also improve assessment of inter-individual variation in body size or phenotype that can alter CNR (El-Sabaawi et al. 2015, Allgeier et al. 2015). Response surfaces finally provide a better means to address continuous and non-linear (allometric) relationships between intake quantity and dissociation of nutrients (Raubenheimer and Simpson 1994; see above); such relationships are rarely considered in studies of CNR and may better relate quantities of nutrients removed from the environment by consumers to quantities released back into the environment as waste (Metcalfe et al. 2014).

By exploring biochemically explicit consumer nutrient wastes we hope to inspire scientists to broaden the diversity of wastes analysed in CNR-related studies, especially over extended time scales and among a greater diversity of systems. Although the amount of a specific element released by consumers may be similar, the impact of wastes on ecosystem nutrient dynamics may vary widely over time, especially for organic wastes that are chemically heterogeneous and not immediately available for uptake by plants or microbes due to slower mineralization (Liess and Håglund 2007, Hawlena and Schmitz 2010, Hawlena et al. 2012). Although poorly understood, the timing and speed of waste mineralization is crucial to discern the role of consumers in nutrient turnover at the ecosystem level, including the role of consumers as potential net sinks of inorganic nutrients on short time scales or net sources on longer time scales (Vanni et al. 2013). Comparisons of CNR across major phylogenetic groups are currently limited to excretion of biochemically homogeneous dissolved nutrients (e.g. ammonium) among aquatic organisms (Vanni et al. 2002, Allgeier et al. 2015). Biochemical resolution is not necessary to discern the short-term impacts of these inorganic wastes and is already considered in the CNR concept; however, further phylogenetic comparisons will be constrained by how wastes vary across taxa, including specific forms of excreted N (e.g. uric acid versus urea versus ammonium).
and biochemical complexity of organic wastes. Indeed, ecologists are increasingly aware of both the physiological and ecological significance of diverse waste products, including food fragments, feces, carcasses, eggs, and exuvia (Fig. 1), as well as the potential delayed effects of these wastes in ecosystems (He and Wang 2008, Vanni et al. 2013, Clay et al. 2013, Halvorson et al. 2015a). Resolving the biochemistry of wastes could provide the most comprehensive way of comparing or linking CNR across taxa and ecosystems (Sardans et al. 2012, Zou et al. 2016), including bridging the divide in applicability of ES and NG to terrestrial and aquatic ecosystems, respectively.

**INTEGRATIVE APPROACHES IN NUTRITIONAL ECOLOGY**

ES and NG developed in parallel as separate frameworks in nutritional ecology, and despite promising synergies between the two, scientists have rarely considered their integration to advance nutritional ecology (but see Raubenheimer and Simpson 2004, Hawlena and Schmitz 2010, Harrison et al. 2014, Cease et al. 2016). Both ES and NG improve our understanding of nutritional regulation by moving beyond previous single-currency approaches to simultaneously considering multiple nutrients/elements (Sterner and Elser 2002, Simpson and Raubenheimer 2012). Notably, the nutritional state-space plots from NG, which provide a methodological cornerstone to our integration, can be applied beyond multiple elements or nutrients commonly used in ES or NG, e.g. by extending beyond C, N, and P to include sodium or calcium when these elements constrain consumer growth and fitness (e.g. Pivnick and McNeil 1987, or Nie et al. 2015, Prater et al. 2016, respectively). State-space plots can be also used in dual stable- or radioisotope tracer studies to label specific components of food resources and track their use by consumers (e.g. Boersma et al. 2016, Halvorson et al. 2016). Finally, the state-space plots of NG take ES beyond ratio approaches to address food resource dilution effects that can alter consumer homeostatic regulation or fitness. For example, NG approaches can clarify ambiguity of diet ratios, such as whether varying nutrient
concentrations in the denominator or numerator term drive differences in nutrient ratios, or when two diets contain the same ratio but different absolute concentrations of the two nutrients (Raubenheimer 1995). Though there are a limited number of empirical studies addressing both ratios and absolute amounts of elements (e.g. Middleton and Frost 2014), ES theory does not preclude these studies and further work is clearly needed. We propose future organism nutrient or elemental budgets be presented in two-dimensional nutrient space plots that incorporate effects of both ratios and amounts of multiple nutrients/elements on consumer regulation and fitness.

The individual organism is of key importance in nutritional ecology, because organism homeostasis drives regulation and fitness responses to diet nutrient composition (Raubenheimer et al. 2012). We present nutrient state-space and response surface plots that place the organism at the centre of nutritional studies by encompassing both pre-ingestive and post-ingestive strategies of homeostatic regulation that are shared across diverse taxa. Therefore, state-space plots could be applied theoretically to any organism, such as heterotrophic microbes or autotrophs, when using relevant nutritional components. New developments in NG state-space models based on nutrient concentrations or proportions of diet mixtures rather than on intake amounts may also make it easier to apply to certain ES-related questions (Raubenheimer 2011, Machovsky-Capuska et al. 2016). Integrative approaches expanding beyond strictly using either elements or macronutrients as currency (Anderson et al. 2004) may also help nutritional ecology address a wider diversity of organisms while taking into account their unique strategies for homeostatic regulation.

Finally, being explicit about both abiotic and biotic environmental factors influencing organism responses to diet nutrients as well as the quantity and quality of food resources available to consumers is an integral piece of forming an integrative framework in nutritional ecology (Raubenheimer et al. 2009, Simpson et al. 2010, Kearney et al. 2010, Hessen et al. 2013). Currencies commonly used at ecosystem
scales, such as elements or energy (Reiners 1986), are useful for describing ecological processes, but may come at a cost of being less explicit about nutritional ecology of individual organisms. We show how resolution of the biochemical form of particular nutrient budget components may improve predictions of the interactions between organisms and their environment. In particular, our integrative approach aims to connect consumer waste composition with nutrient composition of their food resources to better understand how organism homeostatic regulation could feedback to affect the balance of consumer diets in the ecosystem. By comparing organism nutrient budgets under different experimental scenarios, response surface plots can address effects of external factors, such as predator threat or temperature, on organism nutrition (Hawlena and Schmitz 2010, Kearney et al. 2010, Cross et al. 2015). Controlled laboratory experiments provide ideal settings to construct organism nutrient budgets and predict consumer-resource feedbacks that could be tested under field conditions, for instance, by conducting mesocosm or enclosure experiments. ES and NG thus provide many complementary approaches to improve predictions of the interactions between organisms and their environments.

**CONCLUSIONS**

Future advances in nutritional ecology will depend on integrating concepts and tools at the organism-environment interface. By bridging the two distinct frameworks, ES and NG, we provide complementary approaches for a more comprehensive understanding of nutritional ecology. We first suggest that studies may benefit from using state-space and response surface plots grounded in NG to analyse nutrient or elemental budgets of individual organisms. Second, we suggest being more explicit about waste biochemistry, and analyses using response surfaces may improve studies bridging organism homeostatic regulation and ecosystem processes through CNR, a key concept of ES. We used specific examples based on published data to illustrate each of
these integrative approaches, thus representing two clear synergies between ES and NG. Further, linking the two frameworks offers novel insights by addressing the behavioural, physiological, and ecological consequences of organism homeostasis, as well as excess and deficits of multiple nutrients and associated fitness consequences from consuming nutritionally imbalanced food. Integrating ES and NG will require significant methodological adjustments, such as moving beyond the prevailing currencies of either framework and developing new experimental approaches. Ultimately, integrating ES and NG is a valuable step towards bridging the scale of organism nutrition up to population, community, and ecosystem levels of biological organization.

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REFERENCES


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**FIGURE LEGENDS**

**Figure 1.** Conceptual diagram illustrating how the organismal level (heterotrophic consumer) can be linked to the ecosystem level (environment). Diet represents the relative quantity and quality of nutrients and elements ingested by the organism and waste represents different release components from the organism. Smaller white boxes show pools, arrows define fluxes between pools, and grey text indicates processes. Black arrows within the surrounding white space show flows of nutrients and elements within and across scales: dashed black lines represent nutrients or elements released by the organism and thicker black arrows signify flow of material across scales. Abiotic and biotic factors (bottom) influence processes across all scales.
Figure 2. Graphical description of (a) conceptual nutrient budgets (after Raubenheimer et al. 2009) and (b) empirically determined elemental budgets for the caddisfly *Pycnopsyche lepida* reared on diets of different C:P ratio (original data from Halvorson et al. 2015b). Black points (i) indicate the ingested amounts of nutrients/elements, grey points (a) indicate the absorbed amounts, and white points (g) indicate the amount of nutrients/elements that are allocated to newly built biomass (i.e. growth). Nutrient budget components are ingestion (I), egestion (Eg), metabolic loss (ML), such as post-absorptive excretion and/or respiration, absorption (A), and growth (G). Diagonal lines that intersect with the origin are nutritional rails that reflect a particular ratio of nutrients/elements throughout the nutrient space. The example plot (b) shows average elemental budgets of *P. lepida* reared on a P-limited diet (C:P (molar)=2630, indicated in red) and a P-sufficient diet (C:P (molar)=1590).
Figure 3. Response surfaces of the elemental budget components for (a,c,e) carbon and (b,d,f) phosphorus of the caddisfly *Pycnopsyche lepida* fed three different C:P diets (original data from Halvorson et al. 2015b). The colour scales of the budget components (a, b) egestion, (c, d) metabolic loss, and (e, f) growth are standardized within each element to improve comparison across plots. Solid grey lines describe the ratios of C:P intake across the nutrient space, i.e. the nutritional rails for each of the three diets (C:P(molar), 1=1230, 2=1590, 3=2630).
Figure 4. Response surfaces of *P. lepida* (a) mass-specific growth rate (d⁻¹) as an indicator of fitness and carbon:phosphorus ratios of (b) egestion, (c) final body tissue, and (d) newly grown body tissue (original data from Halvorson et al. 2015b). Solid grey lines describe C:P intake rails for each of the three diets (C:P(molar), 1=1230, 2=1590, 3=2630).
Figure 5. Response surfaces of frass nitrogen (N) waste produced by fifth-stadium *Locusta migratoria* after 8 days of growth as (a) protein, (b) uric acid, and (c) amino acids. *L. migratoria* grew on diets that differed in three protein:carbohydrate ratios (1=1:3, 2=1:1, and 3=3:1) represented as nutritional rails (grey diagonal lines). Note different colour scale for uric acid. Original data from Zanotto et al. (1993).
Figure 6. Modelled microbial N uptake from *L. migratoria* waste over a 32-day period. (a) Cumulative microbial N uptake (%) from waste compartments amino acids, uric acid, or protein, and bulk (average) N uptake rate ignoring waste biochemistry (Null model). (b) Mean cumulative N uptake and (c) mean percent of N taken up by microbes from total wastes produced by *L. migratoria* fed four diets that differed in three protein:carbohydrate (p:c) ratios based on Zanotto et al. (1993) (note that p:c ratios of 7:7 and 21:21 correspond to the nutritional rail number 2 in Fig. 5 and 7).
Figure 7. Response surfaces of (a) total microbial N growth, (b) total microbial N mineralized, (c) proportion of total N waste supporting microbial growth, and (d) the proportion of total N waste mineralized from frass of *L. migratoria* after 8 days of microbial uptake, growth, and mineralization. Uptake/Mineralization over time was modelled from protein, amino acid, and uric acid N contents of frass reported in Zanotto et al. (1993) and subsequent microbial N uptake and mineralization rates according to the integrative ES/NG model (see also Figs. 5, 6); protein:carbohydrate ratios (1=1:3, 2=1:1, and 3=3:1).
**Figure 8.** Percent deviation of the null model ignoring waste biochemistry from microbial N mineralization predicted using the ES/NG model using compound-specific mineralization rates of the three major biochemical forms of N in locust waste. The horizontal line distinguishes overestimated and underestimated N mineralization predicted by the null model relative to the ES/NG model prediction.
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