

LETTER

Phosphorus supply shifts the quotas of multiple elements in algae and *Daphnia*: ionic basis of stoichiometric constraints

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Abstract

The growth rate hypothesis posits that the rate of protein synthesis is constrained by phosphorus (P) supply. P scarcity invokes differential expression of genes involved in processing of most if not all elements encompassing an individual (the ionome). Whether such ionome-wide adjustments to P supply impact growth and trophic interactions remains unclear. We quantified the ionomes of a resource-consumer pair in contrasting P supply conditions. Consumer growth penalty was driven by not only P imbalance between trophic levels but also imbalances in other elements, reflecting complex physiological adjustments made by both the resource and the consumer. Mitigating such imbalances requires energy and should impact the efficiency at which assimilated nutrients are converted to biomass. Correlated shifts in the handling of multiple elements, and variation in the supplies of such elements could underlie vast heterogeneity in the rates at which organisms and ecosystems accrue biomass as a function of P supply.

Keywords

Biomass production, consumer–resource interactions, ecological stoichiometry, ionomics, growth rate hypothesis, nutrient quotas, zooplankton.

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INTRODUCTION

The rate at which organisms produce new biomass is central to understanding population growth and the fluxes of energy and materials in ecosystems (Odum 1968). Moreover, growth rate is a central parameter in models describing organismal fitness because it is under direct selection or is strongly correlated with several other fitness-relevant traits (Stearns 1992). The synthesis of proteins is the primary anabolic process underlying growth (Milo & Phillips 2015; Kafri *et al.* 2016), and protein concentration in a cell is involved in triggering cell division (e.g. Cookson *et al.* 2010). These basic cellular processes underlie the growth of organisms and populations, and the productivity of ecosystems.

Approaches based on temperature, energy or nutrients have shaped the study of biomass production at various scales (reviewed in Ratkowsky *et al.* 1982; van der Ploeg *et al.* 1999). Such seminal efforts resulted in the identification of key extrinsic parameters that have large impacts on biomass production (e.g. Monod 1949). The supply of phosphorus (P) is often important in constraining the rate of biomass production because it is more abundant in biomass than in the inorganic environment (Smil 2000). When extrinsic supply of P is low, cells expend energy in sequestering P from the environment, or recycling/reallocating internal P stores, ultimately compromising rates of biomass production (Droop 1973).

Commonly referred to as the cell quota model, Droop's model is widely used in ecology (Sterner & Elser 2002).

The growth rate hypothesis (GRH; Elser *et al.* 2003) illuminated key cellular mechanisms underlying the relationship between growth and P quota because ribosomal RNA (rRNA) is the dominant cellular sink for P, accounting for c. 50% of total P (see Fig. 1c in Elser *et al.* 2003) in unicellular and small (< 1 mg dry mass) multicellular organisms (Gillooly *et al.* 2005). The GRH predicts three key positive associations between: P quota and rRNA, rRNA and protein synthesis, and protein synthesis and growth. Because the GRH arises from fundamental biochemical processes, it is useful in understanding large scale ecological patterns such as the relationship between P supply and ecosystem productivity (Vollenweider 1968; Smith 1982). Nevertheless, there is considerable variation in such log–log relationships, and studies have found no support for one or more of the tripartite GRH linkages (e.g. Flynn *et al.* 2010; Sherman *et al.* 2017).

The GRH focuses on key material resources (i.e. N, P) required for protein synthesis in situations where energy required for peptide anabolism is not limiting (Loladze & Elser 2011), arguably due to the initial focus of ecological stoichiometry on factors other than energy in impacting ecosystem productivity (Sterner *et al.* 1992a). Nevertheless, even when there is replete supply of nitrogen and phosphorus, growth cannot proceed without sufficient energy for

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transcription of mRNA, and translation of proteins (e.g. Bier 1999). The synthesis of RNA and protein utilises about 35% of available energy in cells (Buttgereit & Brand 1995) which is the basis for a rule of thumb stating that incorporation of one carbon atom into biomass requires *c.* 1 ATP (Milo & Phillips 2015). Ion pumps utilise the next highest amount of energy (*c.* 20%; Buttgereit & Brand 1995) to maintain optimal electrochemical gradients across the cell membrane. Although these values are approximations based on a few observations, they indicate that the extrinsic supply of the *c.* 20 elements represented in biomass (Williams & Frausto da Silva 2005) can substantially impact energy budgets.

Moreover, extrinsic supply of an element alters the processing of multiple other elements inside a cell or organism. Transcriptomic studies capturing changes in the expression of all genes in a genome in response to change in a single element (e.g. P) find that genes unrelated to the processing of P are differentially expressed in autotrophs (e.g. Mission *et al.* 2005) as well as heterotrophs (e.g. Jeyasingh *et al.* 2011). Such responses impact the processing of multiple other elements and associated energy demands (e.g. Malinouski *et al.* 2014). Understanding the biochemical and genetic mechanisms of such correlated shifts in all elements encompassing an individual (i.e. the ionome), and the relevance of shifts to the expression of key traits such as growth rate is the central goal of the field of ionomics (Salt *et al.* 2008).

Aforementioned observations indicate that information (e.g. extrinsic supply, cellular quotas) on the entire suite of elements in a system should have much ecological relevance (e.g. Jeyasingh *et al.* 2014, 2017; Kaspari & Powers 2016; Peñuelas *et al.*, 2019 who refer to the ionome as the 'elementome'). However, the majority of ionomic data is on autotrophs such as algae (e.g. Cunningham & John 2017) and land plants (reviewed in Huang & Salt 2016). Although similar data are becoming available for a few other taxa (e.g. arthropods, Goos *et al.* 2017; fish, Rudman *et al.* 2019; amphibians, Prater *et al.*, 2019), the majority of such work do not characterise ionomic responses to changes in elemental supply. Unlike autotrophs, the responses of metazoan consumer ionomes to changes in inorganic nutrient supply include associated ionomic changes in diet (Jeyasingh & Pulkkinen 2019). Ionomic responses of higher trophic levels to changes in key elements such as P have yet to be characterised and should have much to contribute towards understanding patterns in higher order ecology, such as variation in ecosystem productivity unexplained by classical limiting factors (e.g. P supply in lakes; Smith 1982).

How should ionomes respond to P limitation? Although much work is needed to rigorously answer this question, one general prediction is that quotas of trace metal cofactors (e.g. iron, manganese, zinc), and elements serving structural functions, such as magnesium that stabilises RNA and DNA (e.g. Hartwig 2001) should decrease in P limited conditions. When P limits protein synthesis, the demand for trace metals should decrease because there are fewer protein ligands where these metals serve as cofactors. Several trace metal ions, particularly those involved in Fenton-like reactions can cause oxidative stress when they are not bound to inorganic or organic ligands inside a cell (e.g. Sies 2015) or impede the transport of

major ions (e.g. Kramer *et al.* 1986). Consequently, cells allocate considerable energy to maintain trace metal homeostasis (Lodish *et al.* 2000; Balamurugan & Schaffner 2006; Worms *et al.* 2006), indicating a trade-off in energy allocated to the predominant energy sink, protein anabolism. We posit that such trade-offs in the energy quotient allocated to peptide anabolism has a large role to play in the efficiency at which assimilated N and P are used for generation of new biomass, potentially underlying substantial variation in P use efficiencies among genotypes (e.g. Jeyasingh *et al.* 2009), species (e.g. Seidendorf *et al.* 2010) and ecosystems (e.g. Kelley *et al.* 2018).

We quantified changes in the ionome of the green alga *Scenedesmus obliquus* to inorganic P supply, and fed it to *Daphnia pulicaria* genotypes and observed growth and ionic responses. Specifically, we measured the ionic response of algae to two P-supply conditions (LoP, 5 $\mu\text{mol L}^{-1}$; HiP, 50 $\mu\text{mol L}^{-1}$) and predicted that concentrations of all elements should decrease under P limitation. We fed LoP and HiP *Scenedesmus* to four genotypes of *Daphnia pulicaria* and measured their growth and ionic responses and predicted that daphniid growth will be slower in LoP conditions, and that the concentration of all elements in the *Daphnia* ionome will be lower. Because an element in a biological system never acts independently of other elements, univariate analyses of each element in the ionome or even dimension reduction multivariate analyses could potentially miss pivotal biological information (Baxter 2015). As such, we identified suites of elements using the nutrient balance approach of Parent *et al.* (2013), and tested for treatment effects (i.e. P supply for algae and *Daphnia*, and P supply and genotype for *Daphnia*). Finally, to understand how ionome-wide changes in response to P supply in both algae and *Daphnia* impact the balance of elements between trophic levels, and its consequences for *Daphnia* growth, we constructed trophic stoichiometric ratios (TSR; Filipiak & Weiner 2014) which is an index of elemental imbalance between resource and consumer as a function of carbon, for each of the other (12) elements measured. We predicted that multidimensional TSRs will be sensitive to P treatment and that TSRs for all elements will be negatively correlated with growth of *Daphnia*.

MATERIALS AND METHODS

Study organisms and experimental setup

A *Scenedesmus obliquus* genotype was cultured in continuous chemostats with P-sufficient (HiP; 50 μM) or P-deficient (LoP; 5 μM) media (Kilham *et al.* 1998). Fresh outflow from stable state chemostats were used for elemental analyses and to feed *Daphnia*. We studied four genotypes of *Daphnia pulicaria* that were hatched from sediment cores from South Center Lake, Chisago County, Minnesota (Frisch *et al.* 2014). Two genotypes were isolated from 4 to 8 cm layer of the sediment core (4-8/2 and 4-8/3 in Frisch *et al.* 2014; hereafter termed as Genotype A and Genotype B respectively), and the remaining two from the 60–64 cm layer (60-64/1 and 60-64/2 in Frisch *et al.* 2014; hereafter termed as Genotype C and Genotype D respectively). We chose to study these genotypes

because they exhibit unique growth responses to dietary P (Frisch *et al.* 2014) and associated ecological consequences (Roy Chowdhury & Jeyasingh 2016). We measured juvenile growth rate (JGR; mm day⁻¹) of <12h-old-neonates under HiP or LoP diet for 5 days. This experiment was replicated thrice for each genotype. To generate ionomes, 12 gravid females per genotype were kept in 1L jars and fed LoP or HiP *Scenedesmus* at a concentration of 1 mg C L⁻¹ day⁻¹ for approximately 2 weeks when juveniles were harvested for elemental analyses (see below). These cultures were replicated thrice for each of the two P treatments (six cultures per genotype) and maintained at c. 20 °C, and 16:8 light: dark cycle.

Elemental analysis

Fresh algae from chemostat outflow was filtered onto pre-combusted and pre-weighed Whatman GF/F glass filters (GE Healthcare Life Sciences, Pittsburgh, PA, USA) or sterile, pre-weighed Whatman cellulose acetate membrane filters (0.45 µm; GE Healthcare Life Sciences, Pittsburgh, PA, USA). Glass fibre filters were dried at 60 °C for 72 h and weighed before analysis. Carbon and nitrogen of algae on glass filters were quantified using an automated analyser (varioMicro Cube; Elementar Americas, Mt. Laurel, NJ, USA). Dried cellulose acetate filters containing algal samples were digested in 800 µL of HNO₃ and 400 µL of H₂O₂, digested for 24 h, and diluted to a volume of 10 mL with ultrapure (Type 1) water. Digested samples were injected into an ICP-OES analyser (iCAP 7400; Thermo Scientific, Waltham, MA, USA) for measurements of all other elements. Each P treatment was replicated thrice.

Fifteen similar-sized premature daphniids were isolated from each of the HiP or LoP cultures, washed thrice with ultrapure (Type 1) water, dried at 60°C for 72 h, and weighed to the nearest µg (Mettler Toledo XP2U, Columbus, OH, USA). Carbon and nitrogen content of *Daphnia* were analysed similar to algae. Samples for ICP-OES were digested in 15 ml trace metal-free polypropylene conical centrifuge tubes (VWR International, Radnor, PA, USA) by adding 200 µL of trace metal grade 67–70% HNO₃ (BDH Aristar® Plus, VWR International, Radnor, PA, USA) and 100 µL of trace metal grade 30–32% H₂O₂ (BDH Aristar® Ultra, VWR International, Radnor, PA, USA). Each sample was then allowed to digest overnight at room temperature (c. 22 °C), until the solution ran clear. All digested *Daphnia* samples were then diluted to a final volume of 5 mL with ultrapure (Type 1) water. Each genotype-by-P treatment group was replicated thrice.

Validation and calibration of the ICP-OES was achieved by using multi-element external reference standards (CCV Standard 1, CPI International, Santa Rosa, CA, USA). Additionally, an in-line Yttrium internal standard (Peak Performance Inorganic Y Standard, CPI International, Santa Rosa, CA, USA) was used to correct for any instrument drift or matrix effects. Digestion blanks for both *Daphnia* and algae, consisting of only diluted HNO₃ and H₂O₂ with no sample were also run to correct for background concentrations. Algal digestion blanks also included a digested cellulose acetate filter. Fifteen of the 28 total elements analysed by the ICP-OES were ultimately excluded from further analysis because the measured

concentrations of the samples were within one standard deviation of our blank controls, indicating that these concentrations were potentially close to or below the limit of detection for the ICP-OES. The remaining 13 elements (C, Ca, Fe, K, Li, Mg, Mn, N, Na, P, S, Sr, Zn) were all above detection limits, and the concentrations of these elements (in µg g⁻¹) were log₁₀-transformed before statistical analyses.

Statistical analyses

To visualise relationships among multiple elements, we first conducted a principal components analyses (PCA) to examine the effects of phosphorus supply (for *Scenedesmus*) or P supply and genotype and their interaction (for *Daphnia*) on log transformed elemental concentrations of the 13 measured elements. The PCA revealed multidimensional relationships between elements and predictor variables, which separated differentially across two principle component axes. Thus, we used these principal components to construct isometric log-ratios balances (henceforth, *ILRs*) that are referred to as nutrient balances. *ILRs* are unbiased estimates of multivariate relationships among elements, which avoid violating common statistical assumptions and can be used to describe and interpret elemental interactions in biomass (Parent *et al.* 2013). Moreover, *ILRs* are amenable to hypothesis testing using general linear models without inflating critical values due to multiple tests for treatment effects on all 13 elements. We included all elements loading on each of the two PC axes to construct *ILRs* for each replicate following Parent *et al.* (2013) using the equation: $ILR = \sqrt{\frac{(rs)}{(r+s)}} \ln \frac{g(c^+)}{g(c^-)}$ where r and s represent the number of elements on the left- and right-hand side of the balance and $g(c^+)$ and $g(c^-)$ represent the geometric mean of the elements on the left- and right-hand side of the balance respectively. After nutrient balances were constructed, we tested for differences between P supply treatment and among genotypes, using a multivariate analysis of variance (MANOVA). Upon finding significant differences (Wilk's Lambda $P < 0.001$), we conducted univariate main effects ANOVAs to examine differences in individual balances between P supply treatments and among *Daphnia* genotypes.

To better understand the growth relevance of ionome-wide shifts in algae and *Daphnia* in response to P supply, we calculated trophic stoichiometric ratios (TSR) for all elements following Filipiak & Weiner (2014): $TSR_x = \frac{(C \div X)_{algae}}{(C \div X)_{Daphnia}}$, where C is carbon content, and X is content of element x (i.e. Ca, Fe, K, Li, Mg, Mn, N, Na, P, S, Sr, or Zn). TSR values substantially greater than 1 indicate stoichiometric mismatch. We performed a PCA to understand the effects of P treatment, *Daphnia* genotype and *Daphnia* growth on ionome-wide TSRs.

RESULTS

The PCA of algal elemental composition revealed two PCs explaining 91.9% of total variation (Fig. 1; Table S1a; Fig. S1). We constructed nutrient balances for each of the two PC axes in *Scenedesmus*; [C | Ca,Fe,K,Li,Mg,Mn,N,Na,P,S,Sr,Zn] along PC1, and [Li,Mg,Mn,N,Na,P,S | C,Ca,Fe,K,Sr,Zn] along PC2. One-way main effect ANOVAs on these

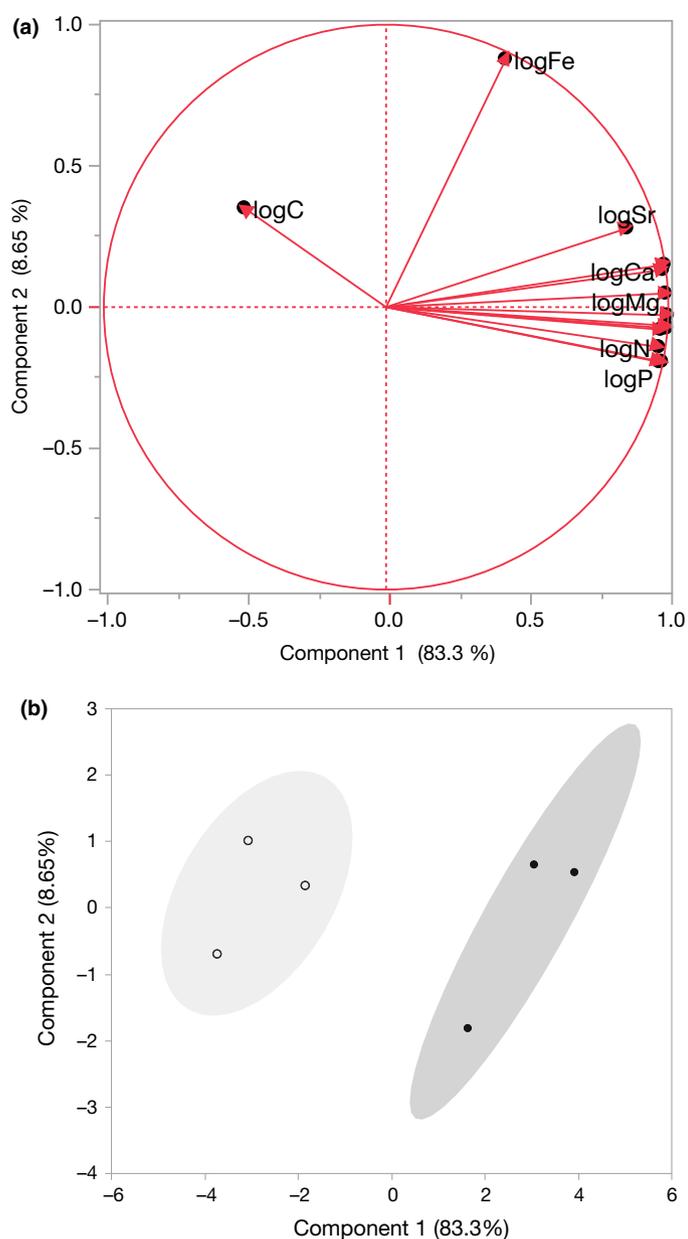


Figure 1 (a) PCA plot representing the multidimensional elemental composition of *Scenedesmus*. See Table S1a for loading matrix. Note that the PCA included all 13 elements, although closely overlapping vectors are not labelled in the figure. Vectors represent linear correlations (loadings) of the individual elements to the two PCs. Vector length indicates the strength of these correlations. (b) PCA plot representing the multidimensional elemental composition of *Scenedesmus* algae under low (open circles) or high (closed circles) phosphorus conditions. Markers represent individual sample locations, while ellipses represent 90% coverage probability for each treatment.

balances revealed significant effects of P supply on the balances along both PC axes (Table 1a).

ANOVA on *Daphnia* juvenile growth rate revealed a significant P treatment by genotype interaction ($F_{\text{trt} \times \text{geno}} = 19.93$; d.f. = 3; $P < 0.00001$). Genotypes A and B, resurrected from recent sediment layers exhibited the typical growth penalty under LoP, whereas genotypes C and D, resurrected from ancient sediment layers did not (Fig. 2).

Table 1 Results of multivariate analyses on isometric log-ratio balances along the first two principal component axes (see Table S1): (a) between *Scenedesmus* grown in high or low phosphorus supply, and (b) between *Daphnia* reared on such algae, and among the four genotypes studied. Degrees of freedom (d.f.), type 3 sum of squares (SS), F-ratio of mean squares (F) and *P*-values are reported for the main effects ANOVAs. Significant effects are indicated by bold face *P*-values. Interactive effects of treatment and genotype in the *Daphnia* data set was not significant. MANOVAs were significant for both algae and *Daphnia* (see text)

Factor	Nutrient balance	SS	d.f.	F	<i>P</i> -value
(a) <i>Scenedesmus</i>					
Treatment	[C Ca,Fe,K,Li,Mg,Mn,N,Na,P,S,Sr,Zn]	41.609	1	39.540	0.003
	[Li,Mg,Mn,N,Na,P,S C,Ca,Fe,K,Sr,Zn]	8.375	1	29.23	0.006
(b) <i>Daphnia</i>					
Treatment	[C Ca,Fe,K,Li,Mg,Mn,N,Na,P,S,Sr,Zn]	23.607	1	101.607	0.000
	[Ca,Fe,Mn,Sr,Zn C,K,Li,Mg,N,Na,P,S]	0.390	1	2.327	0.147
Genotype	[C Ca,Fe,K,Li,Mg,Mn,N,Na,P,S,Sr,Zn]	2.788	3	3.999	0.027
	[Ca,Fe,Mn,Sr,Zn C,K,Li,Mg,N,Na,P,S]	2.245	3	4.466	0.018

Similar to algae, all elements except carbon decreased in *Daphnia* feeding upon LoP algae, although the magnitude of this effect was genotype-specific (Fig. 3; Table S1b; Fig. S2). Nutrient balances along the two PC axes for *Daphnia* resulted in the following balances; [C | Ca,Fe,K,Li,Mg,Mn,N,Na,P,S,Sr,Zn] along PC1, and [Ca,Fe,Mn,Sr,Zn | C,K,Li,Mg,N,Na,P,S] along PC2 (Table 1b). Effects of P treatment on the two nutrient balances constructed revealed significant differences along PC1, whereas no significant effects of P supply was found on the nutrient balance along PC2. On the other hand, significant effects of genotype were observed on nutrient balances along both PC axes because all four genotypes occupied unique positions in the PCA (Fig. 3).

PCA on trophic stoichiometric ratios (TSRs) of all elements measured revealed that TSRs of all elements, except Fe, were negatively correlated with *Daphnia* growth rate (Fig. 4a; Table S2). P treatment invoked a clear separation of ionome-wide TSRs (Fig. 4b) along the first two PCs explaining *c.* 85% of total variation (Table S2). TSR values ranged three orders of magnitude with striking changes in values between HiP and LoP treatments (Table S3). Specifically, while the TSR for P (13.91) in LoP conditions clearly indicates a stoichiometric constraint, three other elements (i.e. Ca, Na, Sr) were also considerably imbalanced (Table S3).

DISCUSSION

We found substantial changes in the algal ionome (Fig. 1) in response to inorganic phosphorus supply, and in the *Daphnia* ionome reared on these two types of algae (Fig. 3). In low P supply, the quotas of multiple other elements in both algae and *Daphnia*, in addition to nitrogen and phosphorus are lowered, while carbon quotas increased (Fig. S1). These

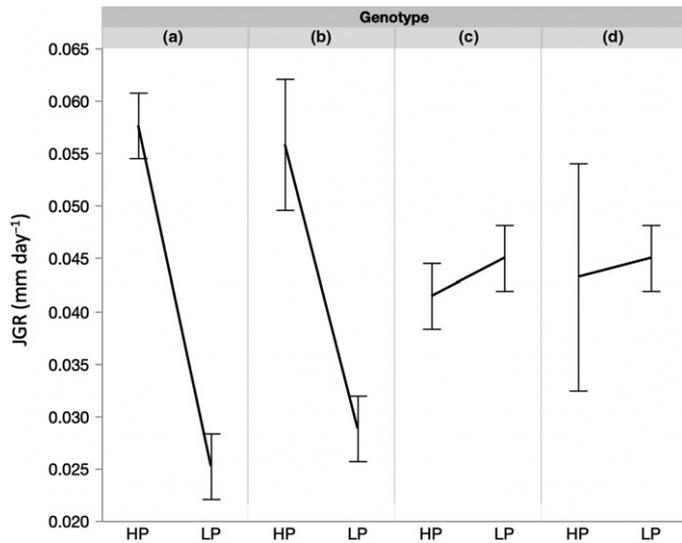


Figure 2 Mean (± 1 SD; $n = 3$) juvenile growth rate (JGR) of the four genotypes studied under high (HP) and low phosphorus (LP) diet. Genotypes A and B were isolated from 4 to 8 cm layer of the sediment core (4–8/2 and 4–8/3 in Frisch *et al.* 2014), and genotypes C and D are from the 60–64 cm layer (60–64/1 and 60–64/2 in Frisch *et al.* 2014). JGR was measured as the change in length of < 12 h-old-neonates under HiP or LoP diet over 5 days, and represented as mm day^{-1} .

observations indicate that the well documented slower growth rates of taxa in low P supply may not strictly be driven by P constrains on ribosome biogenesis (Elser *et al.* 2003) and allocation of ATP towards handling of excess carbon (reviewed in Sterner & Hessen 1994; Hessen & Anderson 2008), but also in dealing with several other elements (Fig. S2) because such energetic demands should alter energy available for peptide anabolism. As such, the predictions of the N:P-explicit growth rate hypothesis (Loladze & Elser 2011) can be sensitive to changes in the supply of other elements, and/or the energetic efficiencies at which different taxa deal with other elements that may be rendered replete due to P limitation of growth.

Low supply of inorganic phosphorus decreased the concentration of 10 elements, including P in algae, whereas C content increased using the traditional multiple comparisons approach (Fig. S1). Nutrient balance analyses (Parent *et al.* 2013) along the first PC axes also confirmed a significant treatment effect on the concentrations of these elements (Table 1a). These results are consistent with prior work reporting higher C quotas, and lower quotas of N and P in P limited *Scenedesmus* (reviewed in Sterner & Hessen 1994), lending support to GRH predictions. Lower quotas of other elements discovered in this study indicate that, as P constrains protein synthesis and decreases protein quotas, the demand for other elements should also decrease (Fig. 1). One possibility is that lower quotas of macromolecules (e.g. RNA, protein) in low P conditions render elements that perform key structural (e.g. Mg in RNA; Hartwig 2001) and functional (e.g. trace metal cofactors in proteins; Nelson & Cox 2012) roles, replete. Handling such excess ions may require energy depending on the concentration gradient across the cell wall. For example the concentration of sodium ($23.4 \mu\text{M}$) in the medium used (Kilham *et al.* 1998) is roughly 50% higher than the Na concentrations observed in LoP *Scenedesmus* cells ($9.39 \mu\text{M}$; Fig. S1). In such conditions cells may allocate considerable ATP towards maintaining Na homeostasis by actively pumping Na out (e.g. *c.* 3Na^+ pumped per ATP hydrolysed; Feher 2016), impacting the ATP quotient allocated to the ribosome for protein anabolism, resulting in slower protein synthesis. In other words, the commonly observed slower protein synthesis and growth rates in algae experiencing low P supply may not only be due direct P-limitation of protein synthesis as posited by the GRH but also because of correlated shifts in the handling of other elements.

Daphnia feeding on P limited algae with lower concentrations of all elements and higher concentration of C responded by growing slower (Fig. 2) and adjusting their ionomes in a genotype-specific manner (Fig. 3; Fig. S2). Ionome-wide shifts were identical to that of algae (Table 1a), as indicated by the nutrient balances constructed using principal component loadings along the first axis explaining 65% of variation

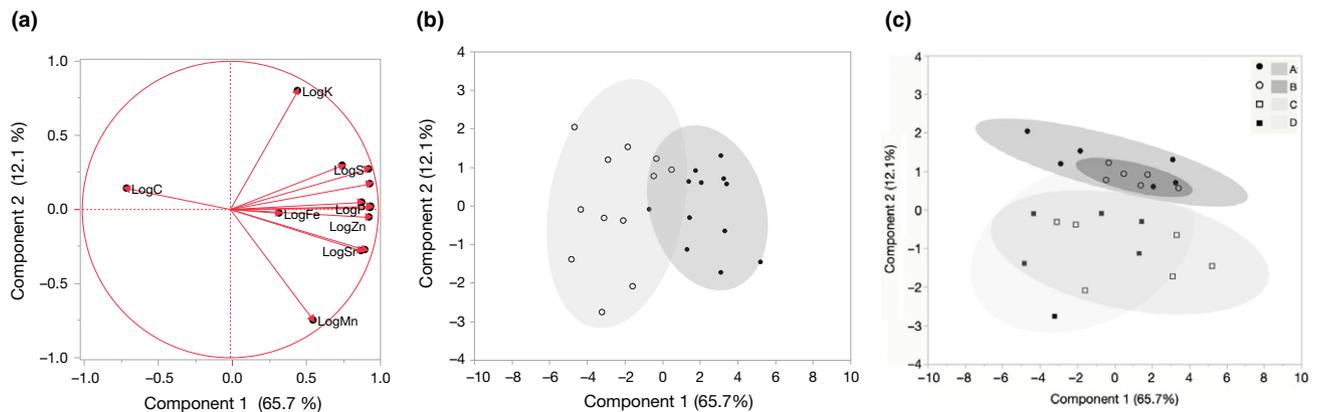


Figure 3 (a) PCA plot representing the multidimensional elemental composition of *Daphnia*. See Table S1b for loading matrix. Note that the PCA included all 13 elements, although closely overlapping vectors are not labelled in the figure. Vectors represent linear correlations (loadings) of the individual elements to the two PCs. Vector length indicates the strength of these correlations. (b) PCA plot showing the locations of *Daphnia* ionomes reared on high (HiP, closed circles) or low (LoP, open circles) phosphorus *Scenedesmus*. (c) Locations of the four (A, B, C, D) *Daphnia* genotypes. Markers represent individual sample locations, while ellipses represent 90% coverage probability for each treatment or genotype.

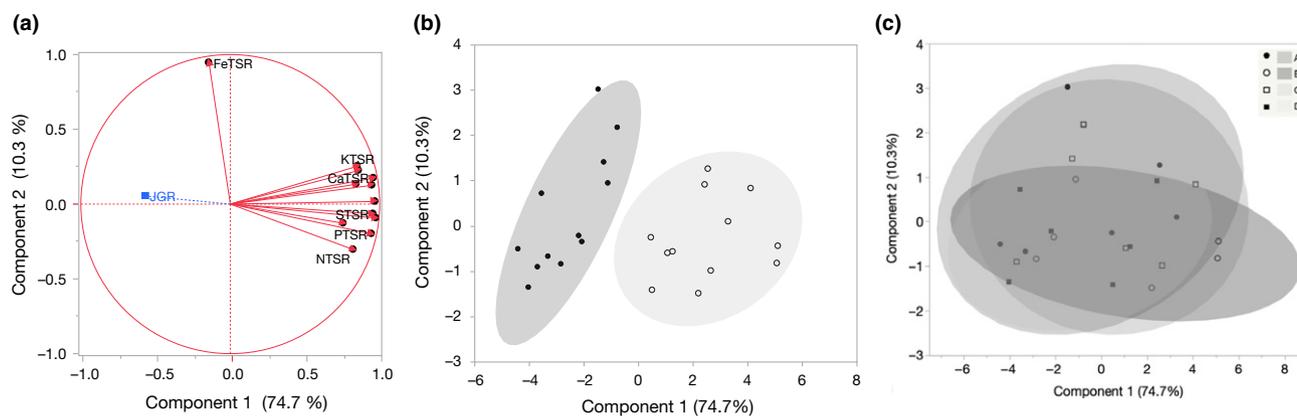


Figure 4 (a) PCA plot representing multidimensional trophic stoichiometric ratios (TSR), and the relationship between TSRs and *Daphnia* growth (JGR; blue square and vector). See Table S2 for loading matrix. Note that the PCA included all 12 TSRs, although closely overlapping vectors are not labelled in the figure. Vectors represent linear correlations (loadings) of the individual TSRs to the two PCs. Vector length indicates the strength of these correlations. (b) PCA plot showing the locations of TSRs in high (HiP, closed circles) or low (LoP, open circles) phosphorus conditions. (c) Locations of the four genotypes studied. Markers represent individual sample locations, while ellipses represent 90% coverage probability for each treatment or genotype.

(Table S1), and revealed significant effects of P treatment and genotype (Table 1b). This means that resource and consumer ionomes are qualitatively similar in responding to large changes in P supply. Given that P impacts growth via fundamental mechanisms shared by all taxa, such responses may be general at the organismal level, although we note that tissue-level responses to P limitation increases concentrations of some elements (e.g. leaf; Baxter *et al.* 2008). Higher C content of daphniids observed under LoP is consistent with prior work reporting that *Daphnia* feeding on LoP algae are faced with excess C that is stored as lipids (e.g. Sterner *et al.* 1992b), disposed via increased respiration (e.g. Jeyasingh 2007; Ruiz *et al.* 2018), or excreted as dissolved organic carbon (DOC; e.g. Darchambeau *et al.* 2003). Such mitigation requires energy, and thus should contribute to lower growth rates under P limitation (Hessen & Anderson 2008). Lower concentrations of several other elements (i.e. beyond those directly involved in protein synthesis such as N and P) observed in this study (Fig. S2) indicates that as P constrains protein synthesis, the demand for such elements should decrease, triggering daphniids to alter the processing of multiple elements in the ionome, potentially impacting the ATP quotient allocated to power protein anabolism.

Although ionomes of all four *Daphnia* genotypes responded similarly to P supply (Fig. 3b), genotypes occupied unique positions in the PCA (Fig. 3c). The nutrient balance approach employed enabled quantitative tests for the effect of genotype on the ionome and indicated significant differences in nutrient balances constructed using the loadings of the first two PC axes (Table 1b). This result is perhaps not surprising in light of distinct growth responses of the study genotypes to P treatment (Fig. 2). Frisch *et al.* (2014) found that genotypes A and B, resurrected from *c.* 20-year-old sediments, exhibited strong growth penalties under P limitation that is typically reported in the literature (reviewed in Sterner & Schulz 1998), whereas genotypes C and D, arising from *c.* 700-year-old sediments, exhibited remarkably stable growth rates regardless of dietary P supply (Fig. 2). Differences in growth responses to P supply

is driven, at least in part, by substantial transcriptomic differentiation between these two age classes (Roy Chowdhury *et al.* 2015), that also impacts the quantity and quality of algae via consumer-driven P recycling (Roy Chowdhury & Jeyasingh 2016). Results from this study indicate that such unique growth and associated ecological impacts among genotypes are not only due to changes in P processing but also correlated shifts in the processing of multiple other elements. We note that rigorously teasing apart such variation would require more genotypic replicates than the four we studied.

Trophic stoichiometric ratios of 12 elements revealed strong negative correlations with juvenile growth rate (JGR) with all TSRs except that of Fe (Fig. 4; Table S2). The Fe TSR result is consistent with recent studies reporting no relationship between dietary Fe and JGR of daphniids (Lind & Jeyasingh 2018; Jeyasingh & Pulkkinen 2019). Negative correlations between JGR and TSRs for N and P were consistent with theory (Elser & Urabe 1999), although we found evidence for stronger stoichiometric imbalances in three other elements (Fig. 4). While the TSR for P in LoP (13.91) indicated stoichiometric constraint, explaining slower JGR per the growth rate hypothesis (Elser *et al.* 2003), data indicate that Ca, Na and Sr were considerably more imbalanced (Table S3). We note that such stronger trophic imbalances in these three elements does not necessarily mean that the growth penalty observed in LoP conditions is driven by these elements. These elements were considerably imbalanced in HiP treatment as well, and these elements are largely acquired from the medium by *Daphnia* via osmoregulatory organs (Porcella *et al.* 1969; Bianchini & Wood 2008). Nevertheless, transport and processing of these elements requires energy, which may be higher in LoP as indicated by the higher TSR values and thus impact the amount of energy available for protein assembly, and growth. Although it is unlikely daphniids in our experiments were limited by Ca, Na or Sr because the medium is rich in these elements (Kilham *et al.* 1998) and was held constant in both HiP and LoP treatments, the supplies of these elements are quite variable among natural ecosystems (Lecuyer 2014),

and is known to constrain *Daphnia* production directly (e.g. Hessen *et al.* 2000) or in an interactive fashion with P (e.g. Prater *et al.* 2016).

In conclusion, ionome-wide observations in a simple resource–consumer system underscore the utility of the growth rate hypothesis in making sense of general patterns in growth and trophic interactions. Our data show that changes in the supply of a single element, P, alter suites of elements in both resource and consumer reflecting complex physiological adjustments. Furthermore, the extent to which such adjustments impact growth will be dependent upon the supplies of multiple other elements. We know little about the extent of spatiotemporal variation in the supplies of elements other than those conventionally studied (e.g. P in freshwaters, Fe in oceans), although studies indicate substantial spatiotemporal variation in the supplies of such elements (e.g. Sterner 2008) that are also experiencing anthropogenic shifts (e.g. Björnerås *et al.* 2017; Dugan *et al.* 2017). We also know little about phylogenetic diversity in the ionomes of taxa (i.e. the demands for elements). There is much evidence indicating that ionomes of genotypes and species are distinct, so much so that ionic data can be used to infer the physiological status (e.g. Baxter *et al.* 2008), or geographical source of a sample (e.g. Li *et al.* 2013). Placing our observations upon such prior work, and ecological advances reporting the prevalence of co-limitation in ecosystems (e.g. Harpole *et al.* 2011) suggests that quantifying the dynamics of a wider suite of elements represented in biology will advance our understanding of heterogeneous ecological processes that arise from complex biochemical processes involving multiple elements.

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AUTHOR CONTRIBUTIONS

PDJ conceived the study. PRC, JMG, PRL and RES performed the experiments. PDJ analysed the data and wrote the paper with input from all authors.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.wm37pvmj9>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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