



Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions

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Humans are modifying the availability of nutrients such as nitrogen (N) and phosphorus (P), and it is therefore important to understand how these nutrients, independently or in combination, influence the growth and nutrient content of primary producers. Using meta-analysis of 118 field and laboratory experiments in freshwater, marine and terrestrial ecosystems, we tested hypotheses about co-limitation of N and P by comparing the effects of adding N alone, P alone, and both N and P together on internal N (e.g. %N, C:N) and P (e.g. %P, C:P) concentrations in autotroph communities. In particular, we tested the following predictions. First, if only one nutrient was limiting, addition of that nutrient should decrease the concentration of the other nutrient, but addition of the non-limiting nutrient would have no effect on the internal concentration of the limiting nutrient. If community co-limitation was occurring then addition of either nutrient should result in a decrease in the internal concentration of the other nutrient. Community co-limitation could also result in no change – or even an increase – in N concentrations in response to P addition if P stimulated growth of N fixers. Finally, if biochemically dependent co-limitation was occurring, addition of a limiting nutrient would not decrease, and could even increase, the concentration of the other, co-limited nutrient. We found no general evidence for the decrease in the internal concentration of one nutrient due to addition of another nutrient. The one exception to this overall pattern was marine systems, where N addition decreased internal P concentrations. In contrast, P addition increased internal N concentrations across all experiments, consistent with co-limitation. These results have important implications for understanding the roles that N and P play in controlling producer growth and internal nutrient accumulation as well as for managing the effects of nutrient enrichment in ecosystems.

Synthesis On a global scale, humans have doubled nitrogen (N) inputs and quadrupled phosphorus (P) inputs relative to pre-industrial levels. N and P fertilization influences autotroph internal nutrient concentrations and ratios and thereby affects a variety of community and ecosystem processes, including decomposition and consumer population dynamics. It is therefore critical to understand the effects of nutrient additions on the growth and nutrient concentrations of primary producers. We used meta-analysis to evaluate the responses of autotroph internal N and P concentrations to additions of N, P, and N+P and make inferences about limitation and co-limitation of N and P across marine, terrestrial, and freshwater ecosystems. We found little evidence for single-nutrient limitation, highlighting the fact that multiple nutrients generally limit primary production.

Understanding the mechanisms underlying resource limitation of primary production is essential given human domination of key biogeochemical cycles (Vitousek et al. 1997b), including a doubling of nitrogen (N) inputs and

a quadrupling of phosphorus (P) inputs relative to pre-industrial levels (Vitousek et al. 1997a, Falkowski et al. 2000). Limitation of growth and/or production by each potentially limiting nutrient (e.g. N versus P) has traditionally been

evaluated separately due to the long-held paradigms of single-nutrient limitation of primary production in most ecosystems (Vitousek and Howarth 1991, van der Ploeg et al. 1999, Howarth and Marino 2006). More recently, however, it has become clear that multiple resources may simultaneously limit primary production (Elser et al. 2007, Saito et al. 2008, Harpole et al. 2011), especially at the community level (Arrigo 2005, Danger et al. 2008).

Both N and P are essential elemental nutrients for growth at the biochemical level. P availability is closely associated with the ribosomal RNA machinery necessary for protein synthesis (Elser et al. 2000), and N is essential for the building blocks of those proteins (Sterner and Elser 2002). Furthermore, P serves as a key energy currency (i.e. ATP) within cells. The interactive roles played by these nutrients suggest the possibility of synergistic effects of N availability on P use and vice versa, resulting in “biochemically dependent co-limitation” (sensu Saito et al. 2008). For instance, as N becomes more available, plants may up-regulate synthesis of phosphatase enzymes, enhancing their ability to take up P (Treseder and Vitousek 2001, Menge and Field 2007, North et al. 2007, Fujita et al. 2010) and seaweeds may increase synthesis of transport proteins, increasing P uptake efficiency (Perini and Bracken 2014). P-mediated acceleration of litter decomposition enhances soil N mineralization, which can control community-level N uptake (Güsewell et al. 2002).

Furthermore, at the community level, co-limitation can occur when different organisms in the primary producer community are limited by different nutrients (Arrigo 2005). For example, in marine phytoplankton assemblages in nutrient-depleted waters, growth of N-fixing species is enhanced by P addition (Karl et al. 1997, Wu et al. 2000), whereas growth of non-N-fixing species is enhanced by N addition (Suzumura and Ingall 2004). Similarly, intermediate-aged forests, which contain both early-successional species that harbor symbiotic N-fixers and late-successional species that lack N fixers (Gehring et al. 1999, Menge et al. 2008), are often co-limited by N and P (Gehring et al. 1999).

In contrast to these mechanisms for biochemically dependent and community co-limitation, Davidson and Howarth (2007) recently suggested that “synergistic” effects of N and P on plant production (Elser et al. 2007) could simply arise from alternation between N limitation and P limitation as one nutrient, then the other, is incorporated into a plant’s biochemical pathways. Evaluating the effects of N and P addition on internal N and P concentrations may provide insights into nutrient limitation and co-limitation and particularly into whether sequential, single-nutrient limitation (Davidson and Howarth 2007) or “true” multiple-nutrient limitation (Ågren et al. 2012) controls biomass responses to simultaneous addition of N and P (Shaver and Chapin 1980, Bowman 1994).

In general, the internal N and P concentrations in primary producers result from interactions between the availability of those nutrients in the surrounding environment and the characteristics of particular primary producer taxa (Chapin et al. 1996, Demars and Edwards 2007, Borer et al. 2013). Internal concentrations and ratios of N and P are often used to estimate the degree to which nutrient availability limits plant growth because higher concentrations typically indicate higher availability, and the internal N:P ratio can be used as an indicator of whether N or P is limiting in a given location

or for a given individual (Koerselman and Meuleman 1996, Verhoeven et al. 1996, Fong et al. 1998, Han et al. 2005).

Internal N and P concentrations also constrain and mediate a variety of community- and ecosystem-level processes. For example, rates of herbivory are related to the N and P content of primary producers (Hillebrand et al. 2009), and trophic structure is strongly dependent on producers’ internal N and P concentrations as well as the dietary requirements of the consumers (Cebrian et al. 2009). Decomposition rates (Enríquez et al. 1993) and disease dynamics (Clasen and Elser 2007, Borer et al. 2010) can also be influenced by N and P stoichiometry in autotrophs. Stoichiometric homeostasis – the ability of a species to maintain a consistent elemental composition despite variation in elemental supply – in internal N and P concentrations is associated with species’ dominance and community stability (Yu et al. 2010), further highlighting the importance of internal N and P concentrations for processes at the community and ecosystem levels. Thus, a predictive understanding of community- and ecosystem-level processes will likely be improved by understanding how the availability of different nutrients affects the relative internal concentrations of those nutrients in individual primary producers and in communities.

Because internal N and P concentrations are typically reported as proportions or percentages of dry mass, if N alone is limiting, adding N should increase the internal N concentration but decrease the internal P concentration, and vice versa. In particular, given that one resource (e.g. P) is limiting, the framework predicts that adding this nutrient should enhance growth, but it should additionally result in decreased concentrations of non-limiting nutrients (e.g. N) relative to initial values, as internal concentrations of both N and P decline along a resource–consumption vector (Shaver and Chapin 1980; Fig. 1A). Thus, if growth is limited by a single nutrient, addition of that nutrient should decrease the internal concentration(s) of the other nutrient(s). In a diverse producer assemblage, a decrease in the internal concentrations of both nutrients should be indicative of community co-limitation, as different species in the assemblage are limited by different limiting nutrients (Arrigo 2004). Finally, if adding one nutrient enhances uptake of another nutrient, adding the first nutrient would not decrease, and could even enhance, internal concentrations of the non-limiting nutrient (Fig. 1B).

To test these predictions, we compiled data describing the effects of experimental additions of N alone, P alone, and N and P together on the internal nutrient concentrations of primary producers and used this dataset to test the generality of single limitation versus co-limitation in freshwater, marine, and terrestrial ecosystems. Single-nutrient limitation would result in a strong negative effect of N addition on internal P concentrations or of P addition on internal N concentrations (Fig. 1A). Community co-limitation could result in two different scenarios. First, declines in both N in response to P addition and P in response to N addition within a system would be consistent with different species responding to different nutrients (Arrigo 2004). Second, if P addition enhances N fixers (Karl et al. 1997, Wu et al. 2000), then adding P could result in either no effect or a positive effect on N concentrations within a system. No change in internal N or P concentrations in response to N or P additions, and particularly a positive effect of N on internal

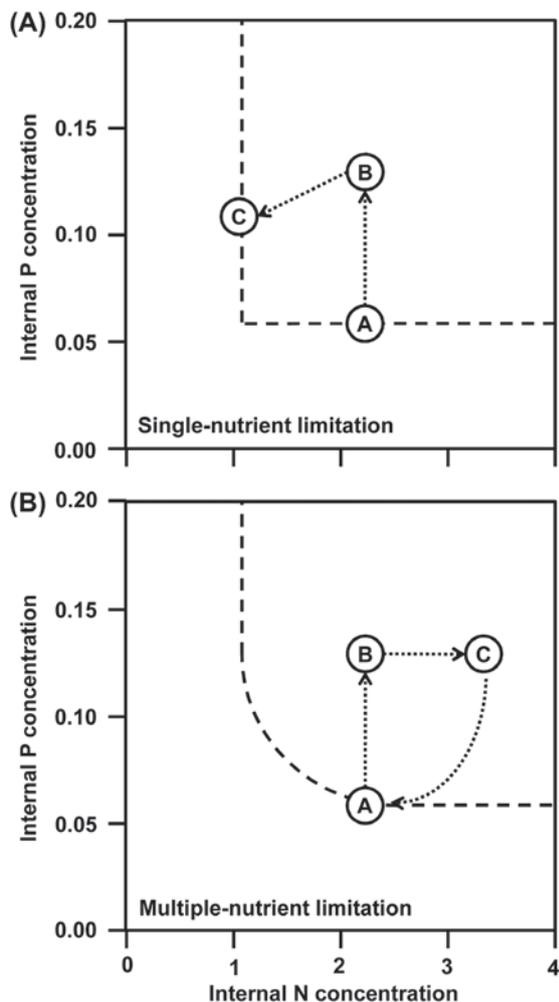


Figure 1. Zero-net-growth isoclines and internal nutrient concentrations for primary producers where single versus multiple nutrients are limiting. In both cases, P limits growth, whereas N is available in excess. Axes are unitless and hypothetical, but N:P approximates the Redfield (1934) ratio. (A) Single-nutrient limitation. In this example, isoclines are orthogonal. Initially, the internal P concentration is insufficient to support growth, whereas internal N is available in excess (Point A). Addition of P, the limiting nutrient, enhances the internal P concentration (Point B), and growth occurs. However, both nutrients are consumed, following a resource consumption vector, ultimately resulting in a decrease in N relative to the original level (Point C). (B) Biochemically dependent co-limitation. In this example, the isocline is curved to reflect smooth transitions between limiting nutrients (Sperfeld et al. 2012). Points A and B are as in Fig. 1A. However, addition of P enhances the internal N concentration by facilitating N uptake (Point C). As growth occurs and N and P are consumed, internal nutrient concentrations return to Point A, and there is no reduction in N due to P addition.

P concentrations or of P on internal N concentrations would support the biochemically dependent co-limitation hypothesis (Iversen et al. 2010; Fig. 1B)

Material and methods

We searched the literature for studies that reported effects of experimental additions of N alone, P alone, and both N and

P together on internal concentrations (percentages, masses per dry tissue mass, or ratios, i.e. C:N or C:P) of N and P in primary producers from freshwater, marine and terrestrial ecosystems. Data ideally represented a complete sample of the producer community, including whole organisms, if possible. Communities in some studies were strongly dominated by one or a few species; for these, data often characterized only the dominant species. Most studies, especially those that reported bulk seston or periphyton samples, characterized the internal N and/or P concentration of the entire producer community.

Most studies included here were gathered as part of the EcoLogical Synthesis of Interactive Experiments (ELSIE) database (Hillebrand et al. 2007, Elser et al. 2007, Gruner et al. 2008), which was designed to evaluate effects of nutrient additions and herbivore manipulations on producer biomass and diversity. When authors reported effects of N addition, P addition, and/or N + P addition on internal concentrations of N and P, those data were often included in the ELSIE database. This subset of ELSIE provided 73 independent experiments, although some nutrient type (N, P or N + P) × nutrient response (N or P concentration) × ecosystem (freshwater, marine or terrestrial) combinations had very low replication.

We therefore conducted further searches of literature databases (e.g. Web of Science, Google Scholar) for terms including bioassay, tissue, nutrient, addition, nitrogen, and phosphorus and gathered an additional 45 experiments, for a total of 118 experiments from 48 published papers and one unpublished study (Supplementary material Appendix 1 Table A1). Of these 118 experiments, 79 reported the effects of N addition on internal N concentrations (e.g. %N, C:N), 62 described effects of P addition on internal N concentrations, and 91 contained data on effects of N + P addition on internal N concentrations. For P, 61 studies reported effects of N addition on internal P concentrations, 60 on effects of P addition on internal P concentrations, and 83 on effects of N + P addition on internal P concentrations. Note that numbers do not add up to 118 because experiments often included more than one nutrient type × nutrient response combination.

Data were extracted from tables or figures in the manuscripts. Data were digitally obtained using the GrabIt! XP add-in for Microsoft Excel and the Engauge digitizer (Mitchell 2002). All studies included internal nutrient concentrations of primary producers in a control treatment where no nutrients were added and at least one (and ideally more than one) nutrient treatment where N, P, and/or N + P were added.

Nutrient additions were compared to controls using log-response ratios (LRR):

$$LRR_N = \ln\left(\frac{N_{nut}}{N_{con}}\right) \quad (1)$$

$$LRR_P = \ln\left(\frac{P_{nut}}{P_{con}}\right) \quad (2)$$

where N_{nut} and P_{nut} represent the internal N and P concentrations in nutrient-added treatments and N_{con} and P_{con} represent the internal N and P concentrations in control treatments without added nutrients. These responses

were evaluated separately for N, P, or N + P additions. The log-response ratio is one of the most widely used metrics in ecological meta-analyses (Hedges et al. 1999). Unlike Hedge's *d* (another common metric), the log-response ratio does not require a measure of sample variability, which allowed us to retain many studies that did not report variances. Furthermore, the log-response ratio shows the least bias of the meta-analysis metrics, and its sampling distribution is approximately normal (Hedges et al. 1999). We used an unweighted analysis, so each experiment had equal influence on the mean.

This database was used to calculate mean LRRs and their bootstrapped 95% confidence intervals (CIs, calculated using the jackboot.sas macro in SAS ver. 9.2) for each effect (i.e. combinations of nutrient addition type, log-response ratio and system type). CIs that did not overlap with zero indicated statistically significant effects of nutrient addition on internal nutrient concentrations. For example, a negative mean LRR for internal N concentrations in response to P addition, (i.e. with a CI that did not overlap zero) would be consistent with a decrease in internal N concentrations due to addition of P. These LRRs and CIs were calculated across all ecosystems and separately for freshwater, marine, and terrestrial systems. LRRs were also compared using *t*-tests (i.e. testing whether mean LRRs differed from zero) and general linear models (GLMs) evaluating whether the effects of nutrient treatments on internal nutrient concentrations differed across ecosystems (SAS ver. 9.2). GLMs evaluated main effects of nutrient treatment (N versus P versus N + P) and ecosystem type (freshwater versus marine versus terrestrial) as well as the interaction between nutrient treatment and ecosystem type. We also evaluated two planned contrasts (1) single-nutrient versus multiple-nutrient treatments and (2) N additions versus P additions. GLM details, results, and parameter estimates are included in the Supplementary material Appendix 2.

Table 1. Responses of primary producers' internal nitrogen and phosphorus concentrations to additions of N, P, and both N and P. Symbols indicate statistically significant ($p < 0.05$) positive (+) and negative (-) effects relative to controls, and 0 denotes no significant effect of nutrient additions.

Nutrients added	Response	
	Internal [N]	Internal [P]
Overall		
N	+	0
P	+	+
N + P	+	+
Freshwater		
N	0	0
P	0	+
N + P	+	+
Marine		
N	+	-
P	0	+
N + P	+	+
Terrestrial		
N	+	0
P	0	+
N + P	+	+

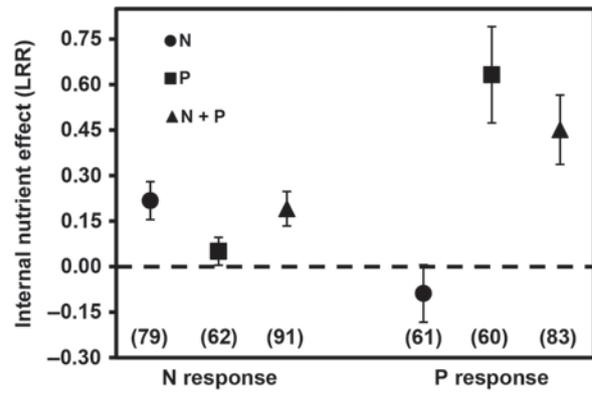


Figure 2. Overall responses of primary producers' internal nitrogen (LRR_N) and phosphorus (LRR_P) concentrations to additions of N, P and both N and P. The responses of internal N concentrations were universally positive ($p < 0.016$), but P responses were mixed. Addition of N had no effect on internal P concentrations ($p = 0.108$), whereas addition of either P or both N and P enhanced P concentrations ($p < 0.001$). Values are mean log-response ratios (LRRs) \pm 95% confidence intervals. Parenthetical numbers indicate sample sizes.

Results

Across all ecosystems, internal N concentrations increased with addition of N alone ($t = 6.4$, $DF = 78$, $p < 0.001$), P alone ($t = 2.14$, $DF = 61$, $p = 0.036$), and N and P together ($t = 6.4$, $DF = 90$, $p < 0.001$; Table 1, Fig. 2), though the magnitude of the increase in tissue N was greater when N was added alone or in combination with P compared to the addition of P alone ($F_{2,224} = 3.6$, $p = 0.028$). These responses were consistent across ecosystem types (nutrient addition \times ecosystem interaction: $F_{4,224} = 1.4$, $p = 0.223$). The response of internal N concentrations to simultaneous addition of N and P did not differ from the response to single nutrient (either N or P) additions (single nutrient

Table 2. Comparisons of nutrient (N, P, and N + P) enrichment effects on internal N and P concentrations across ecosystems. See Supplementary material Appendix 2 for expanded model output, including parameter estimates.

Response	Factor	DF	F-statistic	p-value
Internal [N]	Nutrients added (N vs P vs N + P)	2	3.63	0.028
	Ecosystem (fresh vs mar vs terr)	2	0.29	0.746
	Nutrients \times Ecosystem	4	1.44	0.223
	Contrast: single nutrient vs N + P	1	2.62	0.107
	Contrast: N vs P	1	5.24	0.023
Internal [P]	Error	224		
	Nutrients added (N vs P vs N + P)	2	32.02	< 0.001
	Ecosystem (fresh vs mar vs terr)	2	1.23	0.293
	Nutrients \times Ecosystem	4	1.51	0.200
	Contrast: Single nutrient vs N + P	1	4.61	0.033
	Contrast: N vs P	1	58.99	< 0.001
Error	195			

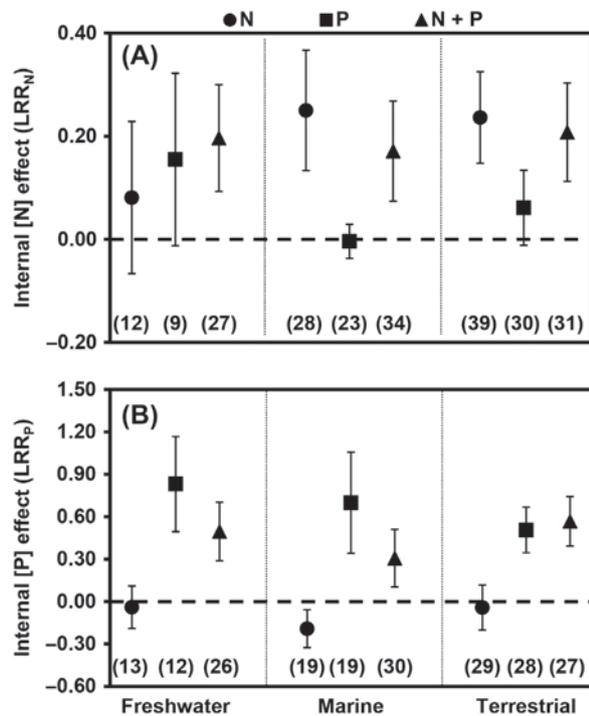


Figure 3. Within-system responses of primary producers' internal nitrogen (LRR_N) and phosphorus (LRR_P) concentrations to nutrient additions. (A) P addition had no effect on N concentrations in marine systems, but otherwise, effects of both N and P addition on internal N concentrations tended to be positive. However, only N + P additions resulted in statistically significant positive responses of internal N concentrations in all three systems. (B) Effects of nutrient addition on P concentrations were also fairly consistent across systems, though N addition decreased internal P concentrations in marine experiments. Both P and N + P additions increased P concentrations in all systems. Values are mean log-response ratios (LRRs) \pm 95% confidence intervals. Parenthetical numbers indicate sample sizes.

versus N + P contrast: $F_{1,224} = 2.6$, $p = 0.107$; Table 2), but the response to additions of N alone was greater than the response to additions of P alone (N versus P contrast: $F_{1,224} = 5.2$, $p = 0.023$).

Within ecosystems (i.e. freshwater, marine, and terrestrial), addition of N alone resulted in enhanced internal N concentrations in marine and terrestrial systems ($p < 0.001$, Table 1, Fig. 3A), but had no effect in freshwater systems ($t = 1.1$, $DF = 11$, $p = 0.316$; Table 1). Addition of P alone tended to enhance internal N concentrations in freshwater and terrestrial systems, but there was no tendency for P to enhance internal N in marine systems, and the effect was not statistically significant in any system (i.e. 95% confidence intervals overlapped zero in all cases, Table 1, Fig. 3A). Thus, in no case was there strong evidence for a decrease in internal N concentrations due to P addition. Responses of internal N concentrations to addition of N and P together were consistently positive in all three systems ($p < 0.003$, Table 1, Fig. 3A). In freshwater systems, internal N concentrations were only enhanced when both N and P were added together ($t = 3.7$, $DF = 26$, $p = 0.001$), with no effect of adding either P alone or N alone (Table 1, Fig. 3A).

Across ecosystems, responses of internal P concentrations to nutrient additions depended on the nutrient added

($F_{2,195} = 32.0$, $p < 0.001$; Table 2); internal P concentrations did not respond to N additions ($t = 1.63$, $DF = 53$, $p = 0.108$), but were enhanced when P ($t = 7.75$, $DF = 52$, $p < 0.001$) or N + P ($t = 7.15$, $DF = 75$, $p < 0.001$) were added (Table 1, Fig. 2). These responses were consistent across ecosystems (nutrient addition \times ecosystem interaction: $F_{4,195} = 1.5$, $p = 0.200$). Simultaneous addition of N and P resulted in an enhancement in internal P concentrations that was greater than the average response to addition of N alone and P alone ($F_{1,195} = 4.6$, $p = 0.033$; Table 2). The same patterns held within systems, with internal P concentrations elevated in response to additions of either P or N + P in all three systems (Table 1, Fig. 3B). N additions decreased internal P concentrations in marine systems ($t = 2.7$, $DF = 18$, $p = 0.014$), but had no effect in freshwater or terrestrial systems ($p > 0.613$, Table 1, Fig. 3B).

Because ~40% of the data on internal nutrient concentrations were collected from study systems dominated by a single species, we compared the results from single-species studies to those from the ~60% of studies that reported community-level data. The effects of nutrient addition on internal N and P concentrations did not differ between single-species and multiple-species samples (N concentration: $F_{1,227} = 0.1$, $p = 0.751$; P concentration: $F_{1,198} = 0.5$, $p = 0.476$). Single-species and multiple-species studies were conducted in all ecosystems, though single-species studies were more common in terrestrial systems (58% of studies) and multiple-species studies were more common in freshwater (76%) and marine (79%) systems.

Because the producer taxa in our database include a phylogenetically diverse assemblage of organisms (Bhattacharya et al. 2004), span a variety of nutrient uptake and accumulation strategies (Chapin et al. 1986, Pedersen and Borum 1996, Valiela et al. 1997, Demars and Edwards 2007), and can differ considerably in their capacity to take up and accumulate nutrients (Valiela et al. 1997, Demars and Edwards 2007), we compared effects of nutrient additions on different producer taxa in freshwater, marine, and terrestrial systems (Fig. 4). Whereas we found no taxon differences in freshwater systems (N concentration: $F_{3,38} = 0.5$, $p = 0.662$; P concentration: $F_{3,41} = 1.1$, $p = 0.351$), responses of both N and P differed between taxa in marine systems (N concentration: $F_{2,76} = 13.1$, $p < 0.001$; P concentration: $F_{2,59} = 7.3$, $p = 0.002$), primarily due to strong responses of macroalgal internal N and P concentrations to nutrient additions. The greater response of macroalgal internal N was associated with N additions (both alone and in combination with P) but not P additions (nutrient 'type', i.e. N versus P versus N + P, by taxon interaction; N concentration: $F_{4,76} = 5.4$, $p < 0.001$). No other nutrient type \times taxon interactions were evident for any system or nutrient response ($p > 0.18$). In terrestrial systems, N responses to nutrient additions did not differ among taxa (N concentration: $F_{3,88} = 1.7$, $p = 0.159$), but P responses differed (P concentration: $F_{3,73} = 4.9$, $p = 0.004$), largely due to stronger responses of bryophytes than other taxa.

Further, because internal N and P concentrations in autotrophs vary with latitude in freshwater, marine, and terrestrial systems (Borer et al. 2013), we evaluated whether the effects of nutrient addition on internal N and P concentrations varied with latitude and found no effect

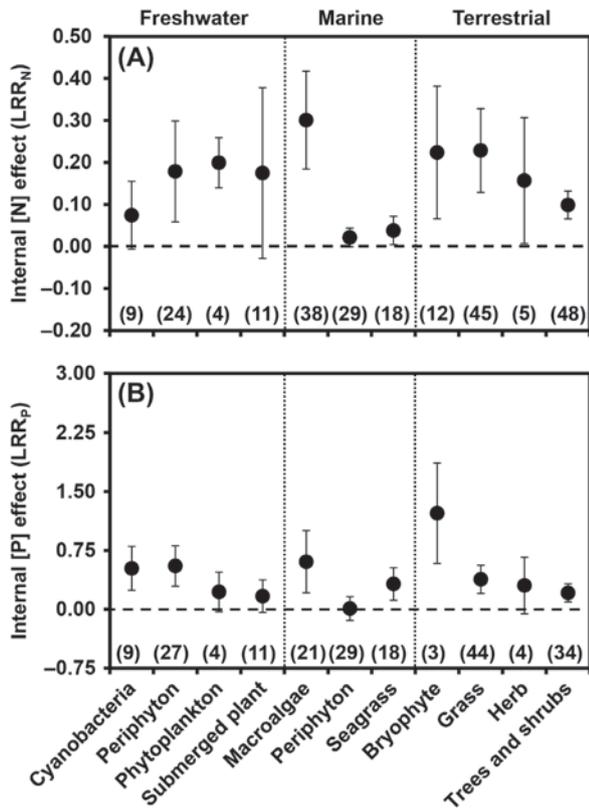


Figure 4. Responses of internal nitrogen (LRR_N) and phosphorus (LRR_P) concentrations of different primary producer functional forms to nutrient additions. (A) Responses of internal N concentrations did not differ between the different freshwater taxa or terrestrial taxa, but in marine systems, macroalgal internal N responses were greater than responses of either periphyton or seagrasses. (B) Responses of internal P concentrations did not differ between the different freshwater taxa, but taxa in both marine and terrestrial systems differed in their internal P responses to nutrient additions. In particular, in marine systems, macroalgae exhibited stronger P responses than periphyton, and in terrestrial systems, responses of bryophytes were stronger than those of any other group. Values are mean log-response ratios (LRRs) \pm 95 confidence intervals, and taxonomic categories represent the most abundant species in the particular system. Parenthetical numbers indicate sample sizes.

(N concentration: $F_{2,227} = 1.9$, $p = 0.149$; P concentration: $F_{2,198} = 1.1$, $p = 0.339$). There were also no differences in the latitudinal distribution of nutrient additions (i.e. N, P or N + P; $p > 0.10$) or systems studied (i.e. freshwater, marine or terrestrial; $p > 0.18$).

Discussion

A strong negative effect of N addition on internal P concentrations or of P addition on internal N concentrations would have been consistent with limitation by a single nutrient. Instead, when all ecosystems were considered together, we found little evidence to support the hypothesis of single nutrient limitation: addition of one nutrient did not cause a decrease in the internal concentration of the other. Furthermore, we observed that autotroph internal N concentrations increased in response to P addition, suggesting

that P availability limits access to N by primary producers in nature. These results are consistent with the hypotheses of biochemically dependent co-limitation and of community co-limitation associated with enhancement of N fixers by P addition. Looking at responses within particular systems, our results support our earlier work indicating that single-nutrient limitation is not common in either terrestrial or freshwater systems and that co-limitation is an important mechanism that mediates the effect of nutrient additions in those systems (Elser et al. 2007, Harpole et al. 2011). In marine systems, N additions resulted in decreases in internal P concentrations. However, there was no effect of P additions on internal N concentrations in marine systems, which could have indicated a potential for community co-limitation associated with responses of different species to N versus P additions (Arrigo 2004). The lack of this effect, combined with substantial increases in biomass in response to additions of N alone (Elser et al. 2007), suggests that single-nutrient limitation by N plays an important role in the marine systems in our database or that prior nutrient history – exposure to and accumulation of P – made P additions in marine systems irrelevant.

Our demonstration of an overall increase in internal N concentrations in response to P addition is particularly striking given that the autotrophs we considered spanned a variety of taxonomic groups with different life-history traits and generation times. Experiments were run for durations ranging from days (phytoplankton) to years (macroalgae and higher plants), and it is likely that longer duration “press” experiments (scaled according to the organisms’ generation times) would produce stronger effects than pulsed short-term nutrient additions, as nutrients tend to be rapidly lost due to immobilization (Prescott et al. 1992) or advection (Williams et al. 2013). Furthermore, the organisms we considered here were subjected to a wide variety of prior exposures to N, P and other potentially limiting resources, all of which have the potential to affect the response of internal N and P concentrations (Fong et al. 2003). And many phytoplankton are more correctly described as mixotrophs, which contribute to both primary and secondary production and obtain nutrients from both the water column and by consuming other organisms (Flynn et al. 2013, Mitra et al. 2014), complicating their responses to nutrient additions.

In general, responses of internal P concentrations were stronger than those of N concentrations, which may reflect the fact that P can be maintained internally as polyphosphate, whereas N must be converted into amino acids (Rhee 1973, Chapin et al. 1990). Physiological demand for and tissue concentrations of P are also typically much lower than N. In general, therefore, more growth is typically necessary to starve an autotroph of P than of N. An exception to this pattern is found in macroalgae, some of which can accumulate substantial amounts of internal nitrate, comprising 15–30% of internal N (Chapman and Craigie 1977, Naldi and Viaroli 2002). The greater responses of macroalgal internal N concentrations to N additions relative to other marine taxa in our dataset may reflect this ability to accumulate inorganic N.

Surprisingly, effects of nutrient additions on internal N and P concentrations were relatively consistent across many of the different taxa within freshwater, marine, and terrestrial

ecosystems, despite large taxonomic and structural differences between the organisms considered. Some groups – especially macroalgae in marine systems and bryophytes in terrestrial systems – seem to be particularly responsive in their internal nutrient concentrations. These species may play disproportionate roles in mediating access of those nutrients to higher trophic levels (Cebrian et al. 2009, Hillebrand et al. 2009). However members of other taxonomic groups responded similarly to each other, despite major structural and phylogenetic differences (e.g. all taxa in freshwater systems, periphyton versus seagrasses in marine systems).

As noted above, the diverse ‘microalgal’ assemblages in marine and freshwater habitats (e.g. phytoplankton, periphyton) likely contained not only autotrophs but also heterotrophic and mixotrophic microorganisms (Pick and Caron 1987, Hillebrand et al. 2002, Duhamel et al. 2012, Flynn et al. 2013, Mitra et al. 2014). For example, especially under low nutrient conditions, nonpigmented picoplankton can dominate plankton assemblages (Pick and Caron 1987, McAndrew et al. 2007, Duhamel et al. 2012), and they may have been the first to respond to nutrient additions due to their superior competitive abilities, especially at low nutrient concentrations (Thingstad et al. 1993). Thus, for mixed assemblages in oligotrophic waters, internal nutrient concentrations likely reflected not only autotrophs but heterotrophs. This may represent a fundamental difference between ‘wet’ systems, where some ‘producer’ groups contained both autotrophs and heterotrophs, and terrestrial systems, where tissues represented primarily autotrophic biomass. However, emerging evidence highlights the importance of microbial and fungal endophytes in the tissues of large terrestrial higher plants, as well (Arnold et al. 2003).

Addition of phosphate can enhance nitrate uptake by phytoplankton (MacIsaac and Dugdale 1972) and plants (Schjørring 1986, Rufty et al. 1993, de Groot et al. 2003), leading to higher internal N concentrations. Several potential mechanisms, at the community, organismal, or biochemical levels, could underlie the enhancement of N uptake following P addition (von Wiren et al. 1997, Güsewell et al. 2002). For instance, in terrestrial systems, P addition can stimulate microbial litter decomposition, leading to increased N availability for plants (Aerts and de Caluwe 1997, Güsewell et al. 2002). However, we found similar effects in freshwater systems, where the majority of studies were conducted on periphyton and phytoplankton communities (Supplementary material Appendix 1). The fact that both terrestrial and freshwater studies contributed to the significant effect of P addition on internal N concentrations across ecosystems suggests that general biochemical mechanisms may be limiting producers’ access to N when P is at low ambient concentrations. These may include decreases in cytokinin concentrations, which can lead to declines in nitrate reductase activity and protein synthesis (de Groot et al. 2003), ultimately decreasing N assimilation into proteins (Schjørring 1986). Regardless of the underlying mechanisms – and it seems likely that more than one operates in the diverse array of community-level experiments analyzed here – our results provide evidence for co-limitation of growth by N and P.

In contrast to the effect of P addition on internal N concentrations, N addition had no overall effect on internal P concentrations across all systems, and even resulted in a

decline in P concentrations in marine systems, suggesting single-nutrient limitation of growth and/or primary production. This decline in P with N additions is consistent with strong effects of single-nutrient N additions on biomass in marine systems (Elser et al. 2007). Internal P concentrations responded consistently only to P addition; the effect of N + P addition on P concentrations was greater than the average of N addition or P addition alone, and the combination of nutrients added (i.e. N, P or N + P) strongly influenced P concentrations. Responses of internal P concentrations to P addition but not to N addition have been found in previous studies (Tessier and Raynal 2003) and may reflect adaptations to meet substantial physiological needs despite low average environmental availability, such as vacuolar P uptake and accumulation (Schachtman et al. 1998). In general, there may be different costs to the accumulation of different nutrients, and this could explain the differential magnitude of increases in internal nutrient concentrations following addition of N and P (Bloom et al. 1985).

Across all systems, adding N enhanced internal N concentrations and adding P enhanced internal P concentrations, suggesting that nutrient uptake in excess of growth requirements is more common than previously thought or that both N and P limitation are common attributes of all systems. Chapin (1980) argues that plants growing in low-nutrient soils have the capacity to take up and accumulate nutrients when there is a flush of availability, resulting in an increase in the internal nutrient concentration (i.e. luxury consumption). An increase in internal nutrient concentrations following nutrient addition has been observed in terrestrial habitats including tundra (Bowman 1994, and reviewed in Chapin 1980), and tropical forest (Vitousek and Farrington 1997), and has been observed for some (Gerloff and Krombholz 1966, Fujita 1985) but not all (McJannet et al. 1995, Bracken 2004) marine and freshwater macrophytes.

Our current analysis goes beyond simple evaluation of the effects of multiple nutrient additions on biomass (Elser et al. 2007, Allgeier et al. 2011; see Supplementary material Appendix 3 for effects of nutrient additions on biomass associated with the expanded dataset analyzed in this study) and clarifies an important issue raised by Davidson and Howarth (2007) in response to this earlier work: that some previous analyses lacked a “mechanistic understanding of how one resource affects the supply of and demand for another resource.” Our results clarify that the greater-than-predicted responses of plant biomass to additions of both N and P observed in this study and others (Elser et al. 2007; Supplementary material Appendix 3; Fig. A1, Table A2) are not only due to stepwise alternation between N and P limitation (Davidson and Howarth 2007), but are likely associated with co-limitation. Furthermore, nutrient identity, not simply relative supply rate, matters; the effects of N additions on internal P concentrations differ from the effects of P additions on internal N concentrations. These insights into the nature of co-limitation, and particularly how changing the availability of one nutrient can control producers’ uptake of another nutrient, have important implications both for our understanding of the roles that these nutrients play in

determining producer growth and for our ability to predict and mitigate effects of excess N and P additions on ecosystems (Vitousek et al. 1997a, Falkowski et al. 2000, Davidson and Howarth 2007, Cebrian et al. 2009).

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References

- Aerts, R. and de Caluwe, H. 1997. Nutritional and plant-mediated controls on leaf litter decomposition of *Carex* species. – *Ecology* 78: 244–260.
- Ågren, G. I. et al. 2012. Nutrient limitation on terrestrial plant growth – modeling the interaction between nitrogen and phosphorus. – *New Phytol.* 194: 953–960.
- Allgeier, J. E. et al. 2011. The frequency and magnitude of non additive responses to multiple nutrient enrichment. – *J. Appl. Ecol.* 49: 96–101.
- Arnold, A. E. et al. 2003. Fungal endophytes limit pathogen damage in a tropical tree. – *Proc. Natl Acad. Sci. USA* 100: 15649–15654.
- Arrigo, K. R. 2005. Marine microorganisms and global nutrient cycles. – *Nature* 437: 349–355.
- Bhattacharya, D. et al. 2004. Photosynthetic eukaryotes unite: endosymbiosis connects the dots. – *BioEssays* 26: 50–60.
- Bloom, A. J. et al. 1985. Resource limitation in plants – an economic analogy. – *Annu. Rev. Ecol. Syst.* 16: 363–392.
- Borer, E. T. et al. 2010. Local context drives infection of grasses by vector borne generalist viruses. – *Ecol. Lett.* 13: 810–818.
- Borer, E. T. et al. 2013. Global biogeography of autotroph chemistry: is insolation a driving force? – *Oikos* 122: 1121–1130.
- Bowman, W. D. 1994. Accumulation and use of nitrogen and phosphorus following fertilization in two alpine tundra communities. – *Oikos* 70: 261–270.
- Bracken, M. E. S. 2004. Invertebrate-mediated nutrient loading increases growth of an intertidal macroalga. – *J. Phycol.* 40: 1032–1041.
- Cebrian, J. et al. 2009. Producer nutritional quality controls ecosystem trophic structure. – *PLoS ONE* 4: e4929.
- Chapin, F. S. III. 1980. The mineral nutrition of wild plants. – *Annu. Rev. Ecol. Syst.* 11: 233–269.
- Chapin, F. S., III et al. 1990. The ecology and economics of storage in plants. – *Annu. Rev. Ecol. Syst.* 21: 423–447.
- Chapin, F. S., III et al. 1986. The nature of nutrient limitation in plant communities. – *Am. Nat.* 127: 48–58.
- Chapin, F. S. III et al. 1996. Plant functional types as predictors of transient responses of Arctic vegetation to global change. – *J. Veg. Sci.* 7: 347–358.
- Chapman, A. R. O. and Craigie, J. S. 1977. Seasonal growth in *Laminaria longicirris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. – *Mar. Biol.* 40: 197–205.
- Clasen, J. L. and Elser, J. J. 2007. The effect of host *Chlorella* NC64A carbon: phosphorus ratio on the production of *Paramecium bursaria* *Chlorella* *Virus-1*. – *Freshwater Biol.* 52: 112–122.
- Danger, M. et al. 2008. Does Liebig's law of the minimum scale up from species to communities? – *Oikos* 117: 1741–1751.
- Davidson, E. A. and Howarth, R. W. 2007. Environmental science: nutrients in synergy. – *Nature* 449: 1000–1001.
- de Groot, C. C. et al. 2003. Interaction of nitrogen and phosphorus nutrition in determining growth. – *Plant Soil* 248: 257–268.
- Demars, B. O. L. and Edwards, A. C. 2007. Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. – *Freshwater Biol.* 52: 2073–2086.
- Duhamel, S. et al. 2012. Light dependence of phosphorus uptake by microorganisms in the subtropical North and South Pacific Ocean. – *Aquat. Microbial Ecol.* 67: 225–238.
- Elser, J. J. et al. 2000. Biological stoichiometry from genes to ecosystems. – *Ecol. Lett.* 3: 540–550.
- Elser, J. J. et al. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. – *Ecol. Lett.* 10: 1135–1142.
- Enríquez, S. et al. 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. – *Oecologia* 94: 457–471.
- Falkowski, P. et al. 2000. The global carbon cycle: a test of our knowledge of earth as a system. – *Science* 290: 291–296.
- Flynn, K. J. et al. 2013. Misuse of the phytoplankton–zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. – *J. Plankton Res.* 35: 3–11.
- Fong, P. et al. 1998. Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga, *Enteromorpha intestinalis* (L. Link). – *J. Exp. Mar. Biol. Ecol.* 231: 63–79.
- Fong, P. et al. 2003. Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. – *Mar. Ecol. Prog. Ser.* 262: 111–123.
- Fujita, R. M. 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. – *J. Exp. Mar. Biol. Ecol.* 92: 283–301.
- Fujita, Y. et al. 2010. Increased N affects P uptake of eight grassland species: the role of root surface phosphatase activity. – *Oikos* 119: 1665–1673.
- Gehring, C. et al. 1999. Response of secondary vegetation in eastern Amazonia to relaxed nutrient availability constraints. – *Biogeochemistry* 45: 223–241.
- Gerloff, G. C. and Krombholz, P. H. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. – *Limnol. Oceanogr.* 11: 529–537.
- Gruner, D. S. et al. 2008. A cross-system synthesis of consumer and nutrient resource control on producer biomass. – *Ecol. Lett.* 11: 740–755.
- Güsewell, S. et al. 2002. Time-dependent effects of fertilization on plant biomass in floating fens. – *J. Veg. Sci.* 13: 705–718.
- Han, W. et al. 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. – *New Phytol.* 168: 377–385.
- Harpole, W. S. et al. 2011. Nutrient co-limitation of primary producer communities. – *Ecol. Lett.* 14: 852–862.
- Hedges, L. V. et al. 1999. The meta-analysis of response ratios in experimental ecology. – *Ecology* 80: 1150–1156.
- Hillebrand, H. et al. 2002. Control of microbenthic communities by grazing and nutrient supply. – *Ecology* 83: 2205–2219.
- Hillebrand, H. et al. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. – *Proc. Natl Acad. Sci. USA* 104: 10904–10909.
- Hillebrand, H. et al. 2009. Herbivore metabolism and stoichiometry each constrain herbivory at different organizational scales across ecosystems. – *Ecol. Lett.* 12: 516–527.
- Howarth, R. W. and Marino, R. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems:

- evolving views over three decades. – *Limnol. Oceanogr.* 51: 364–376.
- Iversen, C. M. et al. 2010. Scaling plant nitrogen use and uptake efficiencies in response to nutrient addition in peatlands. – *Ecology* 91: 693–707.
- Karl, D. et al. 1997. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. – *Nature* 388: 533–538.
- Koerselman, W. and Meuleman, A. F. M. 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. – *J. Appl. Ecol.* 33: 1441–1450.
- MacIsaac, J. J. and Dugdale, R. C. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. – *Deep Sea Res.* 19: 209–232.
- McAndrew, P. M. et al. 2007. Metabolic response of oligotrophic plankton communities to deep water nutrient enrichment. – *Mar. Ecol. Prog. Ser.* 332: 63–75.
- McJannet, C. et al. 1995. Nitrogen and phosphorus tissue concentrations in 41 wetland plants: a comparison across habitats and functional groups. – *Funct. Ecol.* 9: 231–238.
- Menge, D. N. L. and Field, C. B. 2007. Simulated global changes alter phosphorus demand in annual grassland. – *Global Change Biol.* 13: 2582–2591.
- Menge, D. N. et al. 2008. Evolutionary tradeoffs can select against nitrogen fixation and thereby maintain nitrogen limitation. – *Proc. Natl Acad. Sci. USA* 105: 1573–1578.
- Mitchell, M. 2002. Engauge digitizer (v. 4.1). – Sourceforge.
- Mitra, A. et al. 2014. The role of mixotrophic protists in the biological carbon pump. – *Biogeosciences* 11: 995–1005.
- Naldi, M. and Viaroli, P. 2002. Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy). – *J. Exp. Mar. Biol. Ecol.* 269: 65–83.
- North, R. L. et al. 2007. Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. – *Limnol. Oceanogr.* 52: 315–328.
- Pedersen, M. F. and Borum, J. 1996. Nutrient control of algal growth in estuarine waters: nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. – *Mar. Ecol. Prog. Ser.* 142: 261–272.
- Perini, V. and Bracken, M. E. S. 2014. Nitrogen availability limits phosphorus uptake in an intertidal macroalga. – *Oecologia* 175: 667–676.
- Pick, F. R. and Caron, D. A. 1987. Picoplankton and nanoplankton biomass in Lake Ontario: relative contribution of phototrophic and heterotrophic communities. – *Can. J. Fish. Aquat. Sci.* 44: 2164–2172.
- Prescott, C. E. et al. 1992. Immobilization and availability of N and P in the forest floors of fertilized Rocky Mountain coniferous forests. – *Plant Soil* 143: 1–10.
- Redfield, A.C. 1934. On the proportions of organic derivatives in seawater and their relation to the composition of the plankton. – In: Daniel, R. J. (ed.), James Johnson memorial volume. Univ. Press of Liverpool, pp. 176–192.
- Rhee, G.-Y. 1973. A continuous culture study of phosphate uptake: growth rate and polyphosphate in *Scenedesmus* sp. – *J. Phycol.* 9: 495–506.
- Ruffy, T. W., Jr. et al. 1993. Phosphate regulation of nitrate assimilation in soybean. – *J. Exp. Bot.* 44: 879–891.
- Saito, M. A. et al. 2008. Some thoughts on the concept of colimitation: three definitions and the importance of bioavailability. – *Limnol. Oceanogr.* 53: 276–290.
- Schachtman, D. P. et al. 1998. Phosphorus uptake by plants: from soil to cell. – *Plant Physiol.* 116: 447–453.
- Schjørring, J. K. 1986. Nitrate and ammonium absorption by plants growing at a sufficient or insufficient level of phosphorus in nutrient solutions. – *Plant Soil* 91: 313–318.
- Shaver, G. R. and Chapin, F. S., III. 1980. Response to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. – *Ecology* 61: 662–675.
- Sperfeld, E. et al. 2012. Multiple resource limitation theory applied to herbivorous consumers: Liebig's minimum rule vs interactive co-limitation. – *Ecol. Lett.* 15: 142–150.
- Sturner, R. W. and Elser, J. J. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. – Princeton Univ. Press.
- Suzumura, M. and Ingall, E. D. 2004. Distribution and dynamics of various forms of phosphorus in seawater: insights from field observations in the Pacific Ocean and a laboratory experiment. – *Deep Sea Res. I* 51: 1113–1130.
- Tessier, J. T. and Raynal, D. J. 2003. Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. – *J. Appl. Ecol.* 40: 523–534.
- Thingstad, T. F. et al. 1993. Phosphorus cycling and algal-bacterial competition in Sandsfjord, western Norway. – *Mar. Ecol. Prog. Ser.* 99: 239–239.
- Treseder, K. K. and Vitousek, P. M. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. – *Ecology* 82: 946–954.
- Valiela, I. et al. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. – *Limnol. Oceanogr.* 42: 1105–1118.
- van der Ploeg, R. R. et al. 1999. On the origin of the theory of mineral nutrition of plants and the Law of the Minimum. – *Soil Sci. Soc. Am. J.* 63: 1055–1062.
- Verhoeven, J. T. A. et al. 1996. Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. – *Trends Ecol. Evol.* 11: 494–497.
- Vitousek, P. M. and Howarth, R. W. 1991. Nitrogen limitation on land and in the sea: how can it occur? – *Biogeochemistry* 13: 87–115.
- Vitousek, P. M. and Farrington, H. 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. – *Biogeochemistry* 37: 63–75.
- Vitousek, P. M. et al. 1997a. Human alteration of the global nitrogen cycle: sources and consequences. – *Ecol. Appl.* 7: 737–750.
- Vitousek, P. M. et al. 1997b. Human domination of Earth's ecosystems. – *Science* 277: 494–499.
- von Wiren, N. et al. 1997. Regulation of mineral nitrogen uptake in plants. – *Plant Soil* 196: 191–199.
- Williams, S. L. et al. 2013. Additive effects of physical stress and herbivores on intertidal seaweed biodiversity. – *Ecology* 94: 1089–1101.
- Wu, J. et al. 2000. Phosphate depletion in the western North Atlantic Ocean. – *Science* 289: 759–762.
- Yu, Q. et al. 2010. Linking stoichiometric homeostasis with ecosystem structure, functioning and stability. – *Ecol. Lett.* 13: 1390–1399.

Supplementary material (available online as Appendix oik.01215 at <www.oikosjournal.org/readers/appendix>). Appendix 1–3.