

# Ecological Stoichiometry and Multi-element Transfer in a Coastal Ecosystem

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## ABSTRACT

Energy (carbon) flows and element cycling are fundamental, interlinked principles explaining ecosystem processes. The element balance in components, interactions and processes in ecosystems (ecological stoichiometry; ES) has been used to study trophic dynamics and element cycling. This study extends ES beyond its usual limits of C, N, and P and examines the distribution and transfer of 48 elements in 16 components of a coastal ecosystem, using empirical and modeling approaches. Major differences in elemental composition were demonstrated between abiotic and biotic compartments and trophic levels due to differences in taxonomy and ecological function. Mass balance modeling for each element, based on carbon fluxes and element:C ratios, was satisfactory for 92.5% of all element–compartment combinations despite the complexity of the ecosystem model. Model imbalances could mostly be explained by ecological processes, such as increased element uptake during

the spring algal bloom. Energy flows in ecosystems can thus realistically estimate element transfer in the environment, as modeled uptake is constrained by metabolic rates and elements available. The dataset also allowed us to examine one of the key concepts of ES, homeostasis, for more elements than is normally possible. The relative concentrations of elements in organisms compared to their resources did not provide support for the theory that autotrophs show weak homeostasis and showed that the strength of homeostasis by consumers depends on the type of element (for example, macroelement, trace element). Large-scale, multi-element ecosystem studies are essential to evaluate and advance the framework of ES and the importance of ecological processes.

**Key words:** Ecological stoichiometry; Ecosystem model; Element cycling; Carbon flow; Trophic transfer; Homeostasis; Baltic Sea.

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## INTRODUCTION

Element cycling has long been recognized as one of the most fundamental principles explaining ecosystem processes, together with energy (carbon) flows. Even early ecological theory identified complex connections between energy and element cycling, with the focus mostly on food web dynamics (for example, Lindeman 1942; Lotka 1925) and carbon cycling and energy flows (Odum 1957, 1959, 1960). At the same time, Redfield

(1958) recognized that biological processes could be controlled by other elements than C, such as N and P and trace elements. In many recent studies, the role of N and P (and to a lesser extent trace elements) has been explored experimentally and in the field, and it has been demonstrated that the mass balance of multiple chemical elements can affect trophic interactions (for example, Boersma and others 2008; Hillebrand and others 2009; Sterner and Elser 2002) and element cycling (for example, Evans-White and Lamberti 2006; Loladze 2002). Conversely, stoichiometric balance can be influenced by abiotic factors such as irradiance (Finkel and others 2006) and season (Liess and Hillebrand 2005) and biotic factors such as species taxonomy (Karimi and Folt 2006), and food web structure (Fitter and Hillebrand 2009).

The study of the balance of chemical elements in components, interactions, and processes in ecosystems is known as ecological stoichiometry (ES) (Sterner and Elser 2002). A key concept of ES is that stoichiometric imbalances affect physiological processes underlying growth, reproduction, and maintenance (Frost and others 2005) and thus drive ecological processes such as production (Sterner and others 1998), nutrient cycling (Loladze and others 2000), resource use (Sterner and others 1998), competition (Sterner and Elser 2002), and food web dynamics (Elser and others 1998; Hessen 1997). These mismatches in stoichiometry may occur between abiotic and biotic compartments or between trophic levels, causing limitations in particular elements that may control organisms' stoichiometry either from the top down (consumer driven: Evans-White and Lamberti 2006) or from the bottom up (resource driven: Elser and Hassett 1994; Fink and others 2006). Another key concept of ES is homeostasis, that is, the ability of organisms to maintain constant body concentrations despite changing concentrations in the environment and/or their resource supply (Kooijman 1995). Stoichiometric homeostasis is generally assumed to be weak for autotrophs and strong for heterotrophs (Sterner and Elser 2002), so that plant and algae stoichiometry is thought to more closely reflect that of the environment than animals. However, recent work has challenged this assumption. Homeostatic regulation of N and P has been shown to vary widely for vascular plants (Yu and others 2011) and for C:P in zooplankton (DeMott and Pape 2005; Jeyasingh and others 2009). A meta-analysis of stoichiometric homeostasis of C, N, and P in a wide range of organism types (Persson and others 2010) also showed a wide range of responses within both autotrophs and

heterotrophs, though heterotrophs were generally more homeostatic, at least regarding N:P. In a field study of freshwater invertebrates, homeostasis varied depending on whether elements were macronutrients, essential micronutrients, or non-essential elements (Karimi and Folt 2006). Multi-element (and multi-species) data of this sort are unfortunately very scarce.

Although there have been great advances in the field of ES, the focus of most studies is still C, N, and P and their dynamics in pelagic systems. Current ecological theory also fails to stress the close connections between the cycling of chemical elements and the flow of energy in food webs (Loladze and others 2000).

In this paper we tackle these two issues by examining distributions and fluxes of 48 elements in and between 16 components of a shallow coastal ecosystem. In addition to an empirical approach, we calculated element transport using an ecosystem model based on the flow of energy (C) in the ecosystem, connected to C:element ratios. In addition, by extending ES beyond CNP, we investigate if (i) stoichiometric imbalances in fluxes of elements in the ecosystem can be explained by ecological processes, and (ii) differences in organism-resource stoichiometry observed in our field data support or can be explained by theories of homeostasis.

## METHODS

### Context of Study and Study Site

Data used in the present study were obtained in field studies performed within the site-investigation program at the Swedish Nuclear Fuel and Waste Management Co (SKB)'s Forsmark site (Kumblad and Bradshaw 2008). Since 2002, SKB have performed extensive monitoring of this area as a potential site for a geological nuclear waste repository. In this study we focus on interactions within a shallow coastal ecosystem for which we have detailed data describing the pools of 48 elements and fluxes of carbon in and between all major functional components. The application of these data in risk assessment will be addressed in another article.

The study area was Tixlan in the Forsmark area, NW Baltic Proper, Sweden. As part of the SKB site description, the coastal area at Forsmark has been divided into 28 interconnected basins whose delimitations are based on current bathymetry and projections for future drainage areas predicted to appear within the coming 18,000 years due to

glacio-isostatic uplift (Brydsten 2006). Tixlan (Basin 121 in Aquilonius 2010) is an inshore basin with an area of 3.7 km<sup>2</sup>, an average depth of 5.5 m and a volume of 20 million m<sup>3</sup>. It is typical for the area, being semi-exposed with a rocky shore, and rocky shallows with occasional large boulders giving way to a sandy mud substrate.

## Field Collection of Element Concentration Data

All samples used for element analyses were collected near Tixlan island (60°23.550'N, 18°14.740'E) in April 2005, with the exception of the zooplankton and sediment Si samples that were collected in June 2005 and April 2008, respectively.

Sixteen different types of samples (phytoplankton, zooplankton, benthic microalgae, three benthic plants, two benthic herbivores, benthic filter feeders, benthic deposit feeders, planktivorous fish, benthic omnivorous fish, carnivorous fish, sediment and dissolved and particulate matter in the water) were collected at three different localities (replicates) in the bay (Table 1). These represent all the major ecological functional groups of four trophic levels (primary producers, primary, secondary, and tertiary consumers) as well as abiotic components of the coastal ecosystem. The species selected were the most abundant within each functional group. The samples were collected using plastic or Teflon equipment, either from a small boat or by snorkelers and divers. The samples were stored in acid-washed or factory-new plastic containers and frozen whole as soon as possible after collection. Control samples were taken wherever contamination risks were possible and were found in all cases to contain extremely low (background) levels of possible contaminants.

The samples were analyzed for the elemental compositions of Al, As, Ba, Br, C, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Dy, Er, Eu, F, Fe, Gd, Hg, Ho, I, K, Li, Lu, Mg, Mn, Mo, N, Na, Nd, Ni, P, Pb, Pr, Rb, S, Se, Si, Sm, Tb, Th, Ti, Tm, V, Yb, Zn, and Zr. These were chosen to cover a wide range of essential and trace elements, as well as elements of radioecological interest. C and N were analyzed in dried samples with a Leco-CHN analyzer (CHNS-932, EDTA and acetanilide as standards) and P with a segmented flow system (ALPKEM Flow Solution IV) following combustion at 500°C and acidic persulfate oxidation, at the accredited laboratory at the Department of Systems Ecology, Stockholm University. Other elements were analyzed by the laboratories (ISO/IEC 17025) at ALS Scandinavia, using ICP-AES, AFS, and ICP-SFMS. Water (DIM)

**Table 1.** Element Concentration Data and Trophic Levels Used in the Ecosystem Model Components

Ecosystem model components	Trophic level (TL)	Description of samples/raw data for elements other than C
Dissolved matter (DIM)	0	<45 µm fraction
Particulate matter (POM)	0	Fraction retained on a GF/F filter (0.7 µm)
Sediment (SED)	0	Upper 0–6 cm layer
Phytoplankton (Pp)	1	20–60 µm fraction
Benthic plants	1	Average of 3 species: <i>Fucus vesiculosus</i> (Fuc), <i>Potamogeton pectinatus</i> (Pot), <i>Platylella littoralis</i> (Pl)
Benthic microalgae (BMA)	1	Brushed from stones at 1–2 m depth, 20–400 µm size fraction
Zooplankton (Zp)	2	>60 µm fraction, light separated from phytoplankton
Benthic filter feeders	2	<i>Cerastoderma glaucum</i> (Cer). Whole body analysis including shell
Benthic herbivores	2	Average of 2 taxa: <i>Idotea</i> spp. (Ido) and <i>Theodoxus fluviatilis</i> (Theo)
Benthic deposit feeders	2	<i>Macoma balthica</i> (Mac). Whole body analysis including shell
Benthic omnivorous fish	3	<i>Gymnocephalus cernuus</i> (Gym) <sup>a</sup>
Planktivorous fish	3	<i>Rutilus rutilus</i> (Rut) (<110 mm body length) <sup>a</sup>
Piscivorous fish	4	<i>Osmerus eperlanus</i> (Osm) <sup>a</sup>

Concentrations of Cl, F, Br and I were not measured in all components (see Supplementary material). Note that sediment porewater samples were also analysed, but not used in this paper. Porewater data can however be found in the Appendix (Supplementary material).

<sup>a</sup>Analysis of a mid-body section, excluding organs.

samples were acidified with 1 ml nitric acid per 100 ml prior to analysis. Cl and F were leached from the samples using water and for Se, the samples were dissolved by acid hydrolysis using HCl at 120°C for 30 min. All other samples were dried at 105°C and dissolved in HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in a sealed Teflon vial in a microwave oven (modification of USA-EPA methods 200.7 (ICP-AES) and 200.8 (ICP-SFMS)).

These raw data are presented in detail in Kumblad and Bradshaw (2008) and in the Appendix (Supplementary material), but are not described further in this paper. Rather, the data are further explored in terms of the relative amounts of elements in the different ecosystem components.

## Data Handling

In our calculations, all concentrations that were below the limits of detection (LOD) were set to the concentration at LOD to avoid underestimations. For transparency, all measurements below the LOD are indicated in the Appendix (Supplementary material). The abundances of elements relative to other elements in different components of the ecosystem were calculated as percentages (%) of the sum of the masses of all elements. This was done separately for each trophic level. Comparisons of element concentrations in organisms with those of their resources were done with carbon-normal-

ized concentrations (g element g C<sup>-1</sup>), and only for above LOD concentrations. Relative element abundances were also expressed as a chemical formula for the whole ecosystem by converting element masses to moles.

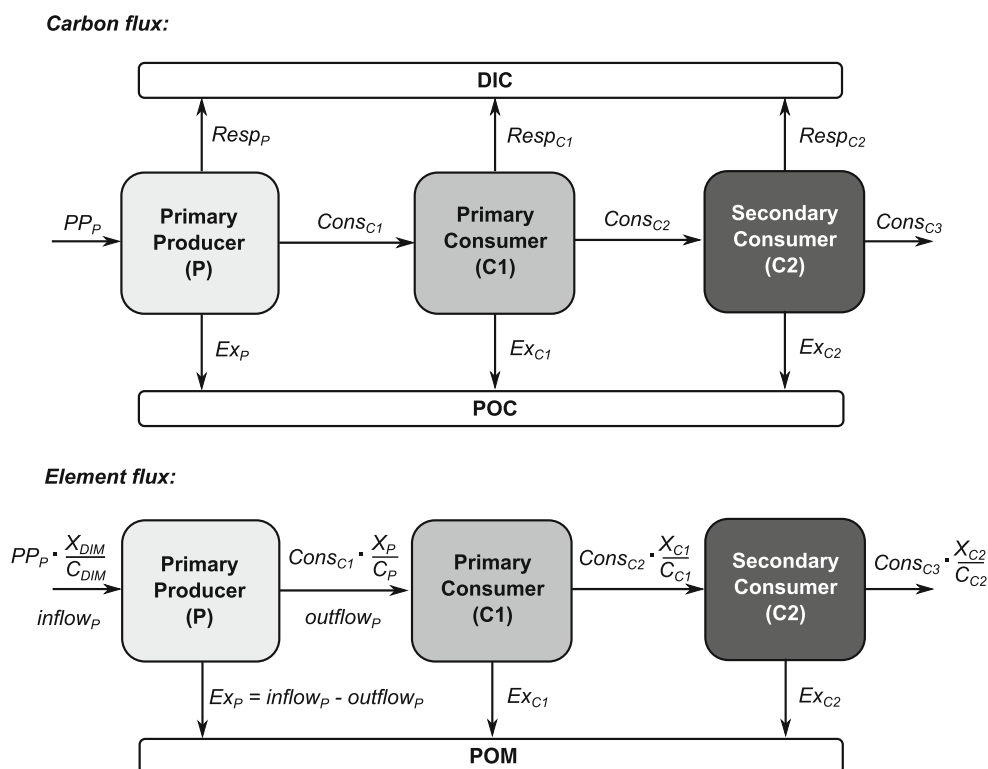
## Modeling of Pools and Fluxes of Carbon and Other Elements

The mass-balance budgets for elements in this study are based on a carbon flow model for Tixlan that was slightly adapted to fit the available site-specific data set of elements.

### Carbon Flow Model for Tixlan

The structure, interactions, and assumptions used for the carbon flow model for Tixlan are described in detail elsewhere (Aquilonius 2010), but are briefly summarized here, including our minor adaptations.

The carbon flow model was based on basin-specific characteristics (for example, hypsography, habitat distributions, water exchange rates) and an assumed food web consisting of *pools* (biotic and abiotic model compartments) and *fluxes* (interactions between model compartments, for example, primary production, respiration, consumption) of carbon in the ecosystem (Figure 1). Bacteria are implicitly included in the in situ measurements of



**Figure 1.** Conceptual description of the calculations of fluxes of carbon (gC basin<sup>-1</sup> y<sup>-1</sup>) and elements (gX basin<sup>-1</sup> y<sup>-1</sup>) driven by primary production (PP), consumption (Cons), respiration (Resp), and excess (Ex; secondary production, feces, excretion, and mortality) between the organisms in the ecosystem. *DIC* dissolved inorganic carbon, *POC* particulate organic carbon, *POM* particulate organic matter.

biomass, concentrations, and metabolic rates. Annual means are used in the model, but these are based on data that describe seasonal fluctuations.

The presence and spatial distribution of benthic and pelagic habitats and dominant organism and abiotic components in these habitats were assessed during extensive field surveys in the Forsmark area during 2004–2007 (Aquiloni [2010](#)). Biomass data ( $\text{g dw m}^{-2}$ ) for each organism were converted to carbon ( $\text{g C m}^{-2}$ ) using conversion factors (obtained from the site or from the literature). The spatial distributions ( $20 \times 20 \text{ m}$  grid) of biomasses were averaged over a year. Species sharing similar ecological functions were combined into functional groups (model compartments) according to [Table 1](#), which were further linked together into a food web model according to [Figure 4](#). Where original field data were lacking, additional data from other surveys in the area or from the literature were used.

The fluxes included in the carbon flow model were biotic (primary production, respiration, and consumption) and abiotic (runoff, advective flow, groundwater inflow, sedimentation, and burial). The abiotic carbon fluxes were not explored further in this paper. A brief outline of the biotic flux calculations is presented below and is conceptually described in [Figure 1](#).

The inflow pathways to biotic compartments were primary production and consumption, and the outflow pathways were respiration, grazing/predation, feces, and excretion of carbon. Primary production (net conversion of dissolved inorganic carbon (DIC) to organic carbon via photosynthesis) was estimated by field surveys or by calculations based on biomass, daily irradiance and nutrient (N and P) availability. Respiration comprises heterotrophic cell respiration and was calculated for each functional group (organism compartment) based on biomass and annual average temperature. Respired carbon was connected to the DIC pool, providing a route for carbon recirculation in the system. The DIC pool was assumed to be in equilibrium with atmospheric carbon. Consumption was calculated with a consumption:respiration factor for the respective functional group. It was assumed that consumers did not discriminate between food sources, but fed in proportion to the availability (biomass) of their food.

The difference between the carbon influx (that is, primary production or consumption) and outflux (that is, grazing/predation and respiration) to/from a compartment was assumed to represent a residual carbon outflux, here called excess. Excess represents secondary production, feces, excretion,

and mortality. Total excess was taken as input to the POC (particulate organic carbon) pool, providing a second route for carbon recirculation in the system. Sedimentation was assumed to be the net flux from POC to sediment, that is, the total excess minus the flux of POC into benthic filter feeders (consumption).

### *Element Budgets*

Mass balance budgets based on the carbon flow model for Tixlan described above, and site-specific element:carbon ratios for each functional group (model compartment) obtained from the field survey were established for all 48 elements, using the same approach as [Kumblad and others \(2006\)](#) ([Figure 1](#)).

The pools of non-carbon elements were calculated using element:carbon ratios ( $X/C$ ) obtained from the field survey, where the carbon pool ( $\text{gC compartment}^{-1} \text{ basin}^{-1}$ ) was multiplied with the  $X/C$ -ratio ( $\text{gX gC}^{-1}$ ), giving an estimate of  $\text{gX basin}^{-1}$  for each compartment.

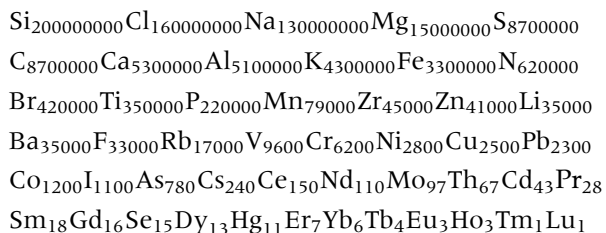
The fluxes of the elements were calculated by assuming that the inflow of an element  $X$  into a compartment was proportional to the inflow of carbon and the  $X/C$ -ratio of the compartment from which the inflow came ([Figure 1](#)). Thus, the carbon flux ( $\text{gC basin}^{-1} \text{ y}^{-1}$ ) was multiplied by the  $X/C$ -ratio ( $\text{gX gC}^{-1}$ ) of the (food) source resulting in an estimate of the elemental influx for each compartment in the basin ( $\text{gX basin}^{-1} \text{ y}^{-1}$ ). Outflow pathways for elements other than carbon were grazing, predation, and excess, that is, residual carbon outfluxes including secondary production, feces, excretion and mortality. As for the elemental influx, the grazing or predation flux was assumed to equal the carbon flux multiplied by the  $X/C$ -ratio ( $\text{gX gC}^{-1}$ ) of the food. As for C, excess was taken as the difference between inflow and outflow and was assumed to be particulate matter which was used as an input to the POM compartment. POM was then either available for reuptake by benthic filter feeders or benthic deposit feeders, or deposited on the sediment.

To analyze if the element models were in balance, the outflow (grazing or predation) of each element from each compartment was compared with the inflow (that is, primary production or consumption) to that compartment. When the outflow of an element exceeded the inflow by a factor of greater than 3 it was defined as being unbalanced. Where this factor was 3 or less it was considered to be within the natural variation of the system.

## RESULTS

### Relative Abundance of Elements in the Ecosystem

The relative abundance of the 48 elements in the coastal ecosystem is best summarized by its chemical formula:



The most common abiotic (Si, Cl, Na, Mg, S, Ca, Al) and biotic (Si, Mg, S, C, Ca, K) elements make up 99% of the element mass, depending on the trophic level (Figure 2A–E). The abiotic part of the ecosystem (TL0; Figure 2E) was clearly dominated by the inorganic ions Cl, Si, and Na (36, 35, and 19% of total abiotic mass, respectively). Large changes in relative element abundance were obvious moving up through the food web. In TL1 many trace elements were represented (for example, Mn, Mg, Fe, Si), but the macroelements C, N, and P became more and more dominant at higher trophic levels (Figure 2A–C). The overall number of dominant elements also decreased, from 12 elements comprising 99% of the mass in TL1 (Figure 2D) to only six elements in TL3 and TL4 (Figure 2A, B).

### Mass-Balance Ecosystem Model for Carbon in Tixlan

In Tixlan the benthic primary producers were responsible for 94% of the total primary production, of which 58% ( $2.2 \times 10^8$  g C) was produced by benthic plants and 36% by benthic microalgae ( $1.4 \times 10^8$  g C) (Figure 4: C). There was a clear dominance of the benthic part of this shallow ecosystem. This was also due to the large carbon pools found in suspended particulate organic matter (POM) and in sediments. Of the total consumption in the system the benthic fauna was responsible for 94%. The system was self-sufficient with regard to carbon as the total primary production ( $3.9 \times 10^8$  g C  $\text{y}^{-1}$ ) was in balance or even slightly exceeded the total consumption ( $2.0 \times 10^8$  g C  $\text{y}^{-1}$ ). The inflow (primary production, consumption, or sedimentation) and outflow pathways (respiration, grazing or predation, feces,

and excretion) of carbon to the compartments considered in the model were well balanced, making the model suitable for further ecosystem analyses.

### Mass-Balance Ecosystem Models for 47 Elements in Tixlan

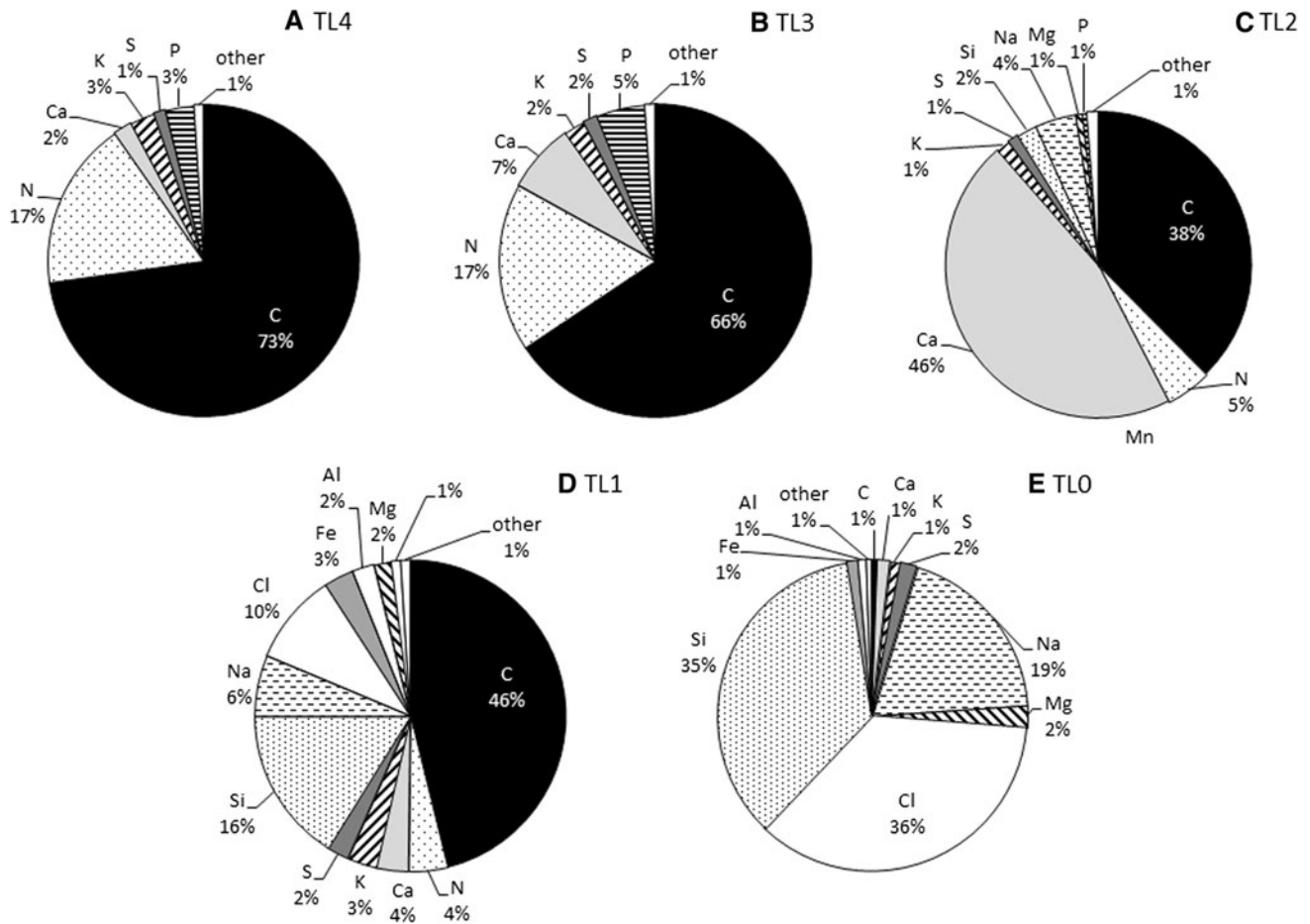
Mass balance budgets based on the carbon flow model for Tixlan were successfully established for all 47 elements. Of all 47 elements modeled, 22 elements (As, Br, C, Cd, Cl, Cr, Cs, Cu, F, Hg, I, K, Li, Mg, Mo, Na, Ni, Pb, Rb, S, Se, Th, V) were in complete balance for all 13 compartments in the model (for an example see Cs; Figure 4). For the remaining 25 elements (Al, Ba, Ca, Ce, Co, Dy, Er, Eu, Fe, Gd, Ho, Lu, Mn, N, Nd, P, Pr, Si, Sm, Tb, Ti, Tm, Yb, Zn, Zr) the models were unbalanced to various degrees (Table 2). In total 92.5% of all compartments were balanced.

Many of the observed imbalances [N, P, Al, Co (Figure 4), Fe, Mn, Si, Ti, and Zn] were associated with the primary producers, especially in phytoplankton and benthic microalgae. Ca was only unbalanced for filter feeders (factor of 26 and 83, from zooplankton and phytoplankton, respectively, Table 2; Figure 4: Ca), represented by the bivalve *C. glaucum*. Lastly, imbalances in actinides and lanthanoids were only observed for zooplankton (factor of 12–51, Table 2) and filter feeders (factor of 9–20).

### Comparison of Organism-Resource Stoichiometry (Homeostasis)

Compared to the element concentrations (carbon-normalized, g element  $\text{g C}^{-1}$ , hereafter referred to as concentrations) in the water, primary producers had higher concentrations of N and P, as well as the trace elements Si, Mn, Fe, Al, Ti, V, and Cd (Figure 3A). They had lower tissue concentrations than the water of many common ions of seawater salts like Na, Cl, K, Ca, S, Mg, Br, Li. The pattern for the transition metals (for example, Cr, Cu, Ni, Zn, Mo, and Pb) was more complex, but most primary producers also had lower concentrations of these than the water.

In the majority of cases, concentrations of elements in the consumers were proportional to the concentrations in the food (Figure 3B, C). TL4 (piscivorous fish) had very similar composition to their food (TL3: benthic and zooplanktivorous fish) for all elements (Figure 3C). Secondary consumers (TL3) had similar or slightly higher element concentrations than their food (TL2: invertebrates)



**Figure 2.** Relative amounts of elements in the different trophic levels (TL): **A** tertiary consumers (TL4), **B** secondary consumers (TL3), **C** primary consumers (TL2), **D** primary producers (TL1), **E** the abiotic components (TL0). The elements shown are those that make up 99% of the total mass of each trophic level. Note that the elements H and O are not included.

**Table 2.** Summary of All Unbalanced Compartments/Elements of the Element Flow Models

Compartment	Elements
Phytoplankton	Co (9), N (6), Mn, P, Si, Zn, Zr (all 14–23), Al, Fe, Ti (all >100)
Benthic microalgae	P (5), Al, Co, Fe, Mn, Ti, Zr (all >17)
Benthic plants	Fe (5), Al (12), Ti (10)
Zooplankton	Ce, Dy, Er, Eu, Gd, Ho, Lu, Nd, Pr, Sm, Tb, Tm, Yb (all 12–16)
Filter feeders <sup>a</sup>	Ba (5), Ca (26)
Filter feeders <sup>b</sup>	Ca (83), Ce, Dy, Er, Eu, Gd, Ho, Nd, Pr, Sm, Tb, Yb (all 9–20)

<sup>a</sup>'Compartment' indicates the model compartment for which the respective element's outflow exceeds its inflow; the figures in brackets state how many times the outflow exceeds the inflow (that is, the imbalance); only differences  $\geq 3$  are shown.

<sup>b</sup>For the pathway zooplankton to filter feeders.

<sup>c</sup>For the pathway phytoplankton to filter feeders.

with regard to the macroelements N, P, S, and K, as well as Hg. For most of the other elements, they had similar or lower body concentrations than their food (Figure 3C). Primary consumers (TL2) had

similar body concentrations to their food when this comprised primary producers (TL1), but lower body concentrations when the food was sediment or POM (Figure 3B).

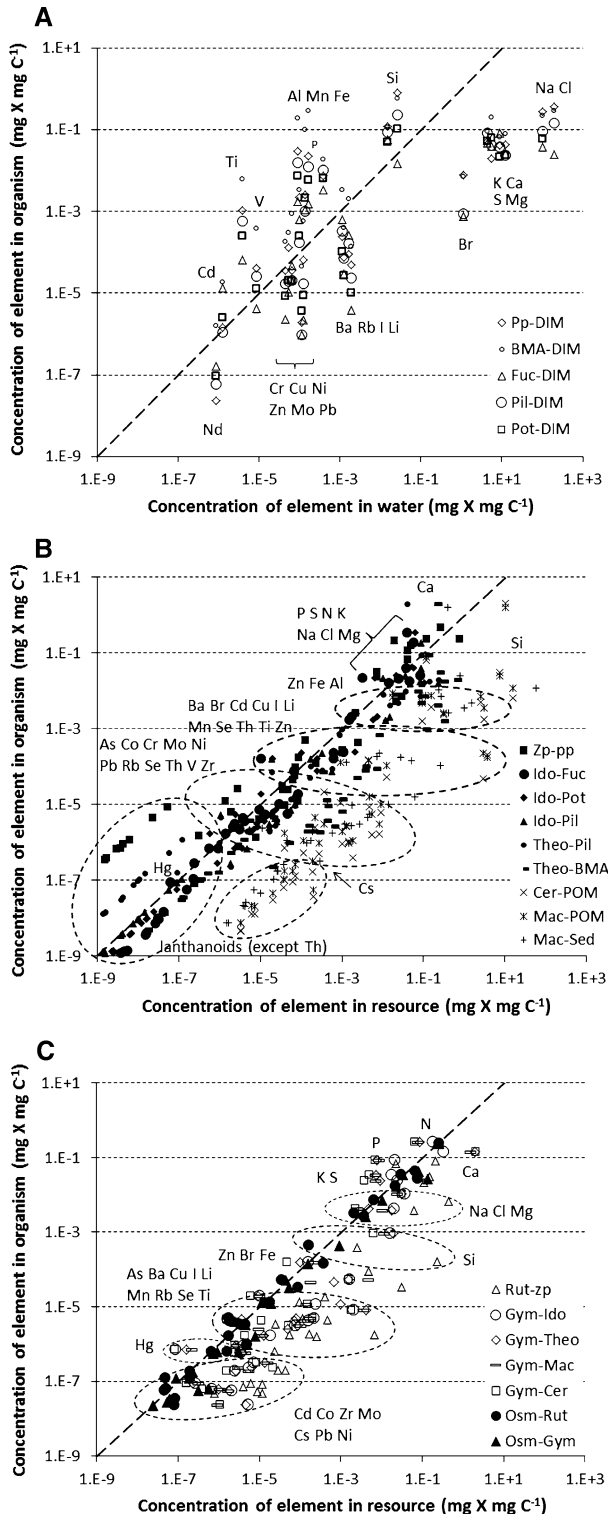


Figure 3. Comparison of element concentrations in organisms with their resources: **A** primary producers-water (DIM); **B** primary consumers, **C** secondary and tertiary consumers. The main element groupings are indicated. Codes in the figure legends are explained in Table 2. Note that not all elements are shown in all graphs as only concentrations above the limit of detection are presented.

## DISCUSSION

### Extending Ecological Stoichiometry Beyond C, N, and P

In this paper we have taken ES beyond its usual limits of C, N, and P dynamics and revealed patterns of distribution of 48 elements in a coastal ecosystem. The distribution of total mass of the elements on a basin-wide scale reflected the usual composition of seawater and the Earth's crust (for example, Fraústo da Silva and Williams 2001; Sterner and Elser 2002) and were typical for a fairly unpolluted coastal Baltic environment (for example, Pohl and Hennings 2006; Notter 1993; Szefer 2002). The abiotic compartments were dominated by Na and Cl, which are the elements that are the major constituents in the dissolved phase of the water (apart from H and O which were not included in this analysis). Silica, an element that is usually very abundant in minerals, soils, and sediments was also common. Unsurprisingly, the biota compartments were dominated by carbon (C), the most essential building block element in all organic material. The essential elements N, P, K, and S were also common in all biota: N and P are both major structural elements in all organisms; K is an essential element for all animals and plants, being necessary for the functioning of ion channels that regulate the flow of ions across cell membranes; and S is a key element in the biochemistry of all living organisms. For almost all elements, the concentrations in the organisms decreased up the food chain (that is, there was no biomagnification). However, for P and N, the reverse was true (enhanced N and P up the food chain).

The largest changes in relative element concentrations were found between trophic levels 0, 1, and 2 (TL0–TL1–TL2). The transfer within these two steps in the food web represents both functional and taxonomic changes. In the first step (TL0–TL1, TL0–TL2), elements are transferred from “abiotic” to “biotic” compartments via photosynthesis or detritus feeding. In water or sediment (TL0) element concentrations are determined mainly by physical processes such as diffusion and adsorption, whereas in living organisms, there is also an active regulation via cellular processes. Elements transferred from TL1 to TL2 move from autotrophs to heterotrophs, which have very different prerequisites and requirements for their energy intake, growth, and maintenance. There are also major taxonomic differences (plant vs animal kingdom). Changes seen between TL2 and TL3 can



be explained by both taxonomic and functional differences; TL2 consisted of invertebrate grazers and detritus- or filter-feeders, whereas TL3 consisted of vertebrate predators.

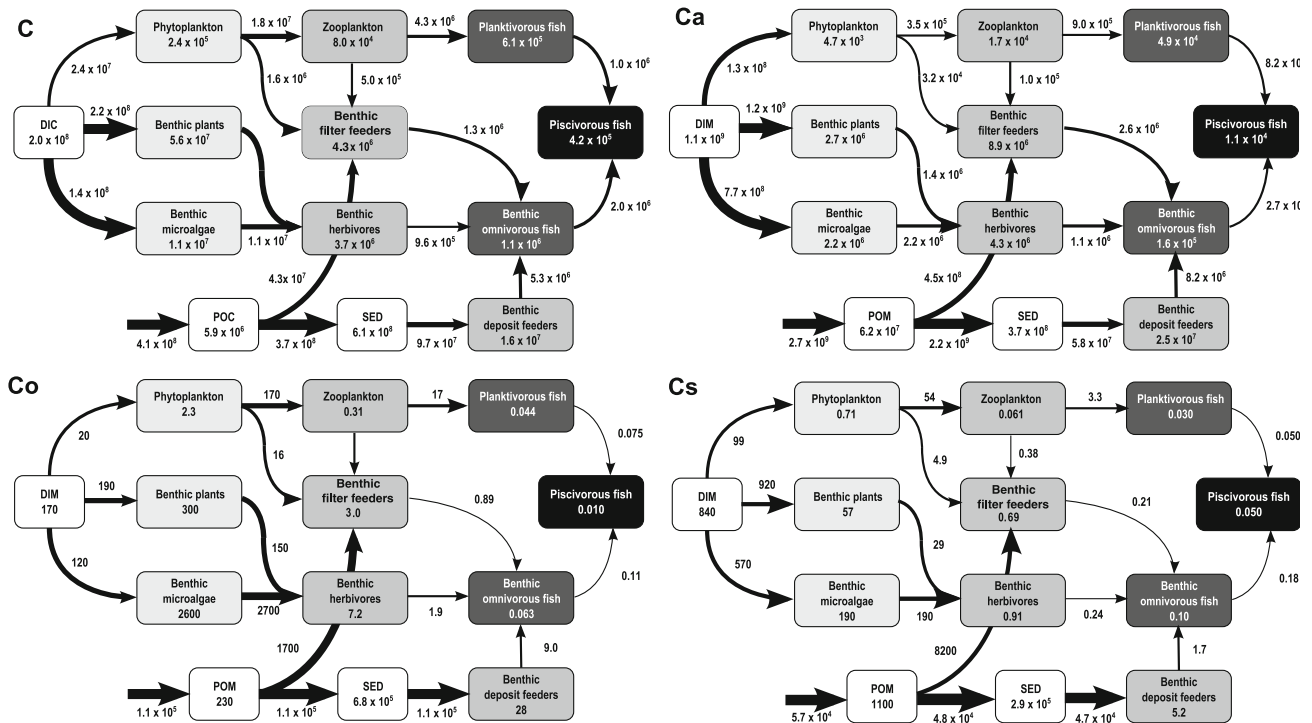
The two elements Si and Ca were particularly abundant in certain trophic levels and deserve special mention. Si comprised 14% of TL1, reflecting the dominance of siliceous diatoms in the phytoplankton at the time of sampling, as well as its important role in many macrophytes (Currie and Perry 2007; Schoelynck and others 2010). Ca made up 46% of TL2, because this group included the shell-bearing bivalves *Macoma baltica* and *Cerastoderma glaucum* and the gastropod *Theodoxus fluviatilis*.

This study provides a snapshot of a dynamic environment, presenting the concentrations of a multitude of elements in a range of organisms from a single time point. In reality, natural variation in concentrations of elements can be large, varying for example with season, life stage of the organism, and so on (for example, Karimi and Folt 2006; Liess and Hillebrand 2005). Element concentrations in short-lived and/or relatively immobile organisms probably reflect their immediate environment, whereas element concentrations in longer-lived and/or more mobile species integrate environ-

mental conditions over time and/or space. However, studies of this kind are limited by the large expense of sample analysis, and as our main interest was in determining patterns across the whole ecosystem, we chose to sample a large number of organisms at the expense of temporal replication.

## Ecological Processes Underlie Stoichiometric Imbalances

We have demonstrated that linking energy flows and element cycling in an ecosystem using stoichiometric relationships between 47 elements and carbon was possible. In only 7.5% of cases were there imbalances, and most of these can be related to underlying biological/ecological processes. Before discussing these further, it should be mentioned that the underlying carbon flow that was used as a driver for the transport of the elements may always be a source of error. However, errors in the carbon model would affect many more elements and compartments than observed, and the carbon model was well-balanced, suggesting a robust basis for further element modeling. The proportion of carbon pools and carbon fluxes is also in line with previous studies of shallow areas of the



**Figure 4.** Pools (g element) and fluxes (g element y<sup>-1</sup>) of C, Ca, Co, and Cs for Tixlan. Element pools and fluxes are calculated from the C model using element:C ratios as illustrated in Figure 1. Shading illustrates the transition from trophic level 1 to 4. Excess is not shown for clarity but is the difference between inflow and outflow for each pool. Total excess = inflow into POM.

Baltic Sea (Kautsky and Kautsky 1995; Kumblad and others 2003).

In the imbalanced cases, none of the organism groups had element concentrations below detection limits or differed much from existing data from the Baltic Sea (summarized in an extensive review by Szefer 2002). Instead, most of the cases of stoichiometric imbalance in the model can be explained by underlying biological and ecological processes.

Many imbalances were associated with the primary producers (Table 2). The majority of these elements are important nutrients (N and P) or trace elements [Co (Figure 4), Fe, Mn, Si and Zn], which often are limiting for aquatic primary producers. Both phytoplankton and benthic microalgae have high rates of primary production during the spring bloom, which occurred during the sampling period. The high incorporation of nutrients and trace elements into new biomass during this short period of time results in temporarily low concentrations in the water. In the model these temporarily low concentrations in the water result in inflows of these elements into the primary producers that are too small to match the estimated outflow, which is based on the average annual grazing rate and the element to carbon ratio in the primary producer. In addition, the rate of primary production could also have been slightly overestimated, which was also the case in earlier model studies from the area (Kumblad and Kautsky 2004). Either of these mechanisms, or a combination, provides a probable explanation for the observed imbalances of the nutrients (N and P) and essential trace elements (Co, Fe, Mn, Si, and Zn). An imbalance in Si (factor of 23) was only observed in phytoplankton, which was dominated by diatoms that have siliceous cell walls. That the observed imbalances relate to the spring bloom is also supported by previous seasonal DIM measurements in the area showing a drop in, for example, dissolved Si in the spring (Nilsson and others 2003; Tröjbom and others 2007 and unpublished field data by SKB). Al, Ti, and Zr, which are not algal micronutrients, were also found to be highly unbalanced in phytoplankton and benthic microalgae. No other concentration data for phytoplankton and benthic microalgae are available for comparison. However, the Al and Ti concentrations of all our other samples are in the same range as other published data (for example, Nilsson and others 2003; Szefer 2002; Tröjbom and others 2007). Sediment contamination of the biota samples is unlikely, as an unrealistically large amount of sediment would be needed to be

included in the samples to explain the observed imbalance.

Ca was only unbalanced for filter feeders, here represented by the bivalve *Cerastoderma glaucum*, which incorporates large amounts of Ca in its shell. In the calculations no distinctions were made for non-carbon elements with regard to their distribution and function within the organisms. This may well be the cause for the observed imbalance in filter feeders as shell-bound Ca has a much longer turnover time and thus lower flux than elements in the soft tissues. Ba was also unbalanced (factor of 5) in only one compartment, the filter feeders. Ba is also known to associate to the bivalve shell matrix (Carré and others 2006; Gillikin and others 2008), so this imbalance is probably linked to the observed Ca imbalance.

Imbalances in actinides and lanthanoids were only observed for zooplankton and filter feeders. Since the biological role of these elements is unknown and the environmental concentrations are low, it is difficult to evaluate if the observed imbalances may be due to model assumptions, sampling methods, chemical analyses or some unknown factor. The model assumptions for zooplankton and filter feeders with regard to the transfer of elements in the food chain are basically the same as for the rest of the compartments, for which unbalanced actinide/lanthanides fluxes have not been observed. Contamination of these samples with POM or sediment is not a probable explanation as concentrations of other sediment-associated elements, such as Al and Ti, were not elevated in these organisms.

Energy or element flow models are associated with many assumptions and uncertainties. Their construction requires definitions of the ecosystem boundaries and classification of its organisms into functional groups. This is not a straightforward task because many organisms have several functions in the ecosystem, not only during different periods of their lifecycle, but also simultaneously. In some cases it may thus be difficult to know the food sources of an organism, or they may have multiple food sources, making these types of calculations and analyses difficult. However, in this model the quality of the input parameters is very high, both because the majority of the data originate from field measurements from the area, and because most data were collected for the purpose of modeling. Despite using a simplified food web, the model conceptualization is robust as it was designed according to well-known ecological principles, and was based on the composition of the food web at the actual site.

## Homeostasis

Most evaluations of homeostasis use data across a gradient of resource concentrations for the same element, often for single or few organisms (see the review by Persson and others 2010). In contrast, this study is a snapshot of element concentrations at a single point in time. However, having data on an unusually large number of elements in multiple compartments of the same ecosystem gave us a unique opportunity to explore homeostasis by comparing organisms and their resources across a wide range of different types of elements for an entire food web, using real field data.

In contrast to the widespread assumption that autotrophs show weak or no homeostasis and rather reflect their surroundings (for example, Sterner and Elser 2002), our data show that primary producers have consistently lower concentrations of the common seawater ions Na, Cl, Ca, Mg, K, S, and Br than the surrounding water, suggesting that osmoregulation is occurring. They also have higher concentrations of some trace elements (Al, Cd, Fe, Mn, Ti) and lower concentrations of others (Cr, Cu, Ni, Mo, Pb, Zn) than water.

Secondary consumers (TL3: fish) also differ in their similarity to their food (TL2: invertebrates), depending on the elements in question. They have similar, or slightly higher, body concentrations with respect to the major essential elements but have lower concentrations of trace elements than TL2. It would appear that these organisms take up the necessary amounts of trace elements from their food within the 'window of essentiality' (Hopkin 1989), that is, to ensure essential levels but avoid toxic body concentrations. Interestingly, Hg also seems to be enriched in TL3 compared to TL2, reflecting possible biomagnification of this element (Gray 2002).

In the case of primary consumers feeding on sediment and POM, element concentrations ( $\text{mg element mg C}^{-1}$ ) are always higher in the food than in the consumers. This is mainly due to the lower C content of sediment and POM; mineralization of the dead organic matter in these components (and presence of large amounts of inorganic matter in the sediment) is presumably the explanation for this.

The higher consumers (TL4) have a very similar composition to their food (TL3). From this dataset it is not possible to distinguish whether this is a function of trophic level or the fact that both TL3 and TL4 are taxonomically similar (they are all fish). However, the element composition of the

primary consumers (TL2) compared to primary producers (TL1) is also similar, despite being taxonomically very different. This supports the theory that 'you are what you eat' (Sterner and Elser 2002) for these steps in the food web.

## CONCLUSIONS

- In this paper we have extended ES beyond its usual limits of pelagic C, N, and P dynamics to 48 elements in a coastal ecosystem. Major differences in elemental composition were demonstrated between abiotic and biotic compartments, and between trophic levels due to a combination of changing taxonomy and ecological function. Moving up the food chain, the relative abundance of macroelements increased, and the number of elements contributing to the majority of the biomass decreased.
- By coupling element:carbon ratios to an ecosystem model based on carbon flow we have (a) demonstrated the power of stoichiometric relationships to predict element transfer in a food web and (b) showed that the majority of stoichiometric imbalances in the model could be explained by ecological/biological processes.
- Our multi-element coastal dataset has allowed us to examine one of the key concepts of ES, homeostasis, on a wider scale than is normally possible with fewer elements and ecosystem components. Our data did not support the theory that autotrophs show weak homeostasis, and showed that the strength of homeostasis by consumers depended more on the type of element (for example, macroelement, trace element) than the trophic level.
- To advance the framework of ES, multi-element studies at the ecosystem scale are essential. New findings from such studies may not only challenge current ecological theories, but may also lay the ground for the next generation of methods for prediction of element transfer in the environment.

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