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New Frontiers in the Study of Element Interactions

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In 1922 the Russian geologist Vladimir Vernadsky coined the term "biogeochemistry" in recognition of the intimate connections that exist between living organisms and the physical components of the Earth. This insight came after many failed attempts to understand the chemical makeup of the Earth's atmosphere and crust through strictly geochemical processes, ignoring the influence of element interactions mediated by living organisms. In his landmark book *Biosfera* (The Biosphere), which was completed in 1926 at Charles University in Prague, Vernadsky wrote:

> A characteristic role is also played by the respiration and feeding of organisms, through which the organisms actively select the materials necessary for life. . . . Living matter builds bodies of organisms out of atmospheric gases such as oxygen, carbon dioxide and water, together with compounds of nitrogen and sulfur, converting these gases into liquid and solid combustibles that collect the cosmic energy of the sun. After death, it restores these elements to the atmosphere by means of life's processes. . . . Such a close correspondence between terrestrial gasses and life strongly suggests that the breathing of organisms has primary importance in the gaseous system of the biosphere; in other words, it must be a planetary phenomenon (Vernadsky 1926).

Since the publication of *Biosfera*, the discipline Vernadsky named has seen impressive growth in its collective knowledge of element cycling, achieved largely through numerous studies of the fluxes, budgets, and chemical transformations of individual elements. Although this line of investigation has been enormously fruitful, a growing number of important questions in ecosystem science involve poorly understood interactions among two or more elements.

The importance of multiple element interactions stems from the fact that pairs and even groups of elements often participate in a series of common biological and geochemical reactions. Because carbon represents a common currency among all life forms, many multiple-element interactions are mediated through the relative ratios of carbon to other nutrients (e.g., Sterner and Elser 2002). For instance, if the availability of a given element limits the rate of carbon fixation by primary producers, other elements that are cycled through biomass production and turnover can be indirectly affected. As an example, research in the Hawaiian Islands has revealed element interactions during ecosystem development that involve a change from nitrogen limitation to phosphorus limitation over geological time scales (Vitousek et al. 2002). Although supplies of N relative to plant demand increase steadily over this progression, the absolute rate of N cycling slows as ecosystems become increasingly P-limited. This stems from the maintenance of relatively conservative N:P ratios in vegetation (Crews et al. 1995) and the dependence of N cycling on organic matter production and turnover.

Despite these insights, growth in scientists' understanding of multiple-element interactions has been restricted by several factors, including the diversity of forms in which a single element can occur (carbon, in particular) and the fact that element ratios in organisms are not always conservative. At present, the degree to which element interactions across a range of ecological systems are controlled by these sources of complexity versus general patterns of organism stoichiometry is simply not known.

Our lack of understanding is highlighted best by the fact that few ecosystem models explicitly include element interactions, a shortcoming that limits their applicability for many scientific and policy issues. For example, the notion of nitrogen saturation in temperate forest ecosystems involves a condition where excess N loading via atmospheric deposition leads to a shift from nutrient limitation by N to limitation by some other element (Aber et al. 1989). A noteworthy ingredient of this shift is its decoupling from the normal mechanisms of ecosystem development demonstrated in Hawaii. An important feature of N saturation is the increasing production and leaching of nitrate from soils, a process that can also cause depletion of positively charged ions such as calcium. Despite growing evidence that this process has begun to affect forests in heavily impacted regions (e.g., Peterjohn, Adams, and Gilliam 1996; Aber et al. 2003), few models have attempted to explicitly capture multiple-element chemical processes in soils (e.g., Gbondo-Tugbawa et al. 2001) and none include the plant-soil feedbacks necessary to simulate the shift in element limitations.

As the biogeochemistry research community is confronted with limitations of single element models, the need for new analytical and conceptual tools will become increasingly apparent. In all fields of science, major breakthroughs often follow technological or conceptual advancements, so a challenge that exists just before those breakthroughs involves recognizing the pending advancements that will be of greatest benefit. The purpose of this chapter is to discuss important limitations in several areas of element interaction research and to highlight several forthcoming conceptual and/or methodological approaches that may help overcome these hurdles. Given the large number of issues that involve multiple element interactions and the diverse array of new approaches becoming available, it would be impossible to conduct an exhaustive review that has relevance for all ecological systems. Instead, our approach is to discuss several persistent challenges and highlight a few recent developments that have promising potential for addressing them. It is our hope that this discussion will inspire new ways of addressing issues beyond the specific examples we offer here.

Current Limitations in Element Interactions Research

Major obstacles for advancing our understanding of element interactions are often manifested as inconsistencies between how we formalize our knowledge into models and the data available to describe the systems being modeled. This discrepancy can be due either to a lack of relevant data, as a result of technical difficulties in making certain measurements, or to a deeper mismatch between parameters and processes included in models and measurable features of the real world. In this section, we discuss examples of several common limitations that influence the applicability of currently available biogeochemistry models, both conceptual and methodological.

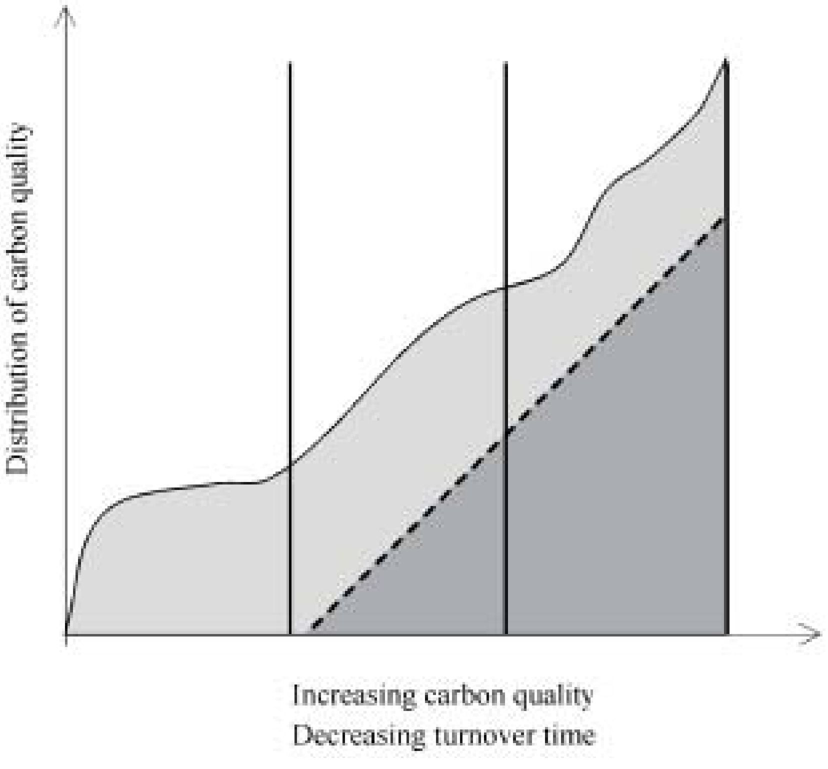
Discrepancies between Model Formulations and Observable Ecosystem Properties

Carbon Dynamics in Terrestrial Soils

A common difficulty in matching model constructs with observations of nature involves the issue of how to capture complex or highly variable properties in a functionally efficient manner. A good example of this challenge involves models of carbon turnover in terrestrial soils, where organic carbon is lumped into a small number of discrete states that are meant to represent a broad continuum of turnover times. Although it is widely acknowledged that what we simplistically refer to as soil carbon contains a complex mixture of chemical compounds with

Box 4.1. Carbon quality and turnover time of soil organic matter

Carbon in soil organic matter is distributed over many different compounds. These compounds have a huge range of degradability traits (qualities) and, consequently, turnover times. In models these different turnover times are represented in different ways. One alternative is to consider the full distribution of turnover times. This approach leads to models that are rather mathematically complex. A simpler approach is to split the distribution of carbon into a small number of discrete classes, as represented in the figure by the vertical lines. The problem is that this partitioning is rather arbitrary and does not correspond to observed fractions. Partitioning the carbon according to some empirical procedure, such as light and heavy fractions, is likely to cut the carbon density distribution in still some other way that is not directly coupled to turnover times. This approach is exemplified with the broken line, where the area under the line would be the light fraction and the area above the line the heavy fraction.



different levels of accessibility for decomposers, the question of how to represent that complexity has been far from obvious.

The method of aggregating soil carbon compounds into distinct groups (such as fast, medium, and slow turnover pools) is attractive because it offers a certain degree of functional utility. Because soil material does not actually exist in such discrete forms, however, it presents the problem of how to parameterize model variables that lack real-world counterparts and cannot be explicitly measured. In addition, there is the challenge of deciding how many compartments such a model should include. Although finer divisions may come closer to mimicking reality, such an approach becomes unwieldy as the number of compartments grows. The use of a few highly aggregated compartments may be functionally desirable, but this approach presents increasing difficulties of linking measurements to corresponding theories within the model (Ågren and Bosatta 1998).

Maintaining the integrity of theories and mechanisms within a model is most necessary where models are expected to predict phenomena that have not been explicitly prescribed or that fall beyond the range of known observations. The most salient examples of this need involve processes in which thresholds play an important role. Net nitrification in forest soils, for example, seems to occur only when the soil C:N ratios fall below approximately 24–25 (MacDonald et al. 2002; Ollinger et al. 2002). In a well-constructed model such a result should come from underlying interactions between soil C and N dynamics without being prescribed a priori.

Element Ratios and Threshold Effects

The difficulty of simulating systems that include important threshold responses may stem from the overriding prevalence of single element nutrient models and models that erroneously assume a linear response to driving environmental variables (i.e., because available data are not sufficient to define a full response surface). An additional challenge arises in systems where biogeochemical cycles are influenced by ecological processes that span multiple levels of organization, particularly those that are usually not included in ecosystem-level studies (e.g., population or community-level feedbacks).

An experimental demonstration of this type of effect was presented by Sommer (1992), who showed how an algae-Daphnia system switched between algae-dominated and grazer-dominated when the dilution rate exceeded a narrowly defined value. At low rates of P turnover, low algal production and high algal C:P ratios severely inhibited grazer growth rates and maintained an algae-dominated community. As P availability rose above a certain critical threshold, however, algal quality improved to the point where consumption rates rose markedly and the experimental ecosystems supported large numbers of grazers. In this system the threshold response was driven by differences in the C:P ratios of algae compared with the requirements of grazers. For models to simulate this dynamic in real ecosystems, they must capture interactions between the biogeochemical processes regulating P cycling, variability in algal P concentrations, and factors controlling algae-grazer competitive relations.

Lack of Data due to Difficulties Making Basic Measurements

Sampling Individual Species within Microbial Communities

In terrestrial plant communities, the ability to sample and analyze tissues from individual organisms has allowed us to gain insights into the functioning and flexibility of species within the community. This type of knowledge has led to important theories about how dynamics of species and ecological communities scale to the level of the entire ecosystem. In microbial communities, which consist of the most numerous and biogeochemically significant organisms on Earth, we have little insight into these relationships because we lack basic methods for sampling and analyzing individual organisms or species.

Microorganisms are arguably the most important detritivores globally and are temporary sources and sinks of plant-essential nutrients. Microbial populations, biomass, and nutrient content are notoriously difficult to measure in soils. Although soil microbial C, N, and P are commonly estimated following fumigation of a soil sample with chloroform, this process solubilizes most cell walls and releases cytoplasmic contents. Because not all microbial cell constituents are recovered, a conversion factor must be used to estimate their nutrient contents (Horwath and Paul 1994). Despite the large amount of effort that has been put toward estimating these conversion factors and understanding how they vary among soils, large uncertainties remain. Moreover, these methods ignore differences *within* microbial communities that may be related to rates of nutrient cycling.

In aquatic systems as well, size-based fractionations might yield samples heavily dominated by individual microbial populations, but separation of populations, or even functional groups, is problematic. Nutrient studies in lake ecosystems are typically based on analysis of particulate matter, but the material sampled can include both living and nonliving components, and the living fraction might contain algae, bacteria, and protozoa in varying abundances. What we lack is an easily applied method to determine the elements held within each individual component of the system.

Determining the Flexibility of Microbial Stoichiometry

In addition to measurements of nutrient stocks in microbial biomass, rates of flows of nutrients through the microbial biomass pool are needed for improved understanding of ecosystem function and multielemental interactions. A common simplification in conceptual and numerical models is that C, N, and P stoichiometry is fixed in the microbial biomass of a given ecosystem, but this is unlikely to be true. If microbial stoichiometry were fixed, then separate functions for each element would not be needed. We know, however, that the processes involved in element linkages can be flexible, which makes the description of these multielemental interactions more complicated. In the case of microbial biomass, for example, each species may have some plasticity in its C:N and C:P ratios because N and P, when available in excess, are taken up and temporarily stored within the biomass. These ratios may also change as species composition changes over relatively short generation time periods in response to varying resources.

In addition to differences among species within a microbial community, differences among organic compounds within the cells of a single species may also affect multiple element interactions. For example, C and N stored in cell walls occur in different ratios and cycle at different rates than do C and N in extracellular enzymes and cellular metabolites used for osmotic regulation. We currently have very little understanding of how much of the N immobilized by soil microorganisms is used for cell growth (requiring proportionally all the C and other elements needed for cell biomass) and how much of the N taken up by microorganisms is subsequently exuded as extracellular enzymes and cellular metabolites (requiring only the N:element ratios of those particular compounds). New approaches are needed to identify the microbial players in a given ecological community and to quantify the importance of various organic compounds with regard to pools and fluxes of elements.

Limitations in Conceptual Understanding

Single versus Multiple Resource Limitation

According to Liebig's law of the minimum, a biological process, such as crop productivity, is limited by the single resource that is in shortest supply relative to demand (von Liebig 1847). If that limitation is alleviated, as in fertilizer amendment to an agricultural field, then that process increases until some other resource becomes limiting. Although Liebig's law is not explicitly included in most biogeochemical models, the concept of "a limiting nutrient" for a given ecosystem is persistent in our thinking. For example, vegetation growing on highly weathered soil substrates is commonly considered P limited, while vegetation on younger substrates is commonly considered N limited (Crews et al. 1995). These generalizations are based on an understanding of soil chemical properties as they vary during soil weathering and are supported by nutrient amendment experiments and patterns of foliar concentrations and nutrient use efficiencies (Vitousek 1984; Vitousek and Farrington 1997; Harrington, Fownes, and Vitousek 2001). Although these simplifications have been instructive, they do not preclude the possibility—indeed, the likelihood—of important multielemental interactions when a wider variety of processes and time scales are considered.

When nutrient manipulation experiments demonstrate a synergistic response to more than one element, the response is usually described by an interaction term resulting from conventional analysis of variance. Although the ANOVA may be necessary for demonstrating statistical significance, the ecological significance is likely to lie in the interpretation of how and why the response of X_1 to X_2 is different when X_3 is present in greater or lesser quantity. This ecological interpretation usually requires expression within a modeling framework and may also require disaggregation of the response variable. For example, if X_1 is plant growth and X_2 and X_3 are N and P concentrations in the soil, it may be necessary to divide the plant biomass growth response into responses of individual species within the community or to analyze separately the responses of roots, stems, and foliage. This simple example ignores much more complex processes, such as herbivory, enzyme production, or symbioses that may be equally important. Although parsimonious explanations and simple models are usually desirable, multielemental interactions clearly require some degree of added complexity in conceptual and numerical models.

Limitations of the Optimization Paradigm

Another common paradigm used in ecosystem models is the notion of optimization, which assumes that organisms optimize the use and allocation of resources in a way that maximizes their functional efficiency. This assumption holds great conceptual value and has been effectively applied to simulating resource limitations in a variety of habitats (e.g., Armstrong 1999; de Mazancourt, Loreau, and Abbadie 1999). There are, however, at least two important pitfalls that must be considered for effective use in element cycling models. These pitfalls involve the question of what processes are being optimized and at what level of organization optimization should occur. Terrestrial ecosystem models, for example, often carry the assumption that optimal resource allocation should lead to a maximization of biological production. Evolutionary pressures, however, favor adaptations that maximize survival and reproduction rather than growth (Arendt 1997). Although growth rate and reproductive success are often related, resource allocation patterns that appear to be suboptimal can be favored when they provide an advantage in terms of competitive interactions with neighboring individuals.

Further, because selection operates on individuals rather than on ecological communities, we should not assume that optimization will necessarily produce maximal functioning over entire ecosystems. As an example, most efforts to predict leaf area index in plant canopies assume that optimization should lead to maximum photosynthetic capacity of the entire canopy. Anten and Hirose (2001), however, suggested that this approach is conceptually flawed because evolutionary mechanisms act not on whole plant canopies, but on the individuals within them. Using a model that contrasted individual and canopy-level photosynthesis, the authors showed that foliar allocation patterns that optimize photosynthesis over the canopy are not evolutionarily stable because at this leaf area index (LAI), any individual within the stand can increase its growth rate by increasing its individual leaf area.

Ecological Interactions and Species Effects

The ways in which individual species influence biogeochemical cycles has been an active area of investigation and debate for many years. Because functional differences between species often involve differences in the proportions and forms in which various elements are used, differences in species composition within ecological communities lead to basic differences in element interactions. For example, Binkley and Valentine (1991) showed differences in soil properties and element cycles among fifty-year-old forest plantations in Connecticut, U.S.A., that corresponded to differences in carbon quality and cation use among the species planted. Understanding differences in element use between species is particularly important for predicting temporal dynamics because species composition in ecological communities is rarely static. Changes over time that result from patterns of disturbance and succession complicate our understanding of element interactions because our understanding of the mechanisms driving those changes is incomplete. In addition, the temporal dynamics of element interactions due to species change is unlikely to coincide directly with the species change itself, because species can have important legacy effects on the environments in which they exist, long after they have been replaced by other species.

Nutrient Availability and Historical Human Land Use

The previous examples involved situations in which population-, community-, and ecosystem-level processes interact in ways that challenge traditional biogeochemistry models. Other examples of how factors that affect element interactions can cross traditional disciplinary boundaries involve processes mediated by

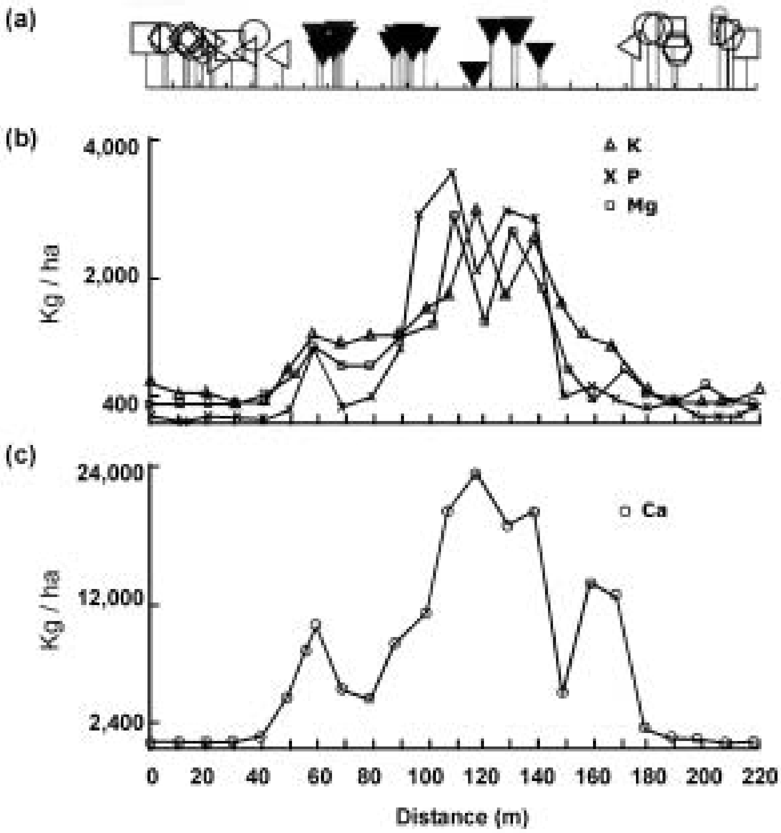


Figure 4.1. A transect through a fine-leafed savanna patch within a broad-leafed savanna, reflecting the long-term legacy of the African Iron Age Tswana people. (a) Distribution of woody plants: solid symbols are fine-leafed trees; (b and c) soil nutrient chemistry (Scholes and Walker 1993).

human social or economic activity. An instructive demonstration of this comes from the nutrient-poor savannas of southern Africa as described by Scholes and Walker (1993). These ecosystems are extensive on the sandy, highly leached and acidic soils that occupy the majority of the region and are typically dominated by broad-leaved vegetation such as *Burkea africana.* Discrete patches of nutrient-rich fine-leaved savanna, however, are occasionally found within these broad-leaved vegetation communities. The distribution of these patches bears no obvious relationship to physical features of the landscape but rather has been tied to archaeological remnants of past human societies.

Settlements of the African Iron Age Tswana people occurred in the region more than 700 years ago but typically lasted for just a few decades in any one location. These settlements led to the accumulation of large amounts of nutrients in soils as a result of firewood collection and the corralling of animals at night. The accumulation of nutrients, in turn, led to a complete switch in the vegetation type and ecosystem dynamics that in some cases have persisted for many centuries (Figure 4.1). It is difficult to understand how relatively short-term nutrient inputs have produced such long-term changes, but one possible explanation is that the added nutrients altered the fundamental plant-soil feedback mechanisms whereby additional phosphorus from decomposing animal tissues made it possible for *Acacias* to become established. Because *Acacias* are di-nitrogen fixers, their presence would further the process of nutrient enrichment. Once established, these sites also attract a variety of native herbivores, which may contribute to the ongoing maintenance of their atypical biogeochemical status. This combination of historical human land use and shifting ecological feedback mechanisms has been characterized quantitatively (Scholes and Walker 1993), but it represents an enormous and, as yet, unmet challenge for process modeling.

New Approaches and Recent Advancements in Element Interactions Research

Although issues discussed in the preceding section represent important and in some cases daunting challenges to element interactions research, it can be both instructive and encouraging to consider areas in which methodological and conceptual breakthroughs have led to rapid and substantial gains in our understanding. As an early example, the biogeochemical theories proposed by Vladimir Vernadsky represented a significant conceptual advancement, yet they could also be viewed as a product of preceding methodological advancements that provided element analyses of atmospheric gases, rocks, and soils.

Many years later, development of methods for retrieving and analyzing gases trapped in ice cores provided a new view of feedbacks between element interactions and climate dynamics that spanned geological time scales (e.g., Mayewski and White 2002). Another example of concordant technological development and scientific understanding can be seen in the technologies associated with trace metal analysis and the role of iron limitation in the open ocean (Ducklow, Oliver, and Smith, Chapter 16, this volume). Development of methods for clean water sampling led to the important discovery that di-nitrogen fixation can be limited by nanomolar levels of iron (e.g., Kolber et al. 1994). In this section we highlight several more recent advancements that may have significant potential to foster rapid changes in element cycling research.

Resolving the Chemical Identity of Element Observations

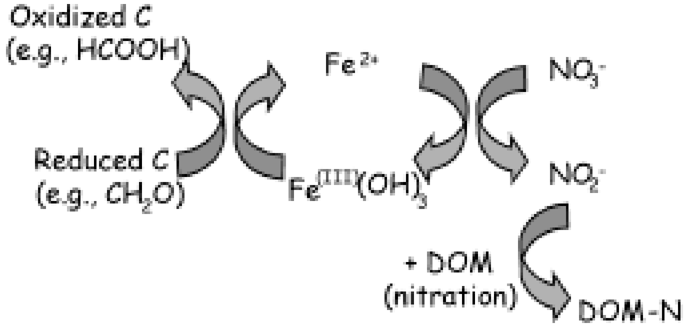
Identification and quantification of materials at a molecular level is a crucial step in the study of element interactions. Most of the major advances in our understanding of processes governing these interactions are grounded in detailed knowledge of chemical compounds. As the preceding sections described, barriers to our understanding of these interactions often arise from an inability to identify and/or measure the distribution of elements across various chemical compounds. For example, we have known for more than twenty years that litter decomposition and associated nutrient transformations are related to the structure and concentration of lignin and nitrogen in plant tissues (e.g., Berg and Staaf 1980; Melillo, Aber, and Muratore 1982; Berg and McClaugherty 2003). Nevertheless, there can still be large unexplained variation in the relationships between lignin, N, and litter decomposition patterns. Recent advances in analytical techniques such as near infrared spectroscopy (NIRS) and pyrolysis-gas chromatography/mass spectrometry-combustion interface-isotope ratio-mass spectrometry (py-GC/MS-C-IRMS) allow a more comprehensive understanding of decomposition at the molecular level and, in particular, the relationship between litter carbon quality and decomposition rate (Joffre et al. 2001; Gleixner et al. 2002).

Solid and Liquid Phase Analyses

For soils and water samples, techniques such as ^{13}C, ^{15}N, and ^{32}P nuclear magnetic resonance (NMR) have been used with varying success across a range of ecosystems. In aquatic ecosystems NMR techniques have been used to better understand the nature of carbon/nitrogen bonding in dissolved organic matter (McKnight et al. 1997). In terrestrial ecosystems at least two studies have combined ^{13}C, ^{15}N, and ^{32}P NMR techniques to evaluate the degree to which N and P mineralization are coupled to C (Gressel et al. 1996). The combined analysis of the structural basis for C, N, and P bonding and change through time focuses on (and challenges) evidence behind long-standing arguments for coupled C-N and decoupled C-P during decomposition/mineralization (McGill and Cole 1981). New techniques involving py-GC/MS-C-IRMS and NMR offer significant insight into decomposition patterns, the preservation of organic matter through time, and the relationship between N availability and decomposition rates (Gleixner, Bol, and Balesdent 1999; Preston 2001; Gleixner et al. 2002; Neff et al. 2002). Despite these advances, there is a clear need to better integrate ecological and molecular techniques and to improve communication between investigators working at multiple scales. Further, to date, there has not been as much advancement in the application of advanced

Box 4.2. Abiotic nitrate immobilization in soils: The ferrous wheel hypothesis

An example of multiple element interactions is the “ferrous wheel hypothesis” for abiotic nitrate immobilization in soils, offered by Davidson, Chorover, and Dail (2003). Carbon compounds derived from photosynthate reduce Fe(III) in soil minerals, producing reactive Fe(II) species that reduce nitrate to nitrite, and nitrite subsequently reacts with receptive moieties in dissolved organic matter (DOM) to produce dissolved organic-N (DON). This process could be an important mechanism by which inorganic-N from atmospheric deposition is converted to organic-N within soils without the large C requirement necessitated by biological assimilation. Iron is abundant as Fe(III) in most well-drained mineral soils, and interactions of nitrate and iron are known to be important in aquatic ecosystems (Senn and Hemond 2002), but little is known about iron speciation and reactivity in soil microsites.



analytical techniques to element interactions at a molecular level. In the future, compound-specific techniques that link structural identification of biomarkers with not only $^{12,13}C$ but also $^{14,15}N$ could significantly advance our understanding of elemental interactions at a molecular level in both aquatic and terrestrial environments.

Gas Phase Analyses

Perhaps the best-known “new advance” in atmospheric measurement techniques involves the development and widespread application of robust open-path infrared gas analyzers. These instruments exponentially increased the number of commercially available eddy-covariance systems and allowed many researchers to measure high-frequency CO_2 and H_2O fluxes at the canopy scale. The wealth of new data these measurements have provided have given us a much better sense of both the variability in CO_2 and H_2O fluxes in natural and agricultural ecosystems and of the mechanisms underlying these variations.

Other techniques are emerging that have already proven helpful for making unique measurements and for making measurements at shorter time scales than were previously possible. Several of these methods involve laser spectroscopy of air samples and the quantification of particular compounds (and in some cases, their isotopic ratios). Tunable diode lasers (TDL) are the best known of these techniques and have been used for canopy measurements of methane, nitrous oxide, and carbon dioxide (Fowler et al. 1995). Recently TDLs have been used for long-term (longer than one year) studies of nitric acid and nitrogen dioxide fluxes at the canopy scale, results from which have been essential for balancing the reactive nitrogen budget in northeastern forests (C. Volpe Horii, unpublished results). Currently, TDLs are beginning to be used for the determination of isotopic ratios during eddy covariance studies for particular compounds of interest (e.g., CO_2; Mcmanus et al. 2002). A next generation of lasers, quantum cascade lasers, promises to bring the analytical capabilities of TDL down to the size of a briefcase, which would free researchers from the need to have a steady supply of liquid nitrogen.

Methods and Models of Atmosphere Biosphere Exchange

Improved conceptual understanding has sometimes been coupled with the simultaneous development of instrumentation and modeling. Emissions of volatile organic carbon (VOC) from vegetation are a case in point. As portable, battery-powered instrumentation for VOC measurements became available, many more plant taxa were sampled across the surface of the globe (Lerdau, Guether, and Monson 1997; Valentini et al. 1997). This increased sampling led to much-improved regional inventories, and these, in turn, allowed better-constrained models of element interactions in the troposphere (Guenther et al. 1995, 1996, 1999). In the early 1990s real-time analyzers for specific compounds allowed identification of the biophysical controls over production and emission. This mechanistic understanding led to the development of mechanistic flux models that were applicable across broad spatial and temporal scales (Harley, Monson, and Lerdau 1999). Such effective models have proven invaluable for determining the redox potential of the atmosphere and the interactions among VOCs, reactive forms of nitrogen, and ozone (Guenther et al. 1999).

It is unlikely that such rapid progress could have been made in the absence of these novel instruments. An additional benefit of this rapid accumulation of knowledge has been an improved understanding and quantification of VOC fluxes with respect to the global carbon cycle (Lerdau, Chapter 9, this volume).

Advancements in Element Analysis for Individuals and Species

In recent years considerable progress has been made in characterization of population sizes, and in some cases detailed size-based or pigment-based characterizations can be made with flow cytometry. Though very helpful in dealing with biomass, flow cytometry does not measure element content. It would be a major breakthrough if flow cytometry could be coupled to a method to estimate element abundance (e.g., x-rays or near infrared spectroscopy [NIRS]). At the molecular level, probes for specific genetic material can now be used to assay particular microbial function groups, such as nitrifying bacteria in soils. In the future continued development of such methods for gaining information about microbial populations (broadly defined, including heterotrophic bacteria, small algae, and others) will greatly improve our knowledge of element cycle linkage in terrestrial and aquatic ecosystems, particularly when biogeochemical measurements of elements can be coupled to population censuses.

A promising example of how this may be achieved involves the combined use of new molecular techniques and stable isotope analyses. This strategy was suggested by Nannipieri, Badalucco, and Landi (1994) as an approach for measuring specific microbial pools in soil, and more recently Radajewski et al. (2000) reported on a specific application. After treating soil with a known ^{13}C source, they were able to extract and separate ^{13}C-labeled DNA and then characterize it (both taxonomically and functionally) by gene probing and sequence analysis. This technique may offer a useful new means of estimating the size of active microbial C pools: by multiplying the ratio of ^{13}C-DNA to total DNA by the soil microbial C pool. In addition, the taxonomic and functional characterization provides a more precise understanding of how microbial communities are affected by various types of added C-substrate.

Molecular Techniques for Nutrient Limitations

One of the most difficult issues associated with element interaction is determining when and how nutrient limitation of organisms occurs. Although it is relatively easy to measure the concentration of an element in a soil sample, it is much more difficult to determine whether that particular element limits a particular biotic activity. A significant methodological advance in the study of element interactions will, we hope, soon come with the development and application of bioreporter probes. Bioreporter organisms are genetically modified cells, so far either bacteria or algae, that have had a fluorescence-producing gene coupled to a regulatory gene that is sensitive to physiological limitation of a single element or resource. Bioreporters have been developed, for example, to measure Fe-limitation (Durham et al. 2002). Bioreporters for other elements are under development.

A similar approach to measurement of element availability is in immunoassays for individual proteins. In Fe-limited algae, the respiratory enzyme flavodoxin substitutes for the Fe-containing ferridoxin when cellular iron is deficient. Immunoassays have been developed that can interrogate the cells from natural populations to determine which of these two enzymes they carry (McKay et al. 1999). Methods development allowing for routine application of bioreporters, immunoassays, and other physiological studies of nutrient availability could mean a major breakthrough, because this approach uses the response of an organism in the habitat itself to measure the availability of nutrients. We look forward to putting a mixture of bioreporters into water samples or soil samples and letting the emitted fluorescence of given wavelengths "report" the ratios of available nutrients in those substrates.

Thus far, this chapter has concentrated largely on the difficulties of identifying microorganisms and their role in element interactions. This emphasis occurs partly because plant species and their element content have generally been easier to identify, at least for aboveground plant parts. Identifying plant roots in the soil profile, however, has been extremely difficult. Ecologists recognize a large variability in rooting depth among plant species (Canadell et al. 1996; Jackson et al. 1996) and, consequently, hypothesize that changes in plant-species composition would have major consequences for the cycling in ecosystems. Testing of this hypothesis requires unequivocal identification of roots in the soil profile. Recently, Jackson et al. (1999) were able to identify roots of tree species at different depths in the soil profile by comparing the root DNA sequences against a reference database developed for aboveground plant parts. Studies like this will become more common in the near future because reference databases are growing at a rapid rate, saving researchers' time and resources.

Scaling Element Interactions from Sites to Regions

A common theme in all fields of science is the question of how to take information collected at one scale and apply it to processes that manifest themselves at another. In ecosystem science this challenge is particularly relevant because processes that are critical to the discipline occur at spatial scales ranging from microscopic to global and at temporal scales ranging from seconds to millennia. In terrestrial systems, methods by which leaf-, plant-, and stand-level observations can be related to landscapes, regions, and continents have been the subject of much discussion (e.g., Ehleringer and Field 1993; Reich, Turner, and Bolstad 1999). Although a variety of approaches have been proposed, there is widespread agreement that remote sensing holds a central and irreplaceable role. Remote platforms provide the only means of viewing large portions of the Earth's surface at regular intervals, and the selective absorption and reflectance of light by plant tissues allows optical sensors to gather tremendous amounts of ecologically relevant information.

Recent Advancements in Remote Sensing

Satellite and aircraft remote-sensing data provide some of the best options for spatial scaling of ecological processes (Ustin, Smith, and Adams 1993). For biogeochemical analysis, however, remote-sensing data generally provide only a fraction of the information scientists require. Many important ecosystem properties cannot be directly detected but must be estimated from algorithms that relate raw reflectance to more useful landscape or vegetation properties. These algorithms are often challenging to derive and can be subject to substantial uncertainties. To date, a large number of remote-sensing applications in ecosystem science have addressed patterns of productivity and carbon uptake, but fewer have explicitly addressed issues of element cycling or, more rarely, multiple element interactions.

One common approach to conducting spatially explicit analyses of ecosystem production has been to use spectral vegetation indices, such as the normalized difference vegetation index (NDVI), that relate to vegetation properties such as cover type or LAI (leaf area per unit ground area). These data can then be used as input to ecosystem models capable of simulating a variety of other processes (e.g., Ollinger, Aber, and Federer 1998). The use of LAI as an estimator of productivity has been most effectively demonstrated across large moisture gradients where substantial variation in LAI can be observed (e.g., Gower, Vogt, and Grier 1992; Fassnacht and Gower 1997). The approach, however, is more limited at finer spatial scales and within regions where moisture regimes and LAI are less variable, but where variation in production nevertheless occurs owing to variation in soil nutrient availability and/or the production efficiency of foliage (Coops and Waring 2001). Additional limitations of LAI-based methods stem from factors that cause both vegetation indices and LAI to exhibit nonlinear, asymptotic relationships with rates of production (Gower, Reich, and Son 1993; Reich, Turner, and Bolstad 1999).

In recent years a variety of new remote-sensing techniques have been developed that may offer improved access to ecosystem structure and function. Synthetic aperture radar and laser altimetry (e.g., light detection and ranging, or LIDAR) provide information on the size and spatial distribution of canopy elements that can provide the basis for biomass estimates. The utility of these techniques is increasingly established, based on data from aircraft and short space flights, but satellites with appropriate sensors and global coverage are not yet deployed. Multiangle remote sensing provides another avenue for approaching canopy structure (Diner et al. 1999). Multiangle data are now available from space-based platforms such as the Multiangle Imaging Spectro Radiometer (MISR). Hyperspatial data, with a spatial resolution of one meter or better, are now available from satellite sensors, and investigators are beginning to explore the potential of new algorithms based on the size, spacing, and dynamics of the crowns of individual trees (Coops and Culvenor 2000).

Another intriguing innovation in remote sensing is the development of aircraft- and satellite-based imaging spectrometers, also known as high-spectral-resolution, or hyperspectral, sensors. Whereas traditional remote-sensing instruments divide the reflectance spectra into a small number of discrete and discontinuous spectral bands, typically averaged over a wide range of wavelengths, hyperspectral sensors measure continuous spectra using a larger number of much narrower bands. This capacity has proven especially useful for biogeochemistry research because spectral information contained in these detailed images can be related to concentrations of specific elements in plant foliage (e.g., Wessman et al. 1988; Martin and Aber 1997; Asner 1998). Spectral detection of biochemical constituents in plant tissue grew initially from work with benchtop spectrometers, which highlighted reflectance features associated with C-H and N-H bonds. Air- and space-borne instruments provide essentially the same type of spectral data but present the added challenge of accounting for signal variation due to atmospheric absorption and variation in canopy structure. In one recent application remote estimates of canopy N concentrations in a temperate forest landscape were combined with extensive field measurements of forest C and N cycling to yield mapped estimates of aboveground net primary production (NPP) and soil C:N ratios (Colorplate 1; Ollinger et al. 2002; Smith et al. 2002). Spatial estimates of leaf N and soil C:N could, in turn, be related to losses of nutrients and dissolved organic carbon from watersheds.

Although hyperspectral instruments have been widely explored with aircraft platforms, the number of sensors in operation is still quite small and the limited opportunities for obtaining data have prevented its widespread use. In addition, the challenges of collecting, storing, and interpreting hyperspectral data are still substantial and will require additional methodological development before the full range of its capabilities and/or limitations is appreciated. Recent development of new satellite sensors (e.g., NASA's Hyperion instrument aboard the Earth Observer 1 [EO-1] platform) make further exploration of this potential promising.

A Challenge for the Future: Resolving Fundamental Patterns of Element Interactions

The world is currently experiencing unprecedented fluxes of biologically and geochemically active elements, but we lack the ability to forecast the consequences of

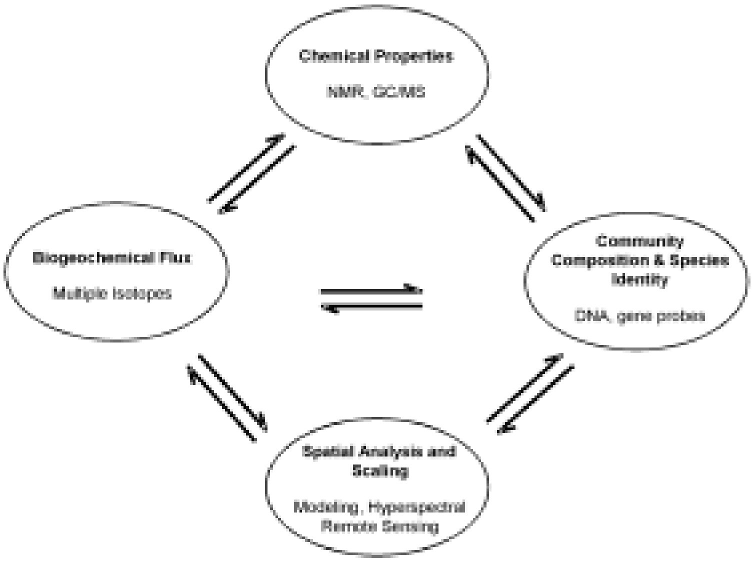


Figure 4.2. Potential for future element interactions research involving combined application of new methodological approaches

these fluxes (Austin et al., Chapter 2, this volume), in part, because our basic understanding of how element interactions are played out is insufficient. We have seen cases where organism stoichiometry provides a useful conceptual framework, but there are also numerous sources of variability that reduce the applicability of simple element ratios. What we presently lack is a basic understanding of the degree to which multiple element interactions follow globally generalizable patterns versus local sources of complexity.

Fundamental biogeochemical mechanisms such as nutrient turnover, atmospheric deposition, and biological N_2 fixation are common across ecosystems, but their relative importance varies tremendously across elements and environmental conditions. Experimentation in element interactions poses unique challenges because, in addition to the inherent complexity of biogeochemical interactions, their drivers are distributed heterogeneously across the globe. For example, whereas patterns of atmospheric nitrogen deposition are driven by human industrial and agricultural activities, patterns of geologically derived elements, such as phosphorus, typically follow the geological origin of the underlying parent material. The biota, which largely controls cycling of elements, also has independent patterns resulting from biogeographic mechanisms. Many large-scale experiments, those of both a manipulative nature and an observational nature, in a range of different ecosystems have added to our understanding of biogeochemistry. We need to move beyond these experiments, however, in trying to explore elemental interactions in a more comprehensive way, making use of a combination of the techniques discussed in the preceding sections (Figure 4.2).

A Suggested Experimental Approach for Basic Element Interactions Research

Single-factor manipulation experiments, statistically analyzed with analysis of variance techniques (e.g., response of element X to an addition of element Y), capture the behavior of a system at only two points and allow only partial understanding of element interactions. Compared with a world in which tremendous heterogeneity and nuanced responses are the norm rather than the exception, it is worth considering whether experimental studies should focus more on multifactorial/multitreatment experiments that more closely resemble the range of conditions present in natural systems. Such experiments would allow scientists to move beyond simple tests of significance toward derivation of full response surfaces, giving better indications of possible thresholds and a continuum of ecosystem responses. Experiments also need to be planned with a longer time frame (minimum of five years) than traditional funding cycles allow because element manipulations can involve long lag times before interactive responses become evident.

We fully recognize, however, that detailed multifactor and treatment experiments are not possible, or even desirable, everywhere. We suggest that parallel series of experiments could be linked to help address the central questions about element interactions. This series of experimental studies might have two tiers of tightly coordinated manipulative experiments and a dense network of monitoring sites. The first tier would be made up of a few multifactorial experiments that would explore major element interactions and their causal mechanisms. At this level the detailed chemical and biological identity techniques already described could be used to explore mechanistic responses to varying treatments in great detail. The first-tier experiments would be complemented by a larger number of less-intensive manipulative experiments distributed along broad gradients of the major biogeochemical drivers. These experiments would provide information on the response surface of the major driving variables and provide validation of the mechanisms suggested by the first-tier experiments.

Finally, a dense network of element cycling monitoring sites established across broad environmental gradients would complement manipulative experiments by providing information across a much wider range of conditions. This network would provide information on a range of biogeochemical parameters and their

Table 4.1. Elements of a proposed experimental framework for examining basic fundamental patterns of element interactions

Site level	*Elements of experimental design*	*Criteria for site or measurement selection*	*Duration and process level detail of measurements*	*Measurements targeted at key uncertainties and utilizing new technologies*
Tier 1	Observational (process based) measurements Multifactorial and/or multilevel manipulative experiments	Representative of mechanisms (and not necessarily climatic, edaphic, or organism/community type) Accessibility	Seconds to years High detail	Molecular and microbial identity/composition •Element pool sizes and fluxes •Ecosystem responses to perturbations (mediated by elemental availability) •Organism-ecosystem interactions
Tier 2	Experiments or observations arrayed along broad gradients of major biogeochemical drivers Broadly distributed experiments or observations that utilize similar measurements/manipulations	Representative of the important biogeochemical drivers (e.g., soil or vegetation type, climatic zones)	Months to decades Intermediate detail	•Ecosystem response to multiple stressors (e.g., N, CO_2, land use) •Elemental effects of change in biological community composition in time and space
Tier 3	Monitoring networks and environmental gradients for large-scale (regional to global) observations of element cycling	Broad geographic distribution	Years to centuries Low detail	•Satellite remote sensing of biosphere properties involving two or more elements

distribution and, we believe, would add tremendous value to regional to global-scale biogeochemical studies. Ecologists have often taken advantage of the natural variation that occurs along environmental gradients, and several efforts are already underway that could be complemented by the addition of element cycling measurements. The International Geosphere-Biosphere Programme (IGBP) has expanded this concept to the global scale with the idea of "transects." They have proposed a global system of about twelve transects, each of which would be thousands of kilometers long, spanning major gradients believed to be susceptible to human-induced changes and climate change. As an example, the Kalahari Transect, mostly in Botswana, is one of three proposed transects spanning the precipitation gradient between the humid tropics and the subtropical deserts. Koch et al. (1995) proposed the following set of benefits that could be gained from these transect efforts:

- The consequences of future changes could be inferred from patterns currently exhibited along the existing gradient.
- Threshold effects may be identified along a continuous environmental gradient.
- A mechanism for extrapolation would be gained that links the site-based work of ecologists to the regional scale of climatologists and policy makers.

Finally, many limitations in trying to quantify elemental interactions exist because of the constraints of time, available labor, and financial resources. Limitations in the availability of financial and human resources preclude replicating key experiments across the globe in a way that would match the existing combinations of drivers. Not only are resources scarce and insufficient, but they also have a skewed distribution, with a large fraction concentrated in developed countries. Most developing countries have smaller gross national products and devote a smaller fraction to research and development than developed countries.

Data Management and Synthesis

Besides collecting new data, we recognize that the scientific endeavor of understanding multiple element interactions needs to improve the management and archiving of existing data. Remarkable progress has occurred as a result of the systematic analysis and synthesis of data that were collected all over the world with different purposes. For example, compilations of existing data sets have given us important new insight into a wide range of topics, including global patterns of N cycling and ^{15}N distributions (Handley et al. 1999), linkages between vegetation N status and C uptake (Reich, Walters, and Ellsworth 1997; Green, Erickson, and Kruger 2003), plant response to altered CO_2 concentrations (Medlyn et al. 2001), and regional to global controls on C fixation (Sala et al. 1988). In many cases, it is not justified to collect new global data sets when reevaluation of existing data is feasible. For multiple scientific endeavors, there are examples of national-level organizations developed to facilitate the organization, storage, and distribution of data. For biodiversity, national services such as CONABIO (Comisión Nacional de Biodiversidad) in Mexico and others now play important roles in managing scientific data. Similar examples exist in many countries for weather data, remote-sensing information, and genetic research. International efforts are being developed under the auspices of the United Nations. Despite these signs of progress, a great deal of biogeochemical data remains broadly distributed and poorly characterized. The lack of centralized data storage and organization limits advances in modeling and synthesis and should be a central focus of new efforts for improved information management at a national or international level.

Conclusions

As with many fields of science, progress in the study of element interactions has often involved an interplay between technological advancements, which provide new information on poorly understood processes, and conceptual breakthroughs, which give us new perspectives on the systems we study and place new emphasis on the need for additional measurements. In this chapter we have highlighted several recent examples of this interaction and have suggested areas in which present knowledge is constrained by data availability, conceptual understanding, or a mismatch between the two.

The status of current ecosystem models illustrates some of the limitations in our present state of knowledge. Despite the well-known role of multiple element interactions in a number of biogeochemical processes, few ecosystem models explicitly include interactions beyond those that involve N and C. To a large extent, this reflects a lack of basic understanding regarding the processes controlling multiple element interactions and the degree to which they can be generalized at various spatial or temporal scales. In other cases model limitations arise either from limitations of the underlying paradigms on which they are based (e.g., whole-system optimization or single-element resource limitation) or from a failure to capture processes that act across multiple levels of organization (e.g., population, community, and ecosystem). Finally, models are frequently limited by a shortage of data available for parameterization or validation. This shortage stems both from the inherent difficulties of measuring certain biogeochemical variables and from discrepancies between variables used in models and those that can be readily measured in the field.

Current gaps in data availability require additional methodological development in a number of areas, including determination of chemical identity, species identity, and approaches for scaling existing information through space and time. Methods for determining chemical identity are important because the chemical properties of organic compounds often influence element interactions in ways that cannot be inferred from element ratios alone. Similarly, because the forms and ratios in which elements are used by organisms can vary among species, methods for determining species identity are important in systems where sampling individuals has historically been problematic (e.g., microbial communities, belowground plant tissues). Examples of recent technological innovations that offer promise in these areas include the use of NMR techniques for analyzing the structure and composition of complex organic compounds, development of bioreporters that produce observable responses to nutrient limitation by specific elements, and combined application of flow cytometry, stable isotopes, and DNA analysis for determining the influence of microbial species or functional groups.

The need to develop methods for scaling information on element interactions stems from the fact that important mechanisms occur at spatial scales ranging from microscopic to global and at temporal scales ranging from seconds to millennia. For example, relating microbial processes that affect nutrient dynamics in soils to patterns of atmosphere-biosphere exchange that affect element cycling globally requires well-validated methods by which information can be translated from one scale to another. Recent improvements in remote-sensing techniques such as laser altimetry and imaging spectroscopy offer great promise in this arena. For these approaches to explicitly address patterns of multiple element interactions, however, particularly with respect to elements that cannot be observed directly (e.g., those occurring in underlying soil substrate), they require further development and integration with other methods.

Lying beneath all of these individual challenges is the fact that the discipline of biogeochemistry still has a limited theoretical basis for understanding multiple element interactions. Although the importance of ecological stoichiometry as a potential controlling mechanism has been appreciated since Redfield's seminal paper on the nutrient ratios of plankton in marine ecosystems (Redfield 1958), we are also well aware of the many factors confounding a simple extension of organism-based ratios to global element cycling patterns. We suggest that one strategy for overcoming this hurdle would be to invest in a series of coordinated experiments intended to examine the response of individual elements to changes in the pools or fluxes of other elements. The structure of these experiments would include both intensive multifactorial manipulations designed to elucidate fundamental mechanisms and spatially distributed studies that focus on specific interactions but across broad environmental gradients. The intended outcome of these experiments, in combination, would be a series of multielement response surfaces and a better understanding of the degree to which element interactions are controlled by globally generalizable patterns versus local sources of complexity.

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