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Stable isotope tracers: Enriching our perspectives and questions on sources, fates, rates, and pathways of major elements in aquatic systems

Patricia M. Glibert ^(D), ^{1*} Jack J. Middelburg ^(D), ² James W. McClelland ^(D), ³ M. Jake Vander Zanden⁴

¹Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, Maryland

²Department of Earth Sciences, Utrecht University, Utrecht, The Netherlands

³Marine Science Institute, University of Texas at Austin, Port Aransas, Texas

⁴Center for Limnology, University of Wisconsin – Madison, Madison, Wisconsin

Abstract

Stable isotope applications have evolved from simple characterizations of isotope composition in organisms and organic matter, to highly complex methodologies on scales ranging from individual compounds and cells, to broad ecosystem-level approaches. New techniques are rapidly evolving, allowing novel, difficult, and inconvenient questions to be addressed. This article aims to provide an overarching perspective on how the field has evolved and where it is going with regard to aquatic systems, some of the oceanographic and limnological concepts derived from these approaches, and important challenges. To this end, we highlight a selection of natural abundance and tracer enrichment studies that represent a wide range of stable isotope applications. These include studies of rate processes and biogeochemical cycling, source tracking, food webs, and paleoenvironments. Our coverage of stable isotope applications is by no means complete, but by highlighting a mixture of classic and new applications across a wide range of research areas, our goal is to convey the power of stable isotope tools and the excitement in this rapidly expanding field, while also encouraging the scrutiny and healthy respect for limitations and assumptions necessary to take full advantage of these powerful tools.

Stable isotope analysis is a tool. Applications of stable isotope methods-from hydrography to physiology, geochemistry, and ecosystem management-are as varied as the creativity of the users and the questions being asked. Applications of stable isotopes have contributed to our understanding of many of the major concepts in oceanography and limnology. In this article, we aim to provide a perspective on stable isotope applications, how the field has evolved and where it is going as it relates to the aquatic realm, some of the concepts derived from these approaches and important challenges. This article is not a review per se-that is a near-impossible task, but by highlighting some classic, as well new approaches and studies, across a wide range of applications, our goal is to convey excitement in this rapidly expanding field, while also encouraging the scrutiny and healthy respect for limitations and assumptions that is necessary to take full advantage of these powerful tools. Examples and studies included herein are not meant to be comprehensive, but rather to be illustrative of the breadth of applications.

This article begins with a synopsis of the vocabulary and fundamental concepts of stable isotope applications. A brief review of the time line in tools and capabilities is then given. Next, focusing on the major stable isotopes of carbon, nitrogen, oxygen, silicon, and sulfur (C, N, O, Si, and S)-but biased toward our experience with C and N-examples of applications and lessons learned from these applications are highlighted. In general, the types of questions/applications fall into several major categories that we will emphasize, including biogeochemical rate processes, source tracking, food webs, and paleoecology/global change (Fig. 1). By painting with a broad, multi-scale, multi-ecosystem, multi-isotope brush, it is hoped that this effort will serve as both an overview and introduction to methodological approaches and may inspire new questions, new applications, new conceptual advances, and cross-fertilization of ideas. The perspective we aim to convey is that the field has advanced from focusing primarily on tool development and testing, to increasing use of isotopic abundance and labeling to address novel, difficult, and inconvenient questions.

Basic principles

Kinetic isotope effects underlie many of the differences in stable isotope ratios that we observe among ecosystem

^{*}Correspondence: glibert@umces.edu

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Fig. 1. Conceptual diagram of the elements for which the most common stable isotopes are used in ecology and the various applications emphasized herein.

components, and fractionation associated with specific biogeochemical reactions, different isotopes, and different physiological states of organisms are often distinct, allowing fundamental processes to be interpreted based on changes in stable isotope values. The nomenclature of stable isotopes is quite varied. Some of this variability relates to specific applications, but this does create potential for confusion. The vocabulary associated with enrichment studies is also different from that of the natural abundance stable isotope literature. Basic terms and relationships are defined and provided here; more detailed reviews of terminology and best practices based on publications of the Commission on Isotopic Abundances and Atomic Weights of the International Union of Pure and Applied Chemistry (IUPAC) can be found in Coplen (2011) and Bond and Hobson (2012).

In general, natural abundance stable isotope ratios are expressed using the lowercase delta (δ) symbol, which represents the deviation in isotopic ratio of a sample relative to a standard minus 1. Thus,

$$\delta = R_{\text{sample}} / R_{\text{standard}} - 1 \tag{1}$$

in which *R* is the isotopic ratio of sample or standard (e.g., ${}^{15}N/{}^{14}N$). The typical standards are atmospheric N for $\delta^{15}N$, the (Vienna) PeeDee Belemnite limestone for $\delta^{13}C$, and Vienna standard mean ocean water for ${}^{18}O$ or ${}^{17}O$.

Many studies report $\delta \times 1000$ to amplify the small differences between samples and standards. In many such studies,

the unit (parts per thousand; $\%_0$, per mil) may be implied rather than given expressly (e.g., Fry 2006). However, δ values may also be reported as parts per hundred (% or percent), parts per million (ppm), or other units, depending on the specific element or application (Coplen 2011).

Differences in δ values between two substances are expressed with an uppercase delta, Δ .

Thus:

$$\Delta_{A-B} = \delta_A - \delta_B \tag{2}$$

Differences in δ or Δ , for example $\Delta \delta^{15}$ N or $\Delta \delta^{13}$ C, may be between reactant and product, food source and consumer, or any other comparison between a measured value and a baseline, however that is defined. Values of Δ may reflect changes in isotope ratios associated with isolated processes or net effects of multiple factors influencing differences in isotope values between any two pools of interest. The Δ term is also used for mass-independent isotope variation (e.g., Δ^{17} O; Δ^{33} S; Δ^{36} S) to express deviations from a specific mass-dependent fractionation law and for *clumped* isotopes (e.g., Δ^{47}) to quantify deviations from a stochastic distribution (Eiler 2007). Clumped isotopes represent a comparatively new field of isotope applications, in which variations in the isotopic composition of both C and O of CO₂ (representing the substitution of ¹³C and/or ¹⁸O in the CO₂ molecule—with a mass of 47) are measured in the same sample, with the advantage that inferences, such as those related to paleotemperatures, can be made without assuming a value for the δ^{18} O value of seawater. It is therefore important for practitioners to define how Δ is being used and what it represents in any given study.

Differences in δ result from equilibrium or kinetic isotope effects. The fractionation factor associated with a particular isotope can be designated as α , the ratio of isotope ratios in product (P) and reactant (quotient Q), or:

$$\alpha = R_{\rm P}/R_{\rm Q} \tag{3}$$

More formally α is often defined as the ratio of $R({}^{i}E/{}^{j}E)_{P}$ and $R({}^{i}E/{}^{j}E)_{Q}$, where *R* is the isotope ratio of heavier isotope ${}^{i}E$ and lighter isotope ${}^{j}E$ of element E in substances P and Q; α thus represents the rate constants for the heavy and light isotopes. As with Δ , there are variations in the literature in how α is defined, specifically which substance, and therefore which isotope, the lighter or heavier, is in the numerator or denominator of each *R* (e.g., Hayes 2004; Fry 2006). Many geochemists prefer the definition above, with the lighter isotope in the denominator, while many chemists and biologists adopt the inverse formulation (Fry 2006). Where the lighter isotope in the denominator, α -1 is defined as ε , the kinetic isotope effect. Thus:

$$\varepsilon = \alpha - 1 \tag{4}$$

This kinetic isotope effect, or isotope discrimination factor, can be thought of as the ratio of rates with which isotopes are converted from reactant to product. This value, as with δ , may be multiplied by a factor, typically 1000 and expressed per mil (%) to magnify small differences (Mariotti et al. 1981; Lajtha and Michener 1994). ε and Δ are approximately equal when δ values fall within the natural abundance range, but they diverge as δ values become increasingly positive or negative (Coplen 2011). As a rule of thumb, ε and Δ can be used interchangeably when δ values fall between +20‰ and -20‰ (Fry 2006). As discussed above for Δ , the isotopic fractionation factor α (as well as ε) can be used with more or less specificity, for example, as related to equilibrium reactions, reactants and products of nonreversible reactions, or net effects of multiple factors, and thus it is important to define what the calculated values specifically represent in a given study. Ultimately, Δ , α , and ε are contextually defined.

For unidirectional reactions in a closed system, kinetic isotopic fractionation follows that of a Rayleigh distillation model (reviewed by Sigman and Casciotti 2001; Sigman et al. 2009; Van Hale and Frew 2010; Fig. 2). As reactant is converted to product, instantaneous isotope fractionation (ε_{inst} ; i.e., the fractionation inherent to the reaction) remains constant, but the observed fractionation (ε_{net}) between the residual reactant pool and the cumulative product changes. In general, the lighter isotope is incorporated into product at a faster rate than the

heavier isotope, causing the residual reactant pool to become progressively more enriched in the heavier isotope with time. Newly formed product remains offset from the residual reactant pool by ε_{inst} , while the isotope ratio of the cumulative product converges on that of the original reactant pool. When all of the heavy and light isotopes in the reactant pool have been converted to product, there is no longer an observable isotope effect. However, even when kinetic fractionation effects are strong, resolving the different processes that contribute to these effects remains challenging.

Applications involving added isotope to trace enrichment during a process generally involve a different set of terms and assumptions (e.g., Dugdale and Goering 1967; Dugdale and Wilkerson 1986; Glibert and Capone 1993; Fry 2006). In this case, the atom fraction or atom% notation is more common than the δ notation. Atom fraction is an absolute measure of the number of atoms of the isotope relative to 100 atoms of the element, the mole fraction of heavy isotope relative to heavy plus light. When expressed as a percent, this value is known as atom percent (at %, or ¹⁵AP, in which the superscript represents the heavy isotope—in this example ¹⁵N). Atom fraction or at %, assuming no measurement error, are always precise, whereas Δ is a relative value. The difference between δ and at % are illustrated for N₂, for which natural atmosphere is the accepted standard. Its δ value is set to 0.0‰, but its at % is 0.366, as atmospheric N_2 has 0.366 atoms of ¹⁵N₂ in 100 atoms of N₂. Atom fraction excess (or in % terms, at % excess, APE) is that atom fraction in a sample minus the measured standard. At % enrichment is the amount of enrichment added in the experiment (e.g., ¹⁵NO₃) relative to the total NO_3 (added + ambient).

The velocity of the rate of uptake or transformation of an isotopic enrichment into a product (V, also termed specific uptake rate, with units of reciprocal time, i.e., h^{-1}), is the excess atom fraction of the target or product pool divided by that of the initial enrichment times the duration of the experiment (Sheppard 1962; Dugdale and Goering 1967):

V = at% excess of product/[at\% enrichment of substrate × time] (5)

Tracer experiments are commonly used in determining the uptake rate of N by phytoplankton. Collos (1987) suggested a modification to Eq. 8 for conditions under which the phytoplankton may be assimilating more than one N compound; this may cause substantial artifacts due to the nature of the measurements of ratios of the isotopic compound. Glibert et al. (1982*b*) also modified this equation to account for the dilution of isotope that occurs due to remineralization of substrate (especially NH₄) during the incubation. The magnitude of errors associated with failure to apply such modifications depends on the type of sample (e.g., laboratory culture vs. field sample), length of incubation, amount of isotope added, and other factors. Kinetic isotope effects in understanding of enrichment studies are typically



Fig. 2. Relationship between isotope fractionation of reactant and product and their consumption, Rayleigh distillation kinetics. The term ε denotes the difference in isotope enrichment between reactant and product. Note that at the initiation of the reaction and near completion of the reaction, this value is difficult to determine as there is either virtually no product at the start and if the reaction has gone to completion, no reactant at the end (ends of these curves have dashed lines).

ignored, as the magnitude of tracer addition generally overwhelms such effects and it is thought that introduced isotopes do not modify metabolic pathways. However, Andriukonis and Gorokhova (2017) have shown that there are profound differences in the growth of algae when provided ¹⁵N enriched media. Such effects may need to be (re)considered when very high isotope enrichment levels are used, as in experiments involving DNA or proteome labeling.

To calculate an absolute flux (denoted as ρ), such as a rate of uptake or transport of a particular N compound, the specific uptake rate (*V*) is multiplied by the concentration of that element in the target pool. For example, for N uptake,

$$\rho = V \times \text{PN} \tag{6}$$

where PN is the particulate N concentration of the sample, ideally measured at the end, rather than beginning of an incubation. In nitrification studies where the added isotope is NH_4 , the velocity of the rate is calculated the same way, but the target pool is the concentration of NO_2 or NO_3 .

In sum, isotope notation, regardless of preferred terms, is the consideration of ratios: ratios of the less abundant to the more abundant isotope, ratios in samples relative to standards, and ratios of enrichment relative to total compound availability. Many final calculated values are, in fact, ratios of ratios of ratios. Moreover, common notations and calculations are derived to amplify the often very small differences between samples and standards. This can make it difficult to resolve sensitivities and/or sources of error. The importance of recognizing sources of error and assumptions in isotopic approaches is a theme returned to repeatedly throughout this article.

The isotope time line

Mass spectrometers have been in use for about a century for isotope analysis, initially largely in laboratories of a few renowned physicists, but the sophistication of isotope applications is rapidly advancing, and tools are quickly evolving (Fig. 3). Pioneering work by Craig (1953) revealed that C isotope differences among organisms are primarily generated during primary production. Through seminal work by Goering et al. (1964, 1966), and then by Dugdale and Goering (1967), tracer studies of N were developed. By applying ¹⁵N tracers, mostly in the form of ¹⁵NO₃ and ¹⁵NH₄, the understanding of environmental regulation of N uptake by phytoplankton was



Fig. 3. Infographic depicting the generalized time line of isotope applications (top) and instrumental developments (bottom).

made possible. From these studies, the theoretical basis for the differentiation of "new" and "regenerated" N was developed (Dugdale and Goering 1967), and several decades of focused work on phytoplankton nitrogenous physiology was made possible. Early natural abundance work showed that C isotopes are transferred to consumers without fractionation (DeNiro and Epstein 1978), while N isotope ratios of consumers become enriched by a few per mil during trophic transfers (DeNiro and Epstein 1981; Minagawa and Wada 1984). The latter studies provided the basis and rationale for the use of N isotopes in trophic transfer studies.

Most measurements made during this era were on "bulk" particulate matter, whether algal suspensions from laboratory cultures or field-filtered participate matter, and analyzed using isotope ratio or emission spectrometers. Size fractionated material (e.g., Glibert et al. 1982*a*; Probyn 1985; Probyn and Painting 1985; Probyn et al. 1990), and that of isolated larger organisms, such as zooplankton and tissue of larger animals, was also possible (e.g., Montoya et al. 1990). These analyses generally required a relatively large sample size, precluding analysis of single microbes, protists, or individual nematodes or zooplankton. While organisms of a few 10 s of micrometers can be physically isolated, lumping of individuals was necessary to obtain the minimum μ g of C or N necessary for isotopic measurements. This limited species-specific isotope ratio

measurements and investigations of individual life stages of these small organisms. Based on work by Carman and Fry (2002), modifications to conventional elemental analysis and mass spectrometry approaches were developed that allowed for both $^{13}\mathrm{C}$ and $^{15}\mathrm{N}$ analysis in very small samples.

Techniques for isolation or extraction of dissolved nutrients, e.g., NH₄, NO₃, NO₂, were a major advance, furthering the ability to resolve rates of transformation of pathways such as denitrification, nitrification, and ammonification. Such methods were chemically and labor-intensive, and even more so in the presence of salt. The ability to resolve the ¹⁵NH₄ and its change over time in experiments in which tracer ¹⁵NH₄ was added led to an expansion in application of isotope dilution approaches in sediment and water-column studies (e.g., Harrison 1978; Blackburn 1979; Caperon et al. 1979; Glibert 1982; Glibert et al. 1982b; Paasche and Kristiansen 1982; Harrison and Harris 1986). Isotope dilution approaches allow the simultaneous resolution of two opposing processes, such as N incorporation and N regeneration in a natural sample. An enrichment of isotope is made, and its incorporation is measured in biomass. However, over time, the atom fraction of the isotope in dissolved form (i.e., the NH₄ in water) will decline because of substrate recycling that favors the lighter isotope (Glibert et al. 1982b). This is due to the fact that those organisms carrying out regeneration, such as microzooplankton, will release NH4 that is not

isotopically labeled as the short time scale of the experiment precludes this. Sigman et al. (1997) developed an extraction method for NO_3 in seawater that soon found widespread use for assessment of the isotope effects associated with nitrification.

Mass spectrometers advanced and the coupling of gas chromatographs (GC) with mass spectrometers were especially important (Fig. 3). The development of the continuous flow interface between an elemental analyzer and isotope ratio mass spectrometer allowed for automation of sampling by the instrument (no longer did individual samples have to be prepped independently for injection into the mass spectrometer). Sample throughput increased many fold and environmental and ecological applications became routine. With more rapid and automated analysis, ecological and biogeochemical studies could now be replicated, and environmental gradients could be better resolved. The coupling of gas chromatographs and mass spectrometers also facilitated the dual analysis of C and N on the same sample.

More affordable mass spectrometers, which included quadrupole mass spectrometers, became available, mostly in the early 1990s (Fig. 3). Quadrupole mass spectrometers led the way to applications of membrane inlet mass spectrometry (e.g., MIMS, Kana et al. 1994), which can now resolve gasses in water with very high precision (e.g., N₂/Ar) and can be used for isotope discrimination as well. A MIMS instrument coupled to a quadrupole mass spectrometer is often used, for example, in applications of the isotope pairing method for denitrification and N₂ fixation (e.g., An et al. 2001; Lundstrum and Acki 2016).

Compound-specific isotope analysis (CSIA) has also been rapidly advancing (Haves et al. 1990; Boschker and Middelburg 2002; Middelburg 2014; Figs. 3-4). Here, rather than bulk isotopic analysis of particulate or aqueous samples, various biomarkers, such as lipids, carbohydrates, or amino acids (AAs), are extracted and their isotope composition compared (Boschker and Middelburg 2002). Biomarkers are compounds that ideally are specific to a species, or more usually a group of organisms. The CSIA approach can be used at natural abundance as well as for tracer applications. When coupled with other advancing methods, such as those of molecular biology, this approach can be very powerful in the analysis of microbial communities, and also of the diet of animals (e.g., McClelland and Montoya 2002; de Kluijver et al. 2012). In food web studies, the ability to resolve AAs aids dietary reconstruction in that the ¹³C and ¹⁵N enrichment of individual AAs between food source and consumer varies widely, but essential AAs have distinct isotope fingerprints which may be passed through food webs without modification, giving clues of source identity. In addition to food web studies, CSIA is finding increasing application in studies of compound biodegradation where contaminants are of concern.

The ability to resolve isotopes of microbes has advanced with the ability to physically isolate microbes by techniques such as flow-cytometry coupled to cell sorting and subsequent isotope analysis. Flow cytometric sorting of phytoplankton or bacterioplankton cells followed by isotope ratio mass spectrometry has been done both for natural abundance studies (e.g., Pel et al. 2003; Bontes et al. 2006 in lakes; Fawcett et al. 2011 in ocean) and tracer applications (e.g., Lipschultz 1995; Bontes et al. 2006). Recently, van Roij et al. (2017) reported a laser ablation combustion isotope ratio mass spectrometer allowing for measurement of ng of particulate organic C. Such a technique was also used to measure $\delta^{13} C$ in single-species dinoflagellate cysts (Sluijs et al. 2017). This technique can, in principle, bridge the gap between traditional isotope data for larger organisms and compound-specific isotope ratio mass spectrometry for microbes (Fig. 4). Resonance Raman microspectrometry in combination with ¹³Cbicarbonate labeling has been introduced to probe single-cell growth rates of phytoplankton (Li et al. 2012; Taylor et al. 2017). Cavity ring-down spectroscopy is also emerging as a new high-throughput, easy-to-use technique providing high-quality isotope ratio data for C in dissolved and particulate organic and inorganic C and methane, for N in nitrous oxide, and H and O in water (Becker et al. 2012; Maher et al. 2013; Erler et al. 2015). The relatively modest investment and robustness of this method will further stimulate the use of stable isotopes in experimental and field studies.

Nanometer-scale secondary ion mass spectrometry (nano-SIMS) provides isotope ratio imaging with a spatial resolution of < 100 nm and has been used together with ^{15}N , ^{13}C , or ^{2}H labeling to study dissolved inorganic C uptake, N₂ fixation, NH₄ and NO₃ assimilation by phytoplankton communities (Ploug et al. 2010; Musat et al. 2012), mixotrophy (Terrado et al. 2017), symbioses (Thompson et al. 2012), C processing by cable bacteria (Vasquez-Cardenas et al. 2015) and consortia of microbes in sediments (Hatzenpichler et al. 2016). When coupled with other highly sensitive tools such as molecular approaches, information on microbial associations and other difficult to resolve pathways is now possible. An additional method gaining rapid application within microbial ecology is that of stable isotope probing (SIP, e.g., Radajewski et al. 2000; Neufeld et al. 2007). In these methods, an enrichment is made with an isotopic compound, e.g., ¹³C or ¹⁵N, and after uptake by natural or cultured organisms, separation of nucleic acids is undertaken using a density gradient ultracentrifugation. Nucleic acids with different densities can thus be separated and their differential incorporation of the label can be measured. Various molecular analyses of these labeled nucleic acids can be undertaken in tandem, using fingerprinting, clone libraries, metagenomics, and so on. By linking function and taxonomic identity, the responses and interactions of microorganisms to ecological conditions can be resolved at very fine scales.

Collectively, these tools are rapidly increasing sophistication in biogeochemical studies, source tracking, food web analysis, and paleoecological studies. As will be shown in the



Fig. 4. Two conceptual depictions of the relationship between organismal insight and the quantitative resolution or instrumental approach necessary. Panel **A** highlights that biogeochemical rate measurements (often based on isotope techniques) provide accurate quantification but no information on the identity of organisms involved. Molecular biology tools provide high-quality information on the presence and potential capabilities of organisms, but limited information on their rates. Compound-specific isotope characterization of biomarkers (DNA, RNA, PLFA, tetraether lipids, D-alanine) in combination with tracer techniques provide rates as well as identity information but with low-to-moderate taxonomic resolution and intermediate accuracy. NanoSIMS can provide individual cell isotope ratio measurements and thus provides high accuracy and high-taxonomic resolution, as well as spatial context. Modified and redrawn from Middelburg (2014) under a creative commons license. Panel **B** highlights how different isotope analysis can be related to either microbial functioning or food web studies and the scale of the organisms for which they are most applicable.

next section, the examples of applications are wide and stable isotopes have become a critical part of a larger toolkit by which ecological, oceanographic, limnological, and paleoecological questions are addressed.

A deeper dive: Insights from classic to cutting-edge isotope applications

Examples that are highlighted here were selected to provide a breadth of approaches, ecosystems, questions addressed, or insights gained. These examples paint the field with a broad brush, a glimpse into this enormous diversity of applications, and are broadly divided in studies related to rate processes and biogeochemical cycling, source tracking based on isotopic values, food web studies, and paleoecological/paleoceanographic studies. These categories are for convenience only, as many approaches and insights span multiple disciplines.

Rate processes and biogeochemistry

Stable isotope applications for the study of rate processes and biogeochemical cycling are so extensive that justice cannot be done to all the insights that have been so gained; only a few snapshots are captured here. Quite simply, isotopes and especially isotope enrichment studies—provide the foundation for our understanding of the biogeochemical pathways of nutrient cycling and the physiological rate processes associated with primary production and nutrient assimilation.

Nitrogen

An enormous literature exists with respect to applications of stable isotopes in studies of oceanic N cycles (see reviews by Sigman and Casciotti 2001; Sigman et al. 2009; Casciotti and Buchwald 2012; Ryabenko 2013). Much is known regarding the isotopic characteristics of the predominant sources and sinks of N, and processes such as N_2 fixation, nitrification, and denitrification.

The fixation of N₂, which has been extensively studied in the ocean (with a particular focus on *Trichodesmium*), introduces bioavailable N at isotopic values near atmosphere; i.e., there is little isotopic discrimination (δ^{15} N ~ 0‰; Minagawa and Wada 1986; Macko et al. 1987; Montoya et al. 2002). Not only does this "light" N₂ (compared to oceanic NO₃, for example) produce a characteristic organic matter isotopic signature, but it can also be traced through the food web (Montoya et al. 2002). Thus, using a mass balance approach, Montoya et al. (2002) were able to estimate the N fixed by diazotrophs according to:

% diazotrophs = 100 ((
$$\delta^{15}N_{m} - \delta^{15}N_{ref}$$
)/($\delta^{15}N_{d} - \delta^{15}N_{ref}$)) (7)

where $\delta^{15}N_m$ is that measured in the sample, $\delta^{15}N_{ref}$ is the reference value for nondiazotophic plankton, and $\delta^{15}N_d$ is that of the diazotrophs. This approach was applied in an assessment of the influence of *Trichodesmium* in the stable isotopic composition of different size fractions along in a study of the subtropical North Atlantic (Mompeán et al. 2013). This study showed that the contribution of atmospheric N was larger than anticipated, and there was a major dependence of the planktonic food web on this N derived by fixation, revealed by the calculated contribution of N from diazotrophs to four different size classes of plankton.

Denitrification, in contrast to N₂ fixation, is a process with strong isotopic discrimination. Denitrifiers, in consuming NO₃ in the water column, use the lighter form of N at a faster rate than the heaver form of N, leaving NO₃ in the dissolved pool that can be substantially enriched with ¹⁵N. Isotope discrimination factors are on the order of 20-30% (Cline and Kaplan 1975; Altabet et al. 1999; Voss et al. 2001). In the sediment, however, denitrifiers are more often limited by available NO₃, and consume most (all) of it, and thus do not leave a residual enriched pool of NO3 and isotopic discrimination is not resolvable (Brandes and Devol 1997; Sigman et al. 2003, 2009). To overcome this problem in resolving sediment denitrification rates, the isotope pairing method may be applied, which is based on measurements of the changes in N2 gas with different isotope compositions $({}^{29}N_2 = {}^{14}N + {}^{15}N_2 = {}^{15}N + {}^{15}N)$ after enriching the overlying water with ¹⁵NO₃ (e.g., Nielsen 1992; Middelburg et al. 1996; Steingruber et al. 2001).

Although the processes of NO₃ assimilation and denitrification have been shown to have fairly strong isotopic effects using N isotope analysis, unraveling those associated with the process of nitrification has proven to be more challenging. This is largely due to the fact that nitrification is carried out through NH₄ oxidation to NO₂ and subsequent NO₂ oxidation to NO₃. The former is due to chemoautotrophic processes of NH₄-oxidizing bacteria and archaea (AOB and AOA), while NO₂ oxidation is due to the chemoautotrophic process of NO₂-oxidizing bacteria (Casciotti and Buchwald 2012). Nitrification also processes N₂O as an intermediate. By using multiple isotopes within the same compound, isotopologues, different processes could be revealed. By resolving both $\delta^{18}O$ and δ^{15} N in N₂O, it was shown that the N₂O produced by AOA incorporates O from O₂, not H₂O, and the δ^{15} N and δ^{18} O produced by AOA is more enriched than that produced by AOB (Santoro et al. 2011). Moreover, dual isotope measurements of both NO₂ and NO₃ have revealed NO₃ regeneration in the euphotic zone and in O₂ deficient zones (e.g., Sigman et al. 2005), and thus nitrification is now recognized to play a significant role in the oceanic N cycle (e.g., Casciotti and Buchwald 2012). Recently, triple isotopes ($\delta^{15}N$, $\delta^{18}O$, and Δ^{17} O of NO₃) have been applied to the study of rates of nitrification and the assimilation of NO3 in streams (Rose et al. 2015) and lakes (Tsunogai et al. 2018). Due to the fact that atmospheric NO₃ generally has positive Δ^{17} O values, while microbially derived NO₃ has Δ^{17} O values ≤ 0 , measurements of Δ^{17} O of NO₃ were found to reliably resolve atmospheric NO₃⁻ and collectively, the isotope analyses yielded gross nitrification and metabolic rates of NO3⁻ where there were detectable quantities of NO₃ in the water column.

Isotopes, together with molecular approaches, have also been used to detect and measure anammox in sediments and water, and these methods have been reviewed by Song and Tobias (2011). It is clear that isotope effects of O or N associated with NO₃ reduction in field studies is variable, complicated by variability in microbial communities. Isotopic effects associated with O may be decoupled from those of N, driven by NO₂ reoxidation where denitrification dominates, or net NO₃ production where anammox dominates (Dähnke and Thamdrup 2015).

Algal assimilation of N also discriminates, with uptake of inorganic nutrients containing lighter isotopes occurring faster than uptake of nutrients containing heavier isotopes. Discrimination factors vary considerably depending on environmental conditions and specific N form, but range between ~ 0‰ and 30‰ (Altabet et al. 1991, 1999; Sigman et al. 1999; Altabet and Francois 2001; Armstrong et al. 2018). Early studies suggested isotope discrimination during the process of NO₃ assimilation (Wada and Hattori 1976), but various subsequent studies attempted to resolve whether the isotopic discrimination was at the transport step (e.g., Montoya and McCarthy 1995) or the enzymatic reduction of NO₃ (Granger et al. 2004). As regeneration also discriminates, with production of the lighter isotope, measured values of dissolved and particulate N represent the net effect of N₂ fixation, denitrification, assimilation, and regeneration, and distinguishing these different effects and contributions remains a daunting challenge.

The processes of algal N assimilation have been extensively studied with tracer enrichments. Tracer enrichment studies, in which isotope is often added at a level of ~ 10% of the ambient source (e.g., Dugdale and Goering 1967; Dugdale and Wilkerson 1986), have contributed extensively to our understanding of new and regenerated production, oceanic f ratios, i.e., that fraction of primary production fueled by NO₃ (e.g., Eppley and Peterson 1979), understanding of relative preferences (e.g., McCarthy et al. 1977; Glibert et al. 1982a; Smith and Nelson 1990), and relationships between the uptake of different N forms and phytoplankton community composition (e.g., Lomas and Glibert 1999; McCarthy 2002; Berg et al. 2003; Glibert et al. 2004). Tracer enrichment studies have also contributed substantially to characterization of physiological parameters such as nutrient kinetic relationships (e.g., Goldman and Glibert 1983; McCarthy et al. 1992; Collos et al. 1997; Lomas and Glibert 1999; Kudela and Cochlan 2000; Fan et al. 2003; Cochlan et al. 2008; Li et al. 2010; Lindehoff et al. 2011), and in the understanding and quantification of processes such as NH₄ repression of NO₃ assimilation (e.g., Wheeler and Kokkinakis 1990; Dugdale et al. 2007; L'Helguen et al. 2008; Glibert et al. 2014, 2016).

In addition to isotope tracer studies carried out with isotope compounds readily purchased from chemical suppliers, much work has been done in using isotopes to understand and quantify the sources and rates of cycling of complex organic material. As one example, Glibert and Bronk (1994) enriched colonies of the diazotroph Trichodesmium with N₂ gas, and after a period of incubation, measured the isotopically labeled dissolved organic N (DON) released and compared those rates with rates of N₂ fixation determined for the same assemblages at the same times of day. These experiments documented that on average, rates of DON release were ca. 50% the rates of N₂ fixation, and that 60-80% of the total organic release was comparatively small molecules. These data showed that release of these organic compounds could be a significant source of new N for the associated bacteria or the non-N2-fixing filaments of the Trichodesmium colonies. The study of the contribution of DON to phytoplankton nutrition has also been advanced through application of labeled complex organic material. Using ¹⁵N dissolved organic material produced in the laboratory (through growth of phytoplankton, bacteria, and plants such as Spartina alterniflora enriched with ¹⁵N media and subsequent extraction of the organics from biomass), and then fed to various algal cultures, plankton communities, and seagrasses, the use of natural DON has been quantified (e.g., Gagnon et al. 2005; See et al. 2006; Bronk et al. 2007;

These and other related studies collectively demonstrate that much of our understanding and insight into biogeochemical processing of N has come from applications of stable isotopes. Whether it is the physiological processes of uptake on the time scale of minutes to hours, to the multiday scales of whole stream studies, to studies integrating over long time scales, our understanding of N cycling pathways is largely a result of creative applications of natural abundance and enrichment stable isotope studies.

Van Engeland et al. 2013 and references therein).

Carbon and oxygen

Plankton photosynthesis and primary production are routinely measured using tracer techniques, although application of stable isotopes is not as common. Among the stable isotope tracer methods are ¹³C and ¹⁸O. The ¹³C method, analogous to that of the radiotracer ¹⁴C, is relatively straightforward, as H¹³CO₃ is added as a tracer and its incorporation into particulate material (and sometimes dissolved organic material) over time is measured (Hama et al. 1993) and yields estimates similar to those determined by ¹⁴C (e.g., Cullen 2001; Pimenov et al. 2008). There are basic differences, however. With ¹⁴C, an absolute amount of isotope incorporation is measured, whereas with ¹³C, a ratio is determined. Thus, if C sources other than the H¹³CO₃ label are incorporated (e.g., organic C substrates), there can be biases leading to underestimates of productivity, but these can be corrected if the final particulate C concentrations is considered (Collos and Slawyk 1985). The ¹³C method can be applied in situ without the concerns associated with use of radioactive isotopes and can, in combination with CSIA, be used to quantify group-specific primary production via measurement of ¹³C incorporation into biomarkers such as alkenones (Popp et al. 2006) or phospholipidderived fatty acids (Dijkman et al. 2009; de Kluijver et al. 2010. 2013). Moreover, it does not only provide information on C fixation, but also on the transfer of recently fixed C to heterotrophic bacteria and other consumers in benthic ecosystems (Middelburg et al. 2000; Oakes et al. 2012) as well as in the plankton (Norrman et al. 1995; Van den Meersche et al. 2004; Pace et al. 2007), including its dependence on ocean acidification (de Kluijver et al. 2010, 2013). Using CSIA coupled with total ¹³C incorporation, Grosse et al. (2015) followed the incorporation of fixed C into neutral carbohydrates, AAs, and fatty acids for several sites in the North Sea and found distinctly different allocations of C under P and N limiting conditions. The application of ¹³C tracers coupled with metabolomics studies as a means to understand functional pathways is rapidly advancing (You et al. 2014 and references therein).

The measurement of productivity with ¹⁸O begins with enrichment of sample water with H₂¹⁸O (Bender et al. 1987, 1999, 2000) and the enrichment of ¹⁸O₂ over time is determined, reflecting the product of the splitting of water during photosynthesis. This method, which is much more labor intensive and technically difficult than that of ¹³C incorporation, is thought to be a better measure of true gross primary production, but there are uncertainties. Key questions are the associated cycling of O₂ in the cells via respiration through the cytochrome oxidase pathway, respiration by the alternative oxidase pathway, photorespiration, and the Mehler reaction (Kana 1992; Robinson et al. 2009; Regaudie-de-Gioux et al. 2014). A recent comparison of estimates of marine primary productivity derived by these and other methods confirms that the highest reported rates of primary production are, in fact, derived from ¹⁸O enrichment methods (Regaudiede-Gioux et al. 2014). The ¹⁸O method has also been used to estimate gross primary production, community metabolism, and net metabolism in a productive agricultural stream in the Midwestern US (Tobias et al. 2007).

An alternative ¹⁸O method for measuring productivity was introduced using a modified MIMS dissolved gas analyzer (Kana et al. 2006). This method overcomes the tedious extraction process used in traditional ¹⁸O mass spectrometry, giving a much more simple method, with quick turnaround, ~ 5 min total, and which cross-calibrates well with the traditional approach (Ferrón et al. 2016). With shipboard processing possible, this allows many more samples to be run, more questions to be addressed. For example, at the Pacific Ocean longterm time series station, where gross primary production ranged from 0.2 μ mol O₂ L⁻¹ d⁻¹ to 1.1 μ mol O₂ L⁻¹ d⁻¹, a standard primary production depth profile with six depths and triplicate samples for each depth could be analyzed in approximately 3-4 h. A recent review (Mader et al. 2017) explored recent developments in the use of stable isotope ratios of O (¹⁸O/¹⁶O and ¹⁷O/¹⁶O) and how isotopic shifts have provided insights into sources and sinks of O, rates of productivity, and other processes such as gas-water exchange.

Hydrogen

The heavy isotope of hydrogen, deuterium, δ^2 H has been used for tracing the water cycle. Transitions between water vapor and liquid water, evaporation and condensation, involve fractionation (reviewed by Fry 2006). During evaporation, the vapor phase is enriched in the lighter isotope, yielding δ H values typically in the range of -10 to -20. With condensation (rain, snow), the isotopically heavy water falls out. Gradients in δ H are thus seen as water vapor moves inland and upland, and larger fractionation patterns are seen in cold regions, i.e., polar areas. Isotope maps of δ H have been created for large regions, and plant and animal δ H reflect these patterns.

Phosphorus

Phosphorus (P) does not have a convenient stable isotope, so studies of P rates and cycling have been hampered by a lack of this tool. Recently, the application of isotopic ratios of O within the PO₄ molecule has advanced biogeochemical studies of the cycling of P (e.g., Jaisi et al. 2011; Payton and McLaughlin 2011; Davies et al. 2014 and references therein). The application of $\delta^{18}O_P$ (e.g., $P^{18}O_4$) has allowed a means to identify biotic and abiotic interactions of P in biogeochemical cycling and metabolism (e.g., McLaughlin et al. 2004, 2006c; Elsbury et al. 2009; Goldhammer et al. 2011). The P-O bonds in inorganic P are resistant to hydrolysis, so in abiotic reactions there is no exchange of O between PO₄ and H₂O, and no fractionation. In contrast, enzymatic reactions cleave the P-O bond, leading to fractionation or kinetic equilibrium of O isotopes either in intracellular fluids or in the external environment (Blake et al. 2005; Jaisi et al. 2011; Davies et al. 2014).

Applications of $\delta^{18}O_P$ have been rapidly advancing. Using $\delta^{18}O_P$ in concert with sediment chemistry, X-ray diffraction, and other approaches, the sediments from an organic-rich, sulfidic (hypoxic) site in the mesohaline portion of the mid Chesapeake Bay were studied to identify sources and pathway of sedimentary P cycling (Josji et al. 2015). These isotopic data led to the conclusion that regeneration of inorganic P from organic matter degradation (remineralization) is the predominant, if not sole, pathway for authigenic P precipitation in the midbay sediments, with implications for diffusion of dissolved P, an important regulator of eutrophication and In Monterey Bay, California, McLaughlin hypoxia. et al. (2006b) found $\delta^{18}O_P$ values in the upper water column to be influenced by both terrestrially derived freshwater and by deeper water. In a similar study Elkhorn Slough, California, samples collected toward the mouth of the estuary suggested that the $\delta^{18}O_P$ values were dominated by oceanic-derived P_i , but further upstream values were reflective of a chemical fertilizer input (McLaughlin et al. (2006a). In a study of wastewater treatment plants in France, Gruau et al. (2005) found that the $\delta^{18}O_P$ for fertilizer and for the wastewater PO₄ were statistically different, but small. On the other hand, Li et al. (2011) were able to show that anthropogenic PO₄ from fertilizers could be traced within freshwater wetlands of the Everglades National Park, Florida. Blake et al. (2001) were able to attribute inputs of PO_4 to groundwater from septic systems based on $\delta^{18}O_{P_1}$.

Clearly, the application of $\delta^{18}O_P$ provides many opportunities for advancement, but such studies are at a comparatively early stage (Davies et al. 2014). Studies that couple this tool with those of stable isotope applications of C and N will greatly advance our understanding of the biogeochemical cycling of nutrients and have broad implications for eutrophication studies.

Sulfur

Sulfur has four stable isotopes: ³²S, ³³S, ³⁴S, and ³⁶S with abundances of 96%, 0.76%, 4.22%, and 0.0136%. Traditionally, S isotopes have been reported as δ^{34} S, differences in the ratio of the two most abundant isotopes $({}^{34}S/{}^{32}S)$ of a sample relative to the standard (Vienna) Canyon Diablo troilite. Although isotope fractionation usually declines with mass, δ^{34} S values show a wide range (about 100‰). This is related to the prominent role of redox reactions within the biogeochemical sulfur cycle (Harrison and Thode 1958: Kaplan et al. 1963; Canfield 2001). While fractionation of S during assimilation is rather limited (a few %), isotope fractionation during sulfate reduction can be more than 55‰, with pure cultures showing lower values than natural populations (Canfield 2001). Fractionation during sulfate reduction depends primarily on the rate of sulfate reduction, the electron donor (H₂ vs. organics), and sulfate concentrations with low activities, high concentration, and organic substrates leading to highest isotope discrimination (Canfield 2001). Most sulfides produced in anoxic waters and sediments is microbially reoxidized to regenerate the energy in reduced sulfur compounds (Jørgensen 1977). Although S isotope fractionation is rather limited during reoxidation (Kaplan and Rittenberg 1964; Fry et al. 1986), reoxidation of sulfide normally results in the formation of intermediate compounds such as elemental sulfur, thiosulfate, sulfite, and so on. These compounds disproportionate, i.e., split into a more reduced (e.g., sulfide) and more oxidized form (e.g., sulfate), with further isotope fractionation as a result (Canfield and Thamdrup 1994; Canfield 2001). As a consequence, isotope differences between sulfide (and sulfide minerals) and sulfate in nature are larger than those expected if only sulfate reduction would be occurring. The combined information of $\delta^{34}S$ and $\delta^{18}O$ of seawater and pore-water sulfate has shown useful to constrain the cryptic S cycle in ocean oxygen minimum zones (Johnston et al. 2014) and to evaluate the relative contribution of anaerobic methane oxidation to sulfate reduction (Antler and Pellerin 2018).

The very negative δ^{34} S values of reduced sulfur compounds are transferred to autotrophs assimilating these substrates, in particular benthic algae and rooted macrophytes (Fry et al. 1982; Trust and Fry 1992). Intertidal cordgrass as well as

seagrasses obtain part of their sulfur via the roots and consequently often has a distinct sulfur isotope composition relative to other coastal and estuarine organic matter sources (Stribling et al. 1998; Holmer and Hasler-Sheetal 2014). Isotopes of S can complement C isotopes to elucidate the contribution of benthic vs. pelagic resources in lacustrine, coastal, and marine systems (Peterson and Howarth 1987; Croisetière et al. 2009). Strong changes in S isotopes along estuarine gradients have also been found, with rapid increases in δ^{34} S as salinities increase from < 1 to ~ 5, but more constant values at higher salinities and this knowledge, together with measures of δ^{34} S in fish muscle, have been used to track the extent to which they remain resident in a habitat or become mobile if and when habitat conditions are less favorable (Fry and Chumchal 2011).

Silica

There has been substantial application of Si isotopes in understanding global Si biogeochemistry and rates of Si incorporation, and a recent review (Sutton et al. 2018) has compiled much of this literature for both marine and freshwater systems. The natural isotopes of Si are ²⁸Si, at 92.23%. ²⁹Si at 4.67%, and ³⁰Si at 3.10%; ³⁰Si is the most commonly used and reported isotope. The major processes fractionating Si are weathering and organismal incorporation. Weathering releases the lighter isotope so the dissolved Si in water becomes lighter. Si assimilation by organisms leaves behind a pool of dissolved Si that is enriched. Most freshwater studies have shown that dissolved δ^{30} Si values are generally derived from chemical weathering and secondary mineral formation, with seasonal biomineralization processes, mainly the uptake of DSi by diatoms, adding a secondary signal (Sutton et al. 2018 and references therein). This general pattern has been shown, for example, for the Nile River (Cockerton et al. 2013), the Yangtze River, where uptake by wetland grasses and rice paddies contribute seasonally (Ding et al. 2004), rivers in Switzerland (Georg et al. 2006), and in Kenva (Hughes et al. 2012). Distributions of δ^{30} Si in marine waters are similarly driven, with active fractionation by diatoms in surface waters being the dominant process in oligotrophic waters. Consequently, productive upwelling systems have among the most highly fractionated δ^{30} Si values reported (Reynolds 2011; Sutton et al. 2018).

There has also been substantial use of ³⁰Si as an enrichment tracer in marine studies (Nelson and Goering 1977), but less so in freshwater. Early studies by Banahan and Goering (1986) showed that uptake rates in coastal, middle, and outer domains of the southern Bering Sea shelf were comparable. Uptake rates from ³⁰Si tracer studies have been reported for midocean gyre studies (Brzezinski and Nelson 1995, 1996; Nelson and Brzezinski 1997) and upwelling regions (Nelson et al. 1981) and the Southern Ocean (Nelson and Gordon 1982). Brzezinski and Kosman (1996) measured Si uptake rates in the Sargasso Sea in spring and found that a substantial

portion of the diatom production occurred near the base of the euphotic zone, in the nitracline.

Source tracking

Natural abundance stable isotope ratios have been widely used to help identify and track biogeochemical sources in the environment (Kendall 1998; Kendall et al. 2008). Different sources of inorganic nutrients or organic matter often have distinct isotopic signatures, and various biological and/or physical processes alter isotope ratios in expected ways (Kendall et al. 2008; Fig. 5). Source tracking has been successful through analysis of dissolved nutrient pools, detrital mixtures, and organisms such as macrophytes (e.g., McClelland et al. 1997; Cole et al. 2004; Cohen and Fong 2006; Benson et al. 2008) mollusks (Fila et al. 2001; McKinney et al. 2001, 2002), or some combination (Gartner et al. 2002; Fry and Allen 2003) whose biomass may reflect source contributions.

Stable isotope ratios of C are often used to distinguish between autochthonous and allochthonous sources of organic matter in freshwater systems (Cole et al. 2011; Cole and Solomon 2012), between pelagic and benthic primary producers (France 1995: Vadeboncoeur et al. 2003: Christianen et al. 2017), and between terrestrial- and marine-derived organic matter in estuaries (Salomons and Mook 1981; Peterson and Fry 1987; Cifuentes et al. 1988; Peterson et al. 1994; Middelburg and Herman 2007). Tank et al. (2011) applied stable isotopes to identify sources of dissolved organic matter (DOM) in lakes of the Mackenzie Delta (western Canadian Arctic) by coupling various measurements of DOM and dissolved organic C (DOC) with the δ^{13} C and δ^{15} N values of DOM and their precursor materials, and found that distinct sources of DOM to adjacent lakes may contribute to differences in the fate and cycling of various C sources.

Stable isotopes are also frequently used to track anthropogenic N in aquatic systems (e.g., Owens 1987; Tucker et al. 1999; Costanzo et al. 2001; Lapointe et al. 2011; Loomer et al. 2014). In particular, increases in $\delta^{15}N$ (relative to a defined baseline or reference site) are often associated with contributions of sewage-derived N (Kendall 1998). The δ^{15} N of inorganic N derived from manure or sewage is often enriched (> 10 %) due to isotopic fractionation that occurs at either the sewage treatment facility or downstream thereof. The $\delta^{15}N$ values of N in raw sewage are not necessarily enriched, but processes such as NH₃ volatilization and denitrification drive the δ^{15} N values of the residual DIN up during treatment and/or processing within the environment. This, in turn, imparts a ¹⁵N-enriched signal to primary producers that take up the sewage-derived N (McClelland et al. 1997; McClelland and Valiela 1998).

In one classic example, Savage and Elmgren (2004) used $\delta^{15}N$ values in the macroalgae *Fucus vesiculosus* to track sewage-derived N in an embayment of the Baltic Sea and quantify effects of reductions in N inputs following implementation of tertiary sewage treatment. They sampled *Fucus*



Fig. 5. Typical δ^{18} O-NO₃ and δ^{15} N-NO₃ ranges for nitrate sources and the processes that alter these values. Modified and redrawn from Kendall (1998) and Kendall et al. (2008)).

along a 36 km transect and documented a gradient of elevated δ^{15} N that extended from peak values near the sewage outfall to ~ 25 km seaward of the outfall. Sewage N was traceable in *Fucus* before and after tertiary treatment was implemented, but δ^{15} N values decreased more rapidly with distance from the outfall and were less enriched along much of the transect after tertiary treatment began, demonstrating a marked decline in the contribution of sewage-derived N to *Fucus*.

A variation of this approach for N source tracking is the deployment of specific organisms for a set length of time over which the isotopic signature of their biomass will change, reflecting the local environment. Costanzo et al. (2001) deployed macroalgae in porous containers for several days, during which time their biomass incorporated the δ^{15} N signature of dissolved N, and were thus able to map a sewage plume in Moreton Bay, Australia. Oysters represent another appropriate organism for such deployment studies, and field trials have been conducted to resolve appropriate length of time for adequate resolution and how different tissues (mantle or gill) vary in isotope incorporation after exposure to different source nutrients/foods (Fertig et al. 2009, 2010).

Stable isotopes are also used to identify and track water itself. For example, differences in $\delta^{18}O$ and $\delta^{2}H$ between groundwater and surface water are frequently used to characterize interactions between these two sources. As but one example, Fan et al. (2016) examined spatial variation in $\delta^{18}O$ and $\delta^{2}H$ of river water and their sources in the Tarim River Basin, an inland river system of Central Asia. They found that headstreams were composed of water originating as precipitation, ice-melt water and groundwater, and using these isotopic ratios together with other measures of particulate material, the various proportions of each of these components were calculated by season. Stable O isotope ratios have also been essential for quantifying freshwater contributions from sea-ice melt vs. meteoric water (i.e., river inputs) in the Arctic Ocean and surrounding seas (e.g., Bauch et al. 1995; Benetti et al. 2016).

Successful source tracking applications depend on substantive isotopic differences between sources of interest, and are most successful when system-specific end-member information is available. However, distinguishing between changes in end members and changes in isotope values that occur along environmental gradients is a perennial challenge (Fry 2002; Middelburg and Herman 2007). For example, resolving δ^{13} C values of organic matter as a consequence of end-member mixing vs. changes in δ^{13} C values of in situ production along estuarine gradients (that mirror changes in DIC- δ^{13} C) is often difficult (Deegan and Garritt 1997).

Aside from the deployment of specific organisms for source tracking that can yield relatively straightforward results, source tracking generally involves a mixing model, but which all too often can turn into a "mixing muddle" (Fry 2006). Applications of multiple isotopes and new approaches to data analysis are accounting for this variability in some studies, but it is still extremely common to apply average source values and treat them as constants.

Whole ecosystem studies

In the past two decades, there have been multiple mesocosm and whole-ecosystem labeling studies designed to measure integrated biogeochemical pathways or flows of C and N through food webs. Examples of whole system tracer enrichment that have lasted up to months can be found in streams, rivers, lakes, estuaries, wetlands, and marshes (e.g., Middelburg et al. 2000; Hamilton et al. 2004; Pace et al. 2004; Gribsholt et al. 2005, 2009; Oakes et al. 2010, 2012; de Goeij et al. 2013). Whole-lake labeling experiments have been instrumental in identifying the role of allochthonous subsidies for lacustrine consumers (e.g., Cole et al. 2002; Pace et al. 2004; Carpenter et al. 2005). Sánchez-Carrillo and Álvarez-Cobelas (2018) have recently reviewed 78 publications in which ¹³C and/or ¹⁵N additions were used at the whole-aquatic-ecosystem scale and insights achieved, as well as constraints and future prospects for such studies.

One such large experiment was carried under the umbrella of the Lotic Intersite Nitrogen eXperiments (LINX; Peterson et al. 2001). The first LINX experiments involved a continuous drip of ¹⁵NH₄ into streams across the US. Water samples, organic matter, and various types of biota-from phytoplankton to insects and riparian vegetation-were collected over time. The second suite of LINX experiments involved ¹⁵NO₃ additions to 72 streams across the US. In addition to the biota, N₂ and N₂O were also quantified. Key findings were that ¹⁵NH₄ was rapidly removed by the biota, while comparatively more ¹⁵NO₃ was transported downstream. The cross-site analysis also showed that small streams were particularly effective at efficiently removing N because they have a higher ratio of streambed to water volume compared to larger streams (e.g., Peterson et al. 2001; Merriam et al. 2002; Mulholland et al. 2002, 2008; Webster et al. 2003; Ashkenas et al. 2004). Böhlke et al. (2004) enriched an agricultural stream with ¹⁵NO₃ to determine reach-averaged rates of denitrification and other N processes. Applying ¹⁵NO₃ labeling to a mountain stream in Idaho, Hall et al. (2009) compared uptake, storage, and export during periods of snowmelt and baseflow. They found snowmelt floods had higher than expected demand for NO₃ because of hyporheic exchange. A more recent study involving NH₄ additions to 13 streams showed the effect of canopy cover on the efficiency with which N was transferred from heterotrophic microorganisms and primary producers to primary consumers (Norman et al. 2017). Whole-estuary experiments, e.g., NISOTREX and NISOTREX II, have been performed with ¹⁵NO₃ enrichment to characterize estuarine N processing under both low- and high-flushing conditions (Holmes et al. 2000; Hughes et al. 2000; Tobias et al. 2003*a*,*b*). Whole-stream ¹³C additions have also been conducted and allowed resolution of the fate of newly fixed C as well as longer term storage and export (Hotchkiss and Hall 2015). Whole-lake enrichments of ¹³C-labeled HCO₃ have also been made and results revealed that terrestrial C did support a portion of higher trophic level production in these systems (Cole et al. 2002, 2011; Carpenter et al. 2005). The interpretation and broader implications of these findings have since been the subject of debate (Brett et al. 2009), though these labeling studies have highlighted the potential role of terrestrialaquatic linkages to supporting aquatic food webs.

Food webs

The movement of matter, energy, and elements to higher trophic levels through the food web is a central aspect of ecology, leading to interest in the possibility of using stable isotopes to elucidate food web relationships in aquatic systems, including estimation of consumer trophic position (TP) and determination of the importance of basal energy sources and dietary relationships (e.g., Peterson and Fry 1987; Vander Zanden and Rasmussen 2001; Middelburg 2014 and references therein). Most commonly, ¹³C and ¹⁵N are applied in such studies, and the common axiom is that for C, "you are what you eat" (i.e., isotope ratios in consumers reflect those of their food), while for N, "you are what you eat +3\%" (i.e., consumers are typically enriched by 2–4% relative to their diet; Smith and Epstein 1970; DeNiro and Epstein 1978, DeNiro and Epstein 1981; Minagawa and Wada 1984; Fig. 6A). Studies using natural abundance stable isotopes to make inferences about energy sources (¹³C) and TP (¹⁵N) have become commonplace, and have been applied to a wide range of aquatic ecosystem types (Middelburg 2014 and references therein). While ¹³C and ¹⁵N have been the dominant stable isotope tracers, studies have used ³⁴S to differentiate food sources in marine (Peterson and Fry 1987) and freshwater systems (Croisetière et al. 2009), and H isotopes to track aquatic-terrestrial linkages (Doucett et al. 2007; Cole et al. 2011; Solomon et al. 2011; Cole and Solomon 2012; Karlsson et al. 2012). Advances in food web studies have also been made through application of $\delta^2 H$, an emerging tool for trophic positioning studies (Vander Zanden et al. 2016). Stable isotope applications to food web studies can generally be binned into those in which TP is characterized, and those in which food sources are identified. The latter can be probed through characterization of isotopic composition of individual foods, or from isoscapes that are derived from substantial records of the isotopic composition of organisms or inorganic nutrients and other materials over broad locations.

Trophic position

Early laboratory experiments showed that animal tissues tend to be more enriched in ¹⁵N than that of their food (DeNiro and Epstein 1981; Minagawa and Wada 1984). This provided the conceptual basis for the use of N isotopes to estimate the number of trophic steps between the base of a food web (i.e., primary producers, trophic level 1) and a consumer of interest. Building on this, field studies began to make qualitative yet powerful inferences about trophic structure of an ecosystem from ¹⁵N measurements across the food web (Hobson and Welch 1992; Kling et al. 1992). Fundamental questions about food chain length, highlighting relationships with ecosystem size, ecosystem productivity, and species diversity were subsequently addressed (Vander Zanden et al. 1999; Post et al. 2000). Such studies have provided evidence for food chain biomagnification of contaminants (Cabana and Rasmussen 1994; Lavoie et al. 2013), and how anthropogenic drivers such



Fig. 6. (A) Classic conceptual food web relationships based on δ^{13} C and δ^{15} N. (B) Schematic showing different trophic levels of the California Current system, the expected proportional prey inputs expected under a wasp-waist model, and far right, the proportional prey to various trophic groups based on isotopic measurements and mixing model results. Panel B reproduced from Madigan et al. (2012) under a creative commons license.

as climate change and introduced species affect food webs (Vander Zanden et al. 1999; Tunney et al. 2014).

While the potential for this approach is apparent, the ability to compare food webs in a quantitative manner is hindered by variation in ¹⁵N at the base of the food chain, analogous to the variation in end-member resolution in source tracking studies. Cabana and Rasmussen (1996) found that the ¹⁵N of long-lived primary consumers such as unionid mussels varied by ~ 13‰ among lakes, with much of the variation explained by anthropogenic nutrient inputs. To address this, Cabana and Rasmussen (1996) expressed the ¹⁵N of higher consumers relative to that of long-lived primary consumers such as mussels, which show little temporal variation in isotopic values, and thus provide a desirable baseline indicator organism. They determined TP as:

$$TP = {}^{15}N_{consumer} - {}^{15}N_{baseline} / TDF + 2$$
(8)

in which TDF is the trophic discrimination factor, the estimated per trophic level isotopic enrichment, which is

commonly assumed to be 3.0 or 3.4 for ¹⁵N. Subsequent studies found that the isotopic baseline in lakes can vary among habitats within an ecosystem, and proposed the establishment of a baseline curve whereby consumer ¹³C values were used to determine the appropriate ¹⁵N baseline (Vander Zanden and Rasmussen 1999; Post 2002). Expressing consumer ¹⁵N relative to that of an isotopic baseline provided ecologists with a relatively simple way to estimate the TP of a consumer, defined as a continuous measure of the number of trophic steps leading to a consumer that is reflective of food web omnivory and actual feeding pathways. Stable isotopes allowed this fundamental ecological concept to be operationalized in a way that has stimulated research interest in trophic relationships and energy flow pathways.

Increasingly, however, it has been recognized that the common axioms of little to no fractionation with trophic transfer for C and approximately +3‰ for N are too simplistic. There is considerable variability associated with these values, and applying them in a generic way can lead to misunderstanding. Middelburg (2014) summarized key factors that affect the accuracy of TP estimates as follows: (1) the assumption that C and N flow through the food web similarly (i.e., no concentration effect) and that per trophic level enrichment of C is limited, (2) variation in the TDF for N, (3) uncharacterized isotopic variation among tissues, and (4) difficulty in estimating appropriate C and N isotopic baselines (Lorrain et al. 2002; Phillips and Koch 2002; Fry 2006; Phillips 2012). Variation in the TDF has received significant attention. While 3.0 is a reasonable approximation of the overall average trophic enrichment factor, the degree of variation is substantial and depends on factors such as the form of excretion, diet, tissue type, taxon, and habitat type (e.g., Vander Zanden and Rasmussen 2001; Post 2002; Vanderklift and Ponsard 2003; Caut et al. 2009; Auerswald et al. 2010; Bunn et al. 2013). Moreover, the variability for herbivores is far greater than that for carnivores (Vander Zanden and Rasmussen 2001). In recent years, Bayesian mixing models have been applied that incorporate many of the uncertainty issues: concentration dependence, variable numbers of sources, uncertainty in sources, and so on (reviewed by Phillips et al. 2014).

Food webs often deviate strongly from simple linear food chains and discrete trophic levels, and stable isotope applications have been used to resolve these complexities. For example, a multiyear stable isotope food web study of the California Current found separation of species into trophic groups based on δ^{13} C and δ^{15} N, and that a wide diversity of prey were incorporated into upper level predators (Madigan et al. 2012; Fig. 6B). Predators did not cluster together as expected if they were uniformly feeding on lower trophic level prey. Bayesian mixing models revealed a high degree of omnivory and more complex trophic linkages, a finding that could have implications for ecosystem stability and resilience to perturbations.

With increasing recognition of the challenges and limitations of the bulk stable isotope approach for estimating TP, analyses of stable N isotope ratios of individual AAs have emerged as a promising technique (McClelland and Montoya 2002; McClelland et al. 2003). McClelland and Montova (2002) found that the stable N isotope ratios of some AAs exhibit high-trophic enrichment, while other showed virtually none. Thus, they proposed that the difference between enriching (i.e., glutamic acid, glu) and nonenriching (phenylalanine, phe) AAs could provide an indicator of consumer TP. This approach cleverly provides a baseline ¹⁵N correction internal to the organism of interest, thereby addressing the issue of accurately defining an external isotopic baseline signature in many ecosystems. Subsequent studies largely confirmed the TDFs reported by McClelland and Montoya (2002). Chikaraishi et al. (2009) reported TDF_{AA} to be $7.6 \pm 1.3\%$ from controlled feeding studies at the time, and proposed the following equation to estimate consumer TP based on AAs (TP_{AA}):

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$$TP_{AA} = \frac{\delta^{15} N_{Glu} - \delta^{15} N_{Phe} - \beta}{TDF_{AA}} + 1$$
(9)

where $\delta^{15}N_{glu}$ and $\delta^{15}N_{phe}$ are ¹⁵N for glutamic acid and phenylalanine, respectively, β is the isotopic difference between the two AAs in primary producers, and TDF_{AA} is the difference in TDF between the two AAs. While it appears that the AAspecific approach may provide higher precision and accuracy than traditional bulk methods for estimating consumer TP (Chikaraishi et al. 2009), a meta-analysis by McMahon and McCarthy (2016) highlighted substantial variability in TDF_{AA} values, and suggest diet quality and mode of N excretion to be factors influencing this variation. Although a truly universal TDF_{AA} is unlikely to exist, efforts to understand the patterns of variation will underpin future advances in the application of this approach for elucidating food webs.

For microbial food webs, it is far more challenging to resolve the isotopic composition among bacteria, archaea, eukaryotic microbes, and detritus (dead organic matter), in particular for N, as there are few biomarkers for tracing N through the microbial domain (Boschker and Middelburg 2002). A biomarker for bacterial peptidoglycan, D-alanine, has been used to trace N uptake and flow through bacteria in coastal waters, seagrass beds, and sediments (Veuger et al. 2006, 2012; Eyre et al. 2016). Stable N isotope analyses of diaminopimelic acid have also been used to track bacterial N (Tobias et al. 2003a,b), although small yields can make this approach challenging. Assimilation of N by heterotrophic microbes feeding on detrital resources leads to elevated δ^{15} N (Caraco et al. 1998) with consequences for the isotope composition, hence trophic level inference, of top consumers (Steffan et al. 2017). However, Guiterrez-Rodriguez et al. (2014) have shown that protozoan trophic steps do not necessarily lead to an increase in δ^{15} N. Polar-lipid derived fatty acids (PLFA) are another suitable biomarker, in this case for ¹³C, for microbial food web studies because of their relatively easy isotope analysis with GC-C-IRMS and because these cell membrane constituents occur in bacteria and eukaryotes and are present in relatively constant amounts in organisms of interest (Boschker and Middelburg 2002; Middelburg 2014). However, specificity of PLFA (for identification of microorganisms) is rather low, and thus bacteria and eukaryotes can be distinguished quite well from each other, but PLFA spectra of many phytoplankton groups overlap (Dijkman et al. 2009). This implies that resolving among phytoplankton groups should be done with utmost care and requires compositional estimators (Van den Meersche et al. 2008; Dijkman et al. 2009).

McCarthy et al. (2007) measured AA distributions in plankton tows, sinking particulate organic matter (POM), and ultrafiltered dissolved organic matter in the central Pacific Ocean and observed differences in δ^{15} N of individual AA indicative of trophic transfers. Their finding underscored that the AAs associated with trophic transfer were also clearly preserved in sinking POM, but with additional changes indicative of subsequent microbial reworking after incorporation into particles. There is much scope for future work on isotope fractionation in microbial biogeochemical cycling (McMahon and McCarthy 2016).

Pathways in food webs

An important aspect of aquatic food web studies involves identifying the basal resources supporting higher trophic levels. Stable isotopes of C have been widely used to track energy sources and pathways into and through aquatic food webs. However, it is important to consider system-specific end-member values when using this tool. For example, C stable isotope ratios are useful for distinguishing between contributions of C4 plants and phytoplankton to higher trophic levels in aquatic systems, whereas it is more difficult to distinguish between C3 plant and phytoplankton contributions. This is particularly true for freshwater systems, where the δ^{13} C values of C3 plants.

While limnology has traditionally focused on planktonic production and processes, C isotope studies have demonstrated a surprisingly important role of periphyton-based production in supporting higher trophic levels in lakes. Periphyton (benthic) and phytoplankton (pelagic) have distinct ¹³C values due to differential fractionation during photosynthesis related to turbulence and boundary layer conditions. In summarizing data from a suite of Canadian lakes, Hecky and Hesslein (1995) found that fish species often have a strongly benthic ¹³C signal. This has been found to be a general pattern, with benthic pathways often comprising the dominant C source supporting fish (Vander Zanden and Vadeboncoeur 2002). Stable isotope studies showing the importance of benthic C to higher trophic levels are part of a broader body of literature highlighting benthic-pelagic coupling in lakes (Schindler and Scheuerell 2002; Vadeboncoeur et al. 2002).

A central question in riverine food web ecology pertains to the importance of autochthonous and allochthonous C sources to riverine consumers (Rounick and Winterbourn 1986). From the earliest studies, it has been recognized that differentiating the role of different habitats and C sources supporting stream food webs can be complicated by a variety of factors: high variability in algal ¹³C related to productivity and flow, the ¹³C of the underlying DIC source, and the difficultly of isolating algae from heterogeneous epilithon (Rosenfeld and Roff 1992). Stable isotope studies in a wide range of riverine systems have demonstrated an important and often outsized role of autochthonous algal C in supporting higher trophic levels. Finlay et al. (1999) reported strong differences in ¹³C between pool and riffle habitats in productive streams, but no difference in unproductive streams. Subsequent studies exploited these differences and highlight previously unrecognized food web linkages, and the role of shallow pools in supporting a range of consumers in streams (Finlay et al. 2002).

Stable isotope studies have also elucidated aquatic to terrestrial linkages in streams. Organic matter, invertebrates, and fish were enriched in ¹⁵N and ¹³C in streams with spawning coho salmon (*Oncorhynchus kisutch*) in Pacific Northwest streams, demonstrating the role of salmon carcasses, which are isotopically distinct, in fueling the food web and increasing productivity (Bilby et al. 1996). Studies have used natural abundance ¹³C and ¹⁵N (Paetzold et al. 2005), as well as ¹⁵N tracer additions (Sanzone et al. 2003) to evaluate the importance of aquatic insects as a resource for riparian arthropods.

Isoscapes

Isoscapes, or spatially explicit maps of isotopic composition (West et al. 2008; Bowen et al. 2010), are also beginning to be developed in marine systems as a tool for reconstruction of broad scale animal movements which have important implications for understanding diet sources. Isoscapes are derived from substantial records of the isotopic composition of organisms or inorganic nutrients and other materials in known locations. In terrestrial systems, large spatial interpolations of isotopic measurements have been made for at least a decade, providing a foundation for research on migration patterns of insects, birds, and mammals (e.g., Flockhart et al. 2013).

Broad marine applications of this approach have been limited by data availability, but there has been recent development of isoscapes of ¹³C and ¹⁵N in coastal, shelf, and lake regions. In Florida Bay, US, the C and N isotopic composition of the seagrass Thalassiosira testudinum was measured across the expanse of the bay (10,000 km²) and over multiple years (Fourgueran et al. 2005). Clear seasonal trends were seen, with δ^{13} C and δ^{15} N values showing maxima in the summer and minima in the winter. Coupled with elemental ratios of the plant material, underlying the seasonal changes in nutrient supply and processes that fractionate the DIN pool. In the Dutch Wadden Sea, the C isotopic composition of 178 species was measured across the entire intertidal area (1460 km²) with high-spatial resolution (Christianen et al. 2017). Benthic microalgae were identified to be the dominant energy source for the majority of consumers at higher trophic levels (from worms and molluscs to fish and birds). Moreover, large spatial heterogeneity in C isotope composition of benthic algae and their consumers was observed, implicating the need for spatially explicit sampling of tidal food webs. Hobson et al. (2012) applied a δ^{13} C and δ^{15} N isoscape approach to the study of fish community structure and large-scale dietary nutrient sources in Lake Winnipeg.

An open ocean isoscape of ¹³C, with one degree and monthly resolution, was developed based on the coupling of a physical-biogeochemical model and predictions of isotopic fractionation of phytoplankton (Magozzi et al. 2017; Fig. 7A). In this model, values of $\delta^{13}C_{PLK}$ (phytoplankton) were predicted from the isotopic composition of dissolved CO₂ ($\delta^{13}C_{CO2}$) and ϵ_p , which was estimated from the concentration of dissolved CO₂, phytoplankton growth rates, size, and



Fig. 7. (**A**) Modeled annually averaged surface-water distribution of the carbon isotope composition of phytoplankton ($\delta^{13}C_{PLK}$, %). Annual average $\delta^{13}C_{PLK}$ values are calculated using a monthly climatology for the period 2001–2010. (**B**) Proposed feeding areas for two Atlantic salmon populations indicated by the strength of correlation between temporal records of sea surface temperature and scale collagen $\delta^{13}C$ values. Each map represents feeding areas for a specific population and cohort: (a) North East Coast 1SW, (b) North East Coast MSW, (c) River Frome 1SW, and (d) River Frome MSW. Panel **A** reproduced from Magozzi et al. 2017) and panel **B** from MacKenzie et al. (2011) under creative commons licenses.

community composition (Magozzi et al. 2017). Such a model may be useful in interpreting foraging range and diet sources in further food web models and overcoming the limitation of poorly resolved spatio-temporal distributions of baseline isotope compositions (Magozzi et al. 2017). An isoscape approach was applied to the study of populations of Atlantic salmon and their feeding grounds (MacKenzie et al. 2011; Fig. 7B). Using the relationship between fractionation of C during photosynthesis and temperature, these variations were applied by correlating the temporal records in tissue isotopes and temperature over the possible spatial range of the species; importantly this analysis did not depend on a priori knowledge of distribution of baseline isotope values.

Paleoenvironments

Fossil foraminifera shells have routinely been analyzed for their C and O isotopic compositions for the purpose of reconstructing past climate conditions; some of this work dates back many decades (e.g., Urey et al. 1951; Epstein et al. 1953; Shackleton 1967). These early studies established that the δ^{18} O and δ^{13} C signals of the shell reflect those of the seawater during calcification. It is generally assumed that the δ^{18} O of the tests primarily reflects the seawater temperature (and paleosalinity) while the δ^{13} C reflects primarily the state of paleoproductivity

and water mass age (e.g., Shackleton and Opdyke 1973). If the shell δ^{18} O in calcite is in equilibrium with seawater, the isotopic discrimination factor between calcite and seawater $(\varepsilon \sim \delta^{18}O_{CaCO3} - \delta^{18}O_{H20})$ is related to the temperature of calcification and therefore, if the δ^{18} O of seawater is known, the δ^{18} O of calcite can be used to reconstruct paleotemperatures (Ravelo and Hillaire-Marcel 2007). The δ^{18} O of foraminifera have been instrumental to reconstruct the cooling of the ocean and climate during the last 50 Ma (e.g., Zachos 2001; Lisiecki and Raymo 2005). However, the assumption that that these isotopic signatures "faithfully record relative changes in isotopic conditions...can be problematic" (D'Hondt and Zachos 1993). These isotope values can be offset due to factors such as shell size, microhabitat differences and/or species or individual variabilities, pore-water chemistry, pH, and other "vital effects" (e.g., McCorkle et al. 1997; Schmiedl et al. 2004; Fontanier et al. 2006; Ishimura et al. 2012). Recently, the isotopic composition of shells of pteropods have been analyzed, and as they secrete aragonite close to the CaCO₃-seawater δ^{18} O equilibrium, their δ^{18} O is suggested to be a good oceanographic proxy of seawater temperature and their δ^{13} C a good proxy of carbonate ion concentration (Keul et al. 2017). Moreover, given their longer life span relative to foraminifera, they may provide a more integrative measure.

Isotopic measurements of sediments have also proven to be useful in understanding paleoceanography, paleolimnology, and past biogeochemical changes, as well as relationships to climate change. Measurements of the bulk δ^{15} N of sediments, while relatively straightforward to obtain, can be complicated to interpret, as this material reflects three components, marine organic matter, potentially terrestrial organic matter, and inorganic N (Robinson et al. 2012; Tesdal et al. 2013). The latter can be significant in organic poor sediments and is due to NH₄ adsorption onto clay materials. The isotopic signal of N in sediments reflects alteration in the isotope signal of the organic matter during sinking, but especially during early burial and early diagenesis (Altabet and Francois 1994). During sinking and diagenesis, organic N becomes increasingly enriched in ¹⁵N relative to that of the surface layer (Robinson et al. 2012). Isolating the source of the isotopic signaturesinking particle alteration or sediment diagenesis—is not easy, but specific biomarkers for C and H analysis have been shown to be useful, whereas analogous biomarkers for N are less useful for various reasons (Altabet 2006).

Based on a compilation of available surface marine sediment δ^{15} N samples in the published literature (http://www.ncdc.noaa.gov/paleo/pubs/nicopp/nicopp.html), values were found to fall in a range from 2.5‰ to 16.6‰, with an average of 6.7‰ (Tesdal et al. 2013). This global data set shows, and as previously described (e.g., Galbraith et al. 2008), lower δ^{15} N values in sediments are found in upwelling regions, while in anoxic regions higher values are found—a result of differential biogeochemical processing of NO₃. Where subseafloor data exist, mostly along the coast, the data are biased toward the more recent Holocene and Late Pleistocene timescales (Tesdal et al. 2013). Moreover, patterns are suggestive of an accelerating N cycling in the late Holocene, indicative of greater rates of denitrification and/or N₂ fixation in the water column.

Past changes in denitrification have been constructed from application of downcore $\delta^{15}N$ analysis. Such data are now available for a number of oceanic denitrification zones. In the Arabian Sea record, transitions from δ^{15} N minima (more denitrification) to maxima (less denitrification) appear to occur abruptly, a few hundred years or less, with minima corresponding to cooler temperature changes (Altabet 2006). Accordingly, climate changes are associated with the strength of the summer monsoon and upwelling favorable winds. This, in turn, influences productivity, altering levels of dissolved O2 and thus denitrification. Using δ^{13} C in conjunction with measures of carbonate, organic C, and N contents of cores of Lake Kinneret, Israel, the lake's productivity and regional climate conditions were reconstructed for the late Holocene and five distinct stages were identified which may be related to changes in geo-political settlements over the past 3000 yr (Dubinski et al. 2003).

Other isotopic signals have also been used to interpret paleoceanographic changes. Diatom-bound organic N has been used to reconstruct past N utilization, with a number of

studies focused on the Southern Ocean (e.g., Sigman et al. 1999; Crosta and Shemesh 2002; Robinson et al. 2004). In contrast to bulk δ^{15} N measurements of sedimentary material, measuring that of just the diatom-bound fraction, while more labor intensive, does not appear to influenced by diagenetic processes (Robinson et al. 2012; Sutton et al. 2018 and references therein).

Diatom ¹⁸O is another useful isotopic signal for paleo studies. It is a reflection of sea surface temperature and this isotopic signal is retained on very long time scales, at least 430 ka (Shemesh et al. 1995). There also appear to be few species effects. This tool was used in the Southern Ocean and showed a ¹⁸O enrichment during the transitions from the last glacial maximum to the Holocene, as well as a post glacial maximum spike, suggestive of melting icebergs.

Another signal in diatoms, that of its Si isotopes (δ^{30} Si), has also been applied in paleo-reconstructions in oceans and in lakes (Panizzo et al. 2016). During biomineralization, diatoms preferentially incorporate ²⁸Si in their frustules, making the ambient water progressively enriched in ³⁰Si (e.g., De La Rocha et al. 1997; De La Rocha 2006), but during dissolution there appears to be an absence of Si fractionation. This isotope signal appears to be faithfully transferred as diatoms sink through the water column, a conclusion based on sediment trap comparisons from Lake Baikal, Siberia (Panizzo et al. 2016). The δ^{30} Si of sponge spicules and radiolarian tests have also been recently used to reconstruct DSi concentrations in deep waters of the Equatorial Pacific (Fontorbe et al. 2017). Both signals indicate a reduction in value about 37 Ma, a likely consequence of changes in the $\delta^{30}\mbox{Si}$ of source DSi and a transition to higher DSi concentrations overall, related to the shift in the deep-water source of the Pacific during the Paleogene.

Analytical challenges remain, however. For example, in applications of δ^{30} Si, δ^{18} O, δ^{13} C, and δ^{15} N studies of diatoms, especially in paleo-reconstruction studies, analytical contamination is a significant problem (Sutton et al. 2018). Inadequate cleaning of the biogenic SiO₂ can introduce substantial isotopic offsets (De La Rocha 2006). Moreover, there is much work to be done to further understand fractionation associated with biogenic Si and water, and disparate fractionation factors have been reported, limiting the usefulness of δ^{18} O as a paleoceanographic proxy (Sutton et al. 2018 and references therein).

Reinforcing challenges, conundrums, and caveats

As repeatedly noted throughout this article, stable isotope applications are based on ratios, ratios of lighter to heavier isotopes (or alternatively heavier to lighter isotopes), ratios of sample to standard, and ratios of these ratios. These ratios may be further manipulated (subtracted, multiplied by arbitrary factors or meaningful environmental parameters) to yield final fractionation values or rate measurements that are subsequently interpreted. The power of isotopes is that small differences allow great insight—into rate processes, sources of nutrients and organic matter to ecosystems, food web structure, and paleoecological or oceanographic conditions. Increasingly sensitive analytical tools are allowing resolution of isotopic differences at finer scales, including smaller and/or individual organisms, discrete compounds and molecules. Yet, by virtue of the fact that isotopic abundances are ratios, they are also very sensitive to errors, and errors magnify, especially when that error is associated with the denominator. When ratios of ratios (of ratios) produce a synthetic value, the origin of errors can be lost. Thus, it is worth underscoring three perennial problems that have been touched on above, but which are often overlooked by both practitioners and consumers of the isotope literature.

- 1. Rate measurements involving isotopic enrichment are particularly sensitive to the isotopic enrichment. This value is in the denominator of the estimate of rate velocity (see Eq. 5). For rate studies, the amount of tracer addedespecially relative to the biomass that will react with this tracer and the time allowed for incubation-will have major effects on final rates estimated. As noted by Glibert and Capone (1993), the decision of how much tracer to add is not always straightforward. While 10% is nominally accepted as a value that will not perturb rates substantially (Dugdale and Goering 1967), this amount may become rapidly depleted if concentrations of N are low and biomass is high (as in the case of a phytoplankton bloom). Using a Monte-Carlo error analysis, Aufdenkampe et al. (2002) estimated how errors in measurement can propagate to a calculated uptake rate. They found that uncertainties in uptake rates could be 6-20%, with higher uncertainties associated with lower uptake rates. Moreover, at higher uptake rates (ρ_{max}) uncertainties are most sensitive to PN concentrations, whereas at lower rates, uncertainties in ¹⁵N enrichment have a larger effect (Aufdenkampe et al. 2002). In a meticulous analysis, Tang and Maggi (2012) have explored/studied the effects of ¹⁵N content on rates of nitrification, denitrification, and dissimilatory NO₃ reduction to NH₄ (DNRA). Specifically, they showed increasing δ^{15} N-NO₃ values generally resulted in a decrease of the rates of the reactions immediately that used NO₃ as a substrate and that this effect propagated to the secondary reaction that consumed the first successor to NO₃, such as NH₄ and N₂O. The magnitudes of such effects were large across the gradient of enrichment levels tested, leading to 50% reduction in nitrification rates and up to 60% reductions in rates of denitrification and DNRA. Collectively these results underscore that the quantity of isotopic enrichment can have profound effects on the reported rate of reaction.
- 2. In applications of source tracking of nutrients or organic matter, variability in end member values is often ignored, and average (assumed constants) are applied instead. Overlapping ranges of source/end-member values need to be

acknowledged, as do effects of biological transformations along environmental gradients. Open, complex, biologically active systems are too often fit with closed system equilibrium equations. Unless source δ values (for whatever element is being compared) are thoroughly characterized, Δ , α , ϵ cannot be established with any accuracy (*see* Eqs. 2– 4). Similarly, in whole system studies, another potentially major source of bias in tracer rate measurements is related to the heterogeneity of tracer distribution within the environment after tracer addition. This may be important in complex, spatially structured communities such as corals and in situ studies of stratified lakes, heterogeneous wetlands and marshes, river beds, and sediment, e.g., in the particular rhizosphere and bioturbated sediments.

3. In food web studies, the extent of variability in fractionation with trophic transfer must be better recognized, and assumptions of no fractionation for C and approximately +3% for N cannot or should not be blindly accepted (see Eqs. 8, 9). Variability in isotopic abundance of food resources must be recognized, even if sampling necessitates a composite or pooled sample. And, as cautioned by Fry (2006, p. 133-134), "a disconcerting possibility is that animals might be consuming most of the important foods, keeping those foods at such low abundances that they are hard to sample... your sampling may have missed something important." Bayesian mixing models and other advanced data assimilation techniques are partially addressing the variability issue, but sophisticated analyses cannot overcome underlying faulty assumptions or resolve the underdeterminancy of many food web studies.

Conclusions

Stable isotope applications have played a starring role in the advancement of modern limnology and oceanography. Advancements in instrumentation have gone hand in hand, and currently available tools, such as nanoSIMS, SIP, resonance Raman microspectrometry, cavity ring down spectroscopy among others, are launching isotope aficionados into a new era of isotope resolution and associated new applications and questions. Finer definition of food webs is possible with CSIA relative to classic bulk measurements. Better resolution of biogeochemical processes is possible, not only with better, faster, more automated analysis, but also with applications of isotopologues and integration of molecular information with isotope resolution. Even as technology improves, successful application of stable isotope techniques will continue to depend as much on the savviness of users as the robustness of the tool itself. However, when treated with a healthy respect, and when resulting data are placed in context with other biological, chemical, or ecological understanding, stable isotope applications will remain pivotal for addressing the many challenging questions that limnologists and oceanographers are tackling.

References

Altabet, M. A. 2006. Isotopic tracers of the marine nitrogen cycle: Present and past, p. 251–293. *In* D. Barceló and A. G. Kostianoy [eds.], Handbook of environmental chemistry, v. 2, part N. Springer. doi:10.1007/698_2_008

Altabet, M. A., W. G. Deuser, S. Honjo, and C. Stienen. 1991. Seasonal and depth-related changes in the source of sinking particles in the North Atlantic. Nature **354**: 136–139. doi: 10.1038/354136a0

Altabet, M. A., and R. Francois. 1994. Sedimentary nitrogen isotope ratios as a recorder for surface ocean nitrate utilization. Global Biogeochem. Cycles **8**: 103–116.

Altabet, M. A., D. W. Murray, and W. L. Prell. 1999. Climatically linked oscillations in Arabian Sea denitrification over the past 1 m.y.: Implications for the marine N cycle. Paleoceanogr. Paleoclimatol. 14: 732–743. doi:10.1029/1999PA900035

Altabet, M. A., and R. Francois. 2001. Nitrogen isotope biogeochemistry of the Antarctic Polar Frontal Zone at 170W. Deep-Sea Res. Part II Top. Stud. Oceanogr. 48: 4247–4273. doi:10.1016/S0967-0645(01)00088-1

An, S., W. S. Gardner, and T. Kana. 2001. Simultaneous measurement of denitrification and nitrogen fixation using isotope pairing with membrane inlet mass spectrometry analysis. Appl. Environ. Microbiol. 67: 1171–1178.

Andriukonis, W., and E. Gorokhova. 2017. Kinetic ¹⁵N-isotope effects on algal growth. Sci. Rep. **7**: 44181. doi:10.1038/ srep44181

Antler, G., and A. Pellerin. 2018. A critical look at the combined use of sulfur and oxygen isotopes to study microbial metabolisms in methane-rich environments. Front. Microbiol. 9: 519. doi:10.3389/fmicb.2018.00519

Armstrong, C. T., D. L. Erdner, J. W. McClelland, M. P. Sanderson, D. M. Anderson, C. J. Gobler, and J. L. Smith. 2018. Impact of nitrogen chemical form on the isotope signature and toxicity of a marine dinoflagellate. Mar. Ecol. Prog. Ser. 602: 63–76. doi:10.3354/meps12619

Ashkenas, L. R., S. L. Johnson, S. V. Gregory, J. Tank, and W. M. Wollheim. 2004. A stable isotope tracer study of nitrogen uptake and transformation in an old-growth forest stream. Ecology 85: 1725–1739.

Auerswald, K., M. H. O. M. Wittmer, A. Zazzo, R. Schaeufele, and H. Schnyder. 2010. Biases in the analysis of stable isotope discrimination in food webs. J. Appl. Ecol. 47: 936–941. doi:10.1111/j.1365-2664.2009.01764.x

Aufdenkampe, A. K., J. J. McCarthy, C. Navarette, M. Rodier, J. Dunne, and J. W. Murray. 2002. Biogeochemical controls on new production in the tropical Pacific. Deep-Sea Res. Part II Top. Stud. Oceanogr. 49: 2619–2648.

Banahan, S., and J. J. Goering. 1986. The production of biogenic silica and its accumulation on the southeastern Bering Sea shelf. Cont. Shelf Res. **5**: 199–213.

Bauch, D., P. Schlosser, and R. G. Fairbanks. 1995. Freshwater balance and the sources of deep and bottom waters in the

Arctic Ocean inferred from the distribution of $H_2^{18}O$. Prog. Oceanogr. **35**: 53–80.

Becker, M., N. Andersen, B. Fiedler, P. Fietzek, A. Körtzinger, T. Steinhoff, and G. Friedrichs. 2012. Using cavity ringdown spectroscopy for continuous monitoring of $\delta^{13}C(CO_2)$ and fCO_2 in the surface ocean. Limnol. Oceanogr.: Methods **10**: 752–766. doi:10.4319/lom.2012.10.752

Bender, M., J. Orchardo, M.-L. Dickson, R. Barber, and S. Lindley. 1999. *In vitro* O₂ fluxes compared with ¹⁴C production and other rate terms during the JGOFS Equatorial Pacific experiment. Deep-Sea Res. Part I Oceanogr. Res. Pap. **46**: 637–654.

Bender, M. L., and others. 1987. A comparison of four methods for determining planktonic community production. Limnol. Oceanogr. 32: 1085–1098. doi:10.4319/ lo.1987.32.5.1085

Bender, M. L., M.-L. Dickson, and J. Orchardo. 2000. Net and gross production in the Ross Sea as determined by incubation experiments and dissolved O₂ studies. Deep-Sea Res. Part II Top. Stud. Oceanogr. **47**: 3141–3158. doi:10.1016/ S0967-0645(00)00062-X

Benetti, M., G. Reverdin, C. Pierre, S. Khatiwala, B. Tounadre, S. Olafdottir, and A. Naamar. 2016. Variability of sea ice melt and meteoric water input in the surface Labrador Current off Newfoundland. J. Geophys. Res. Oceans **121**: 2841–2855. doi:10.1002/2015JC011302

Benson, E. R., J. M. O'Neil, and W. C. Dennison. 2008. Using the aquatic macrophyte *Vallisneria americana* (wild celery) as a nutrient bioindicator. Hydrobiologia **596**: 187–196.

Berg, G. M., M. Balode, I. Purina, S. Bekere, C. Bechemin, and S. Y. Maestrini. 2003. Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen. Aquat. Microb. Ecol. **30**: 263–274. doi:10.3354/ ame030263

Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: Evidence from stable isotopes. Can. J. Fish. Aquat. Sci. **53**: 164–173.

Blackburn, T. H. 1979. Method for measuring rates of NH₄⁺ turnover in anoxic marine sediments, using a ¹⁵N-NH₄⁺ dilution technique. Appl. Environ. Microbiol. **37**: 760–765.

Blake, R. E., J. C. Alt, and A. M. Martini. 2001. Oxygen isotope ratios of PO₄: An inorganic indicator of enzymatic activity and P metabolism and a new biomarker in the search for life. Proc. Natl. Acad. Sci. USA **98**: 2148–2153.

Blake, R. E., J. R. O'Neil, and A. V. Surkov. 2005. Biogeochemical cycling of phosphorus: Insights from oxygen isotope effects of phosphoenzymes. Am. J. Sci. **305**: 596–620.

Böhlke, J. K., J. W. Harvey, and M. A. Voytek. 2004. Reachscale isotope tracer experiment to quantify denitrification and related processes in a nitrate-rich stream, midcontinent United States. Limnol. Oceanogr. 49: 821–838. doi:10.4319/lo.2004.49.3.0821

- Bond, A. L., and K. A. Hobson. 2012. Reporting stable isotope ratios in ecology: Recommended terminology, guidelines and best practices. Waterbirds **35**: 324–331.
- Bontes, B. M., R. Pel, B. W. Ibelings, H. T. S. Boschker, J. J. Middelburg, and E. Van Donk. 2006. The effects of biomanipulation on the biogeochemistry, carbon isotopic composition and pelagic food web relations of a shallow lake. Biogeosciences 3: 69–83.
- Boschker, H. T. S., and J. J. Middelburg. 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol. Ecol. **40**: 85–95. doi:10.1111/j.1574-6941.2002.tb00940.x
- Bowen, G. J., J. B. West, and T. E. Dawson. 2010. Isoscapes in a rapidly changing and increasingly interconnected world, p. 425–432. *In* J. B. West, G. J. Bowen, T. E. Dawson, and K. P. Tu [eds.], Isoscapes: Understanding movement, pattern, and process on earth through isotope mapping. Springer.
- Brandes, J. A., and A. H. Devol. 1997. Isotopic fractionation of oxygen and nitrogen in coastal marine sediments. Geochim. Cosmochim. Acta 61: 1793–1801. doi:10.1016/ S0016-7037(97)00041-0
- Brett, M. T., M. J. Kainz, S. J. Taipale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proc. Natl. Acad. Sci. USA 106: 21197–21201.
- Bronk, D. A., J. H. See, P. Bradley, and L. Killberg. 2007. DON as a source of bioavailable nitrogen for phytoplankton. Biogeosciences 4: 283–296.
- Brzezinski, M. A., and D. M. Nelson. 1995. The annual silica cycle in the Sargasso Sea near Bermuda. Deep-Sea Res. Part I Oceanogr. Res. Pap. 42: 1215–1237.
- Brzezinski, M. A., and C. A. Kosman. 1996. Silica production in the Sargasso Sea during spring 1989. Mar. Ecol. Prog. Ser. 142: 39–45.
- Brzezinski, M. A., and D. M. Nelson. 1996. Chronic substrate limitation of silicic acid uptake rates in the western Sargasso Sea. Deep-Sea Res. Part II Top. Stud. Oceanogr. 43: 437–453.
- Bunn, S. E., C. Leigh, and T. D. Jardine. 2013. Diet–tissue fractionation of δ¹⁵N by consumers from streams and rivers. Limnol. Oceanogr. 58: 765–773. doi:10.4319/lo.2013.58.3.0765
- Cabana, G., and J. B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature **372**: 255–257.
- Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Natl. Acad. Sci. USA **93**: 10844–10847.
- Canfield, D. E. 2001. Biogeochemistry of sulfur isotopes, p. 607–636. *In* J. W. Valley and D. R. Cole [eds.], Reviews in mineralogy and geochemistry. Mineralogical Society of America.
- Canfield, D. E., and B. Thamdrup. 1994. The production of ³⁴S-depleted sulfide during bacterial disproportionation of elemental sulfur. Science **266**: 1973–1975.

- Caperon, J., D. Schell, J. Hirota, and E. Laws. 1979. Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a ¹⁵N isotope dilution technique. Mar. Biol. **54**: 33–40.
- Caraco, N. F., G. G. Lampman, J. J. Cole, K. E. Limburg, M. L. Pace, and D. T. Fischer. 1998. Microbial assimilation of DIN in a nitrogen rich estuary: Implications for food quality and isotope studies. Mar. Ecol. Prog. Ser. **167**: 59–71.
- Carman, K. R., and B. Fry. 2002. Small-sample methods for δ^{13} C and δ^{15} N analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. Mar. Ecol. Prog. Ser. **240**: 85–92.
- Carpenter, S. R., and others. 2005. Ecosystem subsidies: Terrestrial support of aquatic food webs from ¹³C addition to contrasting lakes. Ecology **86**: 2737–2750.
- Casciotti, K. L., and C. Buchwald. 2012. Insights on the marine microbial nitrogen cycle from isotopic approaches to nitrification. Front. Microbiol. **3**: 356. doi:10.3389/fmicb.2012.00356
- Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors (Δ^{15} N and Δ^{13} C): The effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. **46**: 443–453.
- Chikaraishi, Y., and others. 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnol. Oceanogr.: Methods **7**: 740–750.
- Christianen, M. J. A., and others. 2017. Benthic primary producers are key to sustain the Wadden Sea food web stable carbon isotope analysis at landscape scale. Ecology **98**: 1498–1512.
- Cifuentes, L. A., J. H. Sharp, and M. L. Fogel. 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnol. Oceanogr. **33**: 1102–1115. doi:10.4319/ lo.33.5.1102
- Cline, J. D., and I. R. Kaplan. 1975. Isotopic fractionation of dissolved nitrate during denitrification in the eastern tropical North Pacific Ocean. Mar. Chem. **3**: 271–299. doi: 10.1016/0304-4203(75)90009-2
- Cochlan, W. P., J. Herndon, and R. M. Kudela. 2008. Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseduo-nitzschia australis* (Bacillariophyceae). Harmful Algae **8**: 111–118.
- Cockerton, H. E., F. A. Street-Perrott, M. J. Leng, P. A. Barker, M. S. A. Horstwood, and V. Pashley. 2013. Stable-isotope (H, O, and Si) evidence for seasonal variations in hydrology and Si cycling from modern waters in the Nile Basin: Implications for interpreting the quaternary record. Quat. Sci. Rev. **66**: 4–21. doi:10.1016/j.quascirev.2012.12.005
- Cohen, R. A., and P. Fong. 2006. Using opportunistic green macroalgae as indicators of nitrogen supply and sources to estuaries. Ecol. Appl. **16**: 1405–1420. doi: 10.1890/1051-0761(2006)016[1405:UOGMAI]2.0CO;2
- Cole, J. J., S. R. Carpenter, J. F. Kitchell, and M. L. Pace. 2002. Pathways of organic C utilization in small lakes: Results

from a whole-lake ¹³C addition and coupled model. Limnol. Oceanogr. **47**: 1664–1675. doi:10.4319/lo.2002.47.6. 1664

- Cole, J. J., S. R. Carpenter, J. F. Kitchell, M. L. Pace, C. T. Solomon, and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proc. Natl. Acad. Sci. USA **108**: 1975–1980. doi:10.1073/ pnas.1012807108
- Cole, J. J., and C. T. Solomon. 2012. Terrestrial support of zebra mussels and the Hudson River food web: A multi-isotope, Bayesian analysis. Limnol. Oceanogr. 57: 1802–1815. doi:10.4319/lo.2012.57.6.1802
- Cole, M. L., and others. 2004. Assessment of a delta N-15 isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. J. Environ. Qual. **33**: 124–132.
- Collos, Y. 1987. Calculations of ¹⁵N uptake rates by phytoplankton assimilating one or several nitrogen sources. Int. J. Rad. Appl. Instrum. A 38: 275–282. doi: 10.1016/0883-2889(87)90038-4
- Collos, Y., and G. Slawyk. 1985. On the compatibility of carbon uptake rates calculated from stable and radioactive isotope data: Implications for the design of experimental protocols in aquatic primary productivity. J. Plankton Res. 7: 595–603. doi:10.1093/plankt/7.5.595
- Collos, Y., B. Vaquer, G. Bibent, G. Slawyk, N. Garcia, and P. Souchu. 1997. Variability in nitrate uptake kinetics of phytoplankton communities in a Mediterranean coastal lagoon. Estuar. Coast. Shelf Sci. **44**: 369–375.
- Coplen, T. B. 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Comm. Mass Spectrom. **25**: 2538–2560.
- Costanzo, S. D., M. J. O'Donohue, W. C. Dennison, N. R. Loneragan, and M. Thomas. 2001. A new approach for detecting and mapping sewage impacts. Mar. Pollut. Bull. 42: 149–156.
- Craig, H. 1953. The geochemistry of the stable carbon isotopes. Geochim. Cosmochim. Acta **3**: 53–92. doi: 10.1016/0016-7037(53)90001-5
- Croisetière, L., L. Hare, A. Tessier, and G. Cabana. 2009. Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. Freshw. Biol. **54**: 1006–1015. doi:10.1111/j.1365-2427.2008.02147.x
- Crosta, X., and A. Shemesh. 2002. Reconciling down core anticorrelation of diatom carbon and nitrogen isotopic ratios from the Southern Ocean. Paleoceanogr. Paleoclimatol. **17**: 10-1–10-8. doi:10.1029/2000PA000565
- Cullen, J. J. 2001. Primary production methods. *In* J. H. Steele, S. A. Thorpe, and K. K. Turekian [eds.], Encyclopedia of ocean sciences. Academic Press. pp. 2277–2284.
- D'Hondt, S., and J. C. Zachos. 1993. On stable isotopic variation and earliest Paleocene planktonic formanifera. Paleoceanography **8**: 527–547.

- Dähnke, K., and B. Thamdrup. 2015. Isotope fractionation and isotope decoupling during anammox and denitrificication in marine sediments. Limnol. Oceanogr. **61**: 610–624. doi:10.1002/lno.10237
- Davies, C. L., B. W. J. Surridge, and D. C. Gooddy. 2014. Phosphate oxygen isotopes within aquatic ecosystems: Global data synthesis and future research priorities. Sci. Total Environ. 496: 563–575. doi:10.1016/j.scitotenv.2014.07.057
- de Goeij, J. M., D. van Oevelen, M. J. A. Vermeij, R. Osinga, J. J. Middelburg, A. F. P. M. de Goei, and W. Admiraal. 2013. Surviving in a marine desert: The sponge loop retains resources within coral reefs. Science 342: 108–110.
- de Kluijver, A., K. Soetaert, K. G. Schulz, U. Riebesell, R. G. J. Bellerby, and J. J. Middelburg. 2010. Phytoplanktonbacteria coupling under elevated CO₂ levels: A stable isotope labelling study. Biogeosciences **7**: 3783–3797. doi: 10.5194/bg-7-3783-2010.
- de Kluijver, A., J. Yu, M. Houtekamer, J. J. Middelburg, and Z. Liu. 2012. Cyanobacteria as a carbon source for zooplankton in eutrophic Lake Taihu, China, measured by C-13 labeling andfatty acid biomarkers. Limnol. Oceanogr. **57**: 1245–1254. doi:10.4319/lo.2012.57.4.1245
- de Kluijver, A., K. Soetaert, J. Czerny, K. G. Schulz, T. Boxhammer, U. Riebesell, and J. J. Middelburg. 2013. A 13 C labelling study on carbon fluxes in Arctic plankton communities under elevated CO₂ levels. Biogeosciences **10**: 1425–1440. doi:10.5194/bg-10-1425-2013.
- De La Rocha, C. 2006. Opal-based osotopic proxies of paleoenvironmental conditions. Global Biogeochem. Cycles **20**: GB4S09. doi:10.1029/2005GB002664
- De La Rocha, C. L., M. A. Brzezinski, and M. J. DeNiro. 1997. Fractionation of silicon isotopes by marine diatoms during biogenic silica formation. Geochim. Cosmochim. Acta **61**: 5051–5056.
- Deegan, L. A., and R. H. Garritt. 1997. Evidence for spatial variability in estuarine food webs. Mar. Ecol. Prog. Ser. **147**: 31–47.
- DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42: 495–506.
- DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta 45: 341–351.
- Dijkman, N. A., H. T. S. Boschker, J. J. Middelburg, and J. C. Kromkamp. 2009. Group-specific primary production based on stable-isotope labeling of phospholipid-derived fatty acids. Limnol. Oceanogr.: Methods 7: 612–625.
- Ding, T., D. Wan, C. Wang, and F. Zhang. 2004. Silicon isotope compositions of dissolved silicon and suspended matter in the Yangtze River, China. Geochim. Cosmochim. Acta **68**: 205–216. doi:10.1016/S0016-7037(03)00264-3
- Doucett, R. R., J. C. Marks, D. W. Blinn, M. Caron, and B. A. Hungate. 2007. Measuring terrestrial subsidies to aquatic

food webs using stable isotopes of hydrogen. Ecology **88**: 1587–1592. doi:10.1890/06-1184

- Dubinski, Y., J. Erez, and M. Stiller. 2003. Isotopic paleolimnology of Lake Kinneret. Limnol. Oceanogr. **48**: 68–78. doi: 10.4319/lo.2003.48.1.0068
- Dugdale, R. C., and J. J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. **12**: 196–206. doi:10.4319/lo.1967.12.2.0196
- Dugdale, R. C., and F. P. Wilkerson. 1986. The use of ¹⁵N to measure nitrogen uptake in eutrophic oceans; experimental considerations. Limnol. Oceanogr. **31**: 673–689. doi: 10.4319/lo.1986.31.4.0673
- Dugdale, R. C., F. P. Wilkerson, V. E. Hogue, and A. Marchi. 2007. The role of ammonium and nitrate in spring bloom development in San Francisco Bay. Estuar. Coast. Shelf Sci. 73: 17–29. doi:10.1016/j.ecss.2006.12.008
- Eiler, J. M. 2007. "Clumped-isotope" geochemistry—the study of naturally-occurring, multiply-substituted isotopologues. Earth Planet. Sci. Lett. **262**: 309–327.
- Elsbury, K. E., A. Paytan, N. E. Ostrom, C. Kendall, M. B. Young, K. McLaughlin, M. E. Rollog, and S. Watson. 2009. Using oxygen isotopes of phosphate to trace phosphorus sources and cycling in Lake Erie. Environ. Sci. Technol. **43**: 3108–3114. doi:10.1021/es8034126.
- Eppley, R. W., and B. J. Peterson. 1979. Particulates, organic matter flux and planktonic new production in the deep ocean. Nature **282**: 677–680.
- Epstein, S., H. A. Buchsbaum, H. A. Lowenstam, and H. C. Urey. 1953. Revised carbonate-water isotopic temperature scale. Geol. Soc. Am. Bull. **64**: 1315–1326.
- Erler, D. V., T. M. Duncan, R. Murray, D. T. Maher, I. R. Santos, J. R. Gatland, P. Mangion, and B. D. Eyre. 2015. Applying cavity ring-down spectroscopy for the measurement of dissolved nitrous oxide concentrations and bulk nitrogen isotopic composition in aquatic systems: Correcting for interferences and field application. Limnol. Oceanogr.: Methods 13: 391–401.
- Eyre, B. D., J. M. Oakes, and J. J. Middelburg. 2016. Fate of microphytobenthos nitrogen in subtropical subtidal sediments: A N-15 pulse-chase study. Limnol. Oceanogr. 61: 2108–2121. doi:10.1002.lno.10356
- Fan, C., P. M. Glibert, and J. M. Burkholder. 2003. Characterization of the affinity for nitrogen, uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural blooms and laboratory cultures. Harmful Algae 2: 283–299.
- Fan, Y., Y. Chen, Q. He, W. Li, and Y. Wang. 2016. Isotopic characterization of river waters and water source identification in an inland river, Central Asia. Water 8: 286. doi: 10.3390/w8070286
- Fawcett, S. E., M. W. Lomas, J. R. Casey, B. B. Ward, and D. M. Sigman. 2011. Assimilation of upwelled nitrate by small eukaryotes in the Sargasso Sea. Nat. Geosci. 4: 717–722.
- Ferrón, S., D. A. del Valle, K. M. Björkman, P. D. Quay, M. J. Church, and D. M. Karl. 2016. Application of membrane

inlet mass spectrometry to measure aquatic gross primary production by the 18 O in vitro method. Limnol. Oceanogr.: Methods **14**: 610–622.

- Fertig, B., T. J. B. Carruthers, W. C. Dennison, A. B. Jones, F. Pantus, and B. Longstaff. 2009. Oyster and macroalgae bioindicators detect elevated N in Maryland's coastal bays. Estuaries Coast. 32: 773–786.
- Fertig, B., T. J. B. Carruthers, W. C. Dennison, E. J. Fertig, and M. A. Altabet. 2010. Eastern oyster (*Crassostrea virginica*) δ^{15} N as s a bioindicator of nitrogen sources: Observations and modeling. Mar. Pollut. Bull. **60**: 1288–1298. doi: 10.1016/j.marpolbul.2010.03.013
- Fila, L., R. H. Carmichael, A. Shriver, and I. Valiela. 2001. Stable N isotopic signatures in bay scallop tissue, feces, and pseudofeces in Cape Cod estuaries subject to different N loads. Biol. Bull. **201**: 294–296.
- Finlay, J. C., M. E. Power, and G. Cabana. 1999. Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. Limnol. Oceanogr. 44: 1198–1203. doi: 10.4319/lo.1999.44.5.1198
- Finlay, J. C., S. Khandwala, and M. E. Power. 2002. Spatial scales of carbon flow in a river food web. Ecology 83: 1845–1859.
- Flockhart, D. T. T., L. I. Wassenaar, T. G. Martin, K. A. Hobson, M. B. Wunder, and D. R. Norris. 2013. Tracking multi-generational colonization of the breeding grounds by monarch butterflies in eastern North America. Proc. Biol. Sci. 280: 20131087. doi:10.1098/rspb.2013.1087
- Fontanier, C., A. Mackensen, F. J. Jorissen, P. Anschutz, L. Licari, and C. Griveaud. 2006. Stable oxygen and carbon isotopes of live benthic foraminifera from the Bay of Biscay: Microhabitat impact and seasonal variability. Mar. Micropaleontol. 58: 159–183. doi:10.1016/j.marmicro.2005.09.004
- Fontorbe, G., P. J. Frings, C. L. De La Rocha, K. R. Hendry, J. Carstensen, and D. J. Conley. 2017. Enrichment of dissolved silica in the deep equatorial Pacific during the Eocene-Oligocene. Paleoceanogr. Paleoclimatol. **32**: 848–863. doi:10.1002/2017PA003030
- Fourqueran, J. W., S. P. Escoricia, W. T. Anderson, and J. C. Zieman. 2005. Spatial and seasonal variability in elemental content, δ^{13} C, and δ^{15} N of *Thalassia testudinum* from south Florida and its implications for ecosystem studies. Estuaries Coast. **28**: 447–461.
- France, R. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: Foodweb implications. Mar. Ecol. Prog. Ser. **124**: 307–312.
- Fry, B. 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries **25**: 264–271.
- Fry, B. 2006. Stable isotope ecology. Springer, p. 308.
- Fry, B., R. S. Scalan, J. K. Winters, and P. L. Parker. 1982. Sulfur uptake by salt grasses, mangroves, and seagrasses in

anaerobic sediments. Geochim. Cosmochim. Acta **46**: 1121–1124. doi:10.1016/0016-7037(82)90063-1.

- Fry, B., J. Cox, H. Gest, and J. M. Hayes. 1986. Discrimination between ³⁴S and ³²S during bacterial metabolism of inorganic sulfur compounds. J. Bacteriol. **165**: 328–330.
- Fry, B., and Y. C. Allen. 2003. Stable isotopes in zebra mussels as bioindicators of river-watershed linkages. River Res. Appl. 19: 683–696.
- Fry, B., and M. M. Chumchal. 2011. Sulfur stable isotope indicators of residency in estuarine fish. Limnol. Oceanogr. 56: 1563–1576. doi:10.4319/lo.2011.56.5.1563
- Gagnon, R., M. Levasseur, A. M. Weise, and J. Fauchot. 2005. Growth stimulation of *Alexandrium tamarense* (Dinophyceae) by humic substances from the Manicouagan River (Eastern Canada). J. Phycol. **41**: 489–497.
- Galbraith, E. D., M. Sigman, R. S. Robinson, and T. F. Pederson. 2008. Nitrogen in past marine environments, p. 1497–1535. *In* D. G. Capone, D. A. Bronk, M. R. Mulholland, and E. J. Carpenter [eds.], Nitrogen in the marine environment. Academic Press.
- Gartner, A., P. Lavery, and A. J. Smit. 2002. Use of signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Mar. Ecol. Prog. Ser. **235**: 63–73.
- Georg, R. B., B. C. Reynolds, M. Frank, and A. N. Halliday. 2006. Mechanisms controlling the silicon isotopic compositions of river waters. Earth Planet. Sci. Lett. **249**: 290–306. doi:10.1016/j.epsl.2006.07.006
- Glibert, P. M. 1982. Regional studies of daily, seasonal, and size-fraction variability in ammonium remineralization. Mar. Biol. **70**: 209–222.
- Glibert, P. M., J. C. Goldman, and E. J. Carpenter. 1982*a*. Seasonal variations in the utilization of ammonium and nitrate by phytoplankton in Vineyard Sounds, Massachusetts, USA. Mar. Biol. **70**: 237–249.
- Glibert, P. M., F. Lipschultz, J. J. McCarthy, and M. A. Altabet. 1982b. Isotope dilution models of uptake and remineralization of ammonium by marine plankton. Limnol. Oceanogr. 27: 639–650. doi:10.4319/lo.1982.27.4.0639
- Glibert, P. M., and D. G. Capone. 1993. Mineralization and assimilation in aquatic, sediment, and wetland systems, p. 243–272. *In* R. Knowles and T. H. Blackburn [eds.], Nitrogen isotope techniques. Academic Press.
- Glibert, P. M., and D. A. Bronk. 1994. Release of dissolved organic nitrogen by the marine diazotrophic cyanobacterium *Trichodesmium* spp. Appl. Environ. Microbiol. **60**: 3996–4000.
- Glibert, P. M., C. A. Heil, D. Hollander, M. Revilla, A. Hoare, J. Alexander, and S. Murasko. 2004. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Mar. Ecol. Prog. Ser. **280**: 73–83.
- Glibert, P. M., F. Wilkerson, R. C. Dugdale, A. E. Parker, J. A. Alexander, S. Blaser, and S. Murasko. 2014. Microbial

communities from San Francisco Bay Delta respond differently to oxidized and reduced nitrogen substrates—even under conditions that would otherwise suggest nitrogen sufficiency. Front. Mar. Sci. **1**:1–17. doi:10.3389/fmars.2014. 00017

- Glibert, P. M., and others. 2016. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. Limnol. Oceanogr. **61**: 165–197. doi:10/1002/ lno.10203
- Goering, J. J., R. C. Dugdale, and D. W. Menzel. 1964. Cyclic diurnal variations in the uptake of ammonia and nitrate by photosynthetic organisms in the Sargasso Sea. Limnol. Oceanogr. **9**: 448–451. doi:10.4319/lo.1964.9.3.0448
- Goering, J. J., R. C. Dugdale, and D. W. Menzel. 1966. Estimates of in situ rates of nitrogen uptake by *Trichodesmium* sp. in the tropical Atlantic. Limnol. Oceanogr. **11**: 614–620. doi:10.4319/lo.1966.11.4.0614
- Goldhammer, T., T. Max, B. Brunner, F. Einsiedl, and M. Zabel. 2011. Marine sediment pore-water profiles of δ^{18} O using a refined micro-extraction. Limnol. Oceanogr.: Methods **9**: 110–120. doi:10.4319/lom.2011.9.110
- Goldman, J. C., and P. M. Glibert. 1983. Kinetics of inorganic nitrogen uptake, p. 233–274. *In* E. J. Carpenter and D. G. Capone [eds.], Nitrogen in the marine environment. Academic Press.
- Granger, J., D. M. Sigman, J. A. Needoba, and P. J. Harrison. 2004. Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. Limnol. Oceanogr. 49: 1763–1773. doi:10.4319/ lo.2004.49.5.1763
- Gribsholt, B., and others. 2005. Nitrogen processing in a tidal freshwater marsh: A whole-ecosystem (15)N labeling study. Limnol. Oceanogr. **50**: 1945–1959. doi:10.4319/lo.2005.50.6.1945
- Gribsholt, B., B. Veuger, A. Tramper, J. J. Middelburg, and H. T. S. Boschker. 2009. Long-term N-15-nitrogen retention in tidal freshwater marsh sediment: Elucidating the microbial contribution. Limnol. Oceanogr. 54: 13–22. doi: 10.4319/lo.2009.54.1.0013
- Grosse, J., P. van Breugel, and H. T. S. Boschker. 2015. Tracing carbon fixation in phytoplankton–compound specific and total ¹³C incorporation rates. Limnol. Oceanogr.: Methods **13**: 288–302. doi:10.1002/lom3.10025
- Gruau, G., M. Legeas, C. Riou, E. Gallacier, F. Martineau, and O. Hénin. 2005. The oxygen isotope composition of dissolved anthropogenic phosphates: A new tool for eutrophication research? Water Res. **39**: 232–238. doi:10.1016/j. watres.2004.08.035
- Guiterrez-Rodriguez, A., M. Décima, B. N. Popp, and M. R. Landry. 2014. Isotopic invisibility of protozoan trophic steps in marine food webs. Limnol. Oceanogr. **59**: 1590–1598. doi:10.4319/lo.2014.59.5.1590

- Hall, R. O., Jr., M. A. Baker, C. D. Arp, and B. J. Koch. 2009. Hydrologic control of nitrogen removal, storage, and export in a mountain stream. Limnol. Oceanogr. **54**: 2128–2142. doi:10.4319/lo.2009.54.6.2128
- Hama, T., J. Hama, and N. Handa. 1993. ¹³C tracer methodology in microbial ecology with special reference to primary production processes in aquatic environments, p. 39–83. *In* J. G. Jones [ed.], Advances in microbial ecology, v. **13**. Plenum.
- Hamilton, S. K., J. L. Tank, D. E. Raikow, E. R. Siler, N. J. Dorn, and N. E. Leonard. 2004. The role of instream vs allochthonous N in stream food webs: Modeling the results of an isotope addition experiment. J. North Am. Benthol. Soc. 23: 429–448.
- Harrison, A. G., and H. G. Thode. 1958. Mechanisms of the bacterial reduction of sulfate from isotope fractionation studies. Trans. Faraday Soc. **53**: 84–92.
- Harrison, W. G. 1978. Experimental measurements of nitrogen remineralization in coastal waters. Limnol. Oceanogr. 23: 684–694. doi:10.4319/lo.1978.23.4.0684
- Harrison, W. G., and L. H. Harris. 1986. Isotope-dilution and its effects on measurements of nitrogen and phosphorus uptake by oceanic microplankton. Mar. Ecol. Prog. Ser. **27**: 253–261.
- Hatzenpichler, R., S. A. Connon, D. Goudeau, R. R. Malmstrom, T. Woyke, and V. J. Orphan. 2016. Visualizing in situ translational activity for identifying and sorting slow-growing archaeal–bacterial consortia. Proc. Natl. Acad. Sci. USA **113**: E4069–E4078. doi:10.1073/pnas.1603757113
- Hayes, J. M. 2004. An introduction to isotope calculations; [accessed 2018 January 22]. Available from http://www. whoi.edu/cms/files/jhayes/2005/9/IsoCalcs30Sept04_ 5183.pdf
- Hayes, J. M., K. H. Freeman, B. N. Popp, and C. H. Hoham. 1990. Compound-specific isotopic analyses: A novel tool for reconstruction of ancient biogeochemical processes. Org. Geochem. 16: 1115–1128.
- Hecky, R. E., and R. H. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. J. North Am. Benthol. Soc. **14**: 631–653.
- Hobson, K. A., A. Ofukany, D. X. Soto, and L. I. Wassenaar. 2012. An isotopic baseline (δ^{13} C, δ^{15} N) for fishes of Lake Winnipeg: Implications for investigating impacts of eutrophication and invasive species. J. Great Lakes Res. **38**(Supp 3): 58–65. doi:10.1016/j.jglr.2010.11.008.
- Hobson, K. A., and H. E. Welch. 1992. Determinations of trophic relations within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar. Ecol. Prog. Ser. **84**: 9–18.
- Holmer, M., and H. Hasler-Sheetal. 2014. Sulfide intrusion in seagrasses assessed by stable sulfur isotopes—a synthesis of current results. Front. Mar. Sci. 1: 64. doi:10.3389/fmars.2014.00064
- Holmes, R. M., B. J. Peterson, L. A. Deegan, J. E. Hughes, andB. Fry. 2000. Nitrogen biogeochemistry in the oligohaline zone of a New England estuary. Ecology 81: 416–432.

- Hotchkiss, E. R., and R. O. Hall Jr. 2015. Whole-stream ¹³C tracer addition reveals distinct fates of newly fixed carbon. Ecology **96**: 403–416. doi:10.1890/14-0631.1
- Hughes, H. J., S. Bouillon, L. André, and D. Cardinal. 2012. The effects of weathering variability and anthropogenic pressures upon silicon cycling in an intertropical watershed (Tana River, Kenya). Chem. Geol. **308–309**: 18–25. doi: 10.1016/j.chemgeo.2012.03.016
- Hughes, J. E., L. A. Deegan, B. J. Peterson, R. M. Holmes, and B. Fry. 2000. Nitrogen flow through the food web in the oligohaline zone of a New England estuary. Ecology 81: 433–452. doi:10.1890/0012-9658(2000)081[0433:NFTTFW] 2.0.CO;2
- Ishimura, T., U. Tsunogai, S. Hasagawa, F. Nakagawa, T. Oi, H. Kitazato, H. Suga, and T. Toyofuku. 2012. Variation in stable carbon and oxygen isotopes of individual benthic formanifera: Tracers for quantifying the magnitude of isotopic disequilibrium. Biogeosciences **9**: 4353–4367. doi:10.5194/bg-9-4353-2012
- Jaisi, D. P., R. K. Kukkadapu, L. M. Stout, T. Varga, and R. E. Blake. 2011. Biotic and abiotic pathways of phosphorus cycling in minerals and sediments: Insights from oxygen isotope ratios in phosphate. Environ. Sci. Technol. **45**: 6254–6261. doi:10.1021/es200456e
- Johnston, D. T., B. C. Gill, A. Masterson, E. Beirne, K. L. Casciotti, A. N. Knapp, and W. Berelson. 2014. Placing an upper limit on cryptic marine sulphur cycling. Nature **513**: 530–533. doi:10.1038/nature13698
- Jørgensen, B. B. 1977. Sulphur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol. Oceanogr. **22**: 814–832.
- Josji, S. R., R. K. Kukkadapu, D. J. Burdige, M. E. Bowden, D. L. Sparks, and D. P. Jaisi. 2015. Organic matter remineralization predominates phosphorus cycling in the mid-bay sediments in the Chesapeake Bay. Environ. Sci. Technol. 49: 5887–5896. doi:10.1021/es5059617
- Kana, T. M. 1992. Relationship between photosynthetic oxygen cycling and carbon assimilation in *Synechococcus* WH7803 (Cyanophyta). J. Phycol. 28: 304–308. doi: 10.1111/j.0022-3646.1992.00304.x
- Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennett, and J. C. Cornwell. 1994. Membrane inlet mass spectrometry for rapid high-precision determination of N_2 , O_2 and Ar in environmental samples. Anal. Chem. **66**: 4166–4170.
- Kana, T. M., J. C. Cornwell, and L. Zhong. 2006. Determination of denitrification in the Chesapeake Bay from measurements of N accumulation in bottom water. Estuaries Coast. 29: 222–231. doi:10.1007/BF02781991
- Kaplan, I. R., K. O. Emery, and S. C. Rittenberg. 1963. The distribution and isotopic abundance of sulphur in recent marine sediments off southern California. Geochim. Cosmochim. Acta 27: 297–331.

- Kaplan, I. R., and S. C. Rittenberg. 1964. Microbiological fractionation of sulphur isotopes. J. Gen. Microbiol. 34: 195–212.
- Karlsson, J., M. Berggren, J. Ask, P. Bystrom, A. Jonsson, H. Laudon, and M. Jansson. 2012. Terrestrial organic matter support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers. Limnol. Oceanogr. 57: 1042–1048. doi:10.4319/lo.2012.57.4.1042
- Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments, p. 519–576. *In C.* Kendall and J. J. McDonnell [eds.], Isotopic tracers in catchment hydrology. Elsevier Science B.V.
- Kendall, C., E. M. Elliott, and S. D. Wankel. 2008. Tracing anthropogenic inputs of nitrogen to ecosystems. pp. 375– 450. *In* R. Michener and K. Lajtha [eds.], Stable isotopes in ecology and environmental science. Wiley Blackwell Publishing. 375–450.
- Keul, N., K. T. C. A. Peijnenburg, N. Andersen, V. Kitidis, E. Goetze, and R. R. Scheneider. 2017. Pteropds are excellent recorders of surface temperature and carbonate ion concentration. Sci. Rep. 7: 12645. doi:10.1038/s41598-017-11708-w
- Kling, G. W., B. Fry, and W. J. O'Brien. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology **73**: 561–566.
- Kudela, R., and W. P. Cochlan. 2000. Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California. Aquat. Microb. Ecol. **21**: 31–47.
- L'Helguen, S., J.-F. Maguer, and J. Caradec. 2008. Inhibition kinetics of nitrate uptake by ammonium in size fractionated oceanic phytoplankton communities: Implications for new production and f-ratio estimates. J. Plankton Res. **10**: 1179–1188.
- Lajtha, K., and R. H. Michener. 1994. Introduction, p. xi–xix. *In* K. Lajtha and R. H. Michener [eds.], Stable isotopes in ecology and environmental science. Blackwell Scientific Publications.
- Lapointe, B. E., K. Thacker, C. Hanson, and L. Getten. 2011. Sewage pollution in Negril, Jamaica: Effects on nutrition and ecology of coral reef macroalgae. Chin. J. Oceanol. Limnol. 29: 775–789.
- Lavoie, R. A., T. D. Jardine, M. M. Chumchal, K. A. Kidd, and L. M. Campbell. 2013. Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. Environ. Sci. Technol. 47: 13385–13394.
- Li, J., P. M. Glibert, and M. Zhou. 2010. Temporal and spatial variability in nitrogen uptake kinetics during harmful dino-flagellate blooms in the East China Sea. Harmful Algae **9**: 531–539.
- Li, M., and others. 2012. Rapid resonance Raman microspectroscopy to probe carbon dioxide fixation by single cells in microbial communities. ISME J. 6: 875–885. doi:10.1038/ ismej.2011.150

- Li, X., Y. Wang, J. Stern, and B. H. Cu. 2011. Isotopic evidence for the source and fate of phosphorus in Everglades wetland ecosystems. Appl. Geochem. 26: 688–695. doi: 10.1016/j.apgeochem.2011.01.027
- Lindehoff, E., E. Graneli, and P. M. Glibert. 2011. Nitrogen uptake kinetics of *Prymnesium parvum* (Haptophyceae). Harmful Algae **12**: 70–76.
- Lipschultz, F. 1995. Nitrogen specific uptake rates of marine phytoplankton isolated from natural populations of particles by flow cytometry. Mar. Ecol. Prog. Ser. **123**: 245–258.
- Lisiecki, L. E., and M. E. Raymo. 2005. A Pliocene-Pleistocene stack of 57 globally distributed benthic δ^{18} O records. Paleoceanogr. Paleoclimatol. **20**: PA1003. doi:10.1029/2004PA001071
- Lomas, M. W., and P. M. Glibert. 1999. Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. Limnol. Oceanogr. 44: 556–572. doi:10.4319/lo.1999.44.3.0556
- Loomer, H. A., K. D. Oakes, S. L. Schiff, W. D. Taylor, and M. R. Servos. 2014. Use of stable isotopes to trace municipal wastewater effluents into food webs within a highly developed river system. River Res. Appl. **31**: 1093–1100. doi: 10.1002/rra.2826
- Lorrain, A., Y.-M. Paulet, L. Chauvaud, N. Savoye, A. Donval, and C. Saout. 2002. Differential δ^{13} C and δ^{15} N signatures among scallop tissues: Implications for ecology and physiology. J. Exp. Mar. Biol. Ecol. **275**: 47–61.
- Lundstrum, A., and L. R. Acki. 2016. Oxygen interference with membrane inlet mass spectrometry may overestimate denitrification rates calculated with the isotope pairing technique. Limnol. Oceanogr.: Methods **14**: 425–431.
- MacKenzie, K. M., M. R. Palmer, A. Moore, A. T. Ibbotson, W. R. C. Beaumont, D. J. S. Poulter, and C. N. Trueman. 2011. Locations of marine animals revealed by carbon isotopes. Sci. Rep. 1: 21. doi:10.1038/srep00021
- Macko, S. A., M. L. Fogel, P. E. Hare, and T. C. Hoering. 1987. Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. Geochim. Cosmochim. Acta **50**: 2143–2146.
- Mader, M., C. Schmidt, R. van Geldern, and J. A. C. Berth. 2017. Dissolved oxygen in water and its stable isotope effects: A review. Chem. Geol. **473**: 10–21. doi:10.1016/j. chemgeo.2017.10.003
- Madigan, D. J., A. B. Carlisle, H. Dewar, O. E. Snodgrass, S. Y. Litvin, F. Micheli, and B. A. Black. 2012. Stable isotope analysis challenges wasp-waist food web assumptions in an upwelling pelagic ecosystem. Sci. Rep. 2: 654. doi:10.1038/ srep00654
- Magozzi, S., A. Yool, H. B. Vander Zanden, M. B. Wunder, and C. N. Trueman. 2017. Using ocean models to predict spatial and temporal variation in marine carbon isotopes. Ecosphere **8**: e01763. doi:10.1002/ecs2.1763
- Maher, D. T., I. R. Santos, J. R. F. W. Leuven, J. M. Oakes, D. V. Erler, M. C. Carvalho, and B. D. Eyre. 2013. Novel use

of cavity ring-down spectroscopy to investigate aquatic carbon cycling from microbial to ecosystem scales. Environ. Sci. Technol. **47**: 12938–12945. doi:10.1021/es4027776

- Mariotti, A., J. C. Germon, P. Hubert, P. Kaiser, R. Létolle, A. Tardieux, and P. Tardieux. 1981. Experimental determination of nitrogen kinetic isotope fractionations: Some principles; illustration for the denitrification and nitrification processes. Plant Soil 62: 413–430.
- McCarthy, J. J. 2002. Biological responses to nutrients, p. 219–244. *In* A. R. Robinson, J. J. McCarthy, and B. J. Rothschild [eds.], The sea, v. **12**. John Wiley and Sons.
- McCarthy, J. J., W. R. Taylor, and J. L. Taft. 1977. Nitrogenous nutrition of the plankton in the Chesapeake Bay. I. Nutrient availability and phytoplankton preferences. Limnol. Oceanogr. **22**: 996–1011. doi:10.4319/lo.1977.22.6.0996
- McCarthy, J. J., C. Garside, and J. L. Nevins. 1992. Nitrate supply and phytoplankton uptake kinetics in the euphotic layer of a Gulf Stream warm core ring. Deep-Sea Res. Part A Oceanogr. Res. Pap. **39**: S393–S403.
- McCarthy, M. D., R. Benner, C. Lee, and M. L. Fogel. 2007. Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. Geochim. Cosmochim. Acta **71**: 4727–4744.
- McClelland, J. W., I. Valiela, and R. H. Michener. 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. Limnol. Oceanogr. **42**: 930–937. doi:10.4319/lo.1997.42.5.0930
- McClelland, J. W., and I. Valiela. 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnol. Oceanogr. **43**: 577–585. doi:10.4319/lo.1998.43.4.0577
- McClelland, J. W., and J. P. Montoya. 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. Ecology **83**: 2173–2180.
- McClelland, J. W., C. W. Holl, and J. P. Montoya. 2003. Relating low δ^{15} N values of zooplankton to Nation in the tropical North Atlantic: Insights provided by stable isotope ratios of amino acids. Deep-Sea Res. Part I Oceanogr. Res. Pap. **50**: 849–861.
- McCorkle, D. C., B. H. Corliss, and C. A. Farnham. 1997. Vertical distributions and stable isotopic compositions of live (stained) benthic foraminifera from the North Carolina and California continental margins. Deep-Sea Res. Part I Oceanogr. Res. Pap. **44**: 983–1024.
- McKinney, R. A., W. G. Nelson, C. Wigand, and M. A. Charpentier. 2001. Ribbed mussel nitrogen isotope signatures reflect nitrogen sources in coastal salt marshes. Ecol. Appl. **11**: 203–214.
- McKinney, R. A., J. L. Lake, M. A. Charpentier, and S. A. Ryba. 2002. Using mussel isotope ratios to assess anthropogenic nitrogen inputs to freshwater ecosystems. Environ. Monit. Assess. **74**: 167–192.
- McLaughlin, K., S. Silva, C. Kendall, H. Stuart-Williams, and A. Paytan. 2004. A precise method for the analysis of $\delta^{18}O$ of

dissolved inorganic phosphate in seawater. Limnol. Oceanogr.: Methods **2**: 202–212.

- McLaughlin, K., B. J. Cade-Menun, and A. Paytan. 2006*a*. The oxygen isotopic composition of phosphate in Elkhorn Slough, California: A tracer for phosphate sources. Estuar. Coast. Shelf Sci. **70**: 499–506. doi:10.1016/j. ecss.2006.06.030
- McLaughlin, K., F. Chavez, J. T. Pennington, and A. Paytan. 2006b. A time series investigation of the oxygen isotopic composition of dissolved inorganic phosphate in Monterey Bay, California. Limnol. Oceanogr. **51**: 2370–2379. doi: 10.4319/lo.2006.51.5.2370
- McLaughlin, K., A. Paytan, C. Kendall, and S. Silva. 2006c. Oxygen isotopes of phosphatic compounds—application for marine particulate matter, sediments and soils. Mar. Chem. **98**: 148–155. doi:10.1016/j.marchem.2005.09.004
- McMahon, K. W., and M. D. McCarthy. 2016. Embracing variability in amino acid δ^{15} N fractionation: Mechanisms, implications, and applications for trophic ecology. Ecosphere **7**: e01511. doi:10.1002/ecs2.1511
- Merriam, J. L., W. H. McDowell, J. L. Tank, W. M. Wollheim, C. L. Crenshaw, and S. L. Johnson. 2002. Characterizing nitrogen dynamics, retention and transport in a tropical rainforest stream using an *in situ* ¹⁵N addition. Freshw. Biol. **47**: 143–160.
- Middelburg, J. J. 2014. Stable isotopes dissect aquatic food webs from the top to the bottom. Biogeosciences **11**: 2357–2371. doi:10.5194/bg-11-2357-2014
- Middelburg, J. J., K. Soetaert, and P. M. J. Herman. 1996. Evaluation of the nitrogen isotope-pairing method for measuring benthic denitrification: A simulation analysis. Limnol. Oceanogr. **41**: 1839–1844. doi:10.4319/lo.1996.41.8.1839
- Middelburg, J. J., C. Barranguet, H. T. S. Boschker, P. M. J. Herman, T. Moens, and C. H. R. Heip. 2000. The fate of intertidal microphytobenthos carbon: An *in situ* ¹³Clabeling study. Limnol. Oceanogr. **45**: 1224–1234. doi: 10.4319/lo.2000.45.6.1224
- Middelburg, J. J., and P. M. J. Herman. 2007. Organic matter processing in tidal estuaries. Mar. Chem. **106**: 127–147.
- Minagawa, M., and E. Wada. 1984. Stepwise enrichment of N-15 along food chains: Further evidence and the relation between δ^{15} N and animal age. Geochim. Cosmochim. Acta **48**: 1135–1140. doi:10.1016/0016-7037(84)90204-7
- Minagawa, M., and E. Wada. 1986. Nitrogen isotope ratios of red tide organisms in the East China Sea: A characterization of biological nitrogen fixation. Mar. Chem. **19**: 245–259.
- Mompeán, C., A. Bode, V. M. BenÍtez-Barrios, J. F. Dominguez-Yanes, J. Escánez, and E. Fraile-Nuez. 2013. Spatial patterns of plankton biomass and stable isotopes reflect the influence of the nitrogen-fixer *Trichodesmium* along the subtropical North Atlantic. J. Plankton Res. **35**: 513–525. doi:10.1093/plankt/fbt011
- Montoya, J. P., S. G. Horrigan, and J. J. McCarthy. 1990. Natural abundance of ¹⁵N in particulate nitrogen and

zooplankton in the Chesapeake Bay. Mar. Ecol. Prog. Ser. **65**: 35–61.

- Montoya, J. P., and J. J. McCarthy. 1995. Isotope fractionation during nitrate uptake by phytoplankton grown in continuous culture. J. Plankton Res. **17**: 439–464.
- Montoya, J. P., E. J. Carpenter, and D. G. Capone. 2002. Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. Limnol. Oceanogr. 47: 1617–1628. doi:10.4319/lo.2002.47.6.1617
- Mulholland, P. J., and others. 2002. Can uptake length in streams be determined by nutrient addition experiments? Results from an inter-biome comparison study. J. North Am. Benthol. Soc. **21**: 544–560.
- Mulholland, P. J., and others. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature **452**: 202–205.
- Musat, N., R. Foster, T. Vagner, B. Adam, and M. M. M. Kuypers. 2012. Detecting metabolic activities in single cells, with emphasis on nanoSIMS. FEMS Microbiol. Rev. **36**: 486–511. doi:10.1111/j.1574-6976.2011.00303.x
- Nelson, D. M., and J. J. Goering. 1977. A stable isotope tracer method to measure silicic acid uptake by marine phytoplankton. Anal. Biochem. **78**: 139–147.
- Nelson, D. M., J. J. Goering, and D. W. Boisseau. 1981. Consumption and regeneration of silicic acid in three coastal upwelling systems, p. 242–256. *In* F. A. Richards [ed.], Coastal upwelling. American Geophysical Union.
- Nelson, D. M., and L. I. Gordon. 1982. Production and pelagic dissolution of biogenic silica in the Southern Ocean. Geochim. Cosmochim. Acta 46: 491–500.
- Nelson, D. M., and M. A. Brzezinski. 1997. Diatom growth an productivity in an oligotrophic midocean gyre: A 3-yr record from the Sargasso Sea near Bermuda. Limnol. Oceanogr. 42: 473–486. doi:10.4319/lo.1997.42.3.0473
- Neufeld, J. D., J. Vohra, M. G. Dumont, T. Lueders, M. Manefield, M. W. Friedrich, and J. C. Murrell. 2007. DNA stable isotope probing. Nat. Protoc. **2**: 860–866.
- Nielsen, L. P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microbiol. Lett. **86**: 357–362.
- Norman, B. C., and others. 2017. Drivers of nitrogen transfer in stream food webs. Ecology **98**: 3044–3055. doi:10.1002/ ecy.2009
- Norrman, B., U. L. Zweifel, C. S. Hopkinson, and B. Fry. 1995. Production and utilization of dissolved organic carbon during an experimental diatom bloom. Limnol. Oceanogr. **40**: 898–907. doi:10.4319/lo.1995.40.5.0898
- Oakes, J. M., B. D. Eyre, J. J. Middelburg, and H. T. S. Boschker. 2010. Composition, production, and loss of carbohydrates in subtropical shallow subtidal sandy sediments: Rapid processing and long-term retention revealed by C-13-labeling. Limnol. Oceanogr. 55: 2126–2138. doi: 10.4319/lo.2010.55.5.2126

- Oakes, J. M., B. D. Eyre, and J. J. Middelburg. 2012. Transformation and fate of microphytobenthos carbon in subtropical shallow subtidal sands: A C-13-labeling study. Limnol. Oceanogr. 57: 1846–1856. doi:10.4319/lo.2012.57.06.1846
- Owens, N. J. P. 1987. Natural variations in nitrogen-15 in the marine environment. Adv. Mar. Biol. **24**: 389–451.
- Paasche, E., and S. Kristiansen. 1982. Nitrogen nutrition of the phytoplankton in the Oslofjord. Estuar. Coast. Shelf Sci. 14: 237–249.
- Pace, M. L., and others. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427**: 240–243.
- Pace, M. L., S. R. Carpenter, J. J. Cole, J. J. Coloso, J. F. Kitchell, J. R. Hodgson, J. J. Middelburg, N. D. Preston, C. T. Solomon, and B. C. Weidel. 2007. Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? Limnol. Oceanogr. **52**: 2177–2189. doi: 10.4319/lo.2007.52.5.2177
- Paetzold, A., C. J. Schubert, and K. Tockner. 2005. Aquatic terrestrial linkages along a braided-river: Riparian arthropods feeding on aquatic insects. Ecosystems **8**: 748–759.
- Panizzo, V. N., G. E. A. Swann, A. W. Mackay, E. Vologina, M. Sturm, V. Pashley, and M. S. A. Horstwood. 2016. Insights into the transfer of silicon isotopes into the sediment record. Biogeosciences 13: 147–157. doi:10.5194/bg-13-147-2016
- Payton, A., and K. McLaughlin. 2011. Tracing the sources and biogeochemical cycling of phosphorus in aquatic systems using isotopes of pxygen in phosphate, p. 419–436. *In* M. Baskaran [ed.], Handbook of environmental isotope geochemistry. Advances in Isotope Geochemistry. Springer-Verlag.
- Pel, R., H. Hoogveld, and V. Floris. 2003. Using the hidden isotopic heterogeneity in phyto- and zooplankton to unmask disparity in trophic carbon transfer. Limnol. Oceanogr. 48: 2200–2207. doi:10.4319/lo.2003.48.6.2200
- Peterson, B., B. Fry, M. Hullar, S. Saupe, and R. Wright. 1994. The distribution and stable isotopic composition of dissolved organic carbon in estuaries. Estuaries **18**: 111–121.
- Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. **18**: 293–320. doi:10.1146/annurev.ecolsys.18.1.293.
- Peterson, B. J., and R. W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnol. Oceanogr. 32: 1195–1213. doi:10.4319/lo.32.6.1195
- Peterson, B. J., and others. 2001. Control of nitrogen export from watersheds by headwater streams. Science **292**: 86–90. doi:10.1126/science.1056874
- Phillips, D. L. 2012. Converting isotope values to diet composition: The use of mixing models. J. Mammal. **93**: 342–352. doi:10.1644/11-MAMM-S-158.1

- Phillips, D. L., and P. L. Koch. 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia **130**: 114–125. doi:10.1007/s004420100786
- Phillips, D. L., R. Inger, S. Bearhop, A. J. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in foodweb studies. Can. J. Zool. **92**: 823–835. doi:10.1139/cjz-2014-0127
- Pimenov, N. V., A. M. Zyakum, T. S. Prusakova, O. N. Lunina, and M. V. Ivanov. 2008. Application of ¹³C mineral carbon for assessment of the primary production of organic matter in aquatic environments. Microbiology **77**: 224–227.
- Ploug, H., N. Musat, B. Adam, C. L. Moraru, G. Lavik, T. Vagner, B. Bergman, and M. M. Kuypers. 2010. Carbon and nitrogen fluxes associated with the cyanobacterium *Aphanizomenon* sp. in the Baltic Sea. ISME J. **4**: 1215–1223.
- Popp, B. N., R. R. Bidigare, B. Deschenes, E. A. Laws, F. G. Prhal, J. K. Tanimoto, and R. Wallsgrove. 2006. A new method for estimating growth rates of alkenone-producing haptophytes. Limnol. Oceanogr.: Methods **4**: 114–129.
- Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**: 703–718. doi:10.2307/3071875
- Post, D. M., M. L. Pace, and N. G. Hairston Jr. 2000. Ecosystem size determines food-chain length in lakes. Nature **405**: 1047–1049.
- Probyn, T. A. 1985. Nitrogen uptake by size fractionated phytoplankton populations in the Southern Benquela upwelling system. Mar. Ecol. Prog. Ser. 22: 249–258.
- Probyn, T. A., and S. J. Painting. 1985. Nitrogen uptake by size fractionatedphytoplankton populations in Antarctic surface waters. Limnol. Oceanogr. **30**: 1327–1332. doi:10.4319/ lo.1985.30.6.1327
- Probyn, T. A., H. N. Walron, and A. G. James. 1990. Sizefractionated measurements of nitrogen uptake in aged upwelled waters: Implications for pelagic food webs. Limnol. Oceanogr. **35**: 202–210. doi:10.4319/ lo.1990.35.1.0202
- Radajewski, S., P. Ineson, N. R. Parekh, and J. C. Murrell. 2000. Stable-isotope probing as a tool in microbial ecology. Nature **403**: 646–649.
- Ravelo, A., and C. Hillaire-Marcel. 2007. The use of oxygen and carbon isotopes of foraminifera in paleoceanography. Dev. Mar. Geol. 1: 735–764.
- Regaudie-de-Gioux, A., S. Lasternas, S. Agustí, and C. M. Duarte. 2014. Comparing marine primary production estimates through different methods and development of conversion equations. Front. Mar. Sci. 1: 19. doi:10.3389/ fmars.2014.00019
- Reynolds, B. C. 2011. Silicon isotopes as tracers of terrestrial processes, p. 87–104. *In* M. Baskaran [ed.], Handbook of environmental isotope geochemistry. Springer-Verlag.

- Robinson, C., and others. 2009. Comparison of in vitro and in situ plankton production determinations. Aquat. Microb. Ecol. **54**: 13–34. doi:10.3354/ame01250
- Robinson, R. S., B. G. Brunelle, and D. M. Sigman. 2004. Revisiting nutrient utilization in the glacial Antarctic: Evidence from a new method for diatom-bound N isotopic analysis. Paleoceanogr. Paleoclimatol. 19: PA3001. doi: 10.1029/2003PA000996
- Robinson, R. S., and others. 2012. A review of nitrogen isotopic alteration in marine sediments. Paleoceanogr. Paleoclimatol. 27: PA4203. doi:10.1029/2012PA002321
- Rose, L. A., E. M. Elliott, and M. B. Adams. 2015. Triple nitrate isotopes indicate differing nitrate source contributions to streams across a nitrogen saturation gradient. Ecosystems. 18: 1209–1223. doi:10.1007/s10021--015-9891-8
- Rosenfeld, J. S., and J. C. Roff. 1992. Examination of the carbon base in southern Ontario streams using stable isotopes.J. North Am. Benthol. Soc. 11: 1–10.
- Rounick, J.,. S., and M. J. Winterbourn. 1986. Stable carbon isotopes and carbon flow in ecosystems. Biosciences **36**: 171–177.
- Ryabenko, E. 2013. Stable isotope methods for the study of the nitrogen cycle. *In* E. Zambianch [ed.], Topics in oceanography. Intech Open Science.
- Salomons, W., and W. Mook. 1981. Field observations of the isotopic composition of particulate organic carbon in the southern North Sea and adjacent estuaries. Mar. Geol. **41**: 11–20.
- Sánchez-Carrillo, S., and M. Álvarez-Cobelas. 2018. Stable isotopes as tracers in aquatic ecosystems. Environ. Rev. 26: 69–81. doi:10.1139/er-2017-0040
- Santoro, A. E., C. Buchwald, M. R. McIlvin, and K. L. Casciotti. 2011. Isotopic signature of N₂O produced by marine ammonia-oxidizing archea. Science **333**: 1282–1285.
- Sanzone, D. M., J. L. Meyer, E. Marti, E. P. Gardiner, J. L. Tank, and N. B. Grimm. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. Oecologia **134**: 238–250.
- Savage, C., and R. Elmgren. 2004. Macroalgal (*Fucus vesiculo-sus*) δ^{15} N values trace decrease in sewage influence. Ecol. Appl. **14**: 517–526.
- Schindler, D. E., and M. D. Scheuerell. 2002. Habitat coupling in lake ecosystems. Oikos **98**: 177–189.
- Schmiedl, G., M. Pfeilsticker, C. Hemleben, and A. Mackensen. 2004. Environmental and biological effects on the stable isotope composition of recent deep-sea benthic foraminifera, from the western Mediterranean Sea. Mar. Micropaleontol. **51**: 129–152. doi:10.1016/j. marmicro.2003.10.001
- See, J. H., D. A. Bronk, and A. J. Lewitus. 2006. Uptake of *Spartina*-derived humic nitrogen by estuarine phytoplankton in axenic and non-axenic culture. Limnol. Oceanogr. **51**: 2290–2299. doi:10.4319/lo.2006.51.5.2290
- Shackleton, N. J. 1967. Oxygen isotope analyses and Pleistocene temperatures reassessed. Nature **215**: 15–17.

- Shackleton, N. J., and N. D. Opdyke. 1973. Oxygen isotope and paleomagnetic stratigraphy of equatorial Pacific core V28-238: Oxygen isotope temperatures and ice volumes on a 105 and 106 year scale. Quat. Res. 3: 39–55. doi: 10.1016/0033-5894(73)90052-5.
- Shemesh, A., L. H. Burckle, and J. J. Hays. 1995. Late Pleistocene oxygen isotope records of biogenic silica from the Atlantic sector of the Southern Ocean. Paleoceanogr. Paleoclimatol. 10: 179–196.
- Sheppard, C. W. 1962. Basic principles of the tracer method. Wiley.
- Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes. 1997. Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: An adaptation of the ammonia diffusion method. Mar. Chem. **57**: 227–242. doi:10.1016/s0304-4203(97) 00009-1
- Sigman, D. M., M. A. Altabet, R. Francois, D. C. McCorkle, and J.-F. Gaillard. 1999. The isotopic composition of diatombound nitrogen in Southern Ocean sediments. Paleoceanography 14: 118–134. doi:10.1029/1998PA900018
- Sigman, D. M., and K. L. Casciotti. 2001. Nitrogen isotopes in the ocean, p. 1884–1894. *In* J. H. Steele, S. A. Thorpe, and K. K. Turekian [eds.], Encyclopedia of ocean sciences. Academic Press.
- Sigman, D. M., R. Robinson, A. N. Knapp, A. van Green, D. C. McCorkle, J. A. Brandes, and R. C. Thunell. 2003. Distinguishing between water column and sedimentary denitrification in the Santa Barbara Basin using the stable isotopes of nitrate. Geochem. Geophys. Geosyst. 4: 1040. doi: 10.1029/2002GC000384.
- Sigman, D. M., J. Granger, P. J. DiFiore, M. M. Lehmann, R. Ho, G. Cane, and A. van Geen. 2005. Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin. Global Biogeochem. Cycles 19: GB4022. doi:10.1029/2005GB002458
- Sigman, D. M., K. L. Karsh, and K. L. Casciotti. 2009. Ocean process tracers: Nitrogen isotopes in the ocean. pp. 4138– 4153. *In* J. H. Steele, K. K. Turekian, and S. A. Thorpe [eds.], Encyclopedia of ocean sciences. Academic Press.
- Sluijs, A., L. van Roij, J. Frieling, J. Laks, and G.-J. Reichart. 2017. Single-species dinoflagellate cyst carbon isotope ecology across the Paleocene-Eocene thermal maximum. Geology 46: 79–82.
- Smith, B. N., and S. Epstein. 1970. Biogeochemistry of stable isotopes of hydrogen and carbon in salt marsh biota. Plant Physiol. 46: 738–742. doi:10.1104/pp.46.5.738
- Smith, W. O., and D. M. Nelson. 1990. Phytoplankton growth and new production in the Weddell Sea marginal ice zone in the austral spring and summer. Limnol. Oceanogr. **35**: 809–821. doi:10.4319/lo.1990.35.4.0809
- Solomon, C. T., S. R. Carpenter, M. K. Clayton, J. J. Cole, J. J. Coloso, M. L. Pace, M. J. Vander Zanden, and B. C. Weidel. 2011. Terrestrial, benthic, and pelagic resource use in lakes:

Results from a three-isotope Bayesian mixing model. Ecology **92**: 1115–1125.

- Song, B., and C. Tobias. 2011. Molecular and stable isotope methods to detect and measure anaerobic ammonium oxidation (anammox) in aquatic ecosystems. Methods Enzymol. **496**: 63–89. doi:10.1016/ B978-0-12-386489-5.00003.8
- Steffan, S. A., Y. Chikaraishi, P. S. Dharampal, J. N. Pauli, C. Guedot, and N. Ohkouchi. 2017. Unpacking brown food-webs: Animal trophic identity reflects rampant microbiv-ory. Ecol. Evol. 7: 3532–3541.
- Steingruber, S. M., J. Friedrich, R. Gächter, and B. Wehrli. 2001. Measurement of denitriification in sediments with the 15N isotope pairing technique. Appl. Environ. Microbiol. 67: 3771–3778. doi:10.1128/AEM.67.9.3778.2001
- Stribling, J. M., J. C. Cornwell, and C. Currin. 1998. Variability of sulfur isotopic ratios in *Spartina alterniflora*. Mar. Ecol. Prog. Ser. **166**: 73–81.
- Sutton, J. N., and others. 2018. A review of the stable isotope biogeochemistry of the global silicon cycle and its associated trace elements. Front. Earth Sci. 5: 112. doi:10.3389/ feart.2017.00112
- Tang, F. H. M., and F. Maggi. 2012. The effect of ¹⁵N to ¹⁴N ratio on nitrification, denitrification and dissimilatory nitrate reduction. Rapid Comm. Mass Spectrom. **26**: 430–442. doi:10.1002/rcm.6119
- Tank, S. E., L. F. W. Lesack, J. A. L. Gareis, C. L. Osborn, and R. H. Hesslein. 2011. Multiple tracers demonstrate distinct sources of dissolved organic matter to lakes of the Mackenzie Delta, western Canadian Arctic. Limnol. Oceanogr. 56: 1297–1309. doi:10.4319/lo.2011.56.4.1297
- Taylor, G. T., E. A. Suter, Z. Q. Li, S. Chow, D. Stinton, T. Zaliznyak, and S. R. Beaupré. 2017. Single-cell growth rates in photoautotrophic populations measured by stable isotope probing and resonance Raman microspectrometry. Front. Microbiol. 8: 1449. doi:10.3389/fmicb.2017.01449
- Terrado, R. A., L. Pasulka, A. A.-Y. Lie, V. J. Orphan, K. B. Heidelberg, and D. A. Caron. 2017. Autotrophic and heterotrophic acquisition of carbon and nitrogen by a mixotrophic chrysophyte established through stable isotope analysis. ISME J. 11: 2022–2034. doi:10.1038/ismej.2017.68
- Tesdal, J.-E., E. D. Galbraith, and M. Kienast. 2013. Nitrogen isotopes in bulk marine sediment: Linking seafloor observations with subseafloor records. Biogeosciences **10**: 101–118. doi:10.5194/bg-10-101-2013
- Thompson, A., R. A. Foster, A. Krupke, B. J. Carter, N. Musat, D. Vaulot, M. M. Kuypers, and J. P. Zehr. 2012. Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. Science 337: 1546–1550. doi:10.1126/science.1222700
- Tobias, C., M. Cieri, B. J. Peterson, L. A. Deegan, J. Vallino, and J. Hughes. 2003a. Processing watershed-derived nitrogen in a well-flushed New England estuary. Limnol. Oceanogr. 48: 1766–1778. doi:10.4319/lo.2003.48.5.1766

- Tobias, C., A. Giblin, J. McClelland, J. Tucker, and B. Peterson. 2003b. Sediment DIN fluxes and preferential recycling of benthic microalgal nitrogen in a shallow macrotidal estuary. Mar. Ecol. Prog. Ser. 257: 25–36.
- Tobias, C., J. K. Böhlke, and J. W. Harvey. 2007. The oxygen-18 isotope approach for measuring aquatic metabolism in high-productivity waters. Limnol. Oceanogr. **52**: 1439–1453. doi: 10.4319/lo.2007.52.4.1439
- Trust, B. A., and B. Fry. 1992. Sulphur isotopes in plants: A review. Plant Cell Environ. **15**: 1105–1110.
- Tsunogai, U., T. Miyauchi, T. Ohyama, D. D. Komatsu, M. Ito, and F. Nakagawa. 2018. Quantifying nitrate dynamics in a mesotrophic lake using triple oxygen isotopes as tracers. Limnol. Oceanogr. 63: S458–S476. doi:10.1002/lno.10775
- Tucker, J., N. Sheats, A. E. Giblin, C. S. Hopkinson, and J. P. Montoya. 1999. Using stable isotopes to trace sewagederived material through Boston Harbor and Massachusetts Bay. Mar. Environ. Res. 48: 353–375.
- Tunney, T. D., K. S. McCann, N. P. Lester, and B. J. Shuter. 2014. Effects of differential habitat warming on complex communities. Proc. Natl. Acad. Sci. USA **111**: 8077–8082. doi:10.1073/pnas.1319618111
- Urey, H. C., S. Epstein, H. A. Lowenstam, and C. R. McKinney. 1951. Measurement of paleo-temperatures and temperatures of the upper Cretaceous of England, Denmark, and the south-eastern United States. Geol. Soc. Am. Bull. 62: 399–416.
- Vadeboncoeur, Y., M. J. Vander Zanden, and D. M. Lodge. 2002. Putting the lake back together: Reintegrating benthic pathways into lake food web models. BioScience 52: 44–54.
- Vadeboncoeur, Y., E. Jeppesen, M. J. V. Zanden, H.-H. Schierup, K. Christoffersen, and D. M. Lodge. 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnol. Oceanogr. **48**: 1408–1418. doi:10.4319/lo.2003.48.4.1408
- Van den Meersche, K., J. J. Middelburg, K. Soetaert, P. van Rijswijk, H. T. S. Boschker, and C. H. R. Heip. 2004. Carbon-nitrogen coupling and algal-bacterial interactions during an experimental bloom: Modeling a ¹³C tracer experiment. Limnol. Oceanogr. **49**: 862–878. doi:10.4319/ lo.2004.49.3.0862
- Van den Meersche, K., K. Soetaert, and J. J. Middelburg. 2008. A Bayesian compositional estimator for microbial taxonomy based on biomarkers. Limnol. Oceanogr.: Methods 6: 190–199.
- Van Engeland, T., and others. 2013. Dissolved organic matter uptake in a temperate seagrass ecosystem. Mar. Ecol. Prog. Ser. **478**: 87–100.
- Van Hale, R., and R. D. Frew. 2010. Rayleigh distillation equations applied to isotopic evolution of organic nitrogen across a continental shelf. Mar. Freshw. Res. **61**: 369–378.
- van Roij, L., A. Sluijs, J. J. Laks, and G.-J. Reichart. 2017. Stable carbon isotope analyses of nanogram quantities of particulate organic carbon (pollen) with laser ablation nano

combustion gas chromatography/isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. **31**: 47–58.

- Vander Zanden, H. B., D. X. Soto, G. J. Bowen, and K. A. Hobson. 2016. Expanding the isotopic toolbox: Applications of hydrogen and oxygen stable isotope ratios to food web studies. Front. Ecol. Evol. **4**: 20. doi:10.3389/fevo.2016.00020.
- Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature **401**: 464.
- Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer δ^{15} N and δ^{13} C and the trophic position of aquatic consumers. Ecology **80**: 1395–1404.
- Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in delta N-15 and delta C-13 trophic fractionation: Implications for aquatic food web studies. Limnol. Oceanogr. 46: 2061–2066. doi:10.4319/lo.2001.46.8.2061
- Vander Zanden, M. J., and Y. Vadeboncoeur. 2002. Fishes as integrators of benthic and pelagic food webs in lakes. Ecology **83**: 2152–2161.
- Vanderklift, M. A., and S. Ponsard. 2003. Sources of variation in consumer-diet delta(15)N enrichment: A meta-analysis. Oecologia 136: 169–182. doi:10.1007/s00442-003-1270-z
- Vasquez-Cardenas, D., and others. 2015. Microbial carbon metabolism associated with electrogenic sulphur oxidation in coastal sediments. ISME J. **9**: 1966–1978. doi:10.1038/ismej.2015.10
- Veuger, B., D. van Oevelen, H. T. S. Boschker, and J. J. Middelburg. 2006. Fate of peptidoglycan in an intertidal sediment: An in situ ¹³C-labeling study. Limnol. Oceanogr. **51**: 1572–1580. doi:10.4319/lo.2006.51.4.1572
- Veuger, B., D. van Oevelen, and J. J. Middelburg. 2012. Fate of microbial nitrogen, carbon, hydrolysable amino acids, monosaccharides, and fatty acids in sediment. Geochim. Cosmochim. Acta 83: 217–233.
- Voss, M., J. W. Dippner, and J. P. Montoya. 2001. Nitrogen isotope patterns in the oxygen -deficient waters of the eastern tropical North Pacific. Deep-Sea Res. II 48: 1905–1921. doi:10.1016/S0967-0637(00)00110-2
- Wada, E., and A. Hattori. 1976. Natural abundance of ¹⁵N in particulate organic matter in the North Pacific Ocean. Geochim. Cosmochim. Acta **40**: 249–251.
- Webster, J. R., and others. 2003. Factors affecting ammonium uptake in streams: An inter-biome perspective. Freshw. Biol. **48**: 1329–1352.
- West, J. B., A. Sobek, and J. R. Ehleringer. 2008. A simplified GIS approach to modeling global leaf water isoscapes. PLoS One **3**: e2447. doi:10.1371/journal.pone.0002447
- Wheeler, P. A., and S. A. Kokkinakis. 1990. Ammonium recycling limits nitrate use in the oceanic subarctic Pacific. Limnol. Oceanogr. 35: 1267–1278. doi:10.4319/ lo.1990.35.6.1267
- You, L., B. Zhang, and Y. J. Tang. 2014. Application of stable isotope-assisted metabolomics for cell metabolism studies. Metabolites 4: 142–165. doi:10.3390/metabo4020142

Zachos, J. 2001. Trends, rhythms, and aberrations in global climate 65Ma to present. Science **292**: 686–693. doi:10.1126/ science.1059412

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Conflict of Interest

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