

Stable isotopes as one of nature's ecological recorders

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Analyses of the natural variation in stable isotopes of components of ecological systems have provided new insights into how these systems function across paleoecological to modern timescales and across a wide range of spatial scales. Isotope abundances of the molecules in biological materials and geochemical profiles are viewed as recorders that can be used to reconstruct ecological processes or to trace ecological activities. Here, we review key short-, medium- and long-term recording capacities of stable isotopes that are currently being applied to ecological questions. The melding of advances in genetics, biochemical profiling and spatial analysis with those in isotope analyses and modeling sophistication opens the door to an exciting future in ecological research.

Introduction

From studies of the influences of decaying salmon on nitrogen dynamics in Alaskan terrestrial ecosystems [1], to tracing long-distance bird migration routes [2], to reconstructing climate from tree rings [3], stable isotope analyses are an important part of the ecologist's toolbox (Box 1). Since the 1970s, stable isotope analysis of ecological materials has emerged as a quantitative method that has transformed our understanding of ecological systems and of environmental histories [4,5]. A wide range of isotope applications in ecology is emerging through a combination of analytical improvements during the late 1980s and an improved understanding of the factors causing changes in the isotope abundances between substrates and products (a process referred to as fractionation). These applications are usually interdisciplinary in nature, drawing on data and ideas from biochemistry, physiology, ecology, geochemistry and climatology to determine the possible ecological interpretations of isotopic data.

A common theme in ecological applications of stable isotope analysis is the use of isotope ratios as a 'recorder' in biotic and abiotic molecules that is applied to reconstruct ecological processes or trace ecological activities. Among the many examples, carbon isotope ratios ($\delta^{13}\text{C}$) of plant organic matter record environmental effects on photosynthesis [6]; nitrogen isotope ratios

($\delta^{15}\text{N}$) record dietary and trophic-level information in animals [7] and nitrogen fixation in plant-microbe symbioses [8]; and hydrogen ($\delta^2\text{H}$) and oxygen isotope ratios ($\delta^{18}\text{O}$) record water-related dynamics in plants and animals [9,10]. These recorders, the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$ and $\delta^{34}\text{S}$ in biotic or abiotic molecules, provide an 'isotopic signature' that is used to trace the movements of nutrients, compounds, particles and organisms across landscapes and between components of the biosphere, and to reconstruct aspects of dietary, ecological and environmental histories. Owing to the applicability of stable isotope analyses to a range of questions in ecology and evolution, there are many more exciting applications and advances than can be addressed in a single publication. We therefore highlight here the potential for stable isotopes to provide short-, medium- and long-term histories of ecological processes. We do this through consideration of three broad topics: ecosystem processes, animal interactions and spatial patterns.

Although our discussion focuses on isotope abundances at natural levels, isotope labeling studies are also a keen area of current research. For example, double-labeled water injections continue to be the primary method for quantifying water loss and metabolic rates of mammals in natural field settings [11]. At the same time, the addition of ^{15}N -labeled substrates into streams provides one of the most effective means for describing and quantifying the rapid transformations and cycling of nitrogen within aquatic ecosystems [12].

Elucidating aspects of the carbon cycle

Today's atmospheric CO_2 levels oscillate annually, but have a clear upward trajectory as a result of fossil fuel combustion [13]. Photosynthesis, respiration, fossil fuel combustion and land-use changes all contribute to atmospheric CO_2 dynamics, with fluxes simultaneously moving in both directions between the biosphere and the atmosphere via photosynthesis and respiration [14]. Deciphering these CO_2 dynamics requires more than evaluating carbon fluxes between ecosystem compartments. Photosynthesis, respiration and fossil fuel burning all have different isotopic impacts on the atmosphere. Here, measurements of the ^{13}C and ^{18}O contents of CO_2 have provided a constraint that enables one to distinguish between fluxes, thereby allowing, for example, a partitioning of net CO_2 sink fluxes into terrestrial and marine

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Box 1. What are stable isotopes?

Isotopes are elements with unique atomic masses. That is, the isotopes of a given element have the same number of protons, but different numbers of neutrons. Stable isotopes are those isotopes of an element that do not undergo radioactive decay, and most elements have more than one stable isotope. For example, hydrogen has two: ^2H and ^1H (often called deuterium and, rarely, protium respectively), with the lighter isotope being much more abundant. The average

abundances of the common 'light' stable isotopes on Earth are shown in Table I.

The 'light' stable isotopes are of specific interest to ecological studies because these are the primary elements comprising organic matter, but the 'heavy' isotopes (e.g. of strontium) have also been studied as recorders of natural processes. Stable isotope abundances are typically expressed relative to a standard and as ratios of the rare to common isotope in delta (δ) notation (Equation I):

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000\text{‰} \quad [\text{Eqn I}]$$

where R is the molar ratio of the heavy to light isotope of the sample and standard, for example (Equation II):

$$R = \frac{^2\text{H}}{^1\text{H}} \quad [\text{Eqn II}]$$

Stable isotopes are quantified in various ways, but the most common method is with an isotope ratioing mass spectrometer: after conversion of a sample to gaseous form, it is introduced into an ionizing source, the mass spectrometer results are then compared to a standard. Accepted standards used by the international community include V-SMOW (Vienna Standard Mean Ocean Water) for hydrogen and oxygen, PDB (Pee Dee Belemnite) for carbon, atmospheric air for nitrogen and CDT (Canyon Diablo Troilite) for sulfur. These standards can be obtained from the IAEA and are then used to calibrate laboratory standards that are run with samples [67,68].

Table I. Average abundances of stable isotopes that are important for understanding ecological systems

Element	Isotope	Average abundance (%)
Hydrogen	^1H	99.985
	^2H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulfur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{35}S	0.014

components at the global scale [15]. The cyclic, short-term recorder nature of the $\delta^{13}\text{C}$ of CO_2 in Figure 1 reveals the dynamic ecosystem-scale offset between photosynthesis and respiration. Because photosynthesis discriminates against ^{13}C , the seasonally changing balance between photosynthesis and respiration is reflected in the $\delta^{13}\text{C}$ of atmospheric CO_2 , with tropical latitudes having lower amplitudes than do higher latitudes [13,14]. These global perspectives would not have been possible without two fundamental practices that have transformed meteorological and oceanographic sciences, and will continue to transform the ecological sciences: networks for data collection and open data sharing. In addition, interpretations of these isotope patterns only became clear after models of the fractionation processes had been described [16]. New applications of these isotope models have now lead to a better understanding of the controls of ecosystem and regional fluxes, such as the ways in which the hydrological cycle impacts biospheric gas exchange [17], as well as the generality of these patterns that enables scaling across ecosystems [18].

For example, tree rings are medium-term recorders, recording the impacts of weather and climate changes in the cellulose of individual, annually produced rings (Figure 1). Whereas primary productivity impacts are recorded in ring width, the $\delta^{13}\text{C}$ of the rings record the effects of stressors, such as water limitation of photosynthesis [3]. Sugars formed in the leaf initially record the effects of these climate stressors through the impacts of those stressors on stomatal activity and, therefore, on the leaf internal CO_2 concentration (Box 2 [19]). These labile effects on leaf sugars become permanently recorded when sugars are transported to stems and recorded in the cellulose of xylem [20].

Mammalian teeth are another example of long-term recorders of the presence of C3 plant- versus C4 plant-dominated ecosystems across the landscape, an observation that has opened up a new area of ecological research [21]. The $\delta^{13}\text{C}$ values of food sources become locked as carbonates in the enamel of mammalian teeth [22]. MacFadden [23] and Cerling *et al.* [24], among others, have now used $\delta^{13}\text{C}$ of tooth enamel to document evolutionary relationships among mammalian grazers of various taxa based on diet. Although the global spread of C4 ecosystems 6–8 million years ago is now accepted, the drivers of that paleoecological change are still contested. Cerling *et al.* [21], Ehleringer *et al.* [25] and Ehleringer [13] have suggested that C4 ecosystems expanded in response to a global reduction in atmospheric CO_2 , first occurring in the warmest growing-season regions and spreading poleward (Box 2). Pagani *et al.* [26] challenge that interpretation, because they find no significant changes in atmospheric CO_2 over the past 30 million years using a diatom isotope proxy. Huang *et al.* [27] provided isotope evidence to suggest the role of seasonal water availability as a driver for C4 expansion during the last glacial period when atmospheric CO_2 was lowest, whereas Keeley and Rundel [28] have recently proposed that fire is the major driver behind the expansion of C4 grasslands. This debate highlights the significant utility of stable isotopes as recorders of a wide range of ecological processes.

Insights into animal diet, trophic structure, and nutrient transfers

Among the most numerous uses of stable isotopes in ecology have been the applications to dietary patterns. DeNiro and Epstein first pointed out that for animals, 'You

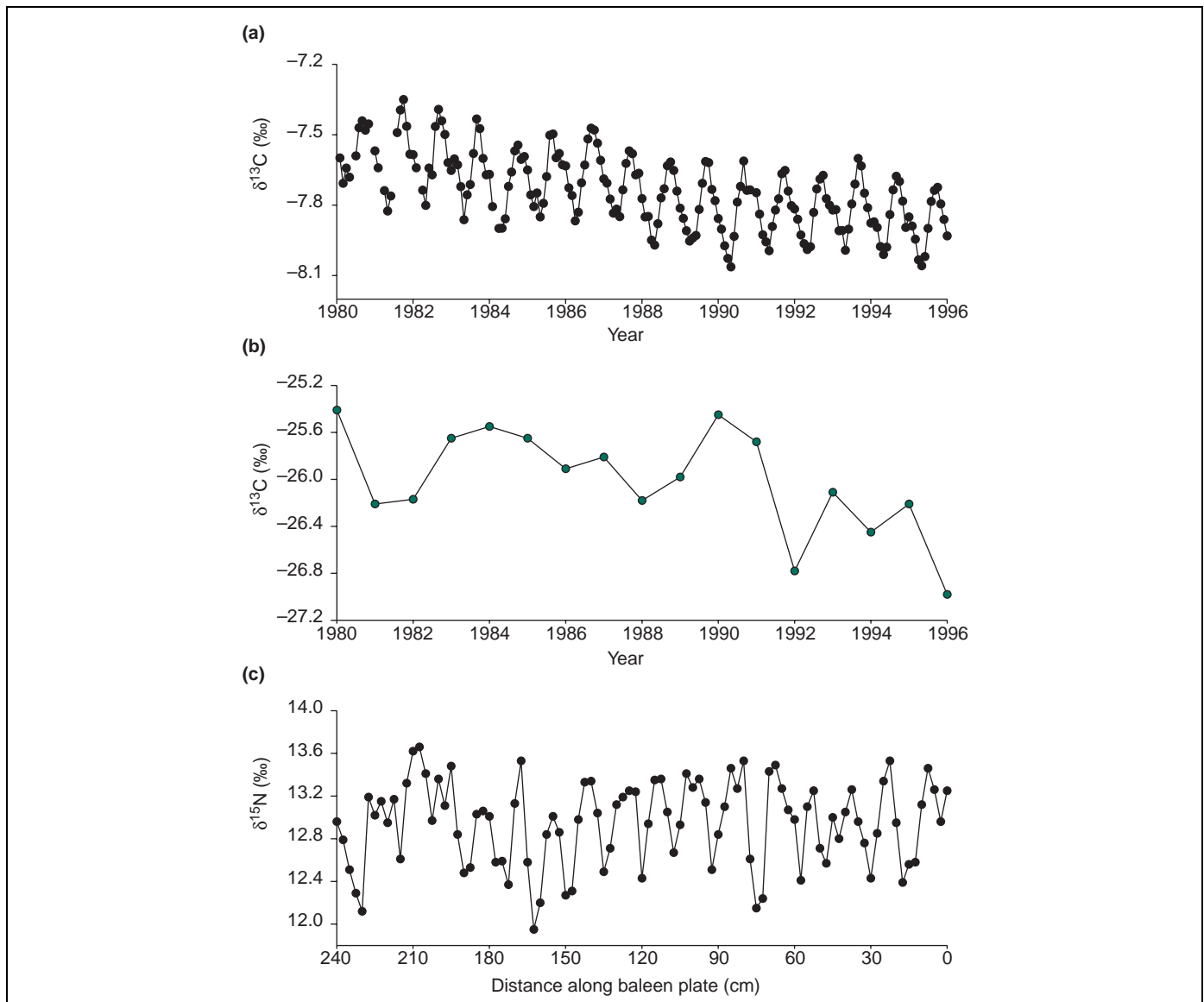


Figure 1. Time courses of representative stable isotope ratios in components of the biosphere. (a) Atmospheric CO₂ δ¹³C at a monthly resolution from the Mauna Loa Observatory; (b) Annual oak *Quercus robur* tree ring cellulose δ¹³C; and (c) Baleen δ¹⁵N at every 2.5 cm from *Balaena mysticetus*. The x-axes on all plots are scaled to represent 16 years of data [approximate for (c) where the x-axis is centimeters along a baleen plate sampled in 1996]. Data from <http://cdiac.esd.ornl.gov/> (a); reproduced with permission from [3] (b) and [35] (c).

are what you eat, isotopically' [29]. This observation has resulted in many studies attempting to unravel the dietary inputs of animals and providing semi-quantitative information about dietary preferences. Applications of stable isotopes to the study of trophic interactions have been abundant, leading to wonderful opportunities for meta-analyses and appropriate words of caution about strict interpretations of the isotope data [7]. The easy availability of isotope analyses has also resulted in studies claiming questionable dietary preference and trophic patterns. Phillips and colleagues [30] have now provided a rigorous mathematical analysis of how to assess the constraints involved in interpreting stable isotope ratio data in dietary and trophic studies, particularly when not all of the dietary sources (end members) are known and when there is isotope overlap among food sources. These studies have been particularly useful in pointing out the

utility of, and limitations to, interpreting dietary isotope ratio data.

The short-term isotope recorders of animal and microbial activities are integrated products of recent metabolism, such as CO₂ in animal breath [22,31–33] and phospholipid fatty acids in soil microbes [5]. In animals, where food storage capacities are greater, evidence suggests that the δ¹³C of respiratory CO₂ largely reflects recent dietary inputs, enabling evaluations of individual meals [34]. The use of open-chamber methods at normal atmospheric CO₂ levels has paved the way for new studies of animal energetics and metabolism [32]. Equally exciting, stable isotope probing approaches to studying soil microbes have provided new opportunities for ecologists to examine functional soil ecology, answering questions such as which sets of soil organisms are activated in response to different resource pulse events [5].

Box 2. Photosynthesis and carbon isotope ratios

The fixation of CO₂ by Rubisco has a competing reaction: that of oxygen fixation and the net release of CO₂ [13]. This is known as photorespiration and decreases net photosynthesis. The competing oxygenase reaction is significant (i.e. photorespiration reduces net photosynthesis) when O₂:CO₂ ratios exceed ca. 400, or ~500 ppmV CO₂ for the modern atmosphere. Therefore, during interglacial and glacial periods, when atmospheric CO₂ was ~280 and 180 ppmV, respectively, significant photorespiration occurred during C3 photosynthesis. A response to this condition was the evolution of C4 photosynthesis, where the site of CO₂ fixation and CO₂ reduction are spatially separated. In this process, CO₂ is enriched in the plant in bundle sheath cells so that photorespiration is suppressed. The isotope effect of this adaptation is enormous: CO₂ fixation occurs in a closed environment so no, or very little, discrimination by Rubisco occurs, as it does in C3 photosynthesis. C4 plants therefore have a δ¹³C value of approximately –12‰ compared with the typical value of –27‰ for C3 plants

Because C4 photosynthesis occurs principally in monocots, particularly the grasses, the isotope distinction between C4 grasses and C3 browse (i.e. leaves of shrubs and trees) is important in modern dietary studies. Because stable carbon isotope ratios are preserved in bioapatite (a primary component of bones and teeth), collagen, muscle, hair and other tissues, it serves as a recorder of diet changes over long and short timescales. For example, elephant hair provides a sensitive recorder of diet change associated with changes in rainfall whereby elephants become predominantly grazers for only a few weeks of the year at the beginning of rainy season [69].

Dynamic short-term dietary activities of mammals become permanently and sequentially recorded in keratin, a medium-term recorder. Here, diet and animal movements at daily to weekly timescales become recorded in the baleen of whales [35] and hair segments of other mammals [22,31,36]. The keratin of feathers is equally important as a recorder of dietary inputs and source water associated with a particular location [37], but because feathers are not continuously produced, their utility in diet reconstruction is less useful than continuously growing protein, such as the keratin that forms fingernails, hair, or baleen. Recently, Cerling *et al.* [38] have highlighted the use of δ¹³C and δ¹⁵N in elephant hairs, in conjunction with global-positioning devices, to reconstruct the diets of individual elephants as they roam across the landscape. This information is relevant to wildlife management at the human interface and can be of significant benefit to conservation efforts. Passey and colleagues [39] have developed new, powerful mathematical techniques that enable ecologists to reconstruct more precisely discrete dietary patterns using the biological record of animal tissues, such as would be expected with migratory events.

A novel interpretation of the long-term records found in soil nitrogen isotopes is the identification of marine nitrogen inputs to the functioning of freshwater riparian ecosystems [1,40,41]. Here, stable isotopes demonstrate the importance of nitrogen and other nutrients derived from decaying salmon on riparian productivity, linking marine animal diets to terrestrial ecosystem function. These studies, and others like them, represent a large-scale perspective on how resources are transported across landscapes. Isotope ratios enable the origins of the nitrogen to be identified, and the quantification of the

effects on the nitrogen cycling of these inland terrestrial ecosystems far from the marine nitrogen source.

The spatial isotope fingerprint of water

The stable isotopes of hydrogen and oxygen in water also serve as important recorders of hydrological and ecological processes. Because of the overriding control of temperature on precipitation isotope ratios, their spatial variation exhibits predictable patterns over the surface of the Earth (Box 3). Networks of data collection, especially the International Atomic Energy Association's (IAEA) isotopes in precipitation network (<http://isohis.iaea.org/>; [42]) have enabled the generation of spatial maps of this variation [43]. Recent model improvements have produced spatially continuous grids that have relatively small confidence intervals for much of the surface of the Earth [44]. An example map produced using this algorithm is shown for North America in Figure 2. The values in this map represent essentially static, multi-decadal expected means. As such, although not representing the dynamic nature of individual storms or between individual years, and lacking such effects as long-distance transport in

Box 3. Isotope hydrology models

Harmon Craig, a pioneering geochemist from the Scripps Institution of Oceanography (<http://sio.ucsd.edu/>), provided the initial key observations of the hydrological cycle that ecologists, hydrologists and climatologists were later to expand upon. Along with the observations of Willi Dansgaard and Irving Friedman, Craig's observations in 1961 of the hydrogen and oxygen isotope patterns in precipitation provided the foundation for most subsequent isotope hydrology studies [70–72]. Isotopic fractionation accompanying the liquid–vapor phase transition was identified as the key process leading to preferential accumulation of the heavy isotopes ²H and ¹⁸O within liquid water, be it in the ocean, lakes, rain droplets in clouds, or leaves of plants [73–75]. Evaporation and condensation have been shown to involve several discrete physical processes with distinct isotope effects, including equilibrium and kinetic phase transitions, and diffusive and advective transport, which together control the relative proportions of each isotopomer (e.g. ²H¹H versus ¹H¹HO) during evaporation [6]. These effects produce the 'global meteoric water line' (GMWL): δ²H = δ¹⁸O * 8 + 10. The GMWL is an empirically derived equation that explains much of the variation in meteoric water and is itself explained by these isotope effects as water moves through the global water cycle [46].

Dansgaard [71] described the four major drivers of variation in precipitation stable isotope ratios: altitude, latitude, distance from coast, and amount of precipitation, with temperature being the dominant factor driving these spatial patterns in isotope ratio variation [76]. Although this synthetic model of the phase change process was developed early, and has largely stood the test of time, our understanding and models of the underlying physical isotope effects continue to be refined (e.g. [77,78]). These ongoing modeling efforts synthesize the evaporation and condensation fluxes at scales from individual storms to global hydroclimatology. A range of approaches has been pursued, from mass-balance models applied to specific storms or atmospheric systems [79], to empirically derived relations used to predict continental- to global-scale water isotope distributions (Figure 2, main text; [44]), to first-principles parameterizations incorporated within state-of-the-art general circulation models (e.g. [75]). Each approach has its own merits, and these analyses provide predictive frameworks within which spatial data on atmospheric water isotope ratios can be interpreted.

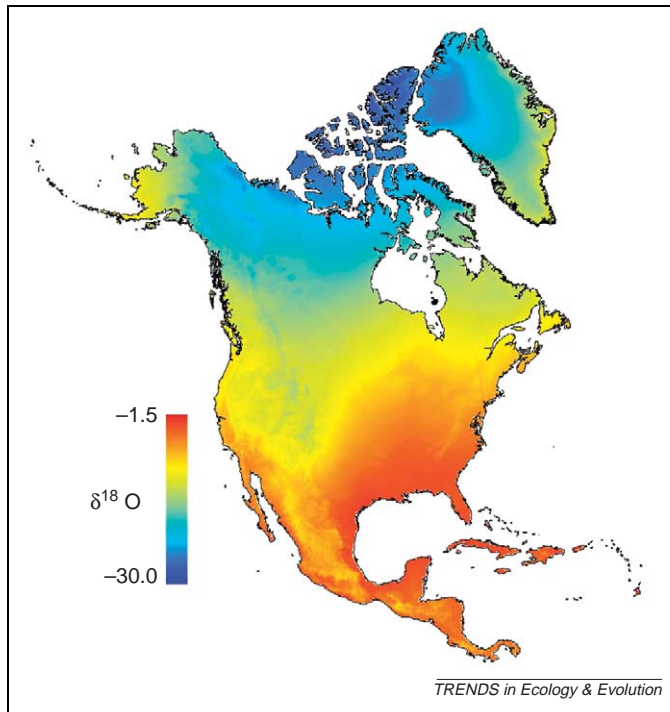


Figure 2. Spatially continuous predictions of precipitation $\delta^{18}\text{O}$ across North America at a 10 arc minute resolution (1/6th of a degree latitude and longitude). Briefly, these continuous grids (produced for the surface of the Earth and available at <http://waterisotopes.org>) are the result of a 'detrended interpolation' method. This method combines empirical relationships between precipitation isotope ratios (primarily obtained from the IAEA network of isotopes in precipitation) and latitude and altitude with a smoothing algorithm for the residual variation (for more information, see [44]).

rivers [45], they can represent 'average' surface waters for a region.

These spatially continuous maps of precipitation isotope ratios now provide the opportunity to evaluate ecological processes across space and time at several scales. For example, if one assumes that the grids are reasonable proxies for plant source water, then the effects of time-varying climate on spatial patterns in leaf water isotope ratios can be modeled. Leaf water stable isotope ratios represent a temporally dynamic component of terrestrial ecosystems and are thus short-term recorders. The application of the Craig-Gordon model (Box 3 [46]) to leaf water enrichment (e.g. [47,48]) was a crucial step in our understanding of leaf water isotopic enrichment. Several recent modifications of these models have been explored to account for their disagreement with empirical data (e.g. [49]) and non-steady state models might yet yield finer temporal scale information (e.g. [50]), enabling the integration of spatial isotope and climate maps to predict spatial patterns in leaf water stable isotope ratios.

Detailed simulation models of the stable isotope ratios of many atmospheric gases depend on estimates of leaf water isotope ratios [51,52], because gases in the atmosphere cycle through plants and, in so doing, acquire an isotopic signature that reflects that passage. CO_2 , for example, exchanges with leaf water at a rate that is dependent on the concentration of carbonic anhydrase, the spatial variation in which has also been modeled at the global scale [53]. This exchange enables this relatively short-term recorder, $\delta^{18}\text{O}$ in atmospheric CO_2 , to be used

to estimate terrestrial productivity, as well as, potentially, the contributions of C3 versus C4 plants over land surfaces.

Leaf water is also the medium in which photosynthesis occurs, a process that can be seen as 'fixing' the labile leaf water signal into more stable carbohydrates, because plant water supplies the oxygen and hydrogen atoms incorporated into plant organic molecules initially by photosynthesis. These products of photosynthesis are precursors to other plant organic compounds, resulting in a range of plant compounds that are medium-term recorders of plant source water isotope ratios and climate. As with $\delta^{13}\text{C}$, the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of cellulose in tree rings has been explored most actively in this regard [20], as have plant lipids [54]. Some recent work on the spatial sources and $\delta^{13}\text{C}$ of *n*-alkanes [55], and their connection to terrestrial biospheric ^{13}C discrimination [56] is also exciting and, if extended to what is known about *n*-alkane hydrogen isotopes (e.g. [57]), could yet yield greater insights into biospheric processes and the effects of environmental perturbations.

Animals also ingest water and incorporate it into stable organic compounds in their tissues [58]. This incorporation has been exploited most extensively to track important ecological factors such as migration routes and metabolic processes in migrating animals [2]. More effort directed at understanding fundamental isotope effects associated with synthesis of organic tissues in animal tissues could provide a wealth of information for animal ecology. A synthetic animal body water isotope ratio model has been published [9] and has been applied primarily to fossil bone and enamel to reconstruct paleoclimates [59]. Significant promise exists for the further development of this field in new analytical techniques that enable the routine isotopic analysis of individual amino acids (e.g. [60]) that could lead to fundamental developments furthering predictive applications in animal isotope ecology.

Exciting futures lie ahead

Technological advances will continue to provide new opportunities and applications for stable isotope studies throughout ecology. Innovations that increase the sampling frequency, decrease sample size, enable compound-specific analysis and field sampling in harsh conditions all promise exciting new avenues of inquiry [61–63]. Combining stable isotope methods with genetic approaches, such as the stable isotope probing of nucleic acids for studying soil microorganism functioning [64], or combining microsatellites and stable isotopes to study the population dynamics of birds [65], are great examples of the tremendous potential in stable isotope methods to answer what were previously difficult or impossible to answer ecological questions. Novel approaches to old questions, such as niche size [66], are also likely to emerge as more ecologists apply stable isotope methods to their areas of research. In addition, the expansion of isotope studies to encompass regional and global scales will continue to grow in importance as ecologists collaborate with atmospheric scientists in tackling questions about the functioning of human-dominated biosphere.

Large spatial scales require networks of data collection as well as the development of data assimilation methods and mechanistic models that can interpret and predict these spatial patterns (e.g. <http://isoscap.es.org>). It seems clear that global networks such as GEWEX (<http://www.gewex.org>) or national networks such as NEON in the USA (<http://www.neoninc.org/>) should be encouraged, as they represent important steps in exploiting the potential wealth of information to be found in the isotopic records of natural systems.

We believe that, in addition to the technological improvements that will continue to advance ecology, the solid theoretical underpinnings built over the past several decades now provide an opportunity to extend the modeling of stable isotope ratios to a wider range of spatial and temporal scales. This future depends, of course, on the continued development of models of isotope fractionation in nature, in conjunction with other measurements, to inform our understanding of changes on continental and global scales.

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References

- Bartz, K.K. and Naiman, R.J. (2005) Effects of salmon-borne nutrients on riparian soils and vegetation in southwest Alaska. *Ecosystems* 8, 529–545
- Rubenstein, D.R. and Hobson, K.A. (2004) From birds to butterflies: animal movement patterns and stable isotopes. *Trends Ecol. Evol.* 19, 256–263
- Loader, N.J. *et al.* (2003) Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings. *Palaeogeogr. Palaeoclimatol.* 196, 395–407
- Robinson, D. (2001) Delta N-15 as an integrator of the nitrogen cycle. *Trends Ecol. Evol.* 16, 153–162
- Staddon, P.L. (2004) Carbon isotopes in functional soil ecology. *Trends Ecol. Evol.* 19, 148–154
- Ehleringer, J.R. *et al.*, eds (1993) *Stable Isotopes and Plant Carbon/Water Relations*, Academic Press
- Post, D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* 83, 703–718
- West, J.B. *et al.* (2005) Legume species identity and soil nitrogen supply determine symbiotic nitrogen-fixation responses to elevated atmospheric [CO₂]. *New Phytol.* 167, 523–530
- Kohn, M.J. (1996) Predicting animal δ¹⁸O: Accounting for diet and physiological adaptation. *Geochim. Cosmochim. Acta* 60, 4811–4829
- Roden, J.S. *et al.* (2000) A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim. Cosmochim. Acta* 64, 21–35
- Nagy, K.A. (2005) Field metabolic rate and body size. *J. Exp. Biol.* 208, 1621–1625
- Elskens, M. *et al.* (2005) Reliability of N flux rates estimated from N-15 enrichment and dilution experiments in aquatic systems. *Glob. Biogeochem. Cycles* 19, Art. No. GB4028, doi:10.1029/2004GB002332
- Ehleringer, J.R. *et al.*, eds (2005) *A History of Atmospheric CO₂ and its Effects On Plants, Animals, and Ecosystems*, Springer
- Flanagan, L.B. *et al.*, eds (2005) *Stable Isotopes and Biosphere–Atmosphere Interactions: Processes and Biological Controls*, Elsevier Academic Press
- Battle, M. *et al.* (2000) Global carbon sinks and their variability inferred from atmospheric O₂ and δ¹³C. *Science* 287, 2467–2470
- Farquhar, G.D. *et al.* (1989) Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 503–537
- Lai, C-T. *et al.* (2005) Canopy-scale δ¹³C of photosynthetic and respiratory CO₂ fluxes: observations in forest biomes across the United States. *Glob. Change Biol.* 11, 633–643
- Pataki, D.E. *et al.* (2003) The application and interpretation of Keeling plots in terrestrial carbon cycle research. *Glob. Biogeochem. Cycles* 17, 221–224
- Scartazza, A. *et al.* (2004) Comparisons of delta C-13 of photosynthetic products and ecosystem respiratory CO₂ and their response to seasonal climate variability. *Oecologia* 140, 340–351
- Roden, J. *et al.* (2000) A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim. Cosmochim. Acta* 64, 21–35
- Cerling, T.E. *et al.* (1997) Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389, 153–158
- Passey, B.H. *et al.* (2005) Carbon isotope fractionation between diet, breath CO₂, and bioapatite in different mammals. *J. Archaeol. Sci.* 32, 1459–1470
- MacFadden, B.J. (2000) Cenozoic mammalian herbivores from the Americas: reconstructing ancient diets and terrestrial communities. *Annu. Rev. Ecol. Syst.* 31, 33–59
- Cerling, T.E. *et al.* (2003) Diets of east African Bovidae based on stable isotope analysis. *J. Mammal.* 84, 456–470
- Ehleringer, J.R. *et al.* (1991) Climate change and the evolution of C₄ photosynthesis. *Trends Ecol. Evol.* 6, 95–99
- Pagani, M. *et al.* (2005) Marked decline in atmospheric carbon dioxide concentrations during the Paleogene. *Science* 309, 600–603
- Huang, Y. *et al.* (2001) Climate change as the dominant control on glacial-interglacial variations in C₃ and C₄ plant abundance. *Science* 293, 1647–1651
- Keeley, J.E. and Rundel, P.W. (2005) Fire and the Miocene expansion of C-4 grasslands. *Ecol. Lett.* 8, 683–690
- DeNiro, M.J. and Epstein, S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42, 495–506
- Phillips, D.L. *et al.* (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia* 144, 520–527
- Ayliffe, L.K. *et al.* (2004) Turnover of carbon isotopes in tail hair and breath CO₂ of horses fed an isotopically varied diet. *Oecologia* 139, 11–22
- Carleton, S.A. *et al.* (2004) Keeling plots for hummingbirds: a method to estimate carbon isotope ratios of respired CO₂ in small vertebrates. *Oecologia* 141, 1–6
- Podlesak, D.W. *et al.* (2005) Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. *Oecologia* 142, 501–510
- Barbosa, L. *et al.* (2005) Reproducibility and reliability of the C-13-acetate breath test to measure gastric emptying of liquid meal in infants. *Nutrition* 21, 289–294
- Hobson, K.A. and Schell, D.M. (1998) Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). *Can. J. Fish. Aquat. Sci.* 55, 2601–2607
- Sharp, Z.D. *et al.* (2003) Hydrogen isotope systematics of hair: archeological and forensic applications. *J. Archaeol. Sci.* 30, 1709–1716
- Bowen, G.J. *et al.* (2005) Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* 143, 337–348
- Cerling, T.E. *et al.* (2006) Stable isotopes in elephant hair document migration patterns and diet changes. *Proc. Natl. Acad. Sci. U. S. A.* 103, 371–373
- Passey, B.H. *et al.* (2005) Inverse methods for estimating primary input signals from time-averaged isotope profiles. *Geochim. Cosmochim. Acta* 69, 4101–4116
- Bilby, R.E. *et al.* (2003) Transfer of nutrients from spawning salmon to riparian vegetation in western Washington. *Trans. Am. Fish. Soc.* 132, 733–745
- Koyama, A. *et al.* (2005) Marine nitrogen in central Idaho riparian forests: evidence from stable isotopes. *Can. J. Fish. Aquat. Sci.* 62, 518–526
- Welker, J.M. (2000) Isotopic (δ¹⁸O) characteristics of weekly precipitation collected across the USA: An initial analysis with application to water source studies. *Hydrol. Proc.* 14, 1449–1464

- 43 Birks, S.J. *et al.* (2002) Maps and animations offer new opportunities for studying the global water cycle. *Eos, Trans. Am. Geophys. Union* 83, 406
- 44 Bowen, G.J. and Revenaugh, J. (2003) Interpolating the isotopic composition of modern meteoric precipitation. *Water Resour. Res.* 39, 1299, doi:10.129/2003WR002086
- 45 Kendall, C. and Coplen, T.B. (2001) Distribution of oxygen-18 and deuterium in river waters across the United States. *Hydrol. Proc.* 15, 1363–1393
- 46 Craig, H. and Gordon, L. (1965) Deuterium and oxygen 18 variations in the ocean and marine atmosphere. In *Stable Isotopes in Oceanographic Studies and Paleotemperatures* (Tongiorgi, E., ed.), pp. 9–130, Consiglio Nazionale Delle Ricerche Laboratorio di Geologia Nucleare
- 47 Dongmann, G. *et al.* (1974) On the enrichment of $H_2^{18}O$ in the leaves of transpiring plants. *Rad. Env. Biophys.* 11, 41–52
- 48 Flanagan, L.B. and Ehleringer, J.R. (1991) Stable isotope composition of stem and leaf water: applications to the study of plant water-use. *Funct. Ecol.* 5, 270–277
- 49 Barbour, M.M. and Farquhar, G.D. (2003) Do pathways of water movement and leaf anatomical dimensions allow development of gradients in $H_2^{18}O$ between veins and the sites of evaporation within leaves? *Plant Cell Environ.* 27, 107–121
- 50 Farquhar, G.D. and Cernusak, L.A. (2005) On the isotopic composition of leaf water in the non-steady state. *Funct. Plant Biol.* 32, 293–303
- 51 Cuntz, M. *et al.* (2003) A comprehensive global three-dimensional model of $\delta^{18}O$ in atmospheric CO_2 : 1. Validation of surface processes. *J. Geophys. Res.* 108, Art. No. 4527
- 52 Hoffmann, G. *et al.* (2004) A model of the Earth's Dole effect. *Global Biogeochem. Cycles* 18, Art. No. GB1008
- 53 Gillon, J. and Yakir, D. (2001) Influence of carbonic anhydrase activity in terrestrial vegetation on the ^{18}O content of atmospheric CO_2 . *Science* 291, 2584–2587
- 54 Sternberg, L.d.S.L. (1988) D/H ratios of environmental water recorded by D/H ratios of plant lipids. *Nature* 333, 59–61
- 55 Conte, M.H. and Weber, J.C. (2002) Long-range atmospheric transport of terrestrial biomarkers to the western North Atlantic. *Glob. Biogeochem. Cycles* 16, 89:81–17
- 56 Conte, M.H. and Weber, J.C. (2002) Plant biomarkers in aerosols record isotopic discrimination of terrestrial photosynthesis. *Nature* 417, 639–641
- 57 Xiong, Y. *et al.* (2005) Characterization of the hydrogen isotopic composition of individual *n*-alkanes in terrestrial source rocks. *Appl. Geochem.* 20, 455–464
- 58 Hobson, K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120, 314–326
- 59 Hedges, R.E.M. *et al.* (2004) Bone as a stable isotope archive for local climatic information. *Quat. Sci. Rev.* 23, 959–965
- 60 McClelland, J.W. *et al.* (2003) Relating low delta N-15 values of zooplankton to N-2-fixation in the tropical North Atlantic: insights provided by stable isotope ratios of amino acids. *Deep-Sea Res.* 50, 849–861
- 61 Bowling, D.R. *et al.* (2003) Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO_2 exchange. *Agri. For. Meteorol.* 118, 1–19
- 62 Castrillo, A. *et al.* (2004) First field determination of the C-13/C-12 isotope ratio in volcanic CO_2 by diode-laser spectrometry. *Optics Exp.* 12, 6515–6523
- 63 Conte, M.H. and Weber, J.C. (2002) Plant biomarkers in aerosols record isotopic discrimination of terrestrial photosynthesis. *Nature* 417, 639–641
- 64 Galvao, T.C. *et al.* (2005) Exploring the microbial biodegradation and biotransformation gene pool. *Trends Biotechnol.* 23, 497–506
- 65 Clegg, S.M. *et al.* (2003) Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). *Mol. Ecol.* 12, 819–830
- 66 Bearhop, S. *et al.* (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *J. Anim. Ecol.* 73, 1007–1012
- 67 Werner, R.A. and Brand, W.A. (2001) Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Commun. Mass Spectrom.* 15, 501–519
- 68 Coplen, T.B. (1996) New guidelines for reporting stable hydrogen, carbon, and oxygen isotope-ratio data. *Geochim. Cosmochim. Acta* 60, 3359–3360
- 69 Cerling, T.E. *et al.* (2004) Orphans' tales: seasonal dietary changes in elephants from Tsavo National Park, Kenya. *Palaeogeogr. Palaeoclimatol.* 206, 367–376
- 70 Craig, H. (1961) Isotopic variations in meteoric waters. *Science* 133, 1702–1703
- 71 Dansgaard, W. (1964) Stable isotopes in precipitation. *Tellus* 16, 436–468
- 72 Friedman, I. *et al.* (1964) The variation of the deuterium content of natural waters in the hydrologic cycle. *Rev. Geophys.* 2, 177–224
- 73 Yurtsever, Y. and Gat, J.R. (1981) Atmospheric waters. In *Stable Isotope Hydrology: Deuterium and Oxygen-18 in the Water Cycle* (Gat, J.R. and Gonfiantini, R., eds), pp. 103–142, International Atomic Energy Agency
- 74 Flanagan, L.B. *et al.* (1991) Comparison of modeled and observed environmental influences on the stable oxygen and hydrogen isotope composition of leaf water in *Phaseolus vulgaris* L. *Plant Physiol.* 96, 588–596
- 75 Mathieu, R. *et al.* (2002) Simulation of stable water isotope variations by the GENESIS GCM for modern conditions. *J. Geophys. Res.* 107, 4037, doi:10.1029/2001JD900255
- 76 Yurtsever, Y. (1975) *Worldwide Survey of Stable Isotopes in Precipitation*, IAEA
- 77 Cappa, C.D. *et al.* (2003) Isotopic fractionation of water during evaporation. *J. Geophys. Res.* 108, 4525, doi:10.1029/2003JD003597
- 78 Jouzel, J. (1986) Isotopes in cloud physics: Multiphase and multistage condensation processes. In *Handbook of Environmental Isotope Geochemistry* (Fritz, P. and Fontes, J., eds), pp. 61–112, Elsevier
- 79 Pierrehumbert, R. (1999) Huascarán $\delta^{18}O$ as an indicator of tropical climate during the Last Glacial Maximum. *Geophys. Res. Lett.* 26, 1345–1348