# On developing a plan for an isotope network within NEON

Report from two BASIN* workshops:
September 16-17, 2004, Park City, Utah
and December 12, 2004, San Francisco, California

Workshop organizers
Dave Williams, Dave Evans, Jim Ehleringer

Workshop participants
Steve Beaupre, Sharon Billings, Gabriel Bowen, Dave Bowling, Renee Brooks, Jeff Chanton, David Dettman, Rick Doucett, Carol Kendall, Jennifer King, Guanghui Lin, Stephen Macko, John Marshall, Dan Murnick, Nathaniel Ostrom, Diane Pataki, Darren Sandquist, Laurel Saito, Alyson Sayer, Jed Sparks, Heidi Steltzer, Paddy Sullyvan, Valery Terwilliger, Bruce Vaughn, Jeff Welker, Blair Wolf

*Biosphere-Atmosphere Stable Isotope Network – an NSF-funded Research Coordination Network

**Executive Summary**

Stable isotope ratio analyses at natural abundance levels play a central role in understanding many ecosystem dynamics, patterns, and processes. These analyses are likely to play a central role in aquatic, atmospheric, and terrestrial components of the National Ecological Observatory Network (NEON) now in the planning stages. As a contribution toward development of NEON, scientists from across the nation interested in ecological applications of stable isotopes have met twice during Fall 2004 to discuss issues that would be of benefit to NEON and other national activities. This document summarizes our ongoing discussions. Here we describe a framework of rationale and plans for development of a network that provides infrastructure, monitoring capacity, research capacity, training, quality control, educational training, and outreach.

The model we describe here requires the establishment of the following elements:

- A **coordination facility** or **facilities** to serve as the backbone infrastructure for high-level technical training and analytical support services, help with technological innovation, facilitate development of QA/QC procedures, and development and distribution of standard reference materials,

- **Regional analytical facilities consisting of multiple isotope ratio mass spectrometers and related peripherals** (networked nationally) to carry out the NEON-related isotope measurements for long-term monitoring, education and scientific investigation,

- A geographically distributed **Isotope Network for Early-Warning Signals (INEWS)** to identify rapidly the changes in the nation's *terrestrial*, *aquatic* and *atmospheric* ecological condition and the potential mechanisms associated with these changes from isotope measurements,

- A comprehensive and multidisciplinary **training and education program** to stimulate innovative, inter-disciplinary applications of stable isotope measurements and ensure that policy and management decisions founded upon isotopic information are made in an educated and informed way.

Although the primary model that we present has a centralized structure and expands on existing multi-agency networks and efforts, we recognize that adopting a de-centralized management and infrastructure may also serve the objectives and meet certain constraints of NEON. We therefore include alternatives to our primary model at key places throughout this document.

The infrastructure and organization presented here may serve as a model for other analytical, monitoring, and education requirements of NEON. More importantly, the organization and analytical, monitoring and educational infrastructure described herein would best be coordinated and even co-located with other NEON analytical infrastructure dedicated to the broader understanding of the chemical, molecular, and biological variation of our nation's ecological resources. Such infrastructure could include elemental analysis, DNA analysis, organic and inorganic chemical analysis, microbial analysis, and animal and plant disease forensics facilities.

## TABLE OF CONTENTS

## I. Introduction

Processes such as land use change, rising levels of atmospheric trace gases, ground water depletion, wastewater treatment and release, and release of organic and inorganic pollutants challenge society to quantify and mitigate potentially damaging alterations to our nation's natural, agricultural and urban environments. Ultimately, human well-being and health and the economic systems that sustain our way of life depend on our abilities to ask and address some critical questions involving these issues. The field of ecology has progressed over the last few decades such that scientists now can quantitatively address many of these questions, which are necessarily large scale both temporally and spatially. Such questions include:

- *To what extent are pollutants entering the food supply and human water sources and what are their origins?*
- *What sources of pollutants in the environment are critical to reduce?*
- *How is groundwater level impacted by aboveground ecosystems and human use?*
- *How do invasive species alter productivity and economic value of the landscape?*
- *What role do certain species play in ecosystems that make them critical for preservation?*
- *What is the geographic origin and migration pattern of disease vectors?*
- *To what extent is climate change altering productivity of natural and manmade ecosystems?*

Perhaps most importantly, *are there methods available that serve as integrative indicators of perturbations to ecosystems, especially ones that highlight important alterations in relatively early stages?* Such questions address issues that impact humans' ability to survive in a healthy, sustainable manner. As such, they move beyond ecology and clearly related disciplines such as hydrology and biogeochemistry, and impact seemingly disparate fields such as the medical sciences, economics, and sociology.

The scope of these questions requires the use of integrative measures that allow us to trace key constituents in the environment and quantitatively indicate the extent to which a process of concern occurs. NEON scientists will necessarily have to employ such measures, as well as educate young investigators in their use to ensure continuity of skills in the future. Increasingly, scientists are employing isotopic analyses to address critical questions such as those described above. Isotope analyses of key ecological and environmental parameters provide measures that *integrate, indicate, record* and *trace* fundamental processes in ecology. Isotope variation present in the important elements of life (H, C, O, N, and S) provide quantitative information about the status of Earth's natural, agricultural and urban ecosystems and the services they supply to humans. Technical advances over the last 15 years in this rapidly advancing and popular analytical approach have allowed ecologists to address increasingly complex problems. As such, isotope analyses will play a major role in the science and education activities of NEON.

This document describes a model for how NEON-related isotope analyses, training, and education activities might be structured within a centralized network built on existing regional capacities. Because of the ability of stable isotope ratio analyses to address questions with such large economic and societal implications for human activities (see above), their use brings together scientists from traditionally separate disciplines to form new interdisciplinary collaborations, benefiting both "hard" sciences as well as the social sciences. Such efforts will benefit a wide range of disciplines while developing our abilities to address issues critical for mankind's continued well-being.

The model we describe here for structuring research that utilizes stable isotopes within NEON requires the establishment of the following elements:

- A **coordination facility** or **facilities** to serve as the backbone infrastructure for high-level technical training and analytical support services, help with technological innovation, facilitate development of QA/QC procedures, and development and distribution of standard reference materials,

- **Regional analytical facilities consisting of multiple isotope ratio mass spectrometers and related peripherals** (networked nationally) to carry out the NEON-related isotope measurements for long-term monitoring, education and scientific investigation,

- A geographically distributed **Isotope Network for Early-Warning Signals (INEWS)** to identify rapidly the changes in the nation's *terrestrial*, *aquatic* and *atmospheric* ecological condition and the potential mechanisms associated with these changes from isotope measurements,

- A comprehensive and multidisciplinary **training and education program** to stimulate innovative, inter-disciplinary applications of stable isotope measurements and ensure that policy and management decisions founded upon isotopic information are made in an educated and informed way.

Although the primary model that we present has a centralized structure and expands on existing multi-agency networks and efforts, we recognize that adopting a de-centralized management and infrastructure may also serve the objectives and meet certain constraints of NEON. We therefore include alternatives to our primary model at key places throughout this document.

The infrastructure and organization presented here may serve as a model for other analytical, monitoring, and education requirements of NEON. More importantly, the organization and analytical, monitoring and educational infrastructure described herein would best be coordinated and even co-located with other NEON analytical infrastructure dedicated to the broader understanding of the chemical, molecular, and biological variation of our nation's ecological resources. Such infrastructure could include elemental analysis, DNA analysis, organic and inorganic chemical analysis, microbial analysis, and animal and plant disease forensics facilities.

## II. What is an isotope?

This is a good starting point for those not familiar with the field. As you recall, the nucleus of each atom contains protons and neutrons. While the number of protons defines the element (e.g., hydrogen, carbon, etc.) and the sum of the protons and neutrons gives the atomic mass, the number of neutrons defines the **isotope** of that element. For example, most carbon (≈ 99 %) has 6 protons and 6 neutrons and is written as $^{12}C$ to reflect its atomic mass. However, about 1 % of the carbon in the Earth's biosphere has 6 protons and 7 neutrons ($^{13}C$) forming the heavy stable isotope of this important element. Stable isotopes do not decay into other elements. In contrast, radioactive isotopes (e.g., $^{14}C$) are unstable and will decay into other elements.

The less abundant stable isotope(s) of an element have one or two additional neutrons than protons, and thus are heavier than the more common stable isotope(s) for those elements. Both heavy and light stable isotopes participate freely in chemical reactions and in biological and geochemical processes, but the rate at which heavy and light stable isotopes react during physical or chemical reactions differs. The chemical bonds and attractive forces of atoms with heavy stable isotopes are stronger than those in the more common, lighter isotopes of an element. As a result, the heavier isotopes react more slowly than the lighter isotopes leading to isotopic separation or *fractionation* between reactant and product in both physical and biological reactions. Fractionation of the heavy and light stable isotopes is important because it a) causes variation in the stable isotope ratio of different source pools in an ecosystem and b) establishes an isotope signal that can indicate the existence or magnitude of key processes involved with elemental cycling.

The stable isotope concentrations of a molecular compound or material are presented in ratio form as the molecular ratio of the heavy-to-light isotopes. Since this ratio is small, we typically present stable isotope abundances relative to an international standard using "delta" notation as:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000, ‰$$

where $\delta X$ is the delta value of the sample for element X (H, O, C, etc.) in parts per thousand ("per mil," ‰) and $R$ is the molar ratio of the heavy (less common) to light (more common) isotope in the sample and in an international standard, respectively. Organic and inorganic standard materials are available through the International Atomic Energy Agency (IAEA) and the National Institute of Standards and Technology (NIST) to facilitate accurate measurement and reporting of isotope ratios for unknown samples and to facilitate cross-lab comparability.

*Isotopes integrate, indicate, record and trace fundamental ecological processes*. Rapid technological advances over the past decade have greatly stimulated the use of isotope analyses by ecologists. This analytical approach is now among the most popular in ecology because of the insights provided by isotope ratios at natural abundance levels.

- Isotopes ***integrate*** ecological processes in space and time. The isotope ratios of plant and animal tissues and organic and inorganic compounds (including gases) in soil represent a temporal integration of significant physiological and ecological processes on the landscape. The timescale of this integration depends on the element turnover rate of the tissue or pool in question. In addition, the isotope ratios of well-mixed environmental reservoirs, such as the atmosphere, streams and aquifers, often represent an integration of source inputs to the system that extend over large spatial scales.

- Isotopes ***indicate*** the presence and magnitude of key ecological processes. Many ecological processes produce a distinctive isotope fingerprint. The presence or absence of such processes and even their magnitude in relation to other processes are indicated by the stable isotope ratio value relative to known background values.

- Isotopes ***record*** biological responses to Earth's changing environmental condition. For cases in which substances or residues accumulate in an incremental fashion, such as in tree rings, animal hair and ice cores, isotope ratios can be used as a record of system response to changing environmental conditions or a proxy record for environmental change.

- Isotopes ***trace*** the origin and movement of key elements and substances. Owing to isotopic fractionations associated with physical and biological reactions, nutrient and element pools within and among ecosystems often differ isotopically. As a result, the source(s) of essential elements and resources acquired by an organism are easily traced using isotope ratios. Strong geographic patterns in isotope signature variation provide the means to trace the movement or origin of a substance or component at landscape to continental scales.

## III. Why develop an isotope network?

NEON activities will address a number of fundamental ecological challenges facing society today. Addressing these challenges in an effective manner will require multi-scale, multi-disciplinary efforts facilitated by emerging technologies, data integration and networked analytical facilities. Isotope measurements can and will play a major role within this large-scale scientific effort both in terms of long-term monitoring and as an essential measurement tool in studies assessing ecological patterns across local to continental scales, because of the additional information represented in the isotopic composition of a compound or material. It is unlikely that all NEON-related isotope analyses, training and education will occur at a single laboratory facility. Instead, analyses likely will take place at multiple locations throughout the United States. A network of isotope ecologists forming, driven by the need to develop training, standardization and monitoring efforts at a national level. The sweeping changes underway in how ecologists conduct research on Earth's ecosystems, embodied in the concept of NEON, highlight the pressing need to establish a national isotope-monitoring network and coordination in training and standardization that will extend well beyond the time frame of individual scientists' careers, and require national-scale, cross-site integration. Specific requirements for NEON-related isotope analyses will be achieved only through a centralized network:

- A national scale monitoring and research effort requires nationwide cross-lab calibration, common protocols and distribution of relevant standards.
- Long-term monitoring and sampling requires adoption of accepted methods and standards achieved only through a coordinated network.
- Interdisciplinary graduate and postdoctoral teaching through centralized short courses is feasible in a network setting.
- Specialized technical training capacities are most efficient when centralized within a network.

Each of these network requirements is addressed in detail in the following sections.

**IV. Addressing the grand challenges in ecology – a central, unique role for isotope measurements**

Isotope analyses provide crosscutting information on the structure, function, and dynamics of ecological and environmental systems. As such, they provide unique information needed to meet ecological challenges facing society in the 21st century. The eight major science themes embraced by NEON–biodiversity, hydroecology, biogeochemical cycles, infectious diseases, climate change, invasive species, land-use, and emerging issues–are all addressed at some level using isotope measurements. Examples of how isotope measurements are used to address these issues are described herein. The examples given below for each of the major NEON science themes demonstrate how isotopes uniquely address the measurement challenges in these areas.

***a) Imprint of invasive species and of changes in biodiversity***

Ecologists now recognize the important linkages between biodiversity and ecosystem function and of the destabilizing impact of invasive species on these relationships. The three examples below highlight how stable isotope ratio analyses are used to understand the importance of biological diversity within ecosystems and the role single species (native and non-native) play in ecosystem dynamics and function.

**Example 1**: *Isotopes indicate functional contribution of plant life form diversity in temperate coniferous forests*. Plant functional type diversity is a useful parameter in ecosystem models and scaling. Functional attributes are generally assumed to relate to life form differences among dominant plant types. Brooks et al. (1997) show how leaf $\delta^{13}C$ values can be used to quantify functional diversity among different plant life forms in boreal forest ecosystems. The different life forms in these forest ecosystems show distinct clustering based on leaf $d^{13}C$ values. Leaf $\delta^{13}C$ values in $C_3$ plants are related to stomatal "openness"-the balance between photosynthetic demand by chloroplasts and supply of $CO_2$ through leaf stomata. Deciduous-tree species consistently showed greater stomatal "openness" (greater $CO_2$ supply through stomata relative to chloroplast demand) compared to evergreen tree species of boreal ecosystems in the Brooks et al. (2000) study. Such isotopic studies establish a critical linkage between an easily measured aspect of community structural diversity and functionally important traits associated with leaf photosynthesis.

**Example 2**: *Isotopes indicate the functional role of a keystone plant species in the Sonoran Desert*. Each summer, saguaro cacti produce large numbers of flowers and fruit that potentially provide a huge pulse of energy, nutrients and water to the animal community. Although their importance has been assumed, until recently the importance of the saguaro's resources to animal consumers has been essentially unknown. Recent work, however, has taken advantage of the isotopic chemistry of saguaros and other cacti to make direct measurements of nutrient transfer between saguaros and animal consumers (Wolf and Martinez del Rio 2000, 2003; Wolf et al. 2002). The $\delta^{13}C$ of avian blood and breath $CO_2$ provide direct measurements of saguaro resource use by individual consumers; these measurements can be scaled to population, community and ecosystems levels. Foraging mode, consumer digestive physiology, and home range/body size all interact to determine the suite of nutrients that consumers derive from cacti fruits.

**Example 3**: *Isotopes* *<u>indicate</u>* *impacts of invasive cheatgrass on semiarid ecosystems of the western US by* *<u>integrating</u>* *soil processes*. Invasion by nonnative species represents one of the most significant components of global change. The introduction of nonnative plant species may decrease ecosystem stability by altering the availability of nitrogen for plant growth by changing litter quality, rates of $N_2$-fixation, or rates of nitrogen cycling and loss. One of the most significant plant invasions in North America has been the establishment and spread of *Bromus tectorum* in arid regions of the Intermountain West. A Rayleigh relationship between soil $\delta^{15}N$ and ln(soil nitrogen content) indicates the impact of invasion on soil nitrogen. Measurements were made in non-invaded, recently invaded (5 yr), and historically invaded (>30 yr) sites on the Colorado Plateau (Figure 1). Invaded sites had consistently greater $\delta^{15}N$ values, indicating that loss of nitrogen was greater than new inputs (Evans and Ehleringer 1993, Evans and Belnap 1999). Furthermore, the Rayleigh relationship was linear for each invasion regime, allowing it to be used as a "clock" to integrate the effects of invasion on soil nitrogen dynamics, and as a tool to indicate the health of ecosystems following invasion.

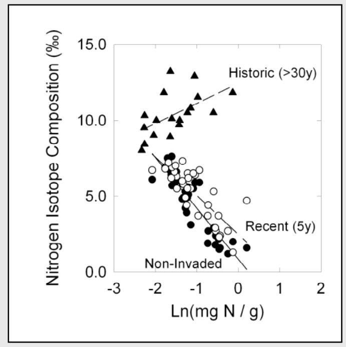


**Figure 1.** Soil nitrogen isotope composition ($\delta^{15}N$) plotted against the natural log of soil N content for 3 sites with different histories of invasion by cheatgrass (Evans and Ehleringer 1993).

***b) Quantifying source changes in <u>hydroecology</u>***

The most critical resource needed to sustain human health and welfare is fresh water. Understanding ecological impacts of drought and human use and alteration of freshwater resources is a key challenge to the ecological and hydrological communities. Isotopes provide excellent tracers in research aimed at understanding the origin, recharge and cycling of water.

**Example**: *Isotopes* *<u>trace</u>* *sources of water used by trees in sensitive desert riparian ecosystems.* Riparian ecosystems of the American Southwest are affected greatly by stream diversion, drought and groundwater pumping.

It is assumed that trees in riparian environments are sustained principally by use of groundwater taken up by deep roots. The stable isotope ratios of hydrogen ($\delta^2H$) and oxygen ($\delta^{18}O$) in water taken up by riparian trees along the San Pedro River in southeastern Arizona show that monsoon rain inputs can be a substantial transpiration source during the growing season (Snyder and Williams 2000), but this is dependent on the depth to groundwater and species (Figure 2). Our understanding of such plant-water interactions in groundwater-dependent systems is essential for devising strategies to provide fresh water to growing human populations in an ecologically sustainable manner.

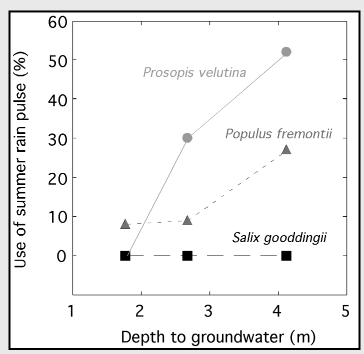


**Figure 2**. Uptake of growing season rainfall by three riparian tree species determined from the stable isotope ratio values of plant xylem water. Redrawn from Snyder and Williams (2000).

***c) Partitioning sources and revealing processes in biogeochemical cycles***

Probably the greatest contribution of isotope analyses to ecology is in studies of terrestrial, aquatic and atmospheric biogeochemical cycling and processes. Isotope ratio analyses are essential in studies of ecosystem carbon and nitrogen cycling and play a dominant role in our understanding of trace element cycling.

**Example 1**: *Isotopes integrate stream nitrate sources and indicate the importance of nitrate removal by denitrification*. In a comparative study of 16 watersheds in the northeastern USA, Mayer et al. (2002) showed how the $\delta^{15}N$ value of stream nitrate elucidates sources and transformations of this nutrient. Nitrate concentrations and $\delta^{15}N$ values were lowest in streams draining forested watersheds, whereas watersheds that had large areas of urban and agricultural land had high $\delta^{15}N$-nitrate values and high nitrate concentrations (Figure 3). Nitrate from urban wastewater and agricultural manure contributed significantly to stream nitrate in these latter watersheds. However, $\delta^{15}N$ values alone are not sufficient to distinguish terrestrial pollutants and soil denitrification from atmospheric nitrate deposition sources to watershed outflow. But the combination of $\delta^{15}N$ and $\delta^{18}O$ values allows these sources to be distinguished (Mayer et al. 2000).

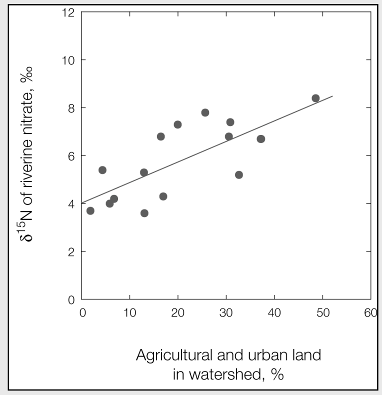


**Figure 3.** Mean nitrogen isotope ratio of nitrate in rivers draining 16 watersheds in the mid-Atlantic and New England states, USA plotted against percent agricultural plus urban land within each watershed. Redrawn from data in Mayer et al. 2002.

**Example 2**: *Isotopes indicate significant inputs of marine nitrogen from spawning salmon into terrestrial ecosystems*. Pacific salmon carry nitrogen from marine environments into terrestrial riparian ecosystems in which they reproduce. The flux of marine-derived nitrogen into terrestrial ecosystems and the nitrogen fertilization of terrestrial vegetation by salmon can be quantified using the stable isotope composition of nitrogen ($\delta^{15}N$). For example, Helfield and Naiman (2001) examined $\delta^{15}N$ values of foliage from riparian plant species in southeast Alaska to determine the percent contribution of salmon-derived nitrogen to vegetation nutrition in stream sections with and without spawning salmon. Marine-derived nitrogen is substantially more enriched than terrestrial nitrogen in the heavy stable isotope ($^{15}N$). Salmon collected by Helfield and Naiman (2001) had an average $\delta^{15}N$ value of 13.4 ‰, whereas foliage of riparian plants in a reference area blocked from spawning salmon had $\delta^{15}N$ values ranging from -3.3 to -0.9 ‰. Three of the four plant species examined by Helfield and Naiman (2001) had significantly higher foliage $\delta^{15}N$ values in salmon spawning areas compared to reference areas (Figure 4), reflecting substantial marine-derived nitrogen input. Based on a simple mixing relationship, Helfield and Naiman (2001) calculated that 22 to 24% of the nitrogen in plants from the salmon spawning area (except for the N-fixer, red alder) was marine-derived from salmon.

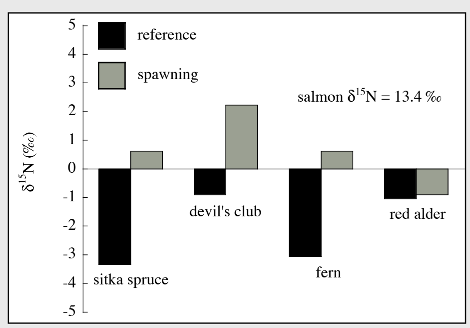


**Figure 4.** Foliar nitrogen isotope values for four riparian plant species collected from a salmon spawning area and from a reference area blocked from salmon access (redrawn from data in Helfield and Naiman 2001)

***d) Identifying geographical origins of infectious diseases and their vectors***

At least two important aspects of the ecology and spread of infectious diseases can be addressed using isotope ratio analyses. There is systematic geographic variation in isotopic signals present in the environment. Because animals isotopically record their diet and source water in hair, bones, feathers, etc., each migratory animal carries an isotopic "tag" identifying its movement across the landscape. Such information is useful for modeling the population ecology and migratory behavior of disease vectors and for understanding the spread of infectious disease. The geographic origin of the disease spores themselves can be detected from isotopes. Point of origin information from isotope ratios is now available for many biological substances.

**Example 1**: *Isotopes trace migratory patterns of birds in relation to geographical origins of avian diseases*. Migratory birds come into contact with diseases and parasites throughout their migratory range. Identifying geographic patterns of migration by individual birds is needed to understand how avian diseases are acquired and spread. Sharp-shinned hawks, for example, migrate many hundreds of kilometers each year in North America. Smith et al. (2004) demonstrate how hydrogen isotope ratios ($\delta^2$H) in sharp-shinned hawk feathers trace the nesting origin of hatching-year individuals trapped in New Mexico in relation to known distributions of avian blood diseases that afflict these hawks. The $\delta^2$H of bird feathers reflects that of local precipitation, which varies in a predictable way across latitudinal gradients. Only sharp-shinned hawks that originated in southwestern North America possessed one key blood parasite, *Haemoproteus janovyi*. Such isotopic information may be essential for resolving complex migratory behavior of birds and the biogeographic spread of avian-born disease.

**Example 2**: *Isotopes can trace the geographic origin of microbial spores*. Because the isotopic composition of precipitation ($\delta^2$H and $\delta^{18}$O) varies in a predictable way across the North American continent, biological substances carry an isotopic "fingerprint" of geographic origins. Microbial spores are no exception. Kreuzer-Martin et al. (2003) demonstrated that the $\delta^2$H and $\delta^{18}$O values of growth media water was incorporated in a very predictable way into spore biomass of the bacterium *Bacillus subtilis* (Figure 5). An isotope fractionation model predicted the $\delta^2$H and $\delta^{18}$O values of growth media water obtained from five regions of the USA within a 95% confidence interval. Stable isotope ratio analyses may be a powerful tool for identifying point-of-origin of microbial products.

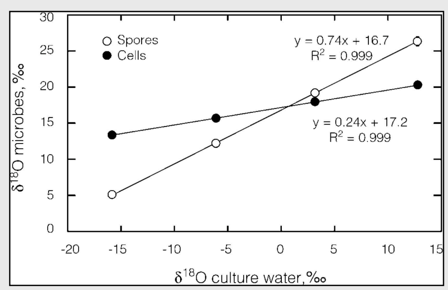


**Figure 5.** Relationships between the $\delta^{18}$O value of microbial biomass and that of the water present in culture media (from Kreuzer-Martin et al. 2003).

***e) Assessing the impact of regional climate change and climate cycles***

Many animals and plants record physiological responses to climate changes within their tissues. Tree rings are the best example from the plant world, but such information is recorded also in baleen from whales, hair from most any animal, tooth enamel, claws, nails and horns.

**Example**: *Isotopes* <u>*record*</u> *the impact of climate changes in tree rings*. In addition to the excellent information recorded in ring-width variation from annual growth increments of wood, the $\delta^{13}C$ of cellulose of each ring records the physiological condition of the plant in relation to annual fluctuations in climate. The $\delta^{13}C$ variations are due to variation in the ratio of leaf internal to ambient $CO_2$ concentration ($c_i/c_a$) during photosynthesis, which is a measure of stomatal "openness." Figure 6 shows annual fluctuations of $c_i/c_a$ calculated from $\delta^{13}C$ of cellulose extracted from wood rings of pine and fir trees and the remarkable correspondence with annual fluctuations in precipitation in Bryce Canyon National Park, UT (Ehleringer and Verville, unpublished). Such information allows researchers to quantify inter-annual variation in the physiological response of vegetation to climate changes.

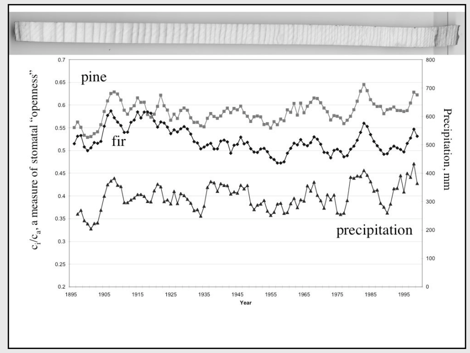


**Figure 6.** Long-term trends in ci/ca ratios (a measure of stomatal openness) derived from the carbon isotope ratios of tree ring cellulose in *Pinus ponderosa* and *Pseudotsuga menziesi* in Bryce Canyon National Park (Ehleringer and Verville, unpublished).

***f) Quantification of <u>land-use</u> <u>change</u> impacts on ecological processes***

Land-use and land-cover changes interact with other global change factors and have impacts on terrestrial, aquatic and atmospheric ecological processes. Urbanization and the intensification of agriculture, mining, water development and other land uses will require careful management if natural ecosystems and their benefits are to be sustained. Isotope ratio measurements are useful for quantifying impacts of land-use changes at regional and global scales.

**Example 1**: *Isotopes* <u>*trace*</u> *sources of pollutants in urban ecosystems*. Urban ecology is attracting attention from a variety of perspectives relating to biodiversity, invasive species ecology, hydroecology, and local and regional climate processes. The expansion of urban areas in the USA is creating unique challenges for the sustainable management of clean water and air. Stable isotope ratio analyses are proving to be useful for understanding sources of atmospheric pollutants in urban settings. Pataki et al. (2003) demonstrate the application of $\delta^{13}C$ and $\delta^{18}O$ analyses for identifying seasonal changes and sources of atmospheric $CO_2$ mixing ratios in the Salt Lake City airshed. Because the $CO_2$ originating from gasoline combustion, natural gas combustion, and respiration from plants and soils each carry unique isotopic signals, their individual contributions to airshed $CO_2$ mixing ratios can be distinguished (Figure 7). A surprisingly large contribution of $CO_2$ from this urban area was from biogenic respiration.

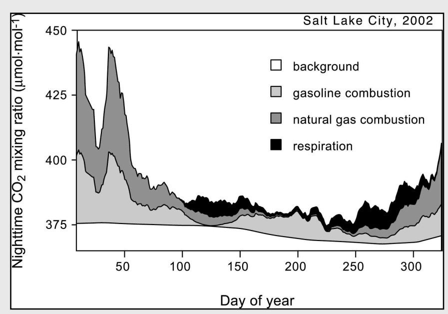


**Figure 7.** Contribution of different anthropogenic and natural sources of $CO_2$ to the Salt Lake City airshed during 2002. Figure from Pataki et al. (2003).

**Example 2.** *Isotopes* *__indicate__* *the dominant source of nitrogen input into arid ecosystems and* *__integrate__* *the effects of land-cover change on soil nitrogen dynamics*. Identifying sources of nitrogen input and the balance between nitrogen input and loss are critical for understanding potential responses of ecosystems to global change. Nitrogen input can occur through atmospheric deposition or biological nitrogen fixation. The primary source of nitrogen input in many arid regions is the biological soil crust; they are dominated by cyanobacteria and lichens that are capable of $N_2$-fixation. The crusts form a continuous cover in undisturbed plant communities, and spatial coverage is often higher than vascular plants. Surface disturbance in arid ecosystems is widespread and results in the elimination of biological soil crusts. Therefore identifying whether nitrogen input is dominated by a physical (atmospheric deposition) or biological ($N_2$-fixation) process is important to determine the potential impacts of surface disturbance on ecosystem nitrogen cycles. Evans and Ehleringer (1993) used a Rayleigh relationship to assess the relative contribution of physical and biological processes in a Pinyon-Juniper community on the Colorado Plateau. The predicted linear relationship between soil $\delta^{15}N$ and ln(soil nitrogen content) was established using soil values (Figure 8). Values for the biological soil crust fell immediately along this relationship while values for atmospheric deposition fell well off the relationship. This indicates that the primary source of nitrogen input was biological $N_2$-fixation, and land-use change may alter the balance between nitrogen input and loss by eliminating this source. This was confirmed by Evans and Belnap (1999), who observed lower soil nitrogen contents and greater soil $\delta^{15}N$ values in disturbed versus undisturbed sites.

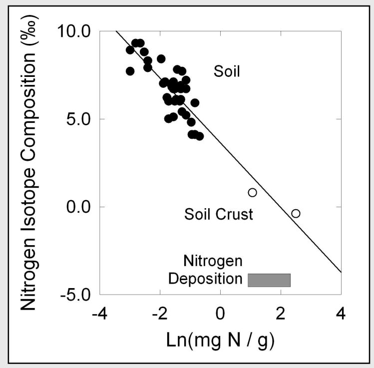


**Figure 8**. Relationship between soil $\delta^{15}N$ and ln(soil nitrogen content) in soils and in two potential sources of N in a cold desert ecosystem in southern Utah.

***g) Addressing __emerging__ __issues__ in ecology***

Because NEON will be a long-term, continental-scale project it will have built-in flexibility to address emerging issues in ecology with national significance. Isotope analyses likely will play a prominent role in identifying these issues and investigating their causes and impacts. This viewpoint is founded on the understanding that isotope ratios *integrate*, *indicate*, *record* and *trace* fundamental processes in ecology and serve to bridge traditionally very different scientific disciplines. Long-term and spatially distributed isotope monitoring of the environment (described below) will illuminate patterns of change in a way not yet achievable. Such long-term, large-scale data sets will bring into focus emerging issues and opportunities that require national attention. Such issues may include a more focused understanding of the impacts of desertification, urbanization, the origin and impact of atmospheric dust and other aerosols, the complex chemistry and transport of reactive trace gases, biogeochemistry of toxic heavy metals in terrestrial and aquatic ecosystems, and ecological issues related to homeland security. Isotope ratio technologies are pre-equipped to address such issues as they emerge.

A key requirement for addressing such emerging issues in ecology is the establishment of *innovation capacity* (described below) for development of new technologies and approaches needed for *future* scientific questions and applications.

***h) Importance of "solid source" isotope measurements in ecology***

So far we have highlighted isotopes applications for some important light elements H, C, N, O, and S that are analyzed using "gas source" isotope ratio mass spectrometers. Thermal ionization mass spectrometers are required for analysis of some other important element tracers used in ecology. Strontium (Sr) is probably the most useful solid source isotope for ecological studies, with important applications to climate change studies (longterm and short term forest disturbances, for example), biogeochemical cycling of Ca of plants, sources of Ca alkalinity in streams, and identifying point-of-origin of animal and plant tissue at landscape and continental scales. Boron (B) is very useful for quantifying seawater incursions into groundwater and tracing sources of human vs animal vs natural contamination of waters. Litium (Li) is also sometimes useful for these purposes.

***i) Importance of radiocarbon measurements in ecology***

Radiocarbon is the rare, radioactive isotope of C that is produced in the stratosphere by the action of cosmic rays. The $^{14}C$ that is produced is subsequently oxidized to carbon dioxide ($CO_2$) and enters the Earth's C cycle in the ocean and on land. Radiocarbon is unstable and will spontaneously decay back to $^{14}N$ with a half-life of 5730 years. The balance of production and decay are such that the natural abundance of $^{14}C$ in atmospheric $CO_2$ was just over one in every trillion ($10^{12}$) C atoms prior to 1950. However, after 1950 additional radiocarbon was produced in the atmosphere as a result of aboveground thermonuclear weapons testing. Concentrations of $^{14}C$ atoms nearly doubled in the troposphere by 1964 at the peak of testing, and have been declining since aboveground testing was banned. Ecosystem carbon pools that exchange $CO_2$ rapidly with the atmosphere have been clearly labeled with bomb $^{14}C$ over the past several decades, which provides information on the exchange rates of these pools. In combination, both radioactive decay and the pulse labeling from weapons testing provide unique information on different time scales about the cycling rates of C through the biosphere that cannot be obtained with stable isotopes.

Development of accelerator mass spectrometry (AMS) technology over the past several decades has made it possible to analyze small quantities of organic materials and $CO_2$ with high precision. However, the capital investment required for AMS is still such that very few, large laboratories in the United States are available to run samples. This has kept the cost of the samples high and has limited the widespread use of $\Delta^{14}C$. Research into ecological processes requires relatively large numbers of samples in order to describe large spatial scales, and to deal with heterogeneity affecting processes cycling carbon at smaller scales. Recently, the development of lower energy AMS has opened up the possibility of lower costs for $\Delta^{14}C$ measurements. The emergence of this technology will have a profound influence on the use of $\Delta^{14}C$ in studies of C cycling, just as the development of automated continuous flow mass spectrometers revolutionized the use of stable isotopes. At this point, the spread of this technology now requires centralized facilities and an expanding pool of educated users who are versed both in the collection and preparation of samples and the interpretation of $\Delta^{14}C$ data.

## V. Structure components of an isotope network

A multi-disciplinary, multi-scale isotope ecology network is emerging. Twenty-nine isotope ecologists representing different regions of the USA, different sub-disciplines in ecology, and academic and government labs (**Appendix A**) assembled for workshops in Park City, Utah in September 2004 and in San Francisco, California in December 2004 to discuss the formation of a national network for isotope ecology. This report is a synthesis of the ideas that emerged from the workshops and related discussions. Isotope networks that already exist at the national level offer useful models for the development of a larger, more diverse NEON-related isotope ecology network. For example, the Biosphere-Atmosphere Stable Isotope Network [BASIN] (http://basinisotopes.org) is an NSF-sponsored Research Coordination Network (RCN) aimed at consolidating stable isotope ratio datasets, establishing common protocols and approaches, networking across sites, and providing training through workshops and symposia, with an initial focus on understanding interactions between terrestrial ecosystems and the atmosphere. BASIN sponsored the NEON-related isotope workshops held in Park City and San Francisco. Participants at these workshops recognized that in NEON there will be a critical need to establish long-term integrated isotope measurement networks to identify multi-decadal patterns of change in terrestrial, aquatic, and atmospheric ecological conditions and processes, with a substantially broader scope than is addressed by existing networks. This document incorporates suggestions and input from each of the two initial workshops.

*Coordination facilities and regional labs*. One model for the structure of an isotope ecology network within NEON includes the establishment of one or a few specialized coordination facilities, centrally located within the conterminous USA, which maintain intimate working connections with smaller regional NEON analytical labs. The coordination facilities should be placed where there is excellent airport access, and where there is a large, highly trained technical workforce to attract and retain the skilled individuals needed to build, manage and operate such facilities. The coordination facilities would function as the distributors of standard materials, high-level technical training, QA/QC procedures, procedures for sample collection, storage, preparation and analysis, and high-level technical support, as well as an innovation center for the development of new standard materials, technologies and analytical procedures. The coordination facility also could house large and/or expensive instrumentation beyond the scope of what is conducted at the regional scale. This could be accelerator mass spectrometers for $^{14}C$ measurements or secondary ion mass spectrometers that will enable NEON scientists to measure the isotopic content of individual microbial cells.

Smaller NEON isotope labs should be established across the different NEON regions because fairly routine analytical capacities are required locally near sampling sites. Scientists working on NEON-related projects require accurate and precise data from an isotope facility with minimal turnaround time. Many stable isotope facilities already exist across all NEON regions of the USA. From practical and economic considerations, it is most logical to enhance regional capacities to meet NEON science goals, rather than centralize all NEON-related isotope analyses into one or a few facilities. Regional labs would carry out the bulk of routine isotopic analyses for core NEON-related science, monitoring, and training needs. Regional labs should be encouraged to participate in and even lead innovation, which can be assisted by the coordination facilities.

However, very specialized procedures (mentioned above) appropriately would be carried out only at coordination facilities.

*Facilities as distinct from an individual investigator's lab*. Regional labs should be distinct from the existing government, university and private labs across the USA. This recommendation is based on several practical realities. First, individual researchers have no great incentive to take on the additional supervision and management needed to support NEON-related activities, which at times would be in conflict with priorities of their own individual research needs. Second, individual faculty and research leaders in government come and go over time; having a sustainable analytical capacity and stationary location would better serve NEON long-term interests. However, placing the NEON regional labs at locations and within institutions where such expertise already exists would benefit the existing labs and NEON related activities. Incentives are needed to attract regional NEON infrastructure to a particular institution or that institution's research park. Such incentives may come in the form of technical and management support from NEON that would add to a synergistic relationship between the existing regional labs and a regional NEON lab. A successful NEON program would be one that developed “buy-in” by the scientific community. Regional labs should be situated where there is a long-standing tradition of scientific interest in broad-scale research studies including long-term monitoring.

*Monitoring versus research needs*. NEON will establish the infrastructure for the scientific and educational requirements needed to meet long-term national-scale ecological challenges. A NEON-related isotope network can serve two major scientific needs: analytical support for traditional scientific inquiry, and sampling and analysis infrastructure for national-scale monitoring of environmental and ecological change. The central NEON coordination facility of facilities and regional NEON labs should have built-in capacities to meet both monitoring, research and innovation objectives. Isotope monitoring should be part of a larger monitoring program within NEON.

*The network as a supplier and user of training*. Training of postdoctoral researchers, graduate students and technicians who will likely carry out the bulk of the sample collection and isotope analyses within NEON, would be greatly aided by a common network. Specialized short courses and workshops on technological aspects of sample collection, preparation, storage, and analysis are most efficiently organized and managed through a network. Such training could be conducted at key regional labs where unique combinations of expertise might exist or can be routinely assembled, or at the central coordination facility or facilities. In either case, some top-down organization of such training activities within the network is critical to ensure that the long-term quality of sample collection, analysis and data interpretation across the USA is maintained at the highest levels.

*Developing transformational interdisciplinary courses for graduate students, postdoctoral researchers, faculty and K-12 educators*. The isotope approach has brought together scientists from traditionally separate biological, physical and social science disciplines. Because of mutual interests in the cycling of key elements and the use of isotopes as a fundamental, interdisciplinary tool, scientists from disciplines as diverse as geochemistry, animal and plant physiology, hydrology, geology, atmospheric science, soil science, geography and Earth system science are collaborating on cross-cutting research aimed at critical environmental and ecological problems.

This transformational role of isotope technology is being expressed further through the emergence of interdisciplinary coursework on isotope applications offered at many universities across the country. Specialized short courses open to students worldwide are now available at several institutions in the USA. Isotope courses are emerging that are taught from a multidisciplinary perspective and often from several instructors with very diverse academic backgrounds. The grand challenges in the field of ecology are continental and global in scope, and require collaboration among disciplines outside the traditional ecological fields. The integrating information captured by isotope analyses provides a natural platform for transformational, interdisciplinary education on large-scale ecological processes. Through a coordinated, but distributed education and training program, the network would serve to bring this vast interdisciplinary knowledge and experience to those who seek to address NEON-related ecological problems using a stable isotope approach or to those who would like to become knowledgeable about how such information is applied.

*An alternative model*. An alternative to the centralized structure involving NEON coordination facilities and integrated regional NEON Inc. labs is to have existing research labs (government, academic, or private) join a loosely organized network organized and enhanced through NEON. Involvement in the network under this model would require minimal vetting and limited top-down facilitation. The advantages of such a structure are that individual lab groups would maintain complete autonomy for innovation, discovery, and mobilization to address emerging issues. The global isotope monitoring networks coordinated through the International Atomic Energy Agency (IAEA) provide good models for this structure.

## VI. Isotope Network for Early-Warning Signals (INEWS)

The capacity for isotope analyses to *integrate*, *indicate*, *record* and *trace* ecological processes presents an unparalleled opportunity to develop a national-scale monitoring network within NEON to detect ecological change at a fundamental level. We propose that NEON establish an *Isotope Network for Early-Warning Signals* (*INEWS*) based on isotope monitoring of *terrestrial*, *aquatic*, and *atmospheric* environmental parameters. Isotope measurements made within this framework have the capacity to transcend all challenge areas in ecology. Such a long-term and large-scale monitoring of the nation's ecological condition can be achieved only through an initiative such as NEON. The potential benefits of such a monitoring network for the country are tremendous and rival the benefits obtained from satellite remote sensing of land-cover changes. Frequent sampling and isotopic analysis of key terrestrial, aquatic and atmospheric environmental parameters can be used to identify anomalies in system behavior that relate to changes in significant ecological processes. Human heart rate, blood pressure and body temperature are screened routinely for potential anomalies that are then investigated in greater detail. In an analogous way, the large-scale ecological condition of the nation's ecological support systems can be monitored and screened using isotope measurements. Such monitoring would serve as an early warning of ecological changes that could be investigated as needed in much greater detail using more sophisticated and intensive methods.

We do not yet have sufficient, long-term isotope data sets for many environmental parameters to interpret potential ecological changes using isotope monitoring. However, there are a few key examples of isotope monitoring networks that are now producing substantial insight into large-scale processes. The International Atomic Energy Agency (IAEA) is sponsoring the Global Network of Isotopes in Precipitation (GNIP; http://isohis.iaea.org/GNIP.asp). GNIP has been surveying the $\delta^2H$ and $\delta^{18}O$ values in precipitation around the world since 1961. The data are used in the fields of hydrology, oceanography, hydrometeorology and ecology and in investigations related to the Earth's water cycle and climate changes. The Biosphere-Atmosphere Stable Isotope Network (BASIN; http://basinisotopes.org) is another example. BASIN has been active only since 1995, but has generated tremendous new insight into carbon and water cycle processes at ecosystem, regional and global spatial scales and on the seasonal and inter-annual fluctuations in carbon and water exchange processes. Other individual investigators are monitoring isotopic composition within the environment. For example, T. Coplen (USGS) has been monitoring the $\delta^{18}O/\delta^2H$ of stream water, and C. Kendall (USGS) has been monitoring the $\delta^{15}N/\delta^{13}C$ of particulate organic matter, at ~40 USGS big river NASQAN (National Stream Quality Accounting Network; http://water.usgs.gov/nasqan/) sites since 1996. For the last year, Kendall also has been monitoring the $\delta^{15}N/\delta^{18}O$ of nitrate at these sites. Such activities should be enhanced and coordinated within a NEON program.

GNIP and BASIN capitalize on existing environmental monitoring networks. Many of BASIN's sampling locations in the USA are also AmeriFlux sites. GNIP sites in the USA are National Atmospheric Deposition Program (NADP) sample collection sites. Sites for a national stable isotope monitoring network within NEON also could be co-located and even co-operated by similar existing environmental monitoring networks within EPA, USGS, NOAA or other government and non-government programs. Establishment and operation of a NEON-related isotope monitoring network presents unique challenges with respect to sample storage and archiving, development of sampling and analysis procedures, data documentation and interpretation, and equipment maintenance. These activities should be coordinated and facilitated at the national level through NEON.

*What information does each isotope provide?*

- $\delta^{13}C$ - an integrated measure of plant stress and plant water use efficiency as reflected in the ratio of leaf internal and external $CO_2$ concentration ($c_i/c_a$). $\delta^{13}C$ is also distinct among plants using different photosynthetic pathways: C3 (the majority of plants) versus C4 (several crops and pasture species), providing an excellent tracer of land use change and history.
- $\delta^{18}O$, $\delta D$ – indicators of hydrologic and climatic processes. These isotopes undergo predictable transformations in the hydrological cycle that allow tracing of water sources and geographical origin, reconstruction of climate, and calculation of energy balance and evaporation.
- $\delta^{15}N$ – an indicator of nutrient sources as well as soil, plant, and aquatic nitrogen transformations and cycling.
- $\delta^{34}S$ – an essential tracer in aquatic and terrestrial food web studies, and important for identifying the origin and processing of organic and inorganic pollutants in atmospheric and aquatic environments.
- $\Delta^{14}C$ –a unique tracer of the age of organic material that stable isotopes alone do not provide. Radiocarbon can be used to estimate the age and turnover time of ecosystem carbon pools, and therefore trace changes in the rate of carbon cycling and sources of

carbon substrates. It is also an excellent indicator of the presence of fossil fuel carbon, in which radiocarbon has all decayed away in contrast to the measurable amount of radiocarbon in modern atmospheric $CO_2$.
- $O_2$/Ar, $O_2/N_2$ ratios – A number of elemental ratios provide critical information about the contribution of ecological processes to biogeochemical cycles. These are not isotope ratios per se but are often measured with similar methodology and instrumentation.

*Indicators of changes in ecosystem physiology and function:*

- Leaf biomass integrates physiology, nutrient and water sources, and energy balance over the period of leaf expansion
- Tree rings provide information about long-term trends and interannual variability in climatic and ecological processes
- Animal material integrates food sources, migration patterns, and physiological processes over varying temporal scales
- Atmospheric water vapor provides information about the contribution of plant transpiration to the atmosphere over ecosystem and regional scales

*Indicators of changes in biogeochemical cycles:*

- Soil organic matter integrates the plant processes described above over longer periods of time, and also reflects soil heterotrophic processes
- Dissolved and suspended organic material in aquatic systems provides large scale integration of terrestrial and aquatic nutrient cycles
- Trace gases such as carbon dioxide reflect the balance of photosynthetic and respiratory processes over large spatial scales

*Indicators of changes in nutrient and pollution sources:*

- Terrestrial organic material records plant uptake of biogenic versus anthropogenic sources of $CO_2$ and nutrients
- Aquatic organic and inorganic material records the sources of nutrient and organic matter inputs integrated over large spatial scales
- Greenhouse gases and atmospheric pollutants reflect the proportional contributions of various biogenic and anthropogenic sources

*An early-warning monitoring network for our nation's terrestrial ecosystems.* Isotope composition of vegetation and soils record integrated impacts of stressors affecting plant metabolism and biogeochemical cycling of key nutrients. Changes in plant and soil $\delta^{13}C$ and $\delta^{15}N$ values identify perturbations to fundamental processes associated with ecosystem water, carbon and nutrient exchange (see Example 2 from section IV-f above; **Appendix B**). Bulk leaf and soil samples are easy to collect and analyze within a monitoring framework. Frequent sampling and $\delta^{13}C$ and $\delta^{15}N$ analysis of plant leaves and soil at strategic positions across the landscape will generate a profoundly useful database of ecological change indicators. Sampling should be biased towards ecosystems that are of great socioeconomic and ecologic value as well as toward ecosystems that are particularly sensitive to environmental and ecological change. Ecotonal areas, urban-wildland transects, agricultural-wildland transects and riparian areas may be particularly valuable within such a monitoring program.

*An early-warning monitoring network for our nation's aquatic ecosystems and watersheds.* The isotope composition of dissolved organic and inorganic constituents within streams integrates and indicates catchment-scale ecological processes and conditions (see example from section IV-c above). A distributed network of stream monitoring sites already exists within the USGS National Water-Quality Assessment (NAWQA) Program. Stable isotope ratio analyses of stream chemical components, which are not included in NAWQA, would provide very useful information on the sources and ecological processes associated with stream chemistry changes. Taken this way, these measurements would integrate watershed-level ecological condition from a sample taken from one single point on the landscape. A geographically distributed, national-scale monitoring network for isotopes in streams throughout the U.S. would warn of potentially important changes taking place in the ecological condition of the nation's watersheds. The key measurements to be made include: $\delta^2$H and $\delta^{18}$O of $H_2O$, the $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S of dissolved organic material and of particulate organic material, the $\delta^{13}$C of dissolved inorganic carbon, the $\delta^{18}$O of dissolved oxygen, the $O_2$/Ar ratio, the $\delta^{15}$N and $\delta^{18}$O of nitrate and $\delta^{15}$N of ammonium (**Appendix B**).

*An early-warning monitoring network for our Nation's airsheds.* The isotope concentration in atmospheric gases integrates sources of biospheric fluxes (see Example 1, section IV-f above) and also indicates changes in carbon, water, and nutrient exchange processes over a large area. The $\delta^{13}$C and $\delta^{18}$O of $CO_2$ is being collected at a limited number of sites across the country through BASIN, but no such isotopic monitoring network is currently in place for other key atmospheric constituents, or in all ecologically important locations involving urban and agricultural lands.  Of relevance for monitoring the nation's airsheds and ecological condition of the landscape are the following compounds in the atmosphere: $\delta^{13}$C and $\delta^{18}$O of $CO_2$, the $\delta^2$H and $\delta^{18}$O of water vapor, the $\delta^{13}$C of particulate organic matter, the $\delta^{15}$N and $\delta^{18}$O of $N_2O$, the $\delta^{13}$C and $\delta^2$H of $CH_4$ and the ratio $O_2/N_2$ (**Appendix B**). Using the same logic for sampling of terrestrial and aquatic systems, atmospheric sampling and monitoring should be done across the different ecological regions of the USA and particularly focused on systems that are potentially sensitive to ecological change. Urban airsheds, agricultural areas, and transition areas with wildland systems should be targeted for greatest scrutiny. The sampling should be coordinated with existing atmospheric monitoring networks associated with AmeriFlux, NOAA, Cooperative Air Sampling Network (CMDL) and NADP.

## VII. Multi-disciplinary education of future generations is the key

Initial conceptions of NEON emphasize training, education and outreach programs aimed at transforming the way ecologists work as well as how the public views the role of ecology in solving national-scale environmental problems. Interdisciplinary approaches, large-scale capacities and integrating methodologies will characterize the field of ecology in the future. *The isotope approach is a bridging technology*; it stimulates professional and scientific transformation by bringing together often-disparate scientific fields. Isotope ratio measurements capture physical, chemical, and biological processes and are essential to the fields of geochemistry, physiology, geology, oceanography, atmospheric science, hydrology and ecology.

The technology and the types of scientific questions that can and will be addressed with isotopes have the potential to unify the cultural abyss that traditionally separates ecology from other natural science disciplines.

*Problems that training and education should solve*. Ecologists, for the most part, are poorly trained in geochemistry, physical chemistry, biochemistry and engineering—areas of expertise needed for fundamental understanding, application and innovation of isotope approaches. Further, ecologists often are separated institutionally from other academic disciplines. Inroads are being made with the emergence of interdisciplinary programs in research and teaching at many universities, but often the diversity of disciplines needed for true scientific integration are found only at large, research-extensive universities. Most 2- and 4-year colleges and universities do not have the resources to assemble expertise in key disciplines needed for the transformation of ecology. A multi-disciplinary training and education program within NEON focusing on isotopes as a unifying approach can help solidify the emerging interdisciplinary focus at large research universities and catalyze interdisciplinary activities for students and faculty at smaller institutions.

*Opportunities that arise from an interdisciplinary education*. The grand challenges facing ecology must be approached from an interdisciplinary perspective. Interdisciplinary education centered on a unifying theme has tremendous potential for generating new and synthetic insight. Isotope ecology courses now exist at many universities and generally are taught from an interdisciplinary perspective. Such courses have been exceedingly successful, especially where they assemble students and faculty from diverse scientific backgrounds. The 2-week intensive summer course taught at the University of Utah (*Stable Isotope Ecology*; http://ehleringer.net/bio7473.html) is one such transforming interdisciplinary course. This lecture / lab course draws faculty instructors and students from around the country and from diverse disciplines including geochemistry, paleoecology, oceanography, animal ecology, hydrogeology, plant ecology and anthropology. The course has catalyzed numerous interdisciplinary research collaborations among former students and faculty. Currently, space limitations allow enrollment of only 20% of the total applicants each year suggesting significant unmet demand.

Several components should be included within a NEON-related training and education program associated with an isotope network:

- *Technical training for laboratory personnel*: An analytical network within NEON will require highly trained laboratory technicians to 1) operate and maintain sophisticated equipment, 2) store and prepare field samples, 3) process, manage and report data, 4) manage QA/QC procedures, and 5) assist and train researchers and educators using regional analytical labs. Centralized training courses and workshops should be implemented to ensure that qualified and skilled technicians are available to carry out the demands of NEON-related isotope research, education and environmental monitoring. The national analytical facility would be an ideal place to host a training course for lab technicians. Isomass Scientific, Inc. (http://www.isomass.com/Training.html) provides a very useful course for beginning and advanced mass spectrometer operators. Such a course could be developed at the NEON national analytical facility specifically to train NEON isotope lab technicians for the variety of tasks needed to maintain a large-scale isotope ecology network. In addition, information about emerging technologies, modified and improved procedures, data reporting protocols, etc. could best be transmitted through annual technical workshops.

- *Graduate, undergraduate, postdoc level courses*: Young researchers in ecology who wish to use the isotope approach should be exposed to a very broad interdisciplinary education on the theory and application of this technology. A NEON-related stable isotope network could offer such courses in various locations around the country. Intensive summer short courses are a very successful (e.g., *Stable Isotope Ecology*; http://ehleringer.net/bio7473.html). These courses could be packaged into basic modules covering different technical and theoretical aspects of stable isotope ecology. Modules could be assembled and delivered by experts in the field supported by regional NEON isotope labs. It is essential that these courses reach out to students and faculty at 2- and 4-year colleges within each region.

- *K-12 education*: The development of a K-12 education program within NEON should highlight the transforming technologies that shape the way ecologists conduct research and understand large-scale processes. Specialists from the field of stable isotope ecology should assist in the development of K-12 curricula within NEON. Many of the variety of isotope samples needed in ecology are easily collected and analyzed. A participatory component of K-12 education might involve collection and analysis of isotope samples within a NEON isotope-monitoring network.

- *Outreach programs*: Demonstrations, workshops, and public participation in science activities are essential components of a successful science outreach program. Community involvement in basic scientific investigation has proven tremendously successful at generating public understanding and a sense of ownership in the scientific activities of public institutions (e.g., *Project Feeder Watch*, http://birds.cornell.edu/pfw/). Some aspects of sample collection needed for broad-scale isotopic monitoring of the environment would be suitable for community participation.

- *Web-based resources*: Web-based information sharing will be an essential aspect of NEON research programs. Web-based resources also would be useful for public outreach and for sharing network information with students, land managers, policy makers, and industry. Isotope information could be provided at a very fundamental level (e.g., http://www.sahra.arizona.edu/programs/isotopes/index.html) with links to more sophisticated topics and information.

**VIII. Standards, standardization, and interlab comparisons to address quality control and quality assurance**

Given the scope and complexity of a NEON-related isotope network, it is essential for its success that every region in the network be organized around a systematic and comprehensive set of operating procedures, isotopic standards, and a mutually agreed upon and flexible QA/QC program. Many field experiments often consist of measurements in single locations with limited temporal and spatial objectives. The very nature of these projects typically does not require investigators to pay as much attention to issues of accuracy, precision and inter-lab calibration as might be prudent or useful. However, when merging large data sets consisting of many different measurements produced by multiple labs, results can be meaningless unless the isotopic data produced are the result of similar sample preparation, similar mass spectrometer operation, identical data reduction, and constrained by the use of lab standards, calibrated to primary reference materials on a regular basis.

Key elements to developing a network of laboratories that can meet the necessary high standard for inter-comparability and long-term quality assurance include:

- Develop a working library of methods for sample collection, sample storage, sample preparation, and isotopic analysis.
- Facilitate development of QA/QC procedures specific to each analysis and devise a data distribution (clearing house) system that will efficiently compile and report results from regional labs.
- Produce, distribute and maintain a set of NEON standard reference materials (i.e. gases, carbonates, organic materials, water samples) appropriate for all core NEON isotopic measurements for use in regional labs.
- Facilitate sample inter-comparisons between labs, where aliquots of a same sample material are analyzed at all regional labs.
- Organize ‘round robin’ calibrations, where reference materials are circulated among labs, aiding both in the production of intermediate standard materials, and laboratory inter-comparison verification.
- Work with the National Institute of Standards and Technology (NIST) and the International Atomic Energy Agency (IAEA) to develop new standard materials, including carbonates and $CO_2$ in atmospheric air, and other materials appropriate for core NEON isotopic measurements.
- Develop and maintain a system-wide data repository system where data can be accessed and analyzed.
- Ensure communication between laboratories for exchange of new ideas, innovation, issue problem-solving, etc. that will occur over time.

The framework for executing many of the above objectives would best be accomplished through a cooperative effort between a coordination facility or facilities and regional labs. In addition, a QA/QC framework will need to be developed by consensus among participating science groups across the country. The coordination facilities would be obvious places to produce NEON standards necessary for isotopic quality control of the proposed measures (see **Appendix C** for a complete list of ecologically related isotope measurements that would require new or additional standards). These are also logical places for a data coordination system to be developed and maintained. Critical to this effort would be incorporating protocols and lessons learned from other large-scale measurement communities. For example, the global carbon isotope community has implemented practices that help solve some of the issues relating to uniformity of scale and inter-comparability of data sets, including web based data management software that links distant labs, and reports on frequent sample inter-comparison projects. (See http://www.cmdl.noaa.gov/ccgg/index.html).

The fundamental organization of such activities may involve components that are *top-down* or *bottom-up* in management structure. Regardless, the balance of these structural components should lead to seamless data integration at a continental scale, without sacrificing participation, innovation, flexibility and creative inquiry at a local level.

## IX. NEON as an opportunity for analytical innovations

The technology associated with isotope ratio measurements has evolved rapidly over the last 15 years. Demands for innovation from the fields of ecology, medical science, atmospheric science, biogeoscience and forensics have been important in the development of automated and rapid techniques for analysis of organic compounds and trace gases. Continuous-flow inlet systems coupled with gas chromatographs and multi-sample prep devices are now widely available. Despite these great advancements, there is an important need for continued innovation to meet demands in these very active fields of research, and especially in ecology. Important questions in ecology can not be addressed with available techniques. Innovation is needed to meet limitations relating to: *timescale and/or time resolution of measurements*, *reactivity of compounds*, *storage of compounds*, and *sampling disturbance*.

Many important ecological processes occur during episodic events over very short duration. Such processes are generally poorly understood because limitations associated with sampling frequency and the short-lived nature of target compounds or issues related to compound storage prevent adequate observation of the phenomena. Future innovations in isotope ratio measurement technology should focus on *in-situ*, continuous monitoring of compounds. Some exciting advancements have been made over the last 2 to 3 years involving use of tunable diode lasers for continuous field measurement of isotopes in $CO_2$ and water vapor. Similar advancements are needed for continuous, non-invasive monitoring of other organic and inorganic trace gases (e.g., $N_2O$, $CH_4$, $NO_x$, $O_2$, $N_2$, isoprenes, etc.) and compounds in aquatic environments (e.g., water, DOC, DIC, nitrate, sulfate, etc.).

Establishment of an innovation facility or at least facilitation of innovation at the local level within NEON would serve to focus energy on solving these and other technological limitations that currently prevent collection of certain types of data. Establish capacity for innovation within the coordination facilities (described above) would create an environment for seamless technological innovation, technology transfer, and positive feedback with NEON scientists who interact with these facilities. However, innovation at the regional level should not be discouraged. Great technical insights and solutions will emerge from regional labs. These innovations should be encouraged and aided to the degree possible by the central innovation facility.

## X. Instrumentation and facility requirements

This document describes a NEON-related stable isotope network needed to support basic research, monitoring and training. We propose that the network have numerous regional labs facilitated through one or several coordination facilities. The coordination facilities will facilitate training, QA/QC development, standard material development and distribution, monitoring networks, and analytical services and focus, to some degree, on innovation. A significant level of resources is needed to establish the coordination facility or facilities and all the regional labs to ensure that they are equipped to meet challenges of NEON-related research and education. Here we provide cost estimates for instituting a NEON-related stable isotope network based on the model laid out in this document.

Coordination facility or facilities

An initial investment (ca. $100k) is required to procure, test, calibrate and store standard materials needed to ensure inter-lab comparability and data quality (see **Section VIII**).

Six isotope ratio mass spectrometers (6 x $180k = $1,080k) are needed to calibrate and monitor standard materials; develop, routinely upgrade and modify sampling and measurement protocols; provide training and support to technicians for regional labs; and fulfill requirements for specialized analytical needs.

Regional facilities

An Isotope Network for Early-Warning Signals (INEWS) for our nation's terrestrial, aquatic and atmospheric environments, NEON-related research, and access to instrumentation for education would require establishment of significant regional analytical capacities. We base our cost estimates on the assumption that monitoring and research each will demand 45% (90% total) of the regional capacity with the remaining 10% of capacity dedicated to teaching and training. **Appendix D** shows detailed cost estimates for establishment of an early warning monitoring system for a single region of NEON and instrumentation for each region required for all functions. Each instrument has the capacity to analyze 12,000 samples total (unknowns and standards) per year. Assuming 45% of these will be for INEWS, then ≈ 4 mass spectrometers are required for each region (4 x $180k each = $720k instrument cost per region).

Human capital and sustained funding

Highly trained technicians are needed to operate and manage the coordination and regional laboratory facilities. One technician is capable of operating and maintaining four mass spec instruments with peripheral devices. This arrangement will also allow time for meeting other demands related to NEON researcher support, data analysis, local training, etc. Based on this ratio, the coordination facility will require two highly trained mass spec scientists (2 x $80k salary+fringe = $160k / yr). One mass spec technician is needed within each region ($60k salary+fringe / yr).

NEON is intended to be a 30 yr program. Measurement technology will advance and original equipment will lose capabilities through attrition and obsolescence. We highly recommend that NEON retain the financial capacity for re-tooling at least once every decade.

**Appendix A.** Participants of the first and second workshops on developing a NEON-related stable isotope network, held in Park City, Utah on September 16-17, 2004 and in San Francisco, California on December 12, 2004.

| Name | Expertise | Institution |
|---|---|---|
| Steve Beaupre | Animal ecophysiology/ecology | University of Arkansas |
| Sharon Billings | Terrestrial biogeochemistry | University of Kansas |
| Gabriel Bowen | Geochemistry, paleoecology | University of Utah |
| Dave Bowling | Atmospheric/biospheric interactions | University of Utah |
| Renee Brooks | Hydroecology | US-EPA, Corvallis, Oregon |
| Jeff Chanton | Atmospheric/biospheric interactions | Florida State Univerity |
| David Dettman | Paleohydrology | University of Arizona |
| Rick Doucett | Aquatic ecology/biogeochemistry | Northern Arizona University |
| Jim Ehleringer | Atmospheric/biospheric interactions | University of Utah |
| Dave Evans | Terrestrial biogeochemistry | Washington State University |
| Carol Kendall | Aquatic biogeochemistry/hydrology | U.S. Geological Survey |
| Jennifer King | Terrestrial biogeochemistry | University of Minnesota |
| Guanghui Lin | Plant physiological ecology | Carnegie Institution of Washington |
| Stephen Macko | Organic geochemistry | University of Virginia |
| John Marshall | Terrestrial Plant Ecology | University of Idaho |
| Dan Murnick | IR instrumentation/engineering | Rutgers University |
| Nathaniel Ostrom | Aquatic biogeochemistry | Michigan State University |
| Diane Pataki | Plant physiological ecology | University of California, Irvine |
| Darren Sandquist | Plant physiological ecology | California State University, Fullerton |
| Laurel Saito | Civil engineering, aquatic ecology | University of Nevada, Reno |
| Alyson Sayer | Terrestrial Ecology | University of Alaska |
| Jed Sparks | Atmospheric/biospheric interactions | Cornell University |
| Heidi Steltzer | Biogeochemsitry | Colorado State University |
| Paddy Sullyvan | Plant physiological ecology | Colorado State University |
| Valery Terwilliger | Biogeography | University of Kansas |
| Bruce Vaughn | Atmospheric chemistry | INSTAAR, Boulder, Colorado |
| Jeff Welker | Hydroecology | University of Alaska |
| Dave Williams | Hydroecology | University of Wyoming |
| Blair Wolf | Animal ecophysiology/ecology | University of New Mexico |

**Appendix B.** Substances and isotopes in terrestrial, aquatic and atmospheric environments that would serve as early-warning signals of ecological change.

| **Compound or material** | **Isotope(s)** | **Ecological information** |
|---|---|---|
| | | |
| **Terrestrial** | | |
| Bulk leaf material from trees | $\delta^{13}C$, $\delta^{15}N$ | Growing season integration of tree nitrogen sources and water availability |
| Bulk soil organic material | $\delta^{13}C$, $\delta^{15}N$ | Long-term integration of soil N and C cycling, and photosynthetic pathway changes |
| | | |
| **Aquatic - stream discharge** | | |
| $H_2O$ | $\delta^{2}H$, $\delta^{18}O$ | Hydrologic cycle changes related to rainfall sources, recharge and evaporation |
| Dissolved organic material | $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ | Long-term and large-scale integration of terrestrial and aquatic nutrient cycles |
| Particulate organic material | $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ | Long-term and large-scale integration of terrestrial and aquatic nutrient cycles |
| $O_2$/Ar ratio | | Extent of biogeochemical sources/sinks of other gases |
| Ammonium | $\delta^{15}N$ | Long-term and large-scale integration of terrestrial and aquatic nutrient cycles |
| Nitrate | $\delta^{15}N$, $\delta^{18}O$, $\delta^{17}O$ | Pollution sources, role of denitrification, biogeochemical processes |
| DO | $\delta^{18}O$ | Photosynthesis/respiration ratios, biogeochemical processes |
| | | |
| **Atmospheric** | | |
| $CO_2$ | $\delta^{13}C$ , $\delta^{18}O$ | Balance between respiration and photosynthesis, $CO_2$ production sources |
| $H_2O$ vapor | $\delta^{2}H$, $\delta^{18}O$ | Air mass origins, evaporation recycling from land surface |
| $N_2O$ | $\delta^{15}N$ , $\delta^{18}O$ | Microbial activities related to nitrification and denitrification and sources |
| $CH_4$ | $\delta^{13}C$, $\delta^{2}H$ | Pathways of methane production and emission, relative role of $CH_4$ oxidation |
| Particulate organic material | $\delta^{13}C$ | Terrestrial carbon cycle processes and sources of organic material |

**Appendix C.** A list of the reference materials needed to provide working standards for addressing common ecological studies using stable isotope ratio analyses.

| **reference materials needed** | **$\delta^2H$** | **$\delta^{18}O$** | **$\delta^{13}C$** | **$\delta^{15}N$** | **$\delta^{34}S$** |
|---|---|---|---|---|---|
| water - isotopically light end of regional precipitation | x | x | | | |
| water - isotopically heavy end of biological range | x | x | | | |
| water - isotopically similar to SMOW | x | x | | | |
| water - isotopically similar to summer rains of our region | x | x | | | |
| water - isotopically similar to winter rains of our region | x | x | | | |
| organic wood - alpha cellulose - isotopically light | | x | x | | |
| organic wood - alpha cellulose - isotopically heavy | | x | x | | |
| organic wood - cellulose nitrate - isotopically heavy | x | | x | | |
| organic wood - cellulose nitrate - isotopically heavy | x | | x | | |
| organic wood - whole wood, low in [N] | | x | x | x | |
| yeast | | x | x | x | |
| soil - soil organic matter | | | x | x | |
| soil - KCl extract | | | | x | |
| whole leaf - C3 isotopically light | | x | x | x | x |
| whole leaf - C3 isotopically heavy | | x | x | x | x |
| whole leaf - C4 | | x | x | x | |
| lignin - isotopically light | | x | x | x | |
| lignin - isotopically heavy | | x | x | x | |
| keratin - isotopically light | x | x | x | x | x |
| keratin - isotopically heavy | x | x | x | x | x |
| collagen - isotopically light | x | x | x | x | x |
| collagen - isotopically heavy | x | x | x | x | x |
| starch - C3 isotopically light | | x | x | | |
| starch - C3 isotopically heavy | | x | x | | |
| animal protein - isotopically light | x | x | x | x | x |
| animal protein - isotopically heavy | x | x | x | x | x |
| carbon dioxide in air - isotopically light | | x | x | | |
| carbon dioxide in air - isotopically heavy | | x | x | | |
| methane in air - isotopically light | x | | x | | |
| methane in air - isotopically heavy | x | | x | | |
| nitrous oxide | | x | | x | |
| nitrate salt - isotopically light | | x | | x | |
| nitrate salt - isotopically heavy | | x | | x | |
| sulfate salt - isotopically light | | x | | | x |
| sulfate salt - isotopically heavy | | x | | | x |
| carbonate - isotopically light | | x | x | | |
| carbonate - isotopically heavy | | x | x | | |

**Appendix D.** Estimated analytical capacity and instrumentation costs required to develop an early warning monitoring system within one NEON region. We assume that this monitoring will account for 45% of analytical capacity, and research (45%) and education (10%) will account for the remainder of needed capacity. The second table predicts the total needed instrumentation costs for each NEON region (monitoring, research, and education).

| Compound or material to be monitored | Isotopes to be analyzed | Sampling frequency (per year) | Number samples (per interval) | Total number of analyses (per year)* |
|---|---|---|---|---|
| | | | | |
| **Terrestrial** | | | | |
| bulk leaf material from trees | $\delta^{13}C$, $\delta^{15}N$ | 1 | 1000 | 1000 |
| bulk soil organic material | $\delta^{13}C$, $\delta^{15}N$ | 1 | 1000 | 1000 |
| standards (20% additional) | | | | 400 |
| **Subtotal** | | | | **2400** |
| | | | | |
| **Aquatic - stream discharge** | | | | |
| $H_2O$ | | 26 | 30 | 780 |
| dissolved organic material | $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ | 26 | 30 | 2340 |
| particulate organic material | $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ | 26 | 30 | 2340 |
| $O_2$/Ar ratio | | 26 | 30 | 780 |
| ammonium | $\delta^{15}N$ | 26 | 30 | 780 |
| nitrate | $\delta^{15}N$, $\delta^{18}O$, $\delta^{17}O$ | 26 | 30 | 1560 |
| dissolved inorganic carbon | $\delta^{13}C$ | 26 | 30 | 780 |
| dissolved oxygen | $\delta^{18}O$ | 26 | 30 | 780 |
| standards (20% additional) | | | | 1872 |
| **Subtotal** | | | | **11232** |
| | | | | |
| **Atmospheric** | | | | |
| $CO_2$ | $\delta^{13}C$, $\delta^{18}O$ | 26 | 30 | 780 |
| $H_2O$ vapor | $\delta^{2}H$, $\delta^{18}O$ | 26 | 30 | 780 |
| $N_2O$ | $\delta^{15}N$ | 26 | 30 | 780 |
| $CH_4$ | $\delta^{13}C$, $\delta^{2}H$ | 26 | 30 | 1560 |
| particulate organic material | $\delta^{13}C$ | 26 | 30 | 650 |
| standards (20% additional) | | | | 936 |
| **Subtotal** | | | | **4680** |
| | | | | |
| **TOTAL monitoring analyses/yr** | | | | **19248** |

*some compounds are analyzed for multiple isotopes simultaneously, others are not. The total number of analyses accounts for this.

**Calculation for the load on one instrument for analyses and number of instruments needed per region:**

Assume 5 days per week and 48 analysis weeks per year (1 month down time) = **240 analysis days total**
Assume one mass spec will analyze 50 samples per analysis day = **12,000 samples per year total**
Assume monitoring accounts for 45% of all analyses = **5,400 monitoring samples analyzed/yr for each mass spec**
19,248 monitoring samples per year / 5,400 sample capacity per year for one mass spec **≈ 4 mass specs per region**

**References cited**

Brooks, J.R., L Flanagan, N. Buchmann, J.R. Ehleringer (1997) Carbon isotope composition of boreal plants: functional grouping of life forms. *Oecologia* 110:301-311.

Evans, R.D. and J. Belnap (1999) Longterm consequences of disturbance on nitrogen dynamics in an arid grassland ecosystem. *Ecology* 80:150-160.

Evans, R. D. and J.R. Ehleringer (1993) Broken nitrogen cycles in aridlands, evidence from $\delta^{15}N$ of soils. *Oecologia* 94:314-317.

Helfield, J.M. and R.J. Naiman (2001) Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. *Ecology* 82:2403-2409.

Kreuzer-Martin, H.W., M.J. Lott, J. Dorigan, and J.R. Ehleringer (2003) Microbe forensics: oxygen and hydrogen stable isotope ratios in *Bacillus subtilis* cells and spores. *Proceedings of the National Academy of Science* 100:815-819.

Mayer, B. et al. (2000) Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: Isotopic constraints. *Biogeochemistry* 57/58:171–197.

Pataki, D.E., D.R. Bowling, and J.R. Ehleringer (2003) Seasonal cycle of carbon dioxide and its isotopic composition in an urban atmosphere: anthropogenic and biogenic effects. *Journal of Geophysical Research* 108: NO. D23, 4735, doi:10.1029/2003JD003865.

Smith, R.B., E.C. Greiner and B.O. Wolf (2004) Migratory movements of sharp-shinned hawks (*Accipitor striatus*) captured in New Mexico in relation to prevalence, intensity, and biogeography of avian hematozoa. *The Auk* 121:837-846.

Snyder, K. and D.G. Williams (2000) Water sources used by riparian trees varies among stream types on the San Pedro River, Arizona. *Agricultural and Forest Meteorology* 105:227-240.

Wolf, B. O. and C. Martinez del Rio (2000) Use of saguaro fruit by White-winged Doves: isotopic evidence of a tight ecological association. *Oecologia* 124:536-543.

Wolf, B. O. and C. Martinez del Rio (2003) How important are columnar cacti as sources of water and nutrients for desert consumers? A review. *Isotopes in Environmental and Health Studies* 39:53-67.

Wolf, B. O., C. Martinez del Rio and J. Babson (2002) Stable isotopes reveal that saguaro fruit provides different resources to two desert dove species. *Ecology* 83:1286-1293.