

Review Article

Tracing plant–environment interactions from organismal to planetary scales using stable isotopes: a mini review

 **Gavin McNicol¹, Zhongjie Yu², Z. Carter Berry³, Nathan Emery⁴, Fiona M. Soper⁵ and Wendy H. Yang⁶**

¹Department of Earth and Environmental Science, University of Illinois at Chicago, Chicago, IL, U.S.A.; ²Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.; ³Schmid College of Science and Technology, Chapman University, Orange, CA, U.S.A.; ⁴Department of Plant Biology, Michigan State University, East Lansing, MI, U.S.A.; ⁵Department of Biology and Bieler School of Environment, McGill University, Montreal, QC, Canada; ⁶Departments of Plant Biology and Geology, University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.

Correspondence: Gavin McNicol (gmcnicol@uic.edu)

Natural isotope variation forms a mosaic of isotopically distinct pools across the biosphere and flows between pools integrate plant ecology with global biogeochemical cycling. Carbon, nitrogen, and water isotopic ratios (among others) can be measured in plant tissues, at root and foliar interfaces, and in adjacent atmospheric, water, and soil environments. Natural abundance isotopes provide ecological insight to complement and enhance biogeochemical research, such as understanding the physiological conditions during photosynthetic assimilation (e.g. water stress) or the contribution of unusual plant water or nutrient sources (e.g. fog, foliar deposition). While foundational concepts and methods have endured through four decades of research, technological improvements that enable measurement at fine spatiotemporal scales, of multiple isotopes, and of isotopomers, are advancing the field of stable isotope ecology. For example, isotope studies now benefit from the maturation of field-portable infrared spectroscopy, which allows the exploration of plant–environment sensitivity at physiological timescales. Isotope ecology is also benefiting from, and contributing to, new understanding of the plant–soil–atmosphere system, such as improving the representation of soil carbon pools and turnover in land surface models. At larger Earth-system scales, a maturing global coverage of isotope data and new data from site networks offer exciting synthesis opportunities to merge the insights of single-or multi-isotope analysis with ecosystem and remote sensing data in a data-driven modeling framework, to create geospatial isotope products essential for studies of global environmental change.

Introduction to stable isotope ecology concepts and methods

Stable isotopes have been used to study plant ecology for more than four decades, with an early focus on organismal physiology and quantifying associated isotope effects, and how they vary along environmental gradients [1,2]. In the last two decades, isotope analysis has expanded to consider the role of plants in biogeochemical cycling and its sensitivity to anthropogenic perturbation, both at fine resolution and at larger spatial scales (Figure 1).

The ratio of heavy-to-light stable isotope abundance of carbon ($^{13}\text{C}/^{12}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$), oxygen ($^{18}\text{O}/^{16}\text{O}$ and $^{17}\text{O}/^{16}\text{O}$), and hydrogen ($^2\text{H}/^1\text{H}$) varies naturally within different plant tissues, and between plants and the soil, water, and atmospheric pools they interact with [5]. Even very subtle differences in the ratio (at the third or greater decimal place) are detectable with both laboratory-based mass spectrometry and, more recently, field-deployable infrared spectroscopy [6,7]. Measured isotope ratios are reported as apparent positive or negative deviations (e.g. $\delta^{13}\text{C}$) in units of per mil (‰) from

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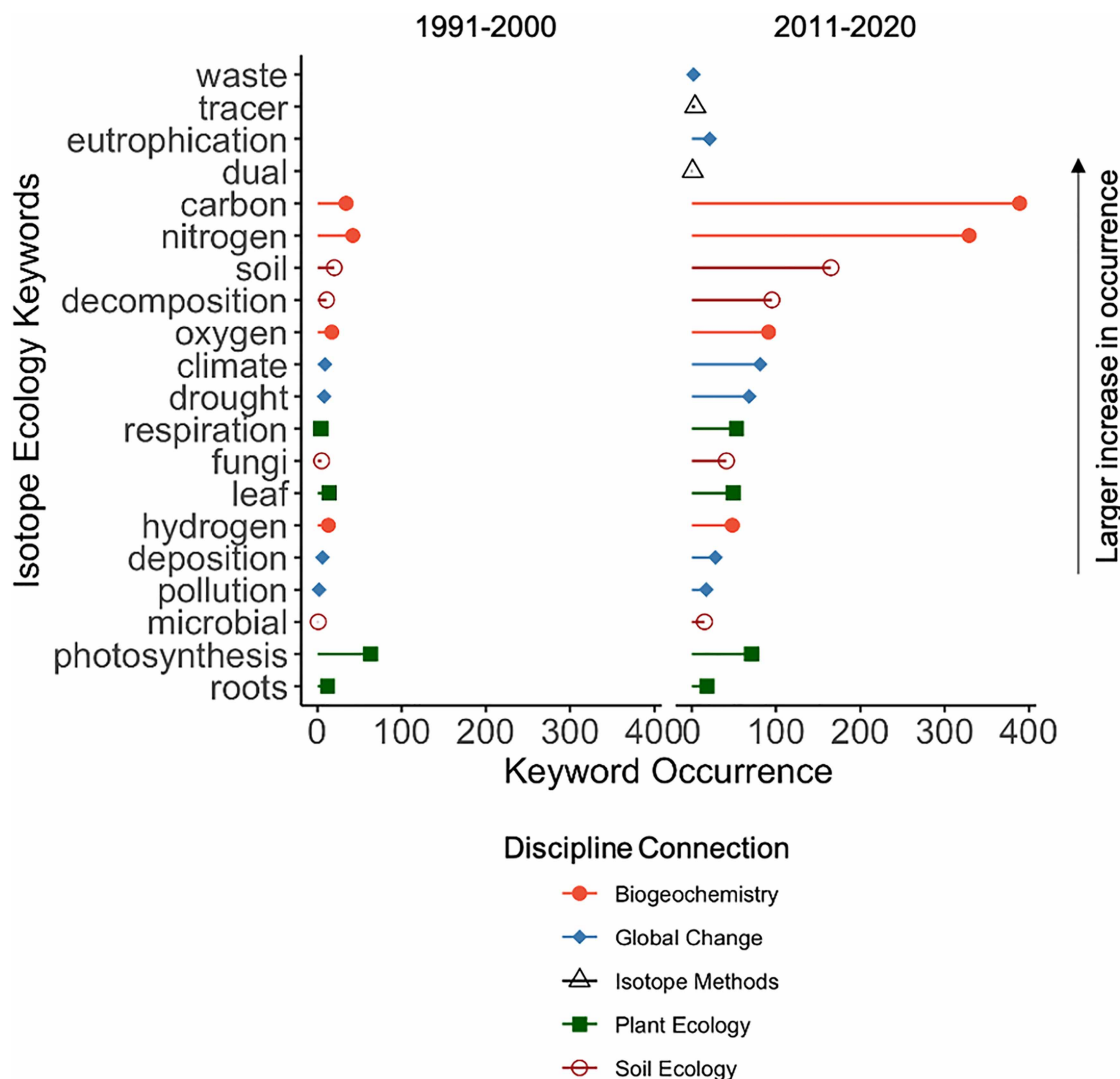


Figure 1. The occurrence of 20 selected keywords in plant ecology studies using stable isotopes in the decades of 1991–2000 and 2011–2020 reflect growing applications in studies of biogeochemical cycles.

Keywords are arranged from the largest increase (top) to smallest increase (bottom) and categorized into disciplines, though several keywords overlap with several disciplines. Connections between plant ecology and biogeochemical cycles of carbon, water, and nitrogen show the largest increases. Soil organisms and processes appear more frequently, including fungi, decomposition, and respiration. Entirely new keywords in the last decade reflect wastewater applications of nitrogen isotopes as well as multi-isotope approaches. The Biblioshiny application [3] in R [4] was first used to search Web of Science for all literature containing the words ‘stable isotope’ and ‘plant’, and ‘carbon’ or ‘nitrogen’ or ‘oxygen’, including ecology, environmental sciences and water, agronomy and forestry disciplines, and excluding zoology, paleontology, and the geosciences. A total of 3783 articles were retrieved and dated back to 1977, with 1247 (roughly one-third) published since 2016. A full set of 8500 keywords was then retrieved from this bibliography using the Web of Science *KeyWords Plus*® algorithm, an augmented method that is preferred over standard keyword or title retrieval. Finally, of the 1374 unique keywords, 20 of interest were identified and their occurrence for the decades of 1991–2000 and 2011–2020 was summed.

known international standards [8]. Environmental patterns in measured stable isotope ratios (isotope values, hereafter) are constrained by kinetic and equilibrium processes that alter the isotopic composition as isotopes move between pools (i.e. have isotope effects; Box 1) [9]. Kinetic isotope effects occur due to differences in unidirectional process rates between the heavy and light isotopes and are often associated with enzymatic reactions

Box 1. Stable isotope terminology used in this paper. Terms denoted by an asterisk were defined by the Commission on Isotopic Abundances and Atomic Weights of the IUPAC [12].

Term	Definition
Isotope (stable)	Two or more forms of the same element that contain equal numbers of protons but different numbers of neutrons in their nuclei, and do not undergo radioactive decay. Isotopes therefore differ in relative atomic mass but not in chemical properties.
Isotopologue*	Molecular species that differ only in isotopic composition (number of isotopic substitutions) and relative molecular mass, e.g. $^{12}\text{C}^{16}\text{O}_2$, $^{13}\text{C}^{16}\text{O}_2$, $^{12}\text{C}^{18}\text{O}^{16}\text{O}$. The term isotopologue is a contraction of 'isotope analogue'.
Isotopomer*	Molecular species having the same number of each isotopic atom (thus, the same relative molecular mass) but differing in their positions, e.g. $^{12}\text{C}^{18}\text{O}^{16}\text{O}$, $^{12}\text{C}^{16}\text{O}^{18}\text{O}$. The term is a contraction of 'isotopic isomer'.
Isotope effect* (or isotopic fractionation)	Alteration of either the equilibrium constant or the rate constant of a reaction (kinetic) if an atom in a reactant molecule is replaced by one of its isotopes.
Isotope balance*	Determination of the amount or isotope-amount ratio of a substance in a mixture by making use of the fact that the sum of the amounts of the isotopes of each constituent of the mixture must equal the total in the system.
Isoflux	Isotopically weighted gas fluxes derived from isotopic balance. Typically measured using micrometeorological techniques (i.e. eddy covariance).
Isoscape	Spatial (geographic) patterns in natural isotope abundances.
Isotope mixing model	An essential mathematical tool for both natural abundance and labeled isotope studies which exploits differences in isotope values and quantities (for n isotopes) to identify and quality relative contributions of two or more (up to $n + 1$) distinct elemental sources [13].

such as photosynthetic assimilation or cellular respiration but also with abiotic processes such as the evaporation of water. In almost all cases, the flux of the lighter isotope is faster (or lighter isotope is favored in reactions) and the flux of the heavier isotope is slower (or heavier isotope is discriminated against) [10]. In contrast, equilibrium isotope effects occur via the passive partitioning of molecules between adjacent pools based on differences in molecular bond strength due, for example, to differences in phases, such as when carbon dioxide (CO_2) dissolves in water [10]. Equilibrium fractionation can also be affected by environmental conditions, particularly temperature. Both kinetic and equilibrium isotope effects are reported in units of ‰ and, in combination, create a complex and dynamic mosaic of isotope values within plants and across the environment. In addition to natural variation arising from kinetic and equilibrium isotope effects, isotopic labeling can be used to artificially enrich a substrate or product pool in the heavy isotope, typically to the percent level. Although labels overwhelm signals from kinetic and equilibrium isotope effects, labeled substrate pools can be traced through plants and into the local environment (pulse-chase experiments), or alternatively, a labeled product pool can be diluted by ongoing reactions at natural isotopic abundances (pool dilution experiments) [11].

Several detailed reviews document how stable isotopes are useful integrators and tracers in plant ecology [9,14–16]. In this mini review, we highlight a select few well-established and emerging isotope applications, with a focus on connecting plants to the planetary, and increasingly human-altered, biogeochemical cycles of C, N, and water (Figure 2).

Carbon

Plant photosynthesis is the largest flux in the global C cycle ($\sim 120 \text{ Pg C y}^{-1}$) and constrains human food, fiber, and fuel production via the productivity of the terrestrial biosphere [17]. Plant biomass $\delta^{13}\text{C}$ reflects the biological regulation of, and environmental conditions for, photosynthesis, providing records of environmental change in the distant and recent past, which may also serve as useful analogues for current global change [9,15]. Plant photosynthetic pathway (C3 vs. C4 vs. CAM) (Box 2), genetics, lifecycle, and morphology, as well as meteorology, soil conditions, and ecological interactions can all alter plant $\delta^{13}\text{C}$, with these environmental and physiological signals being gradually integrated into plant biomass over time [9,18]. As in the study of leaf-level photosynthesis, studies of $\delta^{13}\text{C}$ at larger scales can help inform a more detailed understanding of the constraints on photosynthesis and C cycle responses to future global change.

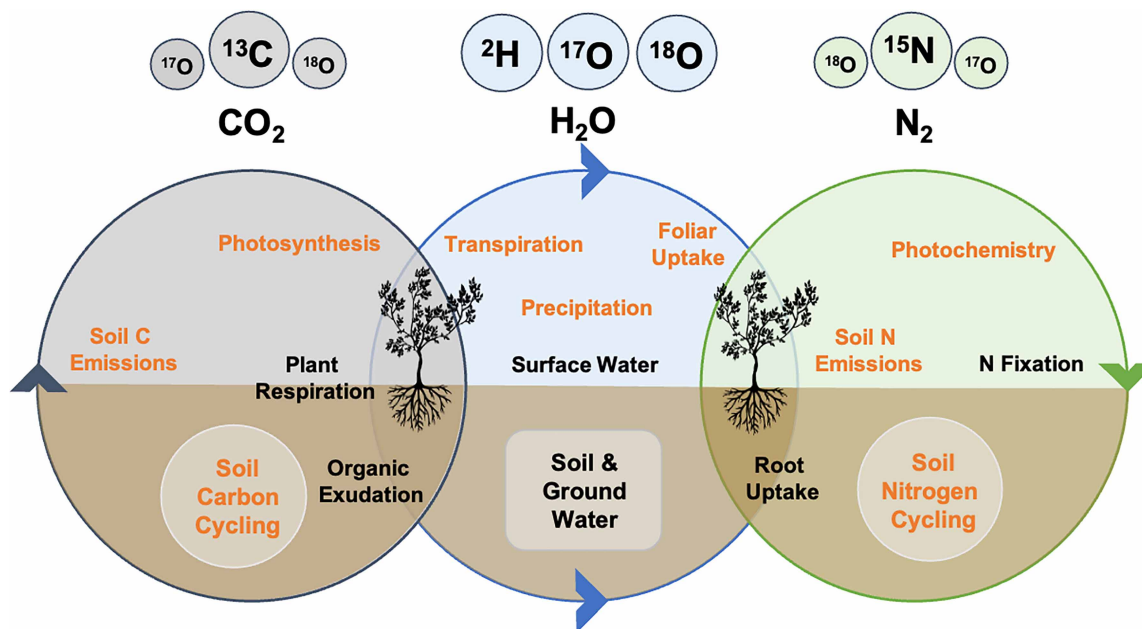


Figure 2. Stable isotopes act as integrators and tracers of organismal to planetary scale biogeochemical cycles of carbon (C), water (H₂O), and nitrogen (N).

The most commonly measured (top, large circles) and less frequently measured (top, small circles) heavy isotopes, and the molecular forms dominant in the atmosphere (top, text), are cycled through terrestrial ecosystems; entering via active assimilation, including via foliar uptake or fixation in root nodules, as well as passive deposition processes, passing through surface and belowground transport pathways, and microbial transformations, and are re-emitted as gases from direct or plant-mediated surface exchanges. Note that, although we only show the heavy isotopes, isotope values are always reported relative to the abundance of more common, lighter isotope (e.g. carbon-12) which is measured simultaneously. Several processes cause isotope effects due to isotopic fractionation (orange) (e.g. from atmospheric CO₂ to plant biomass, and from soil nitrate to nitrous oxide emissions). Other processes (black), often passive, are associated with smaller, negligible, or poorly defined intrinsic isotope effects.

In C₃ plants, which account for ~75% of global photosynthesis [19], variation in the isotopic fractionation between atmospheric $\delta^{13}\text{C}$ ($\sim -8\text{‰}$) and plant biomass $\delta^{13}\text{C}$ (-28‰ to -33‰) is regulated by the balance of diffusive CO₂ supply from the atmosphere to intracellular spaces, and photosynthetic CO₂ demand [20]. This co-limitation model for C₃ photosynthesis, combined with plants' ability to conserve a stable metabolic setpoint, creates a complex sensitivity of plant $\delta^{13}\text{C}$ to environmental conditions, which is most readily interpretable at the extremes [14]. For example, diffusion limits CO₂ supply when plants are water-stressed due to stomatal closure, however, stomata also limit diffusion during high irradiance (such as under strong, direct sunlight). In both these cases of stomatal CO₂ supply limitation, C₃ photosynthesis isotope effects are smaller and plant biomass becomes more enriched in ^{13}C . In agreement with this leaf-level understanding, the first global data-driven model of C₃ isotope effects predicted larger isotope effects in the humid tropics and smaller isotope effects in arid and mountainous regions [18]. However, the model also highlighted the complexity of environmental regulation of C₃ isotope effects at global scales, with precipitation, atmospheric pressure, potential evapotranspiration, soil pH, and leaf N all identified as key model covariates (Figure 3).

C₃ plant isotopes continue to be utilized for the reconstruction of plant function and environmental conditions over time, with tree rings providing a seasonal to annual chronology. Tree ring $\delta^{13}\text{C}$ analysis has been used to interpret interannual changes in forest productivity during periodic drought [21,22] and to understand how other environmental gradients, such as soil texture, can modulate or mitigate drought-related water stress [21,22]. In free air carbon dioxide enrichment (FACE) experiments, tree ring $\delta^{13}\text{C}$ analysis has been used to demonstrate increases in ecosystem-scale water use efficiency under elevated CO₂ [23]. More recently, regional scale tree ring $\delta^{13}\text{C}$ (and $\delta^{18}\text{O}$) has been shown to correlate with forest productivity, estimated by satellite

Box 2. Metabolic, environmental, and isotopic characteristics of the three dominant pathways of plant photosynthesis [15].

Pathway	C3	C4	CAM
Metabolism	Calvin cycle: Production of carbohydrates via 3-carbon intermediates from CO ₂ reduction by the enzyme ribulose biphosphate carboxylase/ oxygenase (Rubisco)	4-carbon-dicarboxylic acid pathway: 'Pumping' of CO ₂ to maintain high pressures in vascular bundle sheath cells where fixation occurs, reducing photorespiration	Crassulacean acid metabolism: Nighttime organic acid formation followed by daylight deacidification and refixation of CO ₂ by Rubisco
Ecology and environment	Globally dominant photosynthetic pathway, especially for trees and shrubs.	Advantage over C3 with low CO ₂ or high irradiance, and higher water- and nitrogen-use efficiency than C3. Tropical and salt grasses; food and biofuel crops.	Succulents under high water stress. ~6% of plants globally, which are typically also capable of C3.
Isotope effects	Fixation by Rubisco: -29‰ Stomatal diffusion (in and out): -4.4‰ Net effect: -20‰ Environmental controls (e.g. temperature, moisture, and irradiance) modify effects but are constrained by plant physiology	Bicarbonate fixation: -5.7‰ Bundle-sheath diffusion: 1.8‰ Net effect: -5‰ Varies with cell leakiness and across a much smaller range than C3 or CAM (-11 to -14‰)	Approx. 1.8‰ per 10% contribution of nighttime uptake (-0.7‰ to -20‰) CAM plant biomass can vary by >20‰ depending on the proportion of day (C3) vs. night-time CO ₂ fixation

remote sensing, which can enable tree ring-based reconstructions of biome productivity over centuries to millennia [24]. Important adjustments have been made to the co-limitation model of C3 photosynthesis and its isotope effects [25,26] to account for metabolic processes such as mesophyll conductance, intracellular respiration, and translocation of compounds into different plant tissues [27]. Although seemingly small, changes in mesophyll conductance and photorespiration isotope effects since the 1970s, caused by rising atmospheric CO₂ and plant water use efficiency (~0.014‰ ppm⁻¹), have been enough to alter global atmospheric δ¹³C [28].

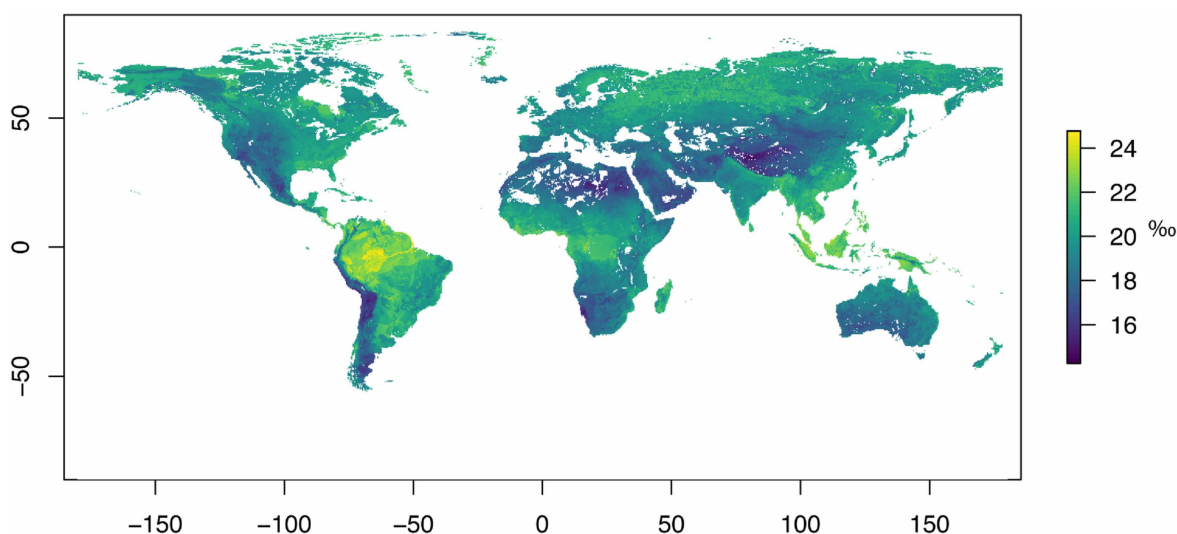


Figure 3. Global patterns in the absolute magnitude of C3 plant photosynthetic carbon isotope effects (δ) in units of per mil (‰), modeled from 3645 C3 leaf observations of δ¹³C. (Reproduced from Cornwell et al. [18] with permission from John Wiley & Sons, Ltd.).

Tracing ^{13}C in the Plant-Soil-Atmosphere System

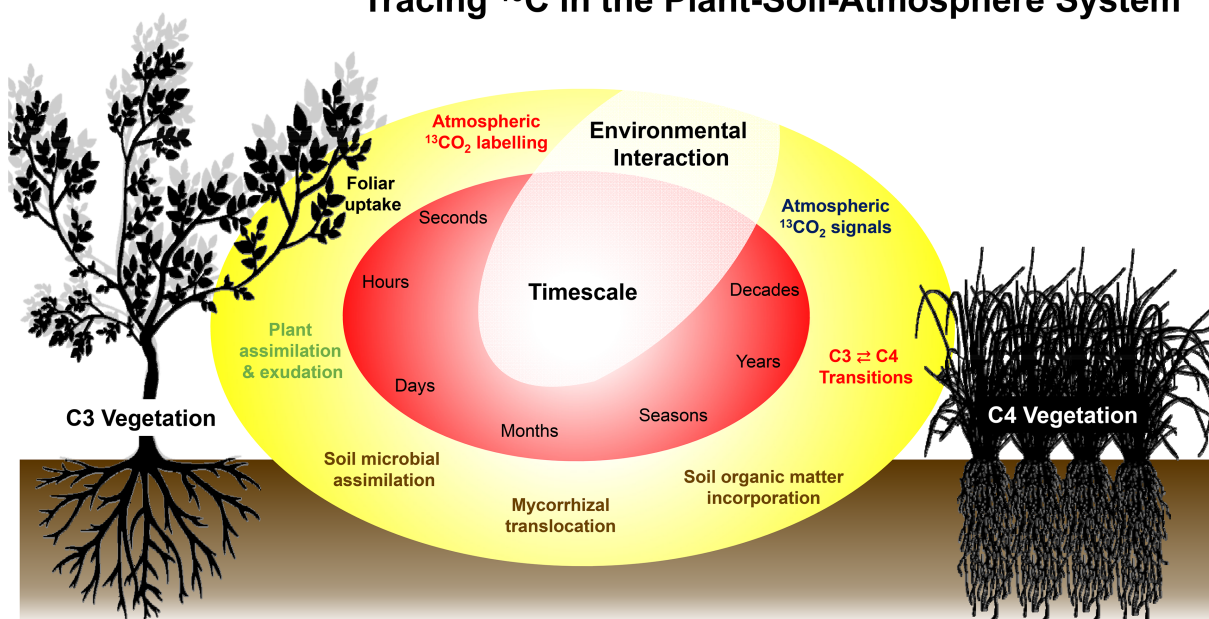


Figure 4. Natural and experimental ^{13}C labeling of plant biomass enables the tracing of C through the plant–soil–atmosphere system.

Isotopic tracing requires a perturbation in the photosynthetically fixed isotope signal with either a synthetic (experimental) label or a major natural shift in photosynthesis, such as a C3–C4 transition (red environmental interactions). Plants can rapidly assimilate and re-respire a portion of this C on timescales of less than a day, while another portion is translocated and exuded into the rhizosphere over the course of several hours to several days. Microbial biomass turnover and active transport by mycorrhizal fungal move C belowground on monthly timescales, while microbial necromass accumulation and decomposition processes over seasons or multiple years lead to the incorporation of C into soil organic matter pools associated with different stabilization mechanisms, and thus of different age and persistence. Finally, over decades or more, the accumulation of belowground C and large-scale shifts in the isotope effects of photosynthesis (e.g. decadal and regional scale shifts from C3 to C4) can lead to changes in the $\delta^{13}\text{CO}_2$ value of even the well-mixed global atmosphere.

Stable C isotopes can also be used to trace plant–soil–atmosphere C cycling (Figure 4). Natural ^{13}C tracer experiments arise from C3 (biomass $\delta^{13}\text{C}$ ca. -28‰) to C4 (biomass $\delta^{13}\text{C}$ ca. -13‰) shifts in the photosynthetic pathway of vegetation [29] which can be associated with succession, invasion, climate change, or land use change [9]. Isotope mass balance is then used to estimate subsequent effects on soil C content, such as C sequestration or loss, which are otherwise difficult to measure directly [27,30]. C3–C4 transitions and their soil C effects have been studied extensively, with recent applications in bioenergy crops to study lifecycle C budgets (e.g. *Panicum virgatum*; [31,32]). New opportunities also exist in data synthesis, demonstrated by a recent global meta-analysis of 47 C3–C4 field studies [33] which used isotope data to model global soil C age-depth distributions and found that recent C inputs account for a small fraction of total soil C globally, raising important questions about soil C residence times. At finer scales, ^{13}C pulse-chase labeling experiments are being used in combination with increasingly sophisticated compound-specific analyses and metabolomics to quantify plant C allocation [34], to trace plant-derived C into actively metabolizing microbial communities (such as mycorrhizal fungi ‘highways’ that share C among trees even of different species [35–37]), and to distinguish different pathways of soil organic matter formation and persistence [38]. Many of these new plant–soil system applications are still rapidly evolving and their target questions remain largely unresolved.

Elevated CO_2 concentrations and increasing temperatures are changing terrestrial ecosystem photosynthesis and respiration, with potential for both positive and negative C cycle and climate feedbacks [39,40]. Isotope analysis can be applied to the study of net ecosystem CO_2 exchanges by pairing high-frequency measurements of canopy $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ mixing ratios with micrometeorological methods used to estimate fluxes of both CO_2 isotopologues (isofluxes; [41]). Isofluxes of CO_2 can also be used to partition net ecosystem exchange measurements into their component photosynthesis and respiration fluxes, providing independent estimates and new insights into cryptic processes such as light-inhibition of respiration [42–44]. Although isoflux-based

partitioning causes little or no ecosystem disturbance, the $\delta^{13}\text{C}$ of net ecosystem exchange still requires careful interpretation due to its mix of different plant and soil microbial signals [45]. Exciting opportunities are also being created by the growth of networked observations of isofluxes. Profiles of atmospheric $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and $\delta^2\text{H}$ measurements, and soil, root, foliar, and litter $\delta^{13}\text{C}$, are now being collected at 47 sites representative of continental United States ecoregions as part of a National Ecological Observatory Network (NEON), with many records already extending >3 years [46].

Water

Vegetation not only moves up to 95% of surface water to the atmosphere but also stores and regulates large pools of water that influence energy, C, and nutrient fluxes [47,48]. Tracing the movement and storage of water in plants and into the environment is made possible with the heavy stable isotopes of oxygen ($\delta^{18}\text{O}$, $\delta^{17}\text{O}$) and hydrogen ($\delta^2\text{H}$) [49]. Water isotopes are useful because their prevalence varies greatly across environmental pools (e.g. bodies of water, streams, woody tissue, leaves, atmosphere, and clouds), allowing us to trace water from one pool to another [50]. In particular, water isotopes allow for better understanding of water movement at the leaf-atmosphere boundary, within plant tissues, and through soil.

Plants exchange water with the atmosphere primarily through leaves and photosynthetic tissues. Water isotopes provide insights into plant function during drought, complementary to those discussed for ^{13}C . Studies combining ^{18}O and ^{13}C measurements reinforce the idea that stomatal closure leads to increased water use efficiency (enriched $\delta^{18}\text{O}$) while also limiting C assimilation (enriched $\delta^{13}\text{C}$) [51,52], with important implications for predicting drought-related plant mortality and parameterizing climate models. Water isotopes also continue to provide new insights about the complex dynamics of water exchange at the leaf-atmosphere interface, which is multidirectional (e.g. foliar water uptake; [53–55]) and varies along spatial and temporal scales [53–55]. For example, a long-held assumption is that leaf water flows exclusively from saturated (within-leaf) to unsaturated (atmospheric) conditions. However, water isotopes have been used to show that the vapor pressure inside leaves is not always saturated [56], complicating models of leaf-atmosphere dynamics. Another avenue of inquiry finds that the flow of hydrogen to cellulose, non-structural carbohydrates, and sugars can produce consistent differences in isotope values, providing new opportunities to study leaf biochemical processes [57,58]. Overall, a finer resolution understanding of leaf water dynamics is valuable for modeling and predicting plant water use, atmospheric exchange, and responses to climate change. Future studies of fine-scale spatial variation in leaf water isotopes must consider the Péclet effect, whereby the inward flow of unenriched water from xylem is counteracted by backward diffusion of enriched water from evaporation [59]. Barbour et al. [60] suggest the use of a spatially explicit model that accommodates water in the liquid and gas phase as well as internal temperature gradients.

As water moves through plants, the possibility of mixing and exchange among plant tissues cannot be overlooked (Figure 5). Use of stable isotopic labeling of water has shown that water flow throughout the plant is not homogenous [61]. Differences across species and environmental contexts have been observed in lateral mixing and the connectivity of xylem in certain locations with different root sections [62,63]. What is becoming clear is that xylem, phloem, cambium, bark, and other supporting cells are likely to have distinct isotope values suggesting significant mixing of water sources across space and time [64–66]. These flows are multidirectional [67] and occur daily [68]. Increasing the spatial and temporal resolution of stable isotope measurements will allow us to resolve these dynamic multidirectional flows within plants.

Perhaps the most common application of stable isotopes of water is sourcing where plants are obtaining their water. Plant strategies of water use, identified with water isotopes, range from almost exclusive reliance on upper soil layer sources [69] to more than half deriving from deeper groundwater sources [70]. Water sources also appear to vary with environmental conditions [71], raising new questions about the plasticity of this response to climate change. For example, as drought threatens ecosystem resilience via reduced or altered water flows, plants can seek out deeper water sources, and this plasticity mitigates plant water impacts over multiple drought years [72]. In plant ecology studies, water samples are obtained from precipitation, multiple soil depths, groundwater, and potentially even fog or dew [9,73]. Using $\delta^{18}\text{O}$ and $\delta^2\text{H}$ to determine the origins of water within a plant relies on the assumption that the isotope value is conserved across pools (i.e. does not fractionate). While some studies have found this to be true [74], an increase in spatial sampling precision has revealed multiple examples of isotopic fractionation (Figure 5), complicating conclusions. Studies have found isotopic fractionation within soil [75], at the soil–root interface [76,77], in the presence of arbuscular mycorrhizal fungi [78], during foliar water uptake [79], and when xylem and bulk tissue water interact [65,66]. Soil

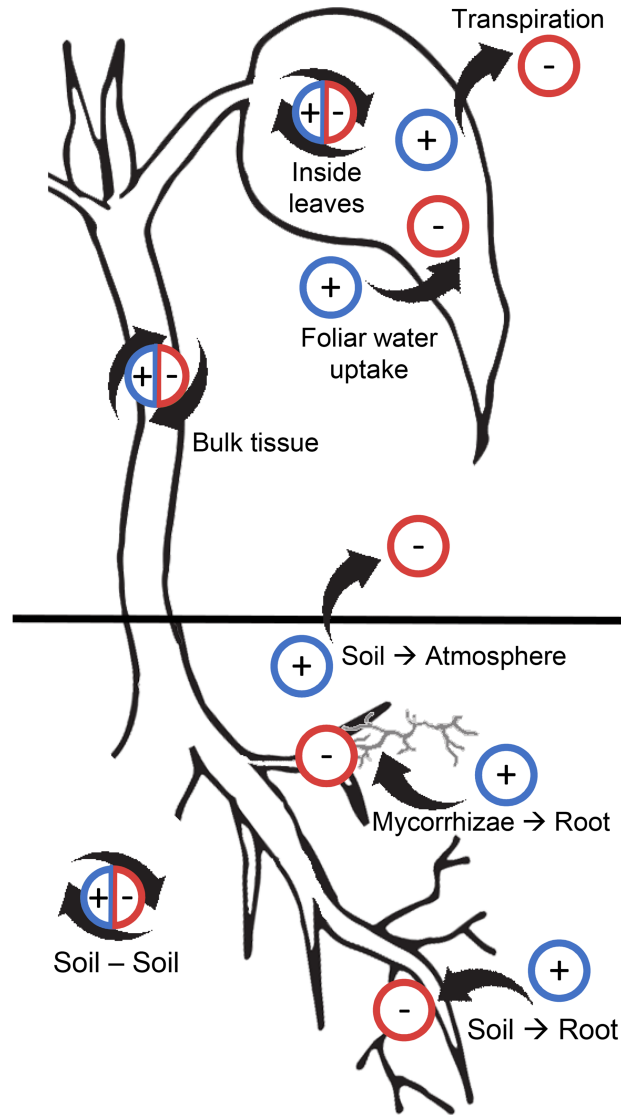


Figure 5. Water movement across substrates, tissues, and the atmosphere can cause isotopic fractionation resulting in isotopic enrichment (plus symbol) or depletion (minus symbol) in the heavy isotopes of H and O.

Fractionation occurs belowground within the soil [75], from soil to the atmosphere [9], and between soil and roots [77]. Internally, isotopic fractionation occurs across bulk tissues [65] and within leaves [59]. Between the leaf surface and the atmosphere, transpiration can cause enrichment [9] and foliar water uptake can lead to depletion [79] in leaf water. Mycorrhizal fungi can also lead to isotopic depletion in roots [78]. These dynamic interactions provide new avenues for research and challenges for studying the movement of water isotopes in plants.

water fractionation tends to be of more concern in shallow, dry soils and can alter water pool isotope values and plant water stable isotope mixtures [80]. Isotopic fractionation, while generally not thought to occur at the soil–root boundary [9], may yet have an influence on plant water isotopic values.

Water isotopes have become a powerful tool for understanding the movement of water through the plant–soil–atmosphere system and how this ecosystem will respond to climate change. New frontiers include increased spatiotemporal sample resolution, allowing investigators to map water movement through soils, plants, and the atmosphere in three dimensions and capturing instantaneous dynamics. New methods that allow for *in situ* quantification of water isotopes using gas-permeable membranes with laser spectrometers [81,82] could lead to constant monitoring of plant water uptake from understudied sources, such as leaf and

bark absorption, without destructive sampling. Spatially, we are starting to isolate water pools of specific cell types in plants [65], particular molecules (e.g. cellulose vs. carbohydrates), and even from water vapor on leaf surfaces [83] and in the soil [84]. Matching these plant-related advances with increased spatial fidelity in adjacent soil environments is a promising research direction, particularly in zones surrounding fine roots, mycorrhizal associations, and in partitioning bound and unbound water [78,85,86].

Nitrogen

Nitrogen (N) is a critical limiting element for plant growth and production. Humans have roughly doubled annual fixation of reactive N since the 19th century [87] and the resulting enrichment of reactive N can cause N fertilization effects, stoichiometric imbalances, decreases in biodiversity and facilitation of invasive plant species, soil acidification, and production of climatically important N trace gases (e.g. nitrous oxide (N_2O)) [88]. Ecosystem responses to increased N availability therefore strongly depend on how N is transported, transformed, and recycled in the plant–soil system.

Nitrogen stable isotopes can be used to trace the N cycle, from uptake in individual roots over the course of days to integrating decadal trends towards increased N availability at continental scales (Figure 6a). For example, ^{15}N enrichment of arbuscular mycorrhizal fungal (AMF) hyphae and associated roots following decomposition of ^{15}N -enriched root litter demonstrated the role of AMF in supplying litter-derived N to plants, with nanometer-scale secondary ion mass spectrometry (NanoSIMS) providing detailed

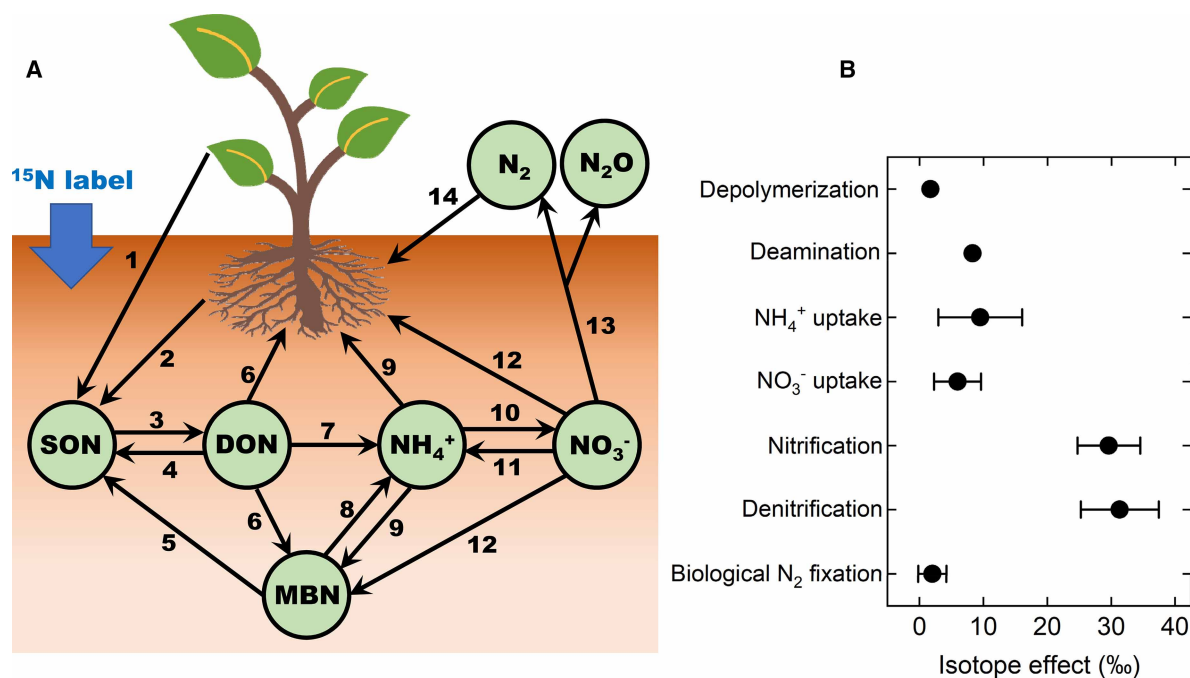


Figure 6. Simplified schematic representation of the soil–plant N cycle and associated N isotope effects.

(a) Major N pools are shown in circles and major fluxes by arrows: (1) aboveground litter and residue input; (2) root litter and exudate input; (3) dissolution and depolymerization of high molecular mass soil organic N (SON); (4) adsorption of dissolved organic N (DON) to mineral soil matrix; (5) microbial death and subsequent stabilization of microbial biomass nitrogen (MBN) into SON; (6) DON uptake; (7) extracellular ammonification; (8) intracellular deamination; (9) NH_4^+ uptake; (10) nitrification; (11) dissimilatory NO_3^- reduction to NH_4^+ ; (12) NO_3^- uptake; (13) denitrification; (14) biological N_2 fixation. Addition of ^{15}N -labeled compounds to a plant or soil N pool and tracing through other reservoirs allows for a quantitative inquiry into the sources and fates of N in the soil–plant system. (b) To understand the distribution of natural abundance N isotopes among different plant and soil pools, several important soil N transformation processes have been studied for their N isotope effects (adapted from [16,98,99]). The distinct fractionating patterns revealed for these processes underpin the integrated nature of natural abundance N isotopes in tracing N dynamics in the plant–soil system.

spatial maps of ^{15}N enrichment within fungal and plant tissues [89]. In addition to SIMS, other mass spectrometry imaging techniques are now enabling spatially resolved interrogation of how plants interact with the biotic and abiotic environment, such as matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) used to trace plant uptake of ^{15}N -labeled nitrate into identifiable metabolites within plant tissues [90]. In agricultural systems, many studies have utilized ^{15}N -labeling to determine the proportion of fertilizer recovered in crops (i.e. fertilizer-use efficiency). A synthetic analysis of these studies unveiled a surprisingly low efficiency (e.g. <50%) in intensively managed cereal crop systems [91,92], highlighting a strong modulation of N transformations and retention by soil microbial communities. To avoid possible stimulation of process rates by the addition of ^{15}N label to the substrate pool, the isotope pool dilution approach utilizes ^{15}N label addition to the product pool to estimate gross production rates of the product [11]. ^{15}N labeling of various soil N pools has been used to measure gross rates of N depolymerization, ammonification, and nitrification [93,94] (Figure 6a). More recently, this approach has been applied to trace gases, notably allowing for field-based measurements of gross N_2O production and consumption rates, which are otherwise difficult to quantify [95–97].

Instead of adding ^{15}N labels to the plant–soil system, natural abundance $\delta^{15}\text{N}$ in various plant and soil reservoirs can be used as a non-invasive and integrated measure of N cycling intensity and ‘openness’ (the ratio of loss to internal recycling). The unique power of natural abundance N isotope measurements stems from the distinct isotopic fractionation during individual N transformation processes mediating the N cycle [100] (Figure 6b). For example, due to a small N isotope effect for the biological fixation of atmospheric N_2 (BNF), N generated through BNF tends to be less enriched than soil-sourced N, allowing for use as a qualitative [101] and under certain specific conditions, a quantitative tracer of BNF (predominantly in agricultural systems) [102]. In contrast, nitrification and denitrification, which often prevail in N-saturated systems, are associated with large kinetic N isotope effects (e.g. >20‰; Figure 6b). As such, elevated $\delta^{15}\text{N}$ values of terrestrial soil and water can be used as an emergent constraint to infer intensity of external N loss (e.g. NO_3^- leaching and N_2O emissions) as well as overall N availability at the ecosystem scale [103]. At the global scale, large datasets of leaf and soil $\delta^{15}\text{N}$ are increasingly available and have been used to make inferences about broader patterns of plant N availability. Using a database of >40 000 samples over four decades, Craine et al. [104] showed that average foliar percent N and $\delta^{15}\text{N}$ have simultaneously decreased; evidence of an overall increase in plant C fixation (due to, for example, increased atmospheric CO_2 and longer growing seasons) that has shifted the balance between plant N demand and supply.

While labeled ^{15}N tracers and natural abundance N isotopes have been extensively used to study the N cycle, they individually suffer from conceptual and technical shortcomings that limit their utility to fully elucidate N cycling complexity (Figure 7). We suggest that combined use of these two isotope techniques (i.e. hybrid isotope tracing) can provide a bridge between mechanistic details and spatiotemporal variability of N cycle dynamics, thereby leading to a more robust understanding of N-plant–environment interactions. Furthermore, recent methodological advances have brought new isotope tracers for probing the plant–soil N cycle that share promising features with both approaches. For example, with two isotopes of N and three of O (i.e. ^{16}O , ^{17}O , and ^{18}O) in a NO_3^- molecule, coupled N and O isotope analysis of NO_3^- is a powerful tool to unveil NO_3^- sources and biogeochemical transformations in the plant–soil system [105]. Historically, $\delta^{17}\text{O}$ of NO_3^- was of minor interest, primarily because the two minor O isotopes in NO_3^- (e.g. ^{17}O and ^{18}O) are altered by O isotopic fractionation processes in a predictable manner. However, in the late 1990s, studies of atmospheric photochemical reactions revealed an anomalously high abundance of ^{17}O in atmospheric NO_3^- (expressed as $\Delta^{17}\text{O}$), as large as 35‰ [106]. Because the production of nonzero $\Delta^{17}\text{O}$ values is strictly a photochemical effect, plant- and microbially processed NO_3^- has $\Delta^{17}\text{O}$ of 0 [107]. Therefore, $\Delta^{17}\text{O}$ is a conservative tracer of atmospheric NO_3^- deposition [108] and can be applied to unambiguously identify the uptake of deposited N into soils and plant tissues (via both root and direct foliar uptake) [109] and to quantify gross nitrification [110,111] and denitrification rates [112] at the watershed scale. Limitations of these applications, however, include the fact that rapid assimilation of atmospheric NO_3^- by microbes and plants can erase the $\Delta^{17}\text{O}$ signal, complicating the tracing of $\Delta^{17}\text{O}$ in the soil–plant system, and that $\Delta^{17}\text{O}$ analysis is limited to O-containing compounds and excludes ammonium (NH_4^+), for example [113].

Isotopomers of N_2O , which vary in the intramolecular position of ^{15}N , are also a potential tool to partition the sources of N_2O among soil nitrifiers and denitrifiers [114,115]. This approach is based on observations from pure culture studies that denitrifiers [116] produce N_2O with lower ^{15}N site preference (S_p , $\delta^{15}\text{N}^\alpha - \delta^{15}\text{N}^\beta$) compared with nitrifiers [117] and fungal denitrifiers [118]. Isotope mapping of S_p against bulk $\delta^{15}\text{N}$ [119],

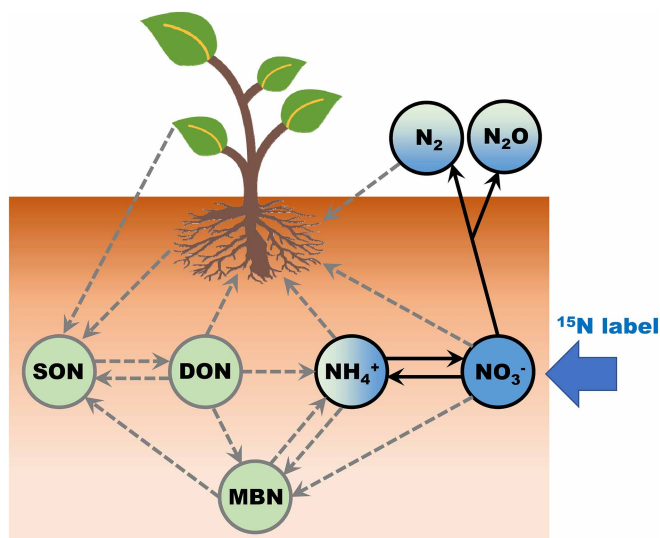


Figure 7. Application of isotope techniques to illuminate the soil–plant N cycle.

Tracing of the plant–soil N cycle is extremely complex due to microsite N variations, the multistep nature of N transformation processes, presence of diverse plant and microbial communities, as well as constant exchange and competition of N between plants and soil microbes. ^{15}N tracer-based methods are powerful tools for tracing short-term sources and fates of N (blue colors) and can help quantify absolute rates of N transformations (black arrows). However, they are often process-centric and may not provide insights into soil-microbe-plant interactions of reactive N over large temporal and spatial scales. In contrast, although natural abundance $\delta^{15}\text{N}$ analyses of soil N pools (green colors) are inherently sensitive to uncertainties in and expression levels of relevant N isotope effects [16,101], including those that are poorly-quantified (e.g. dissimilatory nitrate reduction to ammonia), they can provide an integrated assessment on system N dynamics, including the relative importance of different N cycling pathways in the soil–plant system (gray arrows). Together, these two isotope techniques (i.e. hybrid isotope tracing) as well as novel isotope tracers (e.g. $\Delta^{17}\text{O}$) have the potential to integrate process-level details of N cycling within the broader context of N-plant–environment interactions across spatial and temporal scales.

bulk $\delta^{18}\text{O}$ [120], or most recently, both [121] have been used to partition N_2O sources and estimate N_2O reduction rates. However, uncertainty in the N and O isotopic signatures of the substrates for the source processes [122], variation in isotope effects associated with those processes [123], and variation in the S_p of the atmospheric N_2O endmember [124] render isotope mapping estimates more qualitative than quantitative [125]. Laser spectrometers, if used properly, provide promise for broader use of this approach to improve our understanding of controls on S_p variation across spatiotemporal scales, thereby allowing us to better constrain these estimates [122,126,127,122].

Conclusions and outlook

Plant stable isotopes have been used extensively in reconstructions of past ecological changes, such as C3–C4 vegetation transitions, and provide unique information to infer biophysical environmental causes, such as changes in water availability. Isotopes also record plants' metabolic and physiological plasticity in response to changing conditions, such as maintenance of a metabolic set-point or changes in water or nutrients sources. This makes isotopes additionally useful in understanding mechanisms of contemporary global change, as well as potentially helping to constrain plant functional responses in simulations of future conditions. Innovation in stable isotope ecology is likely to continue via merging multi-isotope analysis or isotopomer analysis with increasingly highly resolved measurements of plant tissue and cellular components, the gas, liquid and solid phases of the soil matrix, and high-frequency micrometeorological data. Merging these parallel data streams will allow investigators to study plant ecology across organismal to planetary scales and will provide a more detailed understanding of plants' role in global biogeochemical cycles.

Summary

- Stable isotope measurements provide unique insights into plant ecology and the role of plants in the global biogeochemical cycles of carbon, nitrogen, and water.
- Natural abundance isotope data and mixing models can record environmental conditions experienced during photosynthesis and can reveal novel plant sources of water and nutrients.
- Isotopic labeling enables the tracing of elemental fluxes in the plant–soil–atmosphere system and quantification of gross process rates.
- Increasing the spatiotemporal resolution of isotope measurements will help future investigators study plant–environment exchanges at physiological timescales.
- Evaluation of regional-to-global isotope patterns using data-driven models and global meta-analyses is supporting new isotope ecology research at Earth-system scales.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

G.M. wrote the introduction and carbon isotope section, Z.C.B. and N.E. co-wrote the water isotope section, and Z.Y., F.M.S., and W.H.Y. co-wrote the nitrogen isotope section. All authors contributed to final edits.

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Abbreviations

AMF, arbuscular mycorrhizal fungal; BNF, biological fixation of atmospheric N₂; CO₂, carbon dioxide.

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