Application of stable isotopes in ecosystem research

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The ecological applications of natural abundance of stable isotopes benefited the understanding of the ecosystem processes. Natural isotopic effects ensuing from difference in physico-chemical properties, fractionation and discrimination of elements such as carbon, hydrogen, oxygen, nitrogen, sulphur, strontium and calcium, have been used to reinforce understanding the various aspects of ecosystem analysis and to revisit some old, baffling processes with new perspectives. Stable isotopes have provided insight into not only the relationship between biodiversity and ecosystem structure and functioning, but also strengthened our knowledge about the possible mechanisms of ecosystem functioning in various ecosystems and biomes. This review elucidates the role of natural abundance of stable isotopes in benefiting ecosystem analysis.

Keywords: Ecosystem analysis, plant–environment interaction, resource facilitation and partitioning, stable isotopes.

STUDIES on the application of stable isotopes (SI) in the ecosystem commenced with the elucidation of some of the most important eco-physiological processes[1,2]. The use of SI in ecological reconstructions has increased exponentially during the last sesquidecade. This growth is attributed to the (a) diversity of isotope signatures available[3] (Table 1), (b) insights into the behaviour of SI, particularly fractionation-led distinct isotopic signatures arising from their routing among fluxes[1,2,4–10], and (c) advances in the isotope mass spectrometry[3].

In the initial years, eco-physiologists used them to appraise photosynthetic mode, water-use and nitrogen-fixing efficiencies[2,7,11]. The same empirical understanding was later used to underpin the details about (semi-)natural ecosystems and processes related to them. It is interesting to note that several advances in ecosystem analysis via SI have given diametrically different answers to several extant theories[12–14]. Their use reinforced understanding about the pattern of diversity, including functional diversity, by underscoring resource partitioning/complementarity among competing/coexisting species[15–17]. In this way SI helped to shore-up the existing understanding about forests[14,18–20], savannas[21–23], grasslands and shrublands[24,25], and ecotones[26–29] for biodiversity conservation underpinning. In addition, SI are also used in the understanding of environmental pollution[30–32] and global change[33–34]. The imprints of SI are now substantively seen providing understanding of plant–plant interactions[35–37], plant–faunal interactions[38–44], plant–soil interface[8,36] and plant–soil–environment interactions[14,18,45–48].

The objective of this article is to synthesize the findings from natural abundances of SI to understand the ecological processes that define configuration and patterns of species and biodiversity conservation strategies through preservation of functional diversity.

Plant communities: understanding structure, function and dynamics

A fundamental goal in ecosystem analysis is to reveal how species competing for limited and identical resources coexist, thus avoiding (resource) competitive exclusion. Ecosystem and community ecologists have underpinned such research with sound habitat–niche pattern theories, stating that coexisting species are capable of partitioning niches and using them complementarily. Underlying assumptions of niche segregation rest on the habitat specialization enjoyed by each species (or functionally similar species), such that different species (or functional groups) are well suited to characteristic habitats or linked to a habitat-defining variable to different degrees for its maintenance. Some of the seminal works to understand the niche partitioning, niche complementarity, resource facilitation and plant–plant interactions among co-occurring plant species under resource-limited conditions have used SI[13,49,50].

Competitive interactions among co-occurring species: resource partitioning and resource facilitation

Focusing on a particular resource effect on plant processes through the aid of SI can help us unravel the types of interactions and how these interactions are influenced by a particular resource availability. Such studies are possible because of the naturally distinctive δ^{15}N signature of ammonium and nitrate due to fractionation

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Table 1. Stable isotope abundance of elements and their major forms used in ecological studies

Elements	Isotopes	Natural abundance (%)	Reference standard
H	^1H, ^2H (D)	99.985, 0.015	Standard Mean Ocean Water – SMOW
C	^{12}C, ^{13}C	98.982, 1.11	Pee Dee Beleminte – PDB
N	^{14}N, ^{15}N	99.632, 0.368	Atmospheric N_2
O	^{16}O, ^{18}O	99.759, 0.204	SMOW, PDB
S	^{32}S, ^{34}S	95.0, 4.22	Vienna Canyon Diablo Troilite (FeS) – VCDT
Ca	^{40}Ca, ^{44}Ca	96.94, 2.086	NIST-SRM980
Sr	^{87}Sr, ^{88}Sr	9.87, 82.53	Absolute ratio measured

during mineralization, nitrification, denitrification and NH_3 volatilization in the soil. These processes imprint soil with different $\delta^{15}N$ signatures, together with significant depth–distribution-related variations in $\delta^{15}N$, which are transferred to the plant during N utilizations[50]. For example, Evans and Ehleringer[15] explained the co-occurrence of four species in nitrogen-limited conditions by adjudging temporal $\delta^{15}N$ variations. Distinctive $\delta^{15}N$-NO_3^- and $\delta^{15}N$–NH_4^+ signatures and significant depth–distribution-related variations in $\delta^{15}N$ evidenced competitive partitioning and compartmentalization of the overall N pool responsible for coexistence, even under resource-poor ecosystems.

Several studies validated uptake of different N forms by co-occurring species to bypass competition. The partition of different N forms, organic versus inorganic, was first recognized by Näsholm *et al.*[8] as a possible mechanism for coexistence. Guehl *et al.*[51] noticed distinct $\delta^{15}N_{leaf}$ signatures in 21 tropical rainforest species, suggesting different N acquisition patterns in co-occurring species. $\delta^{15}N_{leaf}$ studies by Pornon *et al.*[52] demonstrated different N acquisition preferences for NO_3^- and NH_4^+, and temporal complementarity in reducing competition and promoting coexistence among co-occurring species. In other studies, precipitation[53] and plant functional groups[50] were suggested as the important determinants of N partitioning among co-occurring species.

$\delta^{15}N$ isotopic studies by Houlton *et al.*[53] did not find niche portioning for nitrogen consumption among the functionally diverse species in tropical montane forests. They suggested that coexistence among functionally different species is related with the uniform plastic ability to switch among different nitrogen sources; by capitalizing on the most abundant N form. The $\delta^{15}N$ in plant leaves and $\delta^{15}N$ signatures of nitrate and ammonium in the soil suggested that all the species in the driest sites preferred nitrate, whereas ammonium supported the growth of all plant species in the wet sites. Miller *et al.*[16] also suggested uptake plasticity for different N forms by which co-occurring plants reduce competition for nitrogen. Their ^{15}N tracer study showed competitive effect. Under N-limited conditions, inter-specific competition existed for N uptake, wherein neighbours reduced the uptake of particular N form and simultaneously shifted to an alternative N form. Ashton *et al.*[17] also underscored plasticity in the resource use as a dominant mechanism to explain coexistence of species with overlapping resource use. They showed that four alpine species with differing competitive abilities exhibited preferences for different N forms in their fundamental niches (without competitors), but different uptake patterns in their realized niches (with competitors). Competitively superior species showed increased uptake of most of the available NH_4^+ compared to inferior species. In contrast, the competitively inferior species did not alter N uptake pattern in competition[17].

Resource partitioning for water and niche overlap

Different water sources exhibit contrasting δD signatures. However, no isotopic fractionation is observed when water moves from soil to plants. $\delta D_{soil-water}$ varies with depth. δD in surface soil layer is less negative compared to subsurface soils due to evaporative fractionation[9,21,24] (Figure 1). Also, generally $\delta D_{xylem-water}$ is more negative in dry season than in other seasons[54], particularly in the tropical (semi-)arid regions. The $\delta^{18}O$ and δD cues show that coexisting competing plant species escape competition by exploiting different water sources and soil–water at different depths, and adopting different usage strategies[49].

$\delta^{18}O$ and δD signatures of plants and soil–water in subtropical savanna confirmed that grasses acquired water primarily from the upper soil layer (50 cm), whereas trees and shrubs utilized water mostly from deeper layers. More negative $\delta D_{xylem-water}$ (–32‰) of *Prosopis glandulosa* compared to co-occurring *Zanthoxylum fagara* (–26‰), indicated the segregation of water acquisition zones between the two species[21]. Similarly, in Mesic tallgrass prairie, C4 species were dependent on shallow soil–water and C3 species on water of deeper layers[24]. Also, C3 species circumvent competition by changing the water uptake zone seasonally when water availability is limited[24].

A few studies focused on understanding water relation among coexisting species during prolonged dry seasons in the species-rich dry tropical forests. Based on the comparative xylem-, soil- and groundwater δD values, spatial and temporal partitioning of water was noticed among several Barro Colorado tropical forest tree species during dry season[9]. Similarly, in Hawaiian dry forests, $\delta D_{\text{soil–water}}$ and $\delta D_{\text{xylem–water}}$ signatures revealed that among eight tree species, only two relied on deep-layer water, whereas the remaining species showed less negative and overlapping δD values, suggesting possible competition for shallow water[55]. $\delta D_{\text{sap–water}}$ and $\delta^{18}O_{\text{sap–water}}$ discerned summer precipitation partitioning behaviour between coexisting *Pinus edulis* and *Juniperus ostesperma*, where the former depends on summer-derived moisture to a greater extent than the latter[56].

Querejeta *et al.*[13] debunked the notion that trees growing on karst lithology are dependent on groundwater during dry periods. Seasonal changes in the δ^{18}O and δD values of xylem–water, soil–water, rainfall and spring–water revealed a strong seasonal influence on water acquisition by plants growing on these rock types with a strong reliance on water stored in the epikarst zone during dry season.

Resource pulses

Resource partitioning among coexisting species can be explained by resource pulse mechanism[56,57]. Resource pulse is the spatial and temporal variability in the resource supply to plants in discrete pulses after rainfall, when the plants experience relatively high resource availability that is followed by intervening dry interpulse periods of low resource supply[57]. This is critical for (semi-)arid-regions, where season and amount of precipitation primarily govern water availability[56]. The utilization of resource pulses reinforces micro-niche differentiation among coexisting species to escape competition for water and dissolved nutrients.

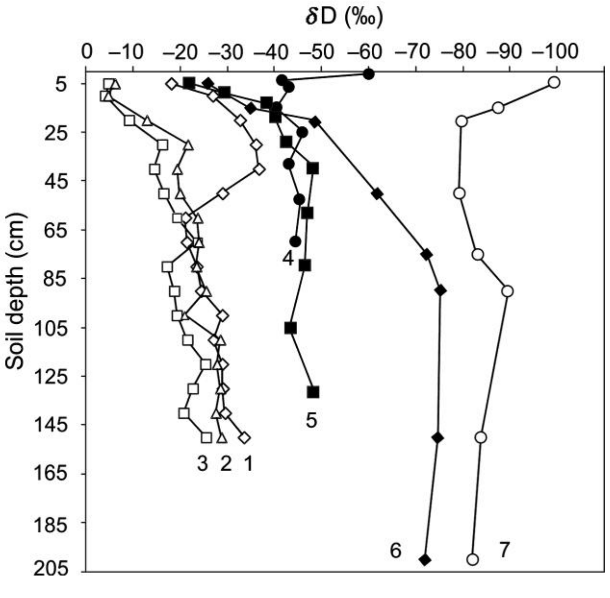

Figure 1. δD (‰) in the soil from: 1. savanna grassland (◇), 2. cluster (Δ), 3. grove (□), 4. shrubland (●), 5. tropical dry forest (■), 6. temperate forest summer (◆), 7. temperate forest (remaining months) (○). 1, 2, 3 after Midwood *et al.*[194,¥]; 4 after Ogle *et al.*[60]; 5 after Stratton *et al.*[55]; 6, 7 after Eggemeyer *et al.*[195,¥]. ¥See Supplementary Material online for references.

Differences in δD and δ^{18}O of waters of xylem, precipitation, deep and shallow soils and groundwater showed that summer monsoon water pulses are an important water source for trees in arid environment[58]. A recent study[59] found resource-pulse discrimination among six shrub species; δD signatures clearly showed that shallow water pulses at the time of onset of summer were used by the shrubs to different degrees to bypass competition.

Ogle *et al.*[60] unravelled bimodal water and nutrient acquisition strategies of *Larrea tridentate* through dimorphic root distribution. Small roots growing up to >10 cm depth allow shrubs to capitalize on water and dissolved nutrients immediately following rainfall, whereas large roots penetrating to depths more than 20–45 cm provide for stable water supply throughout the year. In a sub-alpine forest, Xu *et al.*[61] documented water partitioning among coexisting species. Dominant *Abies faxoniana* relies primarily on groundwater with no change depending on seasons, whereas mid-storey *Betula utilis* and understorey *B. fangiana* mainly rely on rainwater and opportunistically switch to groundwater under stressful conditions.

Hydraulic lift: plant–plant interactions

Hydraulic lift is a process of root-mediated passive movement of soil-water, driven by water potential gradient (between soil and roots) from deep-moist to shallow-drier soil layers[62]. Germane to the plant–plant interactions between co-occurring species, studies used δ^{18}O and δD to understand hydraulic lift[35–37,63]. Redistribution of soil–water in upward, downward and lateral directions via hydraulic lift has significant ecological significance for vegetation in the (semi-)arid region in offsetting water stress, and resource facilitation defining coexistence.

Jackson *et al.*[63] ascertained hydraulic lift as an important component of water use among ten co-occurring woody perennial species of Brazilian Cerrado. The δD values of xylem sap revealed that Cerrado species can partition water niches to escape competitive exclusion in the dry season. Moreira *et al.*[64] in Neotropical savanna and Armas *et al.*[37] in arid costal shrubs showed hydraulic lift-mediated relationships between the species, where facilitative effects of hydraulic lift in transferring water to neighbouring plants were underscored.

$\delta D_{\text{soil–water}}$ and $\delta D_{\text{xylem–water}}$ revealed that hydraulic lift is important in offsetting competition in mesic setting as well[35]. In the seasonally water-limited Mediterranean *Quercus suber* ecosystems, δ^{18}O and δD isotopes in the soil–plant–atmosphere continuum revealed drought-period adaptive plasticity accruing from bimodal root system (morphological trait) and hydraulic trait (ecophysiological trait) that positively contributes to the redistribution of water in the rhizosphere[36].

Stable isotopes proxies for changing community patterns

Differences in the foliar and soil C isotopes are excellent tools to study succession and temporal changes in the boundaries of contrasting ecosystems[28,29]. Any change in the C3 and C4 species/communities has a bearing on soil organic matter (SOM). $\delta^{13}C_{SOM}$ depth–distribution within a soil profile can reveal changes in the distribution of a C3 and C4 plant community. $\delta^{13}C_{leaf-litter}$ of existing vegetation determines the $\delta^{13}C_{SOM}$ in the surface layers of soil profile, whereas $\delta^{13}C$ of subsurface layers represents signatures of past vegetation. These proportional and contrasting changes ($C3:C4_{SOM}$) can be easily used to track the succession between C3 and C4 vegetation[21].

Wang *et al.*[27] used $\delta^{13}C_{SOM}$ values of a prairie–transition–forest biosequence to reveal that C4 prairie (–19‰ to –16‰) was the dominating vegetation before existing forests and other C3 plants (–25.6‰) in the community. Relying on $\delta^{13}C_{SOM/Roots}$, Mcpherson *et al.*[26] provided evidence of woodland expansion into the grasslands in southeastern Arizona *Prosopis juliflora–Quercus emoryi* ecotone. In the Rio Grande plains, $\delta^{13}C_{SOC}$ signatures associated with fine and coarse clay (–20‰ to –15‰) evidenced the presence of C4 grasslands that once occupied these woodlands (–28‰ to –25‰)[21]. In the Amazonian savanna and forests, depth-related $\delta^{13}C$ discontinuity of bulk OM between surface and subsurface suggested that either forest replaced C4 vegetation or a mixture of C3/C4 woody savanna[28]. Bai *et al.*[29] studied the pattern of transformation of C4-dominated grassland to subtropical woodland dominated by *Prosopis glandulosa* in subtropical savanna of southern Texas. They studied spatial variation of soil $\delta^{13}C$ in discrete grasslands (–19‰), shrub clusters (–21‰) and groves (–21.6‰) relative to *P. glandulosa* (–23.3‰). Their study underscored the phases of grassland to woodland transitions; discrete, multispecies shrub clusters organize themselves around and beneath *P. glandulosa*, which coalesce to form groves and finally individual groves merge to form woodlands.

Several studies used depth–distribution $\delta^{13}C$ signatures of SOM to reconstruct palaeoecological vegetation changes[65,66]. Desjardins *et al.*[65] studied variations in $\delta^{13}C$ of SOM with depth and assessed forest–savanna boundary fluctuations in the northern Brazilian Amazon during the Holocene. They found SOM $\delta^{13}C$ values in the upper soil horizons similar to the overlying vegetation types. However, deeper soil layers were different in $\delta^{13}C$ values, which indicated vegetation change in the past. In another study, Biedenbender *et al.*[66] assessed $\delta^{13}C$ and radiocarbon dating of light and heavy SOM to construct landscape pattern of vegetation change. The relative $\delta^{13}C$ comparison of light and heavy SOM helped them reveal the direction and time of vegetation change, ranging from recent over the last few decades to that of vegetation change initiated 5000–6000 years BP. Krull *et al.*[67] used bulk and size-fractionated SOM and POC (particulate organic carbon) $\delta^{13}C$, and ^{14}C data to demonstrate transition of C4 grasslands to *Acacia*-dominated woodlands. Bulk $\delta^{13}C$ indicated a shift from grasses to tree-derived C in the woodlands, whereas differences in $\delta^{13}C$ of POC >53 μm and <53 μm asserted vegetation change from C4- to C3-dominated ecosystem as a recent phenomenon (<100 years).

The invasion of habitats by non-native species is a critical issue, which poses risk to ecosystem processes and services. Recently, invaded communities and habitats were studied with the $\delta^{13}C$ of SOM to gain insight into post-invasion vis-à-vis pre-invasion conditions[68,69]. In a chronosequence study where trees replaced C4 grasslands, $\delta^{13}C_{SOM}$ and $\delta^{15}N_{SOM}$ revealed change in soil C storage pools; new C3-derived OM had accumulated in macroaggregates and POM fractions, whereas older C4-derived OM was associated with microaggregates[70]. Similarly, $\delta^{13}C_{SOM/POM}$ values helped Dalal *et al.*[68] to discern post-invasion effect from native mixed *Acacia harpophylla* and *Casuarina cristata* to *Cenchrus ciliaris* pasture, on pedogenic processes and SOM/SOC composition.

Permanent trait introduction by invading species, especially N-fixing, affects diversity and community composition of the native species through alteration in the resource supply[71]. Bai *et al.*[72] used foliar and soil $\delta^{15}N$ to highlight the introduction of N_2-fixing trait by invading *P. glandulosa* in the subtropical savanna dominated by C4 vegetation. They suggested a facilitative role of N_2-fixing species in promoting the invasion of other woody species, where proximity of N_2-fixers with non-N_2-fixers influenced N-economy of the latter. Another study on sand dune ecosystem invasion by *Acacia longifolia* exhibited similar results[73]. The invading species introduced N_2-fixing trait to the sand dune community, wherein increased N supply benefited native non-N_2-fixing species.

Biogeochemical cycling: improving the ecosystem-based dynamics

The idea about integrating and studying the individual heterogeneous components of biosphere into a single dynamically interacting unit – the biogeochemical unit – was conceived in 1960s and later developed further. All the aspects of C, N, S and biogeochemical cycling of several other elements were reinforced by SI mediation[6].

Carbon biogeochemical processes and carbon isotopes

The use of stable carbon isotope signatures of abiotic and biotic compartments helped strengthen understanding about the processes of C efflux, assimilation/immobilization, allocation and mineralization[34,74,75]. A number of studies examined abiotic and biotic factors and provided reliable descriptors for C flow (metabolite forms) between different continuums[76,77].

Respiratory carbon efflux

The understanding of terrestrial C biogeochemical pathways is reinforced by carbon isotope mediation. The δ^{13}C signatures helped discern net C ecosystem exchange into two fluxes, photosynthetic and respiratory[33]. This helped differentiate total ecosystem respiratory fluxes into above-ground and below-ground fluxes[34]. Within below-ground respiratory fluxes, SI helped distinguish autotrophic from heterotrophic fluxes, and within autotrophic fluxes SI further helped discern the share of root respiration versus rhizomicrobial/rhizosphere respiration[78,79] (Figure 2). Millard *et al.*[78] showed the possibility of ^{13}C discrimination to separate total soil C-efflux into two end-members arising from the root-respired and SOM-derived C-efflux. Such validations are made possible because of the distinction in δ^{13}C fluxes of respired CO_2 (ref. 34), which rely on the gradient of δ^{13}C of metabolites originating from the C isotopic discrimination during its translocation from leaf to roots, and finally to microorganisms in the rhizosphere[80] and litter–SOM interface[81]. In the boreal *Pinus sylvestris* stand, Ekblad and Högberg[82] presented canopy transfer of recently fixed C (photoassimilates) to roots and its subsequent availability as the substrate for below-ground respiratory metabolism, with a time lag of few days. In *Fagus sylvatica* ecosystem, Gavrichkova *et al.*[34] underpinned photosynthateses translocation down to trunk, where they mix with older C pools. Also, a strong 24 h periodicity was found for δ^{13}C in soil respiration, indicating changes in the contribution of the root to the total efflux.

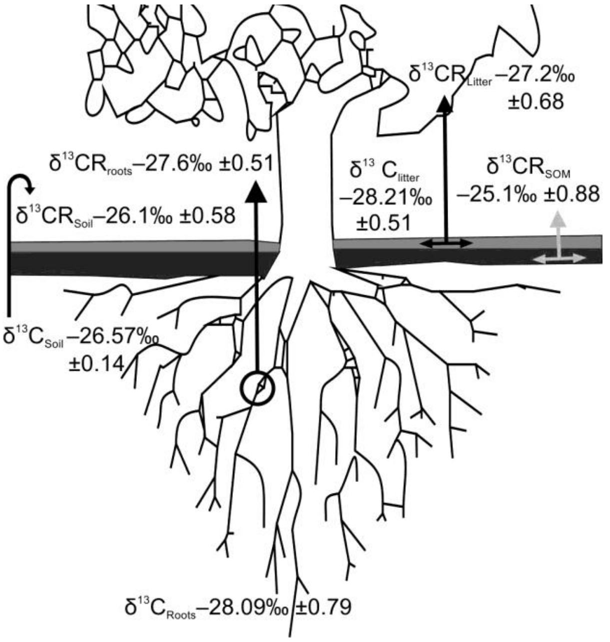

Figure 2. Carbon isotopic values of different components contributing towards total soil respiration (after Millard *et al.*[78]).

One of the most comprehensive studies that modelled whole soil respiratory components on diel scale suggested the coupling of soil-respired $\delta^{13}C_{CO_2}$ with both biotic and abiotic factors[76]. The dependence of root and microbial respiratory metabolism on different substrates was surmised for this, because within a small period of time (h), respiratory carbon δ^{13}C was seen deviating from the mean[76,83]. Šantrůčková *et al.*[84] suggested the role of microbes imparting variations in δ^{13}C of respired carbon, due to their ability to selectively utilize SOC and selective isotopic discrimination during metabolism. This was later found to be related to the priming effect of substrate availability[74,85]. Investigations indicated that rhizosphere $\delta^{13}C_{CO_2}$ is relatively more depleted than δ^{13}C of the root biomass[79]. This was linked to the rhizodeposition with different δ^{13}C values[75], microbial decomposition together with microbial respiration[86], mixing of the metabolites of recent origin with the old metabolites[34], and dynamic shift in the autotrophic and heterotrophic contributions to total soil efflux[83].

Flow of carbon in soil: litter, soil organic matter and microbe interaction

Total soil C reserve and its dynamic behaviour demand understanding of all the processes related with its flow pattern given the important feedback it has on global change. Determination of carbon isotope dynamics has proved useful to gain insight about the mechanism of carbon flow in litter–soil–microbe continuum[75]. This was made possible because of the mechanistic understanding of C isotopic fractionation (during decomposition), while it flows in and out of the immobilization–mineralization cycle[33], its microbial breakdown and subsequent assimilation in SOM[87]. Studies with δ^{13}C signatures elucidated the litter decomposition rates and patterns[85] and SOM movement within soil profile[87].

Rubino *et al.*[87] suggested that about two-third of the C_{litter} input to the mineral soils probably enters in soluble form, while the fraction of C_{CO_2} loss is only one-third of the total C loss from the litter. Later, Ngao and Cotrufo[88], based on the temporal $\delta^{13}C_{CO_2}$ signatures illustrated that among labile, intermediate and recalcitrant C pools, intermediate C pools in the intermediate decomposition phase make the largest contribution to litter respiration. However, Kammer and Hagedorn[89] showed that mineralization is the main pathway of C loss from the decomposing litter during initial decomposition phase.

Fernandez *et al.*[86] presented a δ^{13}C dynamics-based model of C mixing between litter and SOM during decomposition. They found that isotopic signals of decomposing litter depend on the litter quality and the stage of decomposition. $\delta^{13}C$ becomes depleted in comparison to bulk litter samples as decomposition moves forward mainly because of lignin accumulation[90,91]. In advanced decomposition stages, slight enrichment in the isotopic composition was found along with the decay of plant litter-to-SOM continuum[6]. Wedin *et al.*[90] suggested that in advanced decomposition stage, C-enrichment results from the microbial transformation of lignin and non-lignin C, and the incorporation of microbial C in transformed litter. Fernandez *et al.*[86] attributed this to the shifts in $^{13}C_{CO_2}$ during decomposition, acting in tandem with the substrate quality and the ability of microorganisms to selectively discriminate against ^{13}C. Based on the differences in $\delta^{13}C_{CO_2}$ from soil, which include sum of root respiration and microbial respiration, and from SOM-CO_2 arising from its microbial decomposition (Figure 2)[92], the feedback interactions between the supply of organic compound from litter to soil and microorganisms, priming effect on these compounds[74,93] were suggested for short-term increase or decrease in the decomposition rates[85]. A 35-month decomposition study with two cool temperate species[91] and three C pools[88] found a similar substrate control on the litter decomposition. However, selective consumption of various C substrates differing in $\delta^{13}C$ was acknowledged for these enrichments[88].

Dissolved organic carbon (DOC) plays an important ecological role in pedogenesis, mineral weathering and retention. Two most important DOC sources in the soil are litter and humified SOM. However, this question still persists regarding, how and in what proportion they exert their influence on the DOC availability and mobility within the soil? The $\delta^{13}C_{DOC}$ of decomposing litter and humified portion of the soil helps characterize DOC flowpaths and turnover[85], and its major sources as it moves from surface to subsurface soil layers[94]. Two separate studies, in beech and spruce soils[94], and in forest and prairie soils[95] found that the major sources of DOC are more humified fractions of SOM. Both investigations demonstrated less contribution from recent fraction of plant-derived C in the DOC in organic horizon and the greatest fraction of leached DOC to the mineral soil originated from humified OM layer or from Oa horizon. Kammer and Hagedorn[89] illustrated the role of decomposing leaf- and twig-litter as the source of DOC; a track of litter-derived $^{13}C_{DOC}$ signals helped them to suggest that <10% of the DOC leached from the litter layer was recovered at 5 cm depth and the greatest litter DOC fraction was thus retained in the uppermost mineral soil.

Understanding nitrogen dynamics with natural nitrogen isotopic abundances

Understanding nitrogen dynamics and its effect on productivity and species composition has taken precedence after the nitrogen saturation hypothesis was proposed[96]. Nitrogen isotopes were used to reinforce understanding of nitrogen cycling, including denitrification, nitrification and N_2 fixing[97,98]. The potential of foliar $\delta^{15}N$ as an integrator of terrestrial nitrogen cycling to reveal spatial and temporal patterns of nitrogen channeling was appreciated by many studies[6,98] (Table 2); $\delta^{15}N$ ranges of plants within an ecosystem potentially indicate divergent strategies for nitrogen uptake[99].

Martinelli *et al.*[100] conclusively showed the difference in nitrogen cycle in tropical and temperate forest ecosystems; nitrogen cycle in the former is more open compared to the latter. The results of $\delta^{15}N_{leaves}$ of 11,000 plant species suggested the control of mycorrhizal associations over nitrogen availability pattern, in addition to temperature and moisture[101]. However, in the savanna ecosystem, $\delta^{15}N$ signatures indicated that topoedaphic gradient characterizes N-cycling rates.

Studies revisited the nitrogen saturation of forested ecosystems with $\delta^{15}N$ to improve knowledge about response of the forests to cascading effects of nitrogen deposition. By measuring $\delta^{15}N$ isotopes and N concentrations across the depositional gradient, Pardo *et al.*[31] studied the temporal changes in nitrogen saturation in hardwood and conifer forested ecosystems. They hypothesized increased foliar $\delta^{15}N$ with increasing deposition, nitrification and nitrification : mineralization. Their study appreciated the potential of increased nitrification as a source that influences foliar nitrogen compared to deposition along depositional gradient. McLauchlan *et al.*[25] studied $\delta^{15}N$ signatures of 545 herbarium plant species specimens to reconstruct changes in N availability in central North American Great Plains over the past 132 years. Their study strengthened the progressive-N-limitation-type hypothesis stating that decline in nitrogen availability is driven by increased ecosystem N storage as a result of increased atmospheric carbon. They found a decline in foliar N and $\delta^{15}N$ values, suggesting declining N availability in the grasslands despite decades of anthropogenic deposition.

Näsholm *et al.*[8] recognized the ability of boreal plants to bypass the common mineralization pathway of N acquisition. Working on the late successional coniferous forest species, they used ^{15}N, ^{13}C and $^{13}C : {}^{15}N$ ratio to demonstrate that irrespective of different root–fungal associations, boreal plants rely on organic nitrogen. With the aid of $\delta^{15}N$, Averill and Finzi[102] tested the changeover of plant nitrogen cycling from inorganic to organic as a primary mode of nutrition with decrease in mean annual temperature. They found that with increasing elevation, organic nitrogen forms became the dominant nitrogen source in hardwood and coniferous tree species. Foliar $\delta^{15}N$ values varying from –6.4‰ to 5.9‰ in savanna woodland and from –2.3‰ to 8.4‰ in seasonally dry forest suggested diversity of nitrogen-use strategies within both these communities.

Table 2. Foliar nitrogen isotopic composition from different ecosystems

Site	Ecosystem type	δ^{13}N (‰)
Mt Haleakala, Hawaii[53]	Montane tropical forest	–6.89 to 3.21[‡]
Hluhluwe–iMfolozi Park, South Africa[155,¥]	African savanna	–2 to 7.8
Thuringer Scfiefergerbirge, Germany[99]	Montane grassland	0.5 to 5.1
Renberget, Sweden[8]	Boreal forest	–1.5 to 0.3
Texas Agrilife La Copita research area[72]	Subtropical savanna	1.01 to 3.65
French Guyana[51]	Lowland tropical rainforest	–0.3 to 3.5
French Guyana[156,¥]	Tropical rainforest#	0.24 to 3.49
Niwot ridge, Colorado[157,¥]	Dry alpine tundra meadow	–1.41 to 2.34
Castskill mountains, New York[158,¥]	Temperate forest	–1.3 to –0.5
Kakadu National Park (KNP), Australia[104]	Tropical savanna	–0.6 to 2.9
KNP, Australia[104]	Deciduous monsoon forest	1 to 4.7
Desert upland bioregion, Australia[23]	Semi-arid savanna[$]	–0.6 to 8.1
	Semi-arid savanna	0.1 to 5.5
Estação Ecológica des Assis, Brazil[159,¥]	Woodland savanna	–6.4 to 5.9
	Seasonally dry forest	–2.3 to 8.4
Mount Schrankogel, Austria[160,¥]	Alpine-nival ecotone	–5.5 to –3.7
Europe, Alaska, Ontario, Northeastern USA[161,¥]	Hardwood species	~–6.5 to ~2
	Conifer species	~–8.2 to ~4
Different mycorrhizal associations[162,¥]	–	–5 to –1.1
Terrestrial ecosystem range[162,¥]		–11 to 10
Lyman glacier forefront, Washington[163,¥]	Mycorrhizal species	–11.2 to –0.4
	Non-mycorrhizal species	–5.5 to –5
	Fungi	0.8 to 16.6
North Central Oregon, USA	Temperate douglas fir[19]	–2.24 to –0.43
	Tropical species[100]	–9.3to 9.6
	Temperate species[100]	–10.1 to 0.6
Northeastern USA[33]	Conifer and hardwood species^^	~ –5.5 to ~0.1
Bear brook–Hubbard brook forest, Northeastern USA[164,¥]	Hardwood forest	–1.3 to 1.7
Kruger National Park, South Africa[165,¥]	Savanna	3 to 3.2
Hawaii Volcano National Park[166,¥]	Rainforest	–10.1 to 0.7
Ituri River Basin, Democratic Republic of Congo[167,¥]	Tropical rain forest	1.1 to 8.9
North Queensland, Australia[168,¥]	Tropical rainforest	–4.9 to 0.2
Pichavaram estuarine ecosystem, India[140]	Intertidal mangrove forests	5.48 to 7.08
Corrubedo National Park, Spain[69]	Tidal wetland	–0.4 to 3.6
	Saprtina wetland	–0.4 ± 0.8

[‡]Precipitation gradient; [#]Late successional species; [$]Grasses; [^^]Nitrogen deposition gradient.
Superscript numbers represent references; [¥]See supplementary material online for references.

The control of N availability among 330 savanna grasslands sites (South Africa) and the effect of herbivory (grazing) were quantified for plants and soils through the mediation of δ^{15}N (ref. 22). The results of the study suggested that grazing strongly influences nitrogen availability in these savanna sites, rather than mineralization. High nitrogen availability was seen associated with greater grazing pressure along with functional demand of plant types.

Houlton *et al.*[53] resolved an important aspect of nitrogen cycling in tropical rainforests. With δ^{15}N data, they showed that bacterial denitrification is responsible for a large fraction of total ecosystem N loss. Conversely, using a dynamic simulation model of δ^{15}N and N ecosystems, Perakis *et al.*[19] studied the shift in the potential pathway of nitrogen loss from denitrification to NO_3^- leaching (based on soil-water $\delta^{15}N_{NO_3^-}$) with an increase of nitrogen accumulation in temperate ecosystems. Debunking earlier hypothesis of disturbance-led nitrogen loss, they provided evidence which showed that wildfires can foster substantial long-term nitrogen accumulation in ecosystems through post-fire recolonization of symbiotic N_2-fixing vegetation.

Plant N-acquisition: role of mychorrhizal associations

The δ^{15}N signatures helped appreciate the importance of mychorrhizal associations (ericoid, ectomycorrhizal) in the plant nitrogen acquisition[103]. Previous studies had underscored that δ^{15}N and N-cycling are determined by environmental factors. The δ^{15}N study by Schmidt and Stewart[104] found a strong modulating relationship of root specialization from mychorrhizal associations. They found greater NO_3^- availability and high δ^{15}N in monsoon forest soil compared to savanna and tropical woodland soil, indicating stronger mineralization and nitrification in the soil. This resulted in: (i) high δ^{15}N of soil N sources, (ii) high use of NO_3^- and associated lower fractionation against ^{15}N, and (iii) low contribution of mychorrhizal fungi to plant N acquisition and associated lower discrimination against ^{15}N during fungal N transfer. With the aim of understanding δ^{15}N variation drivers, Hobbie *et al.*[103] explored the δ^{15}N patterns associated with the nitrogen forms, fungal symbionts and nitrogen availability in *Pinus sylvestris*. They found that fungal biomass on root tips was enriched in ^{15}N (6‰–8‰) relative to plant biomass. To understand nitrogen together with carbon biogeochemical aspects following woody vegetation clearing, Schmidt *et al.*[23] assessed natural abundance of ^{15}N and ^{13}C of semi-arid dystrophic savanna woody vegetation as integrators of nitrogen and carbon cycles. δ^{15}N values helped reveal that nitrogen input occurs via N_2-fixing microbiotic crusts and nodulating *Acacia coriacea* and *Acacia excelsa*.

Use of sulphur isotope to understand sulphur cycling

Under the prevailing extreme depositional gradients, terrestrial ecosystems are experiencing stresses and it is presumed that even minor perturbation load can jeopardize ecosystem functions via altered biogeochemistry. Amongst all the atmogenic perturbations, sulphur (S) as an acidifying agent, and its direct and indirect effects on terrestrial vegetations are well known[105]. $\delta^{34}S_{SO_4^{2-}}$ stable isotopes are now used to constrain the sources and S fluxes for appreciating the input–output mass balances[106].

A series of investigations undertaken at Hubbard Brook Forest capitalized on δ^{34}S to reinforce S biogeochemistry by studying individual S apportionment in different pools and fluxes, and their interlinkages[105]. Along a strong pollution gradient, δ^{34}S elucidated the process of S retention/release, sources and sinks in 13 forested catchments. The isotopic results indicated that within-catchment, source/sink of S was mainly organically cycled soil S[30]. Mörth *et al.*[107] highlighted the role of O-horizon organic sulphur pools for the sizeable presence of SO_4^{2-} in the run-off.

Since sulphur also originates from weathering, different isotopic values can help discriminate between the potential S sources. Mean precipitation of $\delta^{34}S_{SO_4^{2-}}$ similar to soil $\delta^{34}S_{SO_4^{2-}}$ showed that atmospheric deposition is the main source of sulphur in the Black Forest Catchment, Germany[106]. Mitchell *et al.*[105] elucidated the contribution of weathering in causing spatial variation of δ^{34}S values in soils developed over sizeable S-bearing rock systems. Puig *et al.*[108] used sulphur isotope analysis of stack SO_2, and bulk and throughfall deposition to distinguish the main source of sulphur in scot pine throughfall. They found that most of the throughfall sulphur had its origin in the dry deposition from power plant emissions on the pine canopies.

Natural abundance of strontium isotope (^{87}Sr/^{86}Sr) as a proxy for calcium biogeochemistry

Calcium is an essential element for the growth of forest ecosystems. Among base cations, it is generally the most significant contributor to the cation exchange capacity and the base saturation capacity of soils. It plays a pivotal role in neutralizing acidic inputs and maintains the health of the trees in forest ecosystems. Given its importance, tools to track the sources, pathways and bioavailability of Ca become imperative to evaluate how it behaves in soil–plant–environment systems under perturbations[47,48]. However, ecosystem-specific Ca input and output budgeting is difficult, as some fluxes that are internal to the ecosystem like weathering may release Ca and are hard to measure separately[5].

In ecosystem research, ^{87}Sr/^{86}Sr ratio is used as a proxy to understand Ca biogeochemistry[5,46] (Table 3). Both Ca and Sr are geochemically similar, as both are alkaline-earth elements with similar ionic radius and valence (Ca^{2+} and Sr^{2+}). Sr supply within an ecosystem can be easily ascertained with a great deal of certainty, as atmospheric-fed Sr is less radiogenic compared to weathering-fed Sr[109] (Table 3). Whereas foliar ^{87}Sr/^{86}Sr depends on the binary mixing and lies intermediate between the strontium isotope ratio of weathering and atmospheric sources. However, atmospheric dust imparts spatial and temporal variations in isotopic ratios of both the end-member sources[46] (Figure 3).

Kennedy *et al.*[109] used ^{87}Sr/^{86}Sr and suggested base cation source change during Hawaiian ecosystem development. Weathering remained the important base cation source in young substrates, whereas atmospheric inputs had overriding effects on impoverished older substrates. In an apportioning study, Bélanger and Holmden[110] suggested atmospheric deposition as dominant Ca-source other than mineral weathering in the boreal shield forests, Canada. Berger *et al.*[111] differentiated beech and spruce Ca pump operation using ^{87}Sr/^{86}Sr ratios; beech takes up Ca from deep soil layers (30–50 cm), whereas spruce pumps Ca mainly from 0–10 cm soil layer. Blum *et al.*[47] used ^{87}Sr/^{86}Sr with Ca : Sr ratio to infer that internal Ca recycling between litter, organic horizons, and vegetation dominates Ca uptake in the northern hardwood ecosystems along with the atmospheric deposition. They adjudged this based on ^{87}Sr/^{86}Sr ratio from weathering end-members being higher than foliar and ion exchange complex.

Dendroecological studies coupled with SI cues were undertaken for reconstruction of various temporally discriminated aspects of soil chemical changes in forested ecosystems[5,10]. ^{87}Sr/^{86}Sr signatures in tree rings unfold past provenance quality, site factors and acidification-led nutritional impoverishments[45]. In two separate studies, Poszwa *et al.*[45] and Page *et al.*[10] evaluated Ca availability retrospectively from mineral weathering versus atmospheric

Table 3. $^{87}Sr/^{86}Sr$ isotopic studies to apportion supply of Sr and Ca from two end-member, weathering and atmosphere

Region	Plant species	$^{87}Sr/^{86}Sr$ ratio			Remarks
		Foliar	Atmospheric	Weathering	
Tesuque watershed, New Mexico,	*Populus termuloides*	0.7197	0.71041*	0.7348	75% and 80% Sr from atmosphere
Embudo granite[169,¥]	*Picea engelmannii–*		0.70985[#]		
	Abies lascicarpa	0.7134	0.71130*	0.7529	
			0.70985[#]		
Svartberget[170,¥]	Spruce-*Picea* sp.		0.71586	0.74020	Atmospheric Sr inputs override on acid granites (62–53%) and weathering supply dominates on gneisses, gneissic granite (2–20%)
Kullavna övre	Spruce-*Picea* sp.		0.74448	0.76859	
Kullarna Nedra	Spruce-*Picea* sp.		0.73911	0.76335	
Gneisses, Gneissic granite, Sweden					
Buskbäcken and V Dybäcken-acid granite, Sweden	Spruce-*Picea* sp.		0.714803	0.761463	
Sante Fe National Forest, New Mexico, Granite[171,¥]	*Populus termuloides*		0.71054*	0.73220	More inputs of atmospheric Sr in *Picea* than *Populus*, with no contribution from dry deposition
			0.70992[#]		
	Populus engelmannii		0.71338*	0.72950	
			0.701167[#]		
White mountain national forest,	*Picea rubens*	0.72039	0.71685*	0.72389[S]	32% and 68% Ca atmospheric and weathering originated respectively. Isotope end-member modelling and TF Sr/Ca indicated Sr and Ca in TF mostly derived from canopy leaching
Sillinanite grade metapelites, Northeastern USA[172,¥]	*Abies balsamea*	0.72083	0.71061[#]	0.77472[R]	
	Acer saccharrum	0.72004			
Hawaii basalt chronosequence[173,¥]	*Metrosideros polymorpha*			0.7034[WM]	Temporal sift in Sr source, younger sites predominantly fed with weathering release (85–90%) and mixture of both for sites of intermediate age (200 Ka) to predominantly atmospheric in older sites (84–90%)
0.3–2 Ka		0.7041	0.7090	0.7041[Sol]	
20–4100 Ka		0.7077	0.7090	0.7086[Sol]	
Mauna Loa, Hawaii, Basalt[174,¥]	*Metrosideros polymorpha*	0.70545	0.7096	0.7035	weathering supplied most of the Sr in most of the sites with atmospheric contribution towards 30–50% in wet forests
Strengbach forest catchment-Voges massif, France	*Picea abies*		0.71221*	0.83838	≈50% Sr atmospherically derived in surface soil, whereas in subsurface contribution was dominantly from weathering
Base poor brézouard leucogranite[175,¥]			0.71023[#]		
Kohala peninsula, Hawaii1	*Cenchrus ciliaris*			0.7035[WM]	<5% Sr was atmospherically originated in drier sites
Basalt[176,¥]	*Prosopis pallida*		0.7092	0.7042[††]	Wetter sites ~25 to ~35% atmospheric contribution
Low rainfall	*Pennisetum clandestinum*		0.7092	0.7090[††]	
High rainfall	*Metrosideros polymorpha*				
Hubbard Brook Forest, New Hampshire[177,¥]	*Picea rubens*	0.7200	0.7106	0.7216	~95% of foliar Ca in *Picea* and *Abies*, ~80% in *Fagus*, ~75% in *Betula*, ~60% *Acer* derived from weathering pools
	Abies balsamea	0.7206	0.7170[TF]		
	Fagus grandifolia	0.7199			
	Betula alleghaniensis	0.7202			
	Acer saccharum	0.7200			

(*Contd*)

Table 3. (*Contd*)

Region	Plant species	$^{87}Sr/^{86}Sr$ ratio			Remarks
		Foliar	Atmospheric	Weathering	
Carbonated pleistocene loess-SRF-CB[178,¥]	*Fagus sylvatica*	0.71120	0.70910	0.71207– 0.71364	Sr isotope ratio suggests atmospheric contribution of Ca was 75% and 78% for two ecosystems respectively
Palaeozic ardenne massif shale-HSF-CB[179,¥]	*Fagus sylvatica*	0.714282	0.70924	0.71620– 0.71762	
Palaeozic ardenne massif shale-SW[179,¥]	*Fagus sylvatica*	0.71559	0.70924	0.71710	~56% atmospheric contribution
Lowland tropical rainforest, Costa Rica	*Brosium utile*	0.7042	0.7088	0.7039	≥90% of the foliar Sr was rock derived
Osa basalt[180,¥]	*Schizolobium*	0.7043			
Cone pond ecosystem, New Hampshire Sillmanite grade metapelites[181,¥]	*Picea rubens*	0.72017– $0.71925^{\S}$	0.710	~0.724–0.726 0.719^{FF}	Sr and Ca were predominately obtained by vegetation from forest floor and only subordinately from mineral soil. Ca atmospheric contribution of Sr and Ca was small
Upper Austria	*Picea abies*	0.710	0.707	0.710^{S} 0.718^{SS}	Atmospheric Ca contribution overrides that of weathering contribution
Tertiary sediments[111]	*Fagus sylvatica*	0.705	0.707	0.727^{S} 0.712^{SS}	
Churchill river ecoregion, Saskatchewan, Granite[110]	*Picea mariana*‡	0.71658^{H}, 0.72063^{M}, 0.72820^{L}	$0.71566^{\$}$	$0.726^{(H)1N}$, $0.739^{(H)15N}$ $0.71459^{(H)FF}$ $0.71062^{(H)BH}$ $0.733^{(M)1N}$, $0.750^{(M)15N}$ $0.72239^{(M)FF}$ $0.71891^{(M)BH}$ $0.747^{(L)1N}$, $0.777^{(L)15N}$ $0.72984^{(L)FF}$ $0.73248^{(L)BH}$	Strong influence of topography in sequestering atmospheric calcium. 84–98% of Ca in high, 31–68% of Ca in middle and 37–82% of Ca in low elevation trees originated from atmosphere
	Populus tremuldoies‡	0.71672^{H}, 0.71895^{M}, 0.72730^{L}			
	Pinus banksiana‡	0.71469^{H}, 0.71722^{M}			

*Precipitation (P) and throughfall (TF) together; $^{\#}$Only precipitation; ^{S}Only soil; ^{R}Whole rock; ^{WM}Weathering release; ^{sol}1 M NH_4Ac soil solution; ^{S}surface soil; ^{SS}Subsurface soil; FF: Forest floor; BH, b-horizon (35 cm); H, M, L, High, medium and low elevation. $^{\dagger\dagger}NH_4Cl$ extracted soil labile pool; †With Sr/Ca. §Centennial scale study: 1870–1990. $^{\$}$Average of snow, wet and bulk deposition. ‡Average of foliar and stemwood. Superscript numbers are references; $^{¥}$See supplementary material for references.

deposition by assuming that chemical changes in tree rings reflect the long-term changes in the soil solution ion pools.

Calcium isotopes for understanding calcium biogeochemistry

A few studies presented promising use of Ca isotopes ($\delta^{44/40}$Ca and $\delta^{44/42}$Ca) to directly trace the origin and fate of Ca in terrestrial settings rather than relying on surrogate Sr isotopes[10,14,112–114]. With double-spike TIMS and MC-ICP-MS, substantial progress has been made in past few years to understand fractionation of Ca isotopes in different fluxes of terrestrial settings, plants[18], atmospheric deposits[112], soil[113] and weathering environment[14]. Ca isotopic studies suggested: (a) fractionation (biological) and isotopic gradients from vegetation to soils[10,20], and along the soil depth and soil solution[14], (b) preferential uptake of lighter Ca isotopes from the soil[18], (c) fractionation along the transpiration stream[10,113], and (d) inorganic Ca isotope fractionation magnitude in terrestrial settings being highly dependent on water supply, solution chemistry and transport[115].

Such understanding has attracted interest towards the use of Ca isotopes ($\delta^{44/40}$Ca and $\delta^{44/42}$Ca) to reveal Ca sources, fluxes and interlinkages in various terrestrial units bypassing Sr isotopes[18,113]. Hindshaw *et al.*[20] studied Ca biogeochemical cycling behaviour within alpine soil chronosequences. Their study showed that biological cycling and adsorption dominate the Ca biogeochemical cycle. A recent study in Ca-poor old-growth forested catchment traced Ca source to atmospheric deposition[115]. This study based on the differences in ^{40}Ca enrichment between exchangeable Ca pool and local vegetation suggested the ability of forests to rely on atmospheric deposition and recycling of forest-floor litter accretion for their nutritional requirements, instead of the exchangeable ion pools of mineral soils.

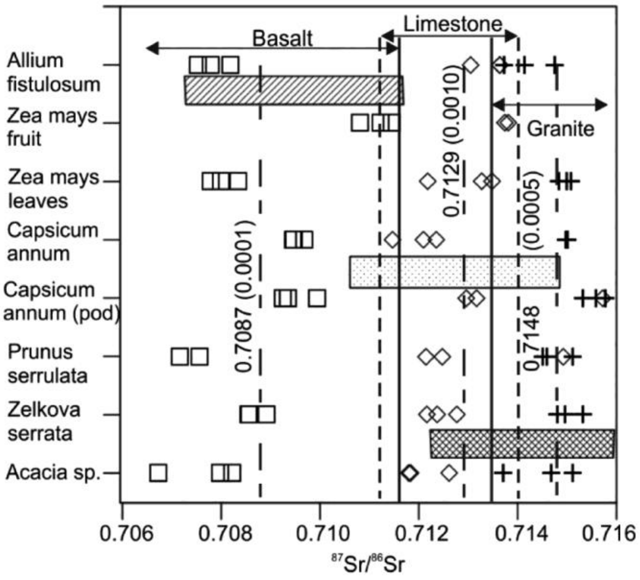

Figure 3. ^{87}Sr/^{86}Sr composition of soil from three Korean bedrock lithologies and plant functional types growing over them (from Stable Isotope Laboratory, KBSI, Ochang).

Ecological interactions: reconstruction of plant–animal associations and habitat use

In the ecosystem management, bracketing ecological interactions between autotrophs and heterotrophs is necessary to prioritize their conservation. Types of interaction, antagonistic or mutualistic, with their period and frequency, reciprocally influence the structure, composition and function of associations for both. To understand the biodiversity effects and ecosystem functioning, learning the functional role of interactions across the trophic levels is imperative[116]. To strengthen existing knowledge, SI are used to recognize the interactions between heterotrophs and autotrophs during different life-stages[117,118], among individual species[119] or within a population[120].

Interactions in terrestrial ecosystems

δ^{13}C, δ^{15}N and δD isotopic variations that result from interpopulation, intrapopulation or individual dietary preferences and allocations helped in understanding biotic interaction between animals and plants[119,121,122] (Figure 4). Within terrestrial ecosystem isotopic signatures, especially carbon and nitrogen, helped identify (a) foraging habitat preferences among coexisting species[123], (b) intrapopulation seasonal[44] and/or ontogenetic[116] changed spatial preference for habitat and individual specialization[119], (c) herbivory from omnivory and carnivory among coexisting population[121], and (d) changed foraging tendencies into grazers or browsers[42].

One of the earliest applications of stable isotopes was seen in the case of *Loxodonta africana* and *Elephas maximus*, where they are used to understand their preference to specific habitats (Table 4). Within the realm of long-term plant–animal associations, C, N and Sr isotopes revealed changes in elephant–vegetation association from browse to graze in response to changing vegetation conditions (diminishing browse cover) in Amboseli National Park, Kenya[124]. Change in the preferences of megaherbivores from browse to graze was different from the earlier diet patterns of elephant populations residing in Tsavo National Park, Kenya[125]. Other studies with $\delta^{13}C_{\mathrm{hairs}}$ (ref. 42) and with $\delta^{13}C_{\mathrm{faeces}}$ (ref. 43) discriminated seasonal specialization for two different populations from Central Kenya and eastern South Africa respectively. Codron *et al.*[43] found two different foraging associations; the north population was consuming more grass (~40%) during the dry season compared to its southern counterpart (~10%).

The latest results of the seasonal elephant–habitat associations in the Kruger National Park, South Africa showed wet-season higher C4 graze associations and dry season C3 browse associations[44]. $\delta^{13}C_{\text{Bone collagen}}$-based long-term study of *E. maximus*[117] did not recognize any change in the foraging association of Asian elephants, but recorded ontogenetic browse and graze shifts among adult and young elephants respectively.

$\delta^{13}C$ signatures of hair, tooth enamel and bone collagen were studied to explore the strength of browse- and graze-associations of 45 African bovids[126]. Similar inquisition about *Hippopotamus amphibius* was expounded by Cerling *et al.*[127], which were earlier considered pure grazers. They recorded relatively depleted (in comparison of other co-existing obligate browsers and grazers) $\delta^{13}C$ and $\delta^{18}O$ isotopic composition in the hairs and tooth enamel, showing their dietary associations with C3 biomass. Isotopic cues further reflected importance of semi-aquatic habitats, which help *Hippopotamus* seasonally modulate diets.

Sponheimer *et al.*[128] analysed $\delta^{13}C$ and $\delta^{15}N$ isotopes in the hair samples of Ganta chimpanzee's (*Pan troglodytes versus*) of Senegal savanna to understand their dietary preferences. The average $\delta^{13}C$ of –22.2‰ (range 21.5‰–23.1‰) of chimpanzee hair suggested that they exploit C3 plant resources almost exclusively, despite the relative abundance of C4 resources in their local environment. Another study from Liberian rainforests observed dietary differences in three different life stages – infant, juvenile and adult – of male and female Ganta chimpanzee, using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ signatures[129]. Hobson *et al.*[130] used hair $\delta^{13}C$ and $\delta^{15}N$ to unravel the trophic segregation and foraging specialization between the two upper Columbia River Basin-inhabiting bear species – black bear (*Ursus americanus*) and grizzly bear (*Ursus arctos*). They found a significant overlap of $\delta^{13}C$ and $\delta^{15}N$ in both the species, for both the sexes and confirmed their herbivore specialization (>91%). Within three grizzly bear conspecifics groups inhibiting the Mackenzie Delta, Edwards *et al.*[120] distinguished groups that practice near to complete herbivory from other omnivorous groups with the aid of $\delta^{13}C_{\text{hair}}$, $\delta^{13}C_{\text{claw}}$ and $\delta^{15}N_{\text{hair}}$.

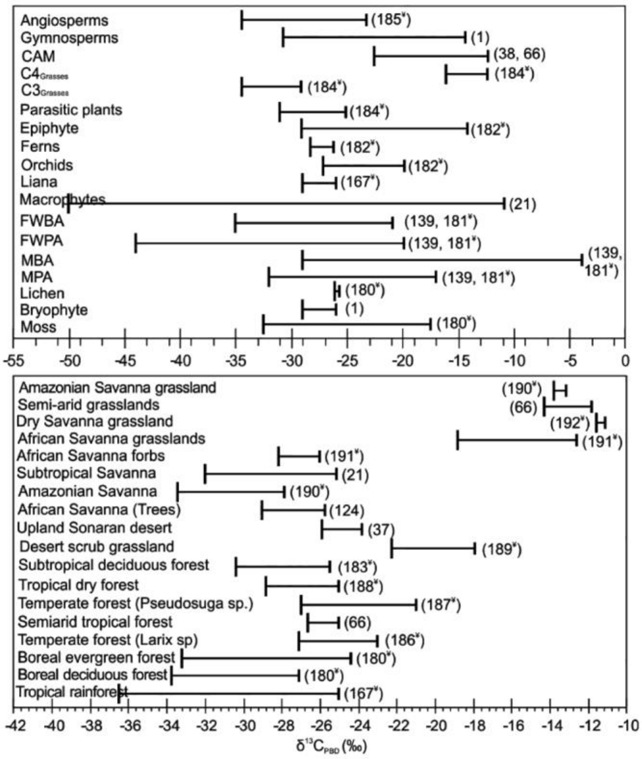

Figure 4. Carbon isotope variations in (***a***) different plant-forms and (***b***) different ecosystems. MPA, Marine planktonic algae; MBA, Marine benthic algae; FWPA, Freshwater planktonic algae; FWBA, Freshwater benthic algae. Numbers in parenthesis are references. ¥ represents references in Supplementary Material (see online).

Treydte *et al.*[131] studied the importance of vegetation growing in old, abandoned cattle ranches (bomas) for common warthog (*Phacochoerus africanus*) in coastal Tanzania. They studied plant, faeces and soil for their $\delta^{15}N$ and $\delta^{13}C$ isotopes. High $\delta^{15}N$ values of warthog faeces and similar high $\delta^{15}N$ values of the vegetation growing in these abandoned ranches suggested that warthogs preferentially fed on the bomas vegetation. $\delta^{13}C$ values of faeces indicated that warthogs ingested on average 83% C4 grasses, especially during dry season.

An example of plant–animal association was recorded for two nectar-feeding bat species, *Leptonycteris curasoae* ($\delta^{13}C$–11.76‰) and *Glossophaga longirostris*[132]. $\delta^{13}C$ of pectoral muscle tissues of both bat species (–11.76‰ to –13.28‰) overlapped with $\delta^{13}C$ of cacti and agave species (–12.47‰). Voigt *et al.*[133] suggested obligate phytophagous feeding behaviour of pteropodid bats. They found $\delta^{15}N$ of wing membrane in pteropodid bats was significantly lower than in obligate insectivorous bats (rhinolophoid, vespertilionid or emballonurid). However, they found $\delta^{15}N$ signatures of pteropodid bats similar to that of Old World stenodermatines bats. This helped them underline that pteropodid bats fulfil their dietary nitrogen requirements from plant matter.

Plant–bird association

Ecological plant–bird associations were examined with SI, particularly δD (ref. 134). SI were used to shore-up either direct association of plants with birds[39] or migration pattern to discern resource dependencies[40]. In the tropical rainforests, whole-blood C and N isotopic analysis of 23 bird species revealed their degree and intensity of association with plant functional groups for dietary requirements[39]. Isotopic differences segregated birds that obtained dietary protein by exclusively forging an association with plants from those that showed omnivory or

Table 4. Association of elephants with resident vegetation in different biomes

Region	Species	Part used	Age-class (years) and types	δ^{13}C (‰)	δ^{15}N (‰)	Remarks
Tsvao National Park, Kenya[125]	*Loxodonta africana*	Bone	0–5	–16.92 to –1.26 (–19.17)	12.86 to 14.27 (13.06)	Similar proportion of C3 and C4 in *L. africana* diet despite conversion of woodlands into grasslands.
		Collagen	5–25	–16.89 to –21.98 (18.67)	9.57 to 15.72 (13.58)	
			25–60	–17.70 to –21.03 (–19.28)	11.58 to 16.61 (14.01)	
		C3 plants		–25.4 to –27.80 (–26.62)		
		C4 plants		–11.90 to –29.0 (–15.17)		
Southern India[117]	*Elephas maximus*	Bone	0–1	–9.4 to –21.5 (16.4)	–	Difference in δ^{13}C of adult and sub-adult showed lower preference for C3 plants by sub-adult. Adults predominantly browsers, whereas younger animals are predominantly grazers.
		Collagen	1–25	–10.5 to –22.7 (15.3)	–	
			26–60	–16.0 to –20.3 (18.6)	–	
			Mother	–13.8	–	
			Foetus	–17.1	–	
		C3 plants		–27.2	–	
		C4 plants		–12.8	–	
Amboseli basin, Kenya[124]	*L. africana*	Bone	Male			Shift in browser to grazer illustrating behavioural and ecological shifts in foraging.
		Collagen	5–20	–15.5 to –12.0 (12.75)	–	
			>20	–12.6 to –19.4 (–15.9)	–	
			Female			
			5–20	–119 to –14.1 (13.14)	–	
			>20	–14.7 to –18.8 (16.68)	–	
Northern Kenya[42]	*L. africana*	Hair	Migratory	–19.04 to –23.14 (21.52)	~6.0 to 10.5 (~7.5)	Short-term diet change noticed. Migratory and resident population occupied two isotopically variable habitats.
			Resident	–18.54 to –24.96 (21.84)	~9.2 to 13.9 (~11.5)	
		Mesic vegetation			1.7 ± 0.5	
		Xeric vegetation			7.9 ± 0.7	
Kruger National Park, Kenya[44]	*L. africana*	Faeces	Dry season	–20.8 to –26.3 (–23.4)		Elephants tend to graze during rainy season when fresh grass cover is maximum and browse in the dry season when the grass cover is reduced.
			Rainy Season	–17.7 to – 24.8 (20.9)		

complete insectivory. For four coastal passerine birds δ^{13}C and δ^{15}N signatures revealed four different habitat fidelity: northern parula–oak forest, white-eyed vireos–shrubland, brown-headed nuthatches–pine forest, and painted bunting–salt marsh habitats[135]. Stable isotopes also identified sexual dimorphism for habitat and molting site fidelity, for instance, capercaillie females use micro-habitat diverse treeline areas, while males are restricted to inner areas of forests[136].

As migratory birds access resources from two or more spatially separated habitats, an insight into the interlink between habitats is critical for their conservation. Isotopic signatures in metabolically inert feathers and hairs, which change after every molt, are used to discern associations that a bird makes with a habitat during its migration, stopover and philopatry[137]. Based on the δ^{13}C values of desert dove (*Zenaida asiatica*) liver and saguaro fruit/nectar, an ecological association was established between the two; during saguaro fruition, most of the carbon incorporated in the dove tissues comes from saguaro[38]. With δ^{13}C and δD, several other studies assigned the preference of migratory birds for breeding and winter habitats[93,138]. Based on the premise that $\delta D_{\text{rainfall}}$ (Figure 5) and to a lesser extent $\delta^{13}C_{\text{plants}}$ change with altitude, distribution of eight species of humming bird communities in the Ecuadorian Andes was assigned[40].

Norris *et al.*[41] used δ^{13}C of red blood cells, plasma and nestling feathers to track habitat use of a long-distance American redstart. They unravelled the carry-over effect of quality of the overwintering Caribbean mangrove dry scrubs to the breeding North-America deciduous mixed forests. Yohannes *et al.*[139] underlined species-specific fidelity during the moulting stopover among nine passerines with two stopovers. The relatively low mean δ^{13}C, but high δ^{15}N values of three warbler and two white-throat species reflected their higher dependence on C3 plants and/or a drier habitat than other warbler species that showed fidelity towards mesic- and C4-habitats.

Associations in aquatic ecosystems

There are uncertainties regarding the predominant association of aquatic heterotrophs for their dietary requirements with autotrophic life-forms (macrophytes, phytoplankton, epiphytic or periphytic microalgae)[140]. Differences in the isotopic signatures helped identify the origin of carbon and hence energy source consumed by aquatic heterotrophs. Differences in the sources and biochemical carbon assimilation pathways and its incorporation in autotroph biomass provide disparate mean δ^{13}C among the various aquatic (plant) life-forms and their detritus[140]. Also, δ^{13}C of aquatic flora alters very little with decomposition and is different from terrestrial autotrophs and terrestrial litter δ^{13}C. Aquatic ecosystem isotopic studies indicated that despite sizeable vascular plant biomass in such systems, δ^{13}C signatures of aquatic animals showed phytoplankton/algal-based associations, depicting their trophic importance[141]. However, this is different from several other studies which reported paradoxical results suggesting the importance of marcophytes[142,143] and

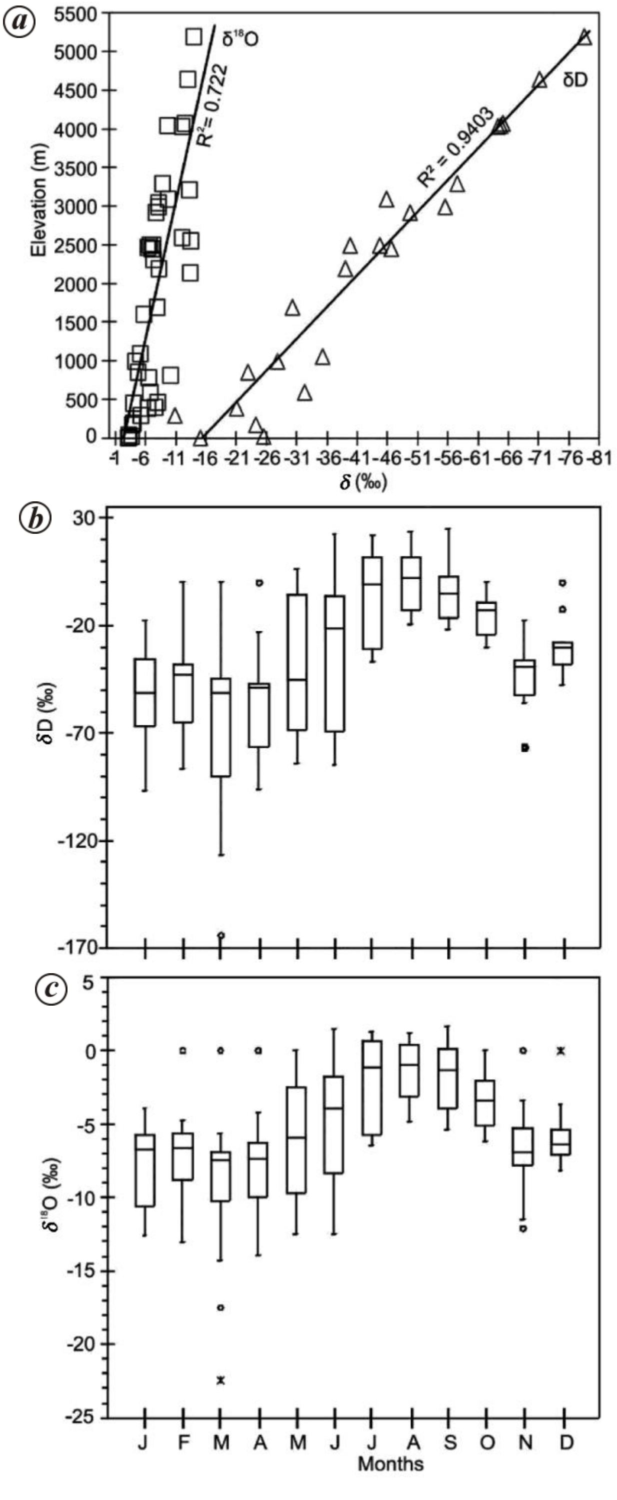

Figure 5. Hydrogen and oxygen isotopic composition of precipitation along the (***a***) elevational gradient and (***b***, ***c***) monthly gradient (after Gonfiantini *et al.*[192]; see Supplementary Material online).

allochthonous dependencies[140]. McClellan *et al.*[118] unravelled the ontogenetic oceanic (small juveniles *Caretta–Sargassum* sp. and nektonic organisms) to neritic shift (large juveniles *Caretta*-demersal foraging) in the habitat and diet of the *Caretta caretta*.

In an interesting study for characterizing the ecological role of dominant brown algae against co-dominant red algae in Northern Brittany habitats, Schaal *et al.*[144] with isotopic cues recorded the preference of aquatic grazers for red algae over dominant brown algae. The importance of aquatic vegetation together with microhabitat specificity during winter for the semi-aquatic beavers inhabiting the subarctic Quebec region was appreciated by Milligan and Humphries[145]. $\delta^{13}C$ analysis distinguished beavers residing in lentic habitat, which primarily depend on aquatic vegetation in winter and autumn for their dietary needs from the beaver population residing in lotic habitats.

Autochthonous subsidies for plant: nutrient transfer from aquatic systems to land

For species-cum-habitat management, understanding the patterns of functional relationships between two (or more) discrete ecosystems to decipher the material flow (subsidies) and ecosystem function is required, where these subsidies (multi-) linkages are inherent and inextricable. Studies using SI as integrator ascertained the reverse linkage of plant nutritional dependence on autochthonous material and nutrient transport from visiting animals. This phenomenon is of importance in the riparian and coastal habitats[146]. Within the riparian habitats, SI helped elucidate the animal-bound N transfer to the surrounding vegetation. In the riparian setting, it was found that vegetation depends on the spawning salmon and its decomposed carcasses for nitrogen supply[147]. Such studies have reported the enrichment of streams with ^{15}N and riparian vegetation shortly after decomposition of the salmon carcasses[146,147].

A number of $\delta^{13}C$ and $\delta^{15}N$ studies asserted the ecological association between sea birds that nest and roost in the coastal regions. They help in insular transport of islands and marine subsidies to the terrestrial vegetations[146–148]. For example, $\delta^{13}C$ and $\delta^{15}N$ signatures revealed the positive influence of seagull guano on the chenopod community by causing spatial variation in the soil chemical properties[149]. The effects of guano deposition on soil chemistry and its consequences for herbaceous plant communities in 11 bird-visited and non-bird visited islands were studied by Wait *et al.*[150]. They found higher species richness and productivity in the bird-visited sites than those not visited by birds. $\delta^{15}N$ signatures indicated the origin of higher soil nitrogen content and that its availability to plants was from guano deposit.

Mizota[146] and García *et al.*[151] presented the negative effect of these subsidies on the species diversity mediated by the altered chemical soil conditions. The nitrogen isotopic study by Mizota revealed the adverse impact of black-tailed gull-transported enhanced N supply on the native *Pinus denisflora* and other species. However, several exotic plants flourished in such areas of enhanced nitrogen supply.

In an interesting study, Hilderbrand *et al.*[152] unravelled the association of bear with salmon and the flow of marine N from salmon to *Picea glauca* via brown bear that feeds on salmon. Aquatic mammals also define productivity of shoreline plants by introducing marine subsidies in the form of nutrients on land masses. Fariña *et al.*[153] demonstrated that the Galapagos sea lion is an effective vector to transport marine nutrients to shoreline plants. Crait and Ben-David[154] documented significant relationship between nitrogen supply in the river otter faeces to soil and subsequent increase in nitrogen concentration in the plants around Yellowstone Lake, USA.

Summary

Stable isotopes play a key role in understanding the key processes of the biosphere. This review highlights how SI help ecologists to understand ecological phenomena like faunal associations, ecohydrology and biogeochemical cycling. SI have also been used in animal ecology, soil ecology, hydrology, climate science and dendrochronological studies to reconstruct past climates. In animal ecology, signatures of stable SI are used to study dietary preferences, including resource polymorphism and ontogenetic specialization, trophic niche-differentiation, energy flow from one trophic level to another within a terrestrial and aquatic food web, trophic structure and both short and long transmigrations, including marine and insect migrations. The use of SI in conjugation with existing ecosystem analysis tools and tracers has the potential to elucidate complex relationships in ecosystem analysis. However, recently introduced Ca isotope needs extensive understanding before it can be used in ecological underpinning. More insights are required regarding fractionating behaviour of calcium isotopes and the factors responsible for the same.

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1. Smith, B. N. and Epstein, S., Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiol.*, 1971, **47**, 380–383.
2. Farquhar, G. D., Ehleringer, J. R. and Hubick, K. T., Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol.*, 1989, **40**, 503–537.
3. Hoef, J., *Stable Isotope Geochemistry*, Springer-Verlag, Berlin, 2009.
4. O'Leary, M. H., Carbon isotope fractionation in plants. *Phytochemistry*, 1981, **20**, 553–567.
5. Åberg, G., Jacks, G., Wickman, T. and Hamilton, P. J., Strontium isotopes in trees as an indicator for calcium availability. *Catena*, 1990, **17**, 1–11.
6. Nadelhoffer, K. J. and Fry, B., Nitrogen isotopes in forest ecosystems. In *Stable Isotopes in Ecology and Environmental Science* (eds Lajtha, K. and Michener, R. H.), Blackwell, UK, 1994, pp. 22–44.

7. Schulze, E. D., Chapin-III, F. S. and Gebauer, G., Nitrogen acquisition and isotope differences among life-forms at the northern treeline of Alaska. *Oecologia*, 1994, **100**, 406–412.
8. Näsholm, T., Ekbald, A., Nordin, A., Giesler, R., Högeberg, M. and Högberg, P., Boreal forest plants take up organic nitrogen. *Nature*, 1998, **392**, 914–917.
9. Meinzer, F. C., Andrade, J. L., Goldstein, G., Holbrook, N. M., Cavelier, J. and Wright, S. J., Partitioning of soil water among canopy trees in a seasonally dry tropical forest. *Oecologia*, 1999, **121**, 293–301.
10. Page, B. D., Bullen, T. D. and Mitchell, M. J., Influence of calcium availability and tree species on Ca isotope fractionation in soil and vegetation. *Biogeochemistry*, 2008, **88**, 1–13.
11. Ehleringer, J. R. and Dawson, T. E., Water uptake by plants: perspective from stable isotopes composition. *Plant Cell Environ.*, 1992, **15**, 1073–1082.
12. Dawson, T. E. and Ehleringer, J. R., Stream side trees that do not use stream water. *Nature*, 1991, **350**, 335–337.
13. Querejeta, J. I., Estrada-Medina, H., Allen, M. F. and Jiménez-Osornio, J. J., Water source partitioning among trees growing on shallow karst soils in a seasonally dry tropical climate. *Oecologia*, 2007, **152**, 26–36.
14. Cenki-Tok, B., Chabaux, F., Schmitt, A. D., Pierret, M. C., Viville, D., Bagard, M. L. and Stille, P., The impact of water–rock interaction and vegetation on calcium isotope fractionation in soil and stream waters of a small forested catchment. *Geochim. Cosmochim. Acta*, 2009, **73**, 2215–2228.
15. Evans, R. D. and Ehleringer, J. R., Water and nitrogen dynamics in an arid environment. *Oecologia*, 1994, **199**, 233–242.
16. Miller, A. E., Bowman, W. D. and Suding, K. N., Plant uptake of inorganic and organic nitrogen : neighbour identity matters. *Ecology*, 2007, **88**, 1832–1840.
17. Ashton, I. W., Miller, A. E., Bowman, W. D. and Suding, K. N., Niche complementarity due to plasticity in resource use: plant partitioning of chemical N-forms. *Ecology*, 2010, **91**, 3252–3260.
18. Holmden, C. and Bélanger, N., Ca isotope cycling in a forested ecosystem. *Geochim. Cosmochim. Acta*, 2010, **74**, 995–1015.
19. Perakis, S. S., Sinkhorn, E. R. and Compton, J. E., δ^{15}N constraints on long-term nitrogen balances in temperate forests. *Oecologia*, 2011, **167**, 793–803.
20. Hindshaw, R. S., Reynolds, B. C., Wiederhold, J. G., Kretzschmar, R. and Bourdon, B., Calcium isotopes in a proglacial weathering environment: Damma glacier, Switzerland. *Geochim. Cosmochim. Acta*, 2011, **75**, 106–118.
21. Boutton, T. W., Archer, S. R. and Midwood, A. J., Stable isotope in ecosystem science: structure function and dynamics of a subtropical savanna. *Rapid Commun. Mass Sp.*, 1999, **13**, 1263–1277.
22. Craine, J. M., Ballantyne, F., Peel, M., Zambatis, N., Morrow, C. and Stock, W. D., Grazing and landscape controls on nitrogen availability across 330 South African savanna sites. *Aust. Ecol.*, 2009, **34**, 731–740.
23. Schmidt, S., Lamble, R. E., Fensham, R. J. and Siddique, I., Effects of woody vegetation clearing on nutrient and carbon relations of semi-arid dystrophic savanna. *Plant Soil*, 2010, **331**, 79–90.
24. Nippert, J. B. and Knapp, A. K., Soil water partitioning contributes to species coexistence in tallgrass prairie. *Oikos*, 2007, **116**, 1017–1029.
25. McLauchlan, K. K., Ferguson, C. J., Wilson, I. E., Ocheltree, T. W. and Craine, J. M., Thirteen decades of foliar isotopes indicate declining nitrogen availability in central North American grasslands. *New Phytol.*, 2010, **187**, 1135–1145.
26. McPherson, G. R., Boutton, T. W. and Midwood, A. J., Stable carbon isotope analysis of soil organic matter illustrates vegetation change at the grassland/woodland boundary in southeastern Arizona USA. *Oecologia*, 1993, **93**, 95–101.
27. Wang, Y., Cerling, T. E. and Effland, W. R., Stable isotope ratios of soil carbonate and soil organic matter as indicators of forest invasion of prairie near Ames Iowa. *Oecologia*, 1993, **95**, 365–369.
28. Sanaiotti, T. M., Martinelli, L. A., Victoria, R. L., Trumbore, S. E. and Camargo, P. B., Past vegetation changes in Amazon savannas determined using carbon isotopes of soil organic matter. *Biotropica*, 2002, **34**, 2–16.
29. Bai, E., Boutton, T. W., Liu, F., Wu, B. and Archer, S. R., Spatial patterns of soil δ^{13}C reveal grassland-to-woodland successional processes. *Org. Geochem.*, 2012, **42**, 1512–1518.
30. Novák, M., Kirchner, J. W., Fottová, D., Prěchová, I., Jaăcková, I., Krám, P. and Hruska, J., Isotopic evidence for processes of sulfur retention/release in 13 forested catchments spanning a strong pollution gradient (Czech Republic Central Europe). *Global Biogeochem. Cycles*, 2005, **19**, GB4012.
31. Pardo, L. H., McNulty, S. G., Boggs, J. L. and Duke, S., Regional patterns in foliar 15N across a gradient of nitrogen deposition in the northeastern US. *Environ. Pollut.*, 2007, **149**, 293–302.
32. Lee, K. S., Bong, Y. S., Lee, D., Kim, Y. and Kim, K., Tracing the sources of nitrate in the Han River watershed in Korea, using δ^{15}N–NO$_3^-$ and δ^{18}O–NO$_3^-$ values. *Sci. Total Environ.*, 2008, **395**, 117–124.
33. Knohl, A., Werner, R. A., Brand, W. A. and Buchmann, N., Short-term variations in δ^{13}C of ecosystem respiration reveals link between assimilation and respiration in a deciduous forest. *Oecologia*, 2005, **142**, 70–82.
34. Gavrichkova, O. *et al.*, Short-term natural δ^{13}C and δ^{18}O variations in pools and fluxes in a beech forest: the transfer of isotopic signal from recent photosynthates to soil respired CO$_2$. *Biogeosciences*, 2011, **8**, 2833–2846.
35. Dawson, T. E., Hydraulic lift and water use by plants: implications for water balance performance and plant–plant interactions. *Oecologia*, 1993, **95**, 565–574.
36. Kurz-Besson, C. *et al.*, Hydraulic lift in cork oak trees in a savanna type Mediterranean ecosystem and its contribution to the local water balance. *Plant Soil*, 2006, **282**, 361–378.
37. Armas, C., Padilla, F. M., Pugnaire, F. I. and Jackson, R. B., Hydraulic lift and tolerance to salinity of semiarid species: consequences for species interactions. *Oecologia*, 2010, **162**, 11–21.
38. Wolf, B. O. and del Rio, C. M., Use of saguaro fruit by white-winged doves: isotopic evidence of a tight ecological association. *Oecologia*, 2000, **124**, 536–543.
39. Herrera, L. G., Hobson, K. A., Rodríguez, G. M. and Hernandez, P., Trophic partitioning in tropical rain forest birds: insights from stable isotope analysis. *Oecologia*, 2003, **136**, 439–444.
40. Hobson, K. A., Wassenaar, L. I., Milá, B., Lovette, I., Dingle, C., and Smith, T. B., Stable isotope as indicator of altitudinal distributions and movement in an Ecuadorean hummingbird community. *Oecologia*, 2003, **136**, 302–308.
41. Norris, D. R., Marra, P. P., Kyser, T. K. and Ratcliffe, L. M., Tracking habitat use of long-distance migratory bird, the American redstart *Setophaga ruticilla*, using stable isotopes of cellular blood. *J. Avian Biol.*, 2005, **36**, 164–170.
42. Cerling, T. E. *et al.*, Stable isotopes in elephant hair document migration patterns and diet changes. *Proc. Natl. Acad. Sci., USA*, 2006, **103**, 371–373.
43. Codron, J., Lee-Thorp, J. A., Sponheimer, M., Codron, D., Grant, R. C. and de Ruiter, D. J., Elephant (*Loxodonta africana*) diets in Kruger National Park, South Africa: Spatial and landscape differences. *J. Mammal.*, 2006, **87**, 27–34.
44. Codron, J. *et al.*, Landscape-scale feeding patterns of African elephant inferred from carbon isotope analysis of feces. *Oecologia*, 2011, **165**, 89–99.
45. Poszwa, A. *et al.*, Retrospective isotopic study of spruce decline in the Vogas Mountains (France). *Water Air Soil Pollut.*, 2003, **3**, 201–222.

46. Drouet, Th., Herbauts, J., Gruber, W. and Demaiffe, D., Strontium isotope composition as tracers of calcium sources in two forested ecosystems in Belgium. *Geoderma*, 2005, **126**, 201–223.
47. Blum, J. D., Dasch, A. A., Hamburg, S. P., Yanai, R. D. and Arthur, M. A., Use of foliar Ca/Sr discrimination and ^{87}Sr/^{86}Sr ratios to determine soil Ca sources to sugar maple foliage in a northern hardwood forest. *Biogeochemistry*, 2008, **87**, 287–296.
48. Song, B. Y., Gautam, M. K., Ryu, J. S., Lee, D. and Lee, K. S., Effects of bedrock on the chemical and Sr isotopic compositions of plants. *Environ. Earth Sci.*, 2015, **74**, 829–837.
49. Donavan, L. A. and Ehleringer, J. R., Water stress and use of summer precipitation in a great basin shrub community. *Funct. Ecol.*, 1994, **8**, 289–297.
50. Kahmen, A., Renker, C., Unsicker, S. B. and Buchmann, N., Niche complementarity for nitrogen: an explanation for the biodiversity and ecosystem functioning relationships? *Ecology*, 2006, **87**, 1244–1255.
51. Guehl, J. M. *et al.*, Functional diversity in an Amazonian rainforest in French Guyana: a dual isotope approach (δ^{15}N and δ^{13}C). *Oecologia*, 1998, **116**, 316–330.
52. Pornon, A., Escarvage, N. and Lamaze, T., Complementarity in mineral nitrogen use among dominant plant species in a subalpine community. *Am. J. Bot.*, 2007, **94**, 1778–1785.
53. Houlton, B. Z., Sigman, D. M., Schuur, E. A. G. and Hedin, L. O., A climate-driven switch in plant nitrogen acquisition within tropical forest communities. *Proc. Natl. Acad. Sci., USA*, 2007, **104**, 8902–8906.
54. Filella, I. and Peñuelas, J., Partitioning of water and nitrogen in co-occurring Mediterranean woody shrub species of different evolutionary history. *Oecologia*, 2003, **137**, 51–61.
55. Stratton, L. C., Goldstein, G. and Meinzer, F. C., Temporal and spatial partitioning of water resources among eight woody species in a Hawaiian dry forest. *Oecologia*, 2000, **124**, 309–317.
56. West, A. G., Hultine, K. R., Burtch, K. G. and Ehleringer, J. R., Seasonal variations in moisture use in Piñon junipor woodlands. *Oecologia*, 2007, **153**, 787–798.
57. Novaplansky, A. and Goldberg, D. E., Effects of water pulsing on individual performance and competitive hierarchies in plants. *J. Veg. Sci.*, 2001, **12**, 199–208.
58. Williams, D. G. and Ehleringer, J. R., Intra and interspecific variations for summer precipitation use in Pinyon-juniper woodlands. *Ecol. Monogr.*, 2000, **70**, 517–537.
59. Knowaljow, E. and Fernãndez, R. J., Differential utilization of a shallow water pulse by six shrub species in Patagonian steepe. *J. Arid Environ.*, 2011, **75**, 211–214.
60. Ogle, K., Wolpert, R. L. and Reynolds, J. F., Reconstructing plant root area and water uptake profiles. *Ecology*, 2004, **85**, 1967–1978.
61. Xu, Q., Li, H., Chen, J., Chang, X., Liu, S. and An, S., Water use pattern of the three species in subalpine forest southwest China: the deuterium isotope approach. *Ecohydrology*, 2011, **4**, 236–244.
62. Caldwell, M. M., Dawson, T. E. and Richards, J. H., Hydraulic lift: consequences of water efflux from roots of plants. *Oecologia*, 1998, **113**, 151–161.
63. Jackson, P. C. *et al.*, Partitioning of soil water among tree species in a Brazilian Cerrado ecosystems. *Tree Physiol*., 1999, **19**, 717–724.
64. Moreira, M. Z. *et al.*, Hydraulic lift in a Neotropical savanna. *Funct. Ecol.*, 2003, **17**, 573–581.
65. Desjardins, T., Filho, A. C., Mariotti, A., Chauvel, A. and Girardin, C., Changes of the forest–savanna boundary in Brazilian Amazonia during the Holocene revealed by stable isotope ratios of soil organic carbon. *Oecologia*, 1996, **108**, 749–756.
66. Biedenbender, S. H., McClaran, M. P., Quade, J. and Weltz, M. A., Landscape patterns of vegetation change indicated by soil carbon isotope composition. *Geoderma*, 2004, **119**, 69–83.
67. Krull, E. S. *et al.*, Recent vegetation changes in central Queensland Australia: evidence from δ^{13}C and ^{14}C analyses of soil organic matter. *Geoderma*, 2005, **126**, 241–259.
68. Dalal, R. C., Cowie, B. A., Allen, D. E. and Yo, S. A., Assessing carbon lability organic matter from δ^{13}C changes following land use change from C$_3$ native vegetation to C$_4$ pastures. *Soil Res.*, 2011, **49**, 98–103.
69. Page, H., Lastra, M., Rodil, I. F., Briones, M. J. I. and Garrido, J., Effects of non-native *Spartina patens* on plant and sediment organic matter carbon incorporation into the local invertebrate community. *Biol. Invasions*, 2010, **12**, 3825–3828.
70. Liao, J. D., Boutton, T. W. and Jastrow, J. D., Organic matter turnover in soil physical fractions following woody plant invasion of grassland: evidence from natural ^{13}C and ^{15}N. *Soil Biol. Biochem.*, 2006, **38**, 3197–3210.
71. Fraterrigo, J. M., Strickland, M. S., Keiser., A. D. and Bradford, M. A., Nitrogen uptake and preference in a forest understorey following invasion by an exotic grass. *Oecologia*, 2011, **167**, 781–791.
72. Bai, E., Boutton, T. W., Liu, F., Wu, X. B., Archer, S. R. and Hallmark, C. T., Spatial variation of the stable nitrogen isotope ratio of woody plants along a topoedaphic gradient in a subtropical savanna. *Oecologia*, 2009, **159**, 493–503.
73. Werner, C., Zumkier, U., Beyschlag, W. and Máguas, C., High competitiveness of a resource demanding invasive acacia under low resource supply. *Plant Ecol.*, 2010, **206**, 83–96.
74. Subke, J. A., Hahn, V., Battipaglia, G., Linder, S., Buchmann, N. and Cotrufo, M. F., Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia*, 2004, **139**, 551–559.
75. Högberg, P. *et al.*, High temporal resolution tracing of photosynthates carbon from the tree canopy to forest soil microorganism. *New Phytol.*, 2008, **177**, 220–228.
76. Maseyk., K. *et al.*, Biotic and abiotic factors affecting the δ^{13}C of soil respired CO$_2$ in a Mediterranean oak woodland. *Isot. Environ. Health Stud.*, 2009, **45**, 343–359.
77. Sun, W., Resco, V. and Williams, D. G., Environmental and physiological controls on the carbon isotope composition of CO$_2$ respired by leaves and roots of a C$_3$ woody legume (*Prosopis velutina*) and a C$_4$ perennial grass (*Sporobolus wrightii*). *Plant Cell Environ.*, 2012, **35**, 567–577.
78. Millard, P., Midwood, A. J., Hunt, J. E., David, W. D. and Boutton, T. W., Partitioning soil surface CO$_2$ efflux into autotrophic and heterotrophic components using natural gradients in soil δ^{13}C in an undisturbed savanna soil. *Soil Biol. Biochem.*, 2008, **40**, 1575–1582.
79. Zhu, B. and Cheng, W., ^{13}C isotope fractionation during rhizosphere respiration of C$_3$ and C$_4$. *Plant Soil*, 2011, **342**, 277–287.
80. Araújo, A. C., Ometto, J. P. H. B., Dolman, A. J., Kruijt, B., Waterloo, M. J. and Ehleringer, J. R., Implications of CO$_2$ pooling on δ^{13}C of ecosystem respiration and leaves in Amazonian forest. *Biogeosciences*, 2008, **5**, 779–795.
81. Buchmann, N., Guhel, J. M., Barigah, T. S. and Ehlinger, J. R., Interseasonal comparison of CO$_2$ concentrations isotopic composition and carbon dynamics in an Amazonian rainforest (French Guiana). *Oecologia*, 1997, **110**, 120–131.
82. Ekblad, A. and Högberg, P., Natural abundance of ^{13}C in CO$_2$ respired from forest soils reveals speed of link between tree photosynthesis and root respiration. *Oecologia*, 2001, **127**, 305–308.
83. Bowling, D. R., Pataki, D. E. and Randerson, J. T., Carbon isotopes in terrestrial ecosystem pools and CO$_2$ fluxes. *New Phytol.*, 2008, **178**, 24–40.
84. Šantrůčková, H., Bird, M. I. and Llyod, J., Microbial processes and carbon isotope fractionation in tropical and temperate grassland soils. *Funct. Ecol.*, 2000, **14**, 108–114.
85. Blagodatskaya, E., Yuyukina, T., Blagodatsky, S. and Kuzyakov, Y., Turnover of organic matter and of microbial biomass under

C_3–C_4 vegetation change: consideration of ^{13}C fractionation and preferential substrate utilization. *Soil Biol. Biochem.*, 2011, **43**, 159–166.

86. Fernandez, I., Mahieu, N. and Cadisch, G., Carbon isotopic fractionation during decomposition of plants materials of different quality. *Global Biogeochem. Cycles*, 2003, **17**, 1075.
87. Rubino, M. *et al.*, Carbon input belowground is the major C flux contributing to leaf-litter mass loss: evidences from a ^{13}C labelled leaf litter experiment. *Soil Biol. Biochem.*, 2010, **42**, 1009–1016.
88. Ngao, J. and Cotrufo, M. F., Carbon isotope discrimination during litter decomposition can be explained by selective use of substrate with differing δ^{13}C. *Biogeosci. Dicuss.*, 2011, **8**, 51–82.
89. Kammer, A. and Hagedorn, F., Mineralization leaching and stabilization of ^{13}C-labelled leaf and twig litter in a beech forest soil. *Biogeosciences*, 2011, **8**, 2195–2208.
90. Wedin, D. A., Tieszen, L. L., Dewey, B. and Pastor, J., Carbon isotope dynamics during grass decomposition and soil organic matter. *Ecology*, 1995, **76**, 1383–1392.
91. Osono, T., Takeda, H. and Azuma, J.-I., Carbon isotope dynamics during leaf litter decomposition with reference to lignin fractions. *Ecol. Res.*, 2008, **23**, 51–55.
92. Millard, P., Midwood, A. J., Hunt, J. E., Barbour, M. M. and Whitehead, D., Quantifying the contribution of soil organic matter turnover to forest soil respiration using natural abundance δ^{13}C. *Soil Biol. Biochem*., 2010, **42**, 935–943.
93. Kaštovaská, E. and Šantrůčková, H., Fate and dynamics of recently fixed C in pasture plant–soil systems under field conditions. *Plant Soil*, 2007, **300**, 61–69.
94. Hagedorn, F., Saurer, M. and Blaser, P., A ^{13}C tracer study to identify the origin of dissolved organic carbon in forested mineral soils. *Eur. J. Soil Sci.*, 2004, **55**, 91–100.
95. Sanderman, J., Baldock, J. A. and Amundson, R., Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. *Biogeochemistry*, 2008, **89**, 181–198.
96. Agren, G. I. and Bosatta, E., Nitrogen saturation of terrestrial ecosystems. *Environ. Pollut*., 1988, **54**, 185–198.
97. Högberg, P., ^{15}N natural abundance in soil–plant systems. *New Phytol*., 1997, **137**, 179–203.
98. Robinson, D., δ^{15}N as an integrator of the nitrogen cycle. *Trends Ecol. Evol*., 2001, **16**, 153–162.
99. Kahmen, A., Wanek, W. and Buchmann, N., Foliar δ^{15}N values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia*, 2008, **156**, 861–870.
100. Martinelli, L. A. *et al.*, Nitrogen stable isotope composition of leaves and soil: tropical versus temperate forest. *Biogeochemistry*, 1999, **46**, 45–65.
101. Craine, J. M. *et al.*, Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol*., 2009, **183**, 980–992.
102. Averill, C. and Finzi, A., Increasing plant use of organic nitrogen with elevation is reflected in nitrogen uptake rates and ecosystem δ^{15}N. *Ecology*, 2011, **92**, 883–891.
103. Hobbie, E. A., Colpaert, J. V., White, M. W., Ouimette, A. P. and Macko, S. A., Nitrogen form availability and mycorhhizal colonization affect biomass and nitrogen isotope patterns in *Pinus sylvestris*. *Plant Soil*, 2008, **310**, 121–136.
104. Schmidt, S. and Stewart, G. R., δ^{15}N values of tropical savanna and monsoon forest species reflect root specializations and soil nitrogen status. *Oecologia*, 2003, **134**, 569–577.
105. Mitchell, M. J. *et al.*, Comparisons of watershed sulphur budget in southeast Canada and northeast US: a new approach and implications. *Biogeochemistry*, 2011, **103**, 181–207.
106. Mayer, B., Feger, K. H., Giesemann, A. and Jager, H. J., Interpretation of sulphur cycling in two catchments in the Black Forest (Germany) using stable sulfur and oxygen isotope data. *Biogeochemistry*, 1995, **30**, 31–58.
107. Morth, C. M., Torssander, P., Kjønaas, O. J., Stuanes, A. O., Moldan, F. and Giesler, R., Mineralization of organic sulfur delays recovery from anthropogenic acidification. *Environ. Sci. Technol*., 2005, **39**, 5234–5240.
108. Puig, R., Ávila, A. and Soler, A., Sulphur isotopes as tracers of the influence of a coal-fired power plant on a scot pine forest in Catalonia (Spain). *Atmos. Environ.*, 2008, **42**, 733–745.
109. Kennedy, M. J., Chadwick, O. A., Vitousek, P. M., Derry, L. A. and Hendricks, D. M. Changing sources of base ions during ecosystem development, Hawaiian Islands. *Geology*, 1998, **26**, 1015–1018.
110. Bélanger, N. and Holmden, C., Influence of landscape on the apportionment of Ca nutrition in a Boreal Shield forest of Saskatchewan (Canada) using ^{87}Sr/^{86}Sr as a tracer. *Can. J. Soil Sci.*, 2010, **90**, 267–288.
111. Berger, T. W., Swoboda, S., Prohaska, T. and Glatzel, G., The role of calcium uptake from deep soils for spruce (*Picea abies*) and beech (*Fagus sylvatica*). *For. Ecol. Manage.*, 2006, **229**, 234–246.
112. Schmitt, A. D. and Stille, P., The sources of calcium in wet atmospheric deposition: Ca–Sr isotope. *Geochim. Cosmochim. Acta*, 2005, **69**, 3463–3468.
113. Wiegand, B. A., Chadwick, O. A., Vitousek, P. M. and Wooden, J. L., Ca cycling and isotopic fluxes in forested ecosystems in Hawaii. *Geophys. Res. Lett*., 2005, **32**, L11404.
114. Ewing, S. A. *et al.*, Non-biological fractionation of stable Ca isotopes in soils of the Atcama Desert, Chile. *Geochim. Cosmochim. Acta*, 2008, **72**, 1096–1110.
115. Farkaš, J., Déjeant, A., Novák, M. and Jacobsen, S. B., Calcium isotope constraints on the uptake and sources of Ca^{2+} in a base-poor forest: a new concept of combining stable ($\delta^{44/42}$Ca) and radiogenic (ε_{ca}) signals. *Geochim. Cosmochim. Acta*, 2011, **75**, 7031–7046.
116. Strauss, S. Y. and Irwin, R. E., Ecological and evolutionary consequences of multispecies plant–animal interactions. *Annu. Rev. Ecol. Evol. Syst.*, 2004, **35**, 435–466.
117. Sukumar, R. and Ramesh, R., Stable isotope ratios in Asian elephant's collagen: implications for dietary studies. *Oecologia*, 1992, **91**, 536–539.
118. McClellan, C. M., Braun-McNeil, J., Avens, L., Wallace, B. P. and Read, A. J., Stable isotopes confirm a foraging dichotomy in juvenile loggerhead sea turtles. *J. Exp. Mar. Biol. Ecol.*, 2010, **387**, 44–51.
119. Bolnick, D. I. *et al.*, The ecology of individuals: incidence and implications of individual specialization. *Am. Nat*., 2003, **161**, 1–28.
120. Edwards, M. A., Derocher, A. E., Hobson, K. A., Branigan, M. and Nagy, J. A., Fast carnivores and slow herbivores: differential foraging strategies among grizzly bears in the Canadian Arctic. *Oecologia*, 2011, **165**, 877–889.
121. DeNiro, M. and Epstein, S., Carbon isotopic evidence for different feeding patterns in two hyrax species occupying the same habitat. *Science*, 1978, **201**, 906–908.
122. Post, D. M., Using stable isotopes to estimate trophic position: models methods and assumptions. *Ecology*, 2002, **83**, 703–718.
123. Yi, X. F., Carbon isotope analysis show dominance of C3 grasses in the feed selected by small herbivores at the alpine meadow at the Tibetan Plateau. *J. Anim. Feed Sci.*, 2006, **15**, 115–120.
124. Koch, P. L., Heisinger, J., Moss, C., Carlson, R. W., Fogel, M. L. and Behrensmeyer, A. K., Isotopic tracking of change in the diet and habitat use of African elephants. *Science*, 1995, **267**, 1340–1343.
125. Tieszen, L. L., Boutton, T. W., Ottichilo, W. K., Nelson, D. E. and Brandt, D. H., An assessment of long-term food habits of Tsavo elephants based on the stable carbon and nitrogen isotope ratios of bone collagen. *Afr. J. Ecol.*, 1989, **27**, 219–226.

126. Sponheimer, M. *et al.*, Diets of southern African bovidae: stable isotope evidence. *J. Mammal.*, 2003, **84**, 471–479.
127. Cerling, T. E. *et al.*, Stable isotope ecology of the common hippopotamus. *J. Zool.*, 2008, **276**, 204–212.
128. Sponheimer, M. *et al.*, Do savanna chimpanzees consume C4 resources? *J. Hum. Evolut.*, 2006, **51**, 128–133.
129. Smith, C. C., Morgan, M. R. and Pilbeam, D., Isotopic ecology and dietary profiles of Liberian chimpanzees. *J. Hum. Evol.*, 2010, **58**, 43–55.
130. Hobson, K. A., Mclellan, B. and Woods, J., Using stable carbon and nitrogen isotopes to infer trophic relationships among black and grizzly bears in the upper Columbia River basin, British Columbia. *Can. J. Zool.*, 2000, **78**, 1322–1339.
131. Treydte, A. C., Bernasconi, S. M., Kreuzer, M. and Edwards, P. J., Diet of the common warthog (*Phacochoerus africanus*) on former cattle grounds in a Tanzanian savanna. *J. Mammal*., 2006, **87**, 889–898.
132. Nassar, J. M., Beck, H., Sternberg, L. S. L. and Fleming, T. H., Dependence of cacti and agaves in nectar-feeding bats from Venezuelan arid zones. *J. Mammal.*, 2003, **84**, 106–116.
133. Voigt, C. C., Zubaid, A., Kunz, T. H. and Kingston, T., Sources of assimilated proteins in old and new world phytophagous bats. *Biotropica*, 2011, **43**, 108–113.
134. Ben-David, M., Flynn, R. W. and Schell, D. M., Annual and seasonal changes in diets of Martens: evidence from stable isotope analysis. *Oecologia*, 1997, **111**, 280–291.
135. Brittain, R. A., Schimmelmann, A. Parkhurst, D. F. and Craft, C. B., Habitat use by coastal birds inferred from stable carbon and nitrogen isotopes. *Estuaries Coasts*, 2012, **35**, 633–645.
136. Blanco-Fontao, B., Obeso, J. R., Banuelos, M. J. and Quevedo, M., Habitat partitioning and molting site fidelity in *Tetrao urogallus cantabricus* revealed through stable isotope analysis. *J. Ornithol.*, 2012, **155**, 555–562.
137. Rocque, D., Ben-David, M., Barry, R. P. and Winker, K., Assigning birds to wintering and breeding grounds using stable isotopes: lessons from two feather generations among three intercontinental migrants. *J. Ornithol*., 2006, **147**, 395–404.
138. Marra, P. P., Hobson, K. A. and Holmes, R. T., Linking winter and summer events in a migratory bird by using stable-carbon isotope. *Science*, 1998, **282**, 1884–1886.
139. Yohannes, E., Hobson, K. A. and Pearson, D. J., Feather stable isotope profiles reveal stopover habitat selection and site fidelity in nine migratory species moving through sub-Saharan Africa. *J. Avian Biol.*, 2007, **38**, 347–355.
140. Prasad, M. B. K. and Ramanathan, A. L., Organic matter characterization in a tropical estuarine–mangrove ecosystem of India: preliminary assessment by using stable isotope and lignin phenols. *Estuarine Coastal Shelf Sci.*, 2009, **84**, 617–624.
141. Moncreiff, C. A. and Sullivian, M. J., Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. *Mar. Ecol. Prog. Ser.*, 2001, **215**, 93–106.
142. Currin, C. A., Newell, S. Y. and Paerl, H. W., The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. *Mar. Ecol. Prog. Ser.*, 1995, **121**, 99–116.
143. Quan, W., Fu, C., Jin, B., Luo, Y., Li, B., Chen, J. and Wu, J., Tidal marshes as energy sources for commercially important nektonic organisms: stable isotope analysis. *Mar. Ecol. Prog. Ser.*, 2007, **352**, 89–99.
144. Schaal, G., Riera, P. and Leroux, C., Trophic ecology in a Northern Brittany (Batz Island, France) kelp (*Laminaria digitata*) forest as investigated through stable isotopes and chemical assays. *J. Sea Res.*, 2010, **63**, 24–35.
145. Milligan, H. E. and Humphries, M. M., The importance of aquatic vegetation in beaver diets and the seasonal and habitat specificity of aquatic–terrestrial ecosystem linkages in a subartic environment. *Okios*, 2010, **119**, 1877–1886.
146. Mizota, C., Nitrogen isotopic pattern of vegetation as affected by breeding activity of black-tailed gull (*Larus crassiostris*): a coupled analysis of feces inorganic soil nitrogen and flora. *Appl. Geochem.*, 2009, **24**, 2027–2033.
147. Ben-David, M., Hanley, T. A. and Schell, D. M., Fertilization of terrestrial vegetation by spawning Pacific salmon: role of flooding and predator activity. *Oikos*, 1998, **83**, 47–55.
148. Drake, D. C., Naiman, R. J. and Bechtold, J. S., Fate of nitrogen in riparian forest soils and trees: an ^{15}N tracer study simulating salmon decay. *Ecology*, 2006, **87**, 1256–1266.
149. García, L. V., Marañón, T., Ojeda, F., Clemente, L. and Redondo, R., Seagull influence on soil properties chenopod shrub distribution and leaf nutrient status in semi-arid Mediterranean islands. *Oikos*, 2002, **98**, 75–86.
150. Wait, D. A., Aubrey, D. P. and Anderson, W. B., Seabird guano influences on desert islands: soil chemistry and herbaceous species richness and productivity. *J. Arid Environ.*, 2005, **60**, 681–695.
151. García, L. V. *et al.*, Protected wading bird species threaten relict centenarian cork oaks in a Mediterranean biosphere reserve: a conservation management conflict. *Biol. Conserv.*, 2011, **144**, 764–771.
152. Hilderbrand, G. V., Hanley, T. A., Robbins, C. T. and Schwartz, C. C., Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. *Oecologia*, 1999, **121**, 546–550.
153. Fariña, J. M., Salazar, S., Wallem, K. P., Witman, J. D. and Ellis, J. C., Nutrient exchanges between marine and terrestrial ecosystems: the case of the Galapagos sea lion *Zalophus wollebaecki*. *J. Anim. Ecol*., 2003, **72**, 873–887.
154. Crait, J. R. and Ben-David, M., Effects of river otter activity on terrestrial plants in trophically altered Yellowstone Lake. *Ecology*, 2007, **88**, 1040–1052.

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