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**Stable isotopes dissect food webs from top to the bottom**

J. J. Middelburg

# Stable isotopes dissect food webs from top to the bottom

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bacteria-zooplankton interactions using biomass and fluxes as currency, or on the higher trophic levels (zooplankton to fishes), and typically use densities, number of individuals and sometimes biomass as model currency. Similarly, most benthic food-web studies are biased towards metazoans, macrophytes and suspended/sedimentary detritus and do not resolve the microbial domain.

It is unfortunate that differences in research traditions (evolutionary ecology vs. biogeochemistry) and methodology (DNA based identity vs. counting, biomass or flux measurements) have so far limited integration of food webs from end-to-end: i.e. from small producers at the bottom to large consumers at the top of the food web. This information is critical for linking changes in the environment (e.g., global warming, eutrophication, hypoxia, ocean acidification) with the functioning of higher organisms because many environmental impacts go via the lower parts of the food webs. Similarly, top predators are thought to impact nutrient cycling in lakes (Carpenter et al., 2001), but are normally ignored in ocean biogeochemistry models. It is for this reason that substantial efforts have been made to integrate different modeling approaches into end-to-end marine ecosystem modeling (Rose et al., 2010; Salihoghu et al., 2013).

Stable isotope analysis (SIA) has emerged as a versatile tool for questions in biogeochemistry, plant and animal physiology, migration patterns, niche definition and shifts, resource use and diet composition, trophic level estimations and food web functioning (Fry, 2006; Bouillon et al., 2011). SIA can be applied to all size classes in food webs, i.e. from aquatic microbes to whales, from soil fungi to elephants, from sediment bacteria to worms and as such may provide an alternative avenue to end-to-end food web studies besides ecosystem modeling (Rose et al., 2010). Most stable isotope applications in biogeochemistry and ecology make use of the small natural variations in stable isotope ratios resulting from physical, chemical and biological processes that cause isotope fractionation. These differences in natural abundance stable isotope ratios are primarily generated during primary production, but subsequent heterotrophic processing also contributes (Craig, 1953).

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Carbon and nitrogen stable isotopes have shown to be powerful tools for elucidating trophic structure and for inferring energy and matter flows in food webs. Stable carbon isotope ratios ( $\delta^{13}\text{C}$ ) of plants vary because of differences in isotopic composition of the inorganic carbon substrate used and the photosynthetic pathway (C3 or C4) involved (Smith and Epstein, 1970). These carbon isotope ratios are reflected in the tissue of animals consuming these plants (De Niro and Epstein, 1978) according to the principle “you are what you eat”, more precisely “you are what you assimilate”. In a subsequent study De Niro and Epstein (1981) showed that stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) of consumers are typically enriched by 2–4 ‰ relative to their diet. Nitrogen isotope ratios can thus be used to estimate trophic position of a consumer (Minagawa and Wada, 1984), while carbon isotope ratios provide information on the eventual carbon/energy sources at the base of the food web (Peterson and Fry, 1987, Post, 2002; Bouillon et al., 2011). This dual-isotope analysis of food webs provides qualitative, objective information about energy flow through food webs and trophic transfers (Fig. 1), and if some assumptions are made allows quantification of food chain length (Peterson and Fry, 1987; van der Zanden and Rasmussen, 2001) and consumer diets (Phillips et al., 2005; Fry, 2013).

Earth scientists pioneered stable isotope techniques, but technological progress, in particular automated continuous-flow, on-line isotope ratio mass spectrometry, has made stable isotope analysis widely available. Stable isotope analysis is now a routine procedure in many laboratories and stable isotope ecology has matured and expanded to such an extent that it is nearly impossible, let alone wise, to review all applications in stable isotope ecology and biogeochemistry. Excellent reviews are available for stable isotope use in (experimental) animal ecology (del Rio et al., 2009; Wolf et al., 2009), plant ecology (Dawson et al., 2001), trophic transfers (Boecklen et al., 2011), estuarine biogeochemistry (Bouillon et al., 2011) as well as several dedicated books (Fry, 2006; Michener and Latjha, 2007).

Here, I will review the use of stable isotopes in food-web reconstruction with the aim to identify avenues for research rather than providing a full account of all studies to

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date. First, I will evaluate the strength and weakness of carbon and nitrogen stable isotope analysis of food webs and discuss how additional isotopes ( $^{34}\text{S}$ ,  $^2\text{H}$  and  $^{14}\text{C}$ ) and quantitative isotope mixing models may contribute to improve food-web reconstructions. Second, most food-web studies are biased towards metazoan consumers and ignore isotopic heterogeneity at the base and concepts such as the microbial loop. The combination of compound-specific isotope analysis of microbial biomarkers and traditional isotope analysis of metazoan consumers is proposed as a fruitful avenue for end-to-end food web analysis complementing model-based approaches. Third, I will review deliberate tracer addition studies of food webs and discuss how they combined with natural abundance studies will allow further resolution of food-web functioning including crossing domains of life. In the final section, I will identify novel research themes and approaches that are now amendable for exploration.

## 2 Food-web analysis based on carbon and nitrogen isotopes

Stable isotope analysis can be done on small or large organisms, producers as well as consumers, and thus allows linking small organisms at the base of the food web with large consumers at the top (Fig. 1). The basic idea is that the isotope ratio of consumer reflects that of its diet after correction for isotopic discrimination. This isotopic discrimination during trophic transfers is very small for carbon isotopes ( $\Delta_{\text{C}} = 0\text{--}1\text{‰}$ , De Niro and Epstein, 1978; Post, 2002; van der Zanden and Rasmussen, 2001) and consumers therefore reflect the weighted isotopic signals of their resources. A simple two-end-member model can be used to estimate the proportion ( $p_{\text{C}}$ ) of carbon derived from resource A:  $p_{\text{C}} = (\delta^{13}\text{C}_{\text{consumer}} - \delta^{13}\text{C}_{\text{resource B}}) / (\delta^{13}\text{C}_{\text{resource A}} - \delta^{13}\text{C}_{\text{resource B}})$ .  $p_{\text{C}}$  can be negative or above 1 if an important food resource has not been sampled or included in the mixing model. A consumer relying for 25% on terrestrial plant detritus with a  $\delta^{13}\text{C}$  of  $-27\text{‰}$  and for 75% on phytoplankton with a  $\delta^{13}\text{C}$  of  $-19\text{‰}$  would have a  $\delta^{13}\text{C}$  of  $-21\text{‰}$ .

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Nitrogen isotope values can also be used for diet studies, but the systematic enrichment of consumers by 2–4 ‰ relative to their diet (Minagawa and Wada, 1984) makes  $\delta^{15}\text{N}$  an excellent measure for identifying trophic transfers and omnivory, and for quantifying trophic position. For a simple linear food chain the trophic position (TP) can be directly estimated from:  $\text{TP} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{resource at base}}) / \Delta_{\text{N}}$ , where  $\Delta_{\text{N}}$  is the enrichment per trophic transfer (2–4 ‰) and  $\delta^{15}\text{N}_{\text{resource at base}}$  is the nitrogen isotope ratio at the base of the food chain. Similarly, the trophic position for a two-source food web can be calculated from:  $\text{TP} = (\delta^{15}\text{N}_{\text{consumer}} - [\rho_{\text{N}} \times \delta^{15}\text{N}_{\text{resource A at base}} + (1 - \rho_{\text{N}}) \times \delta^{15}\text{N}_{\text{resource B at base}}]) / \Delta_{\text{N}}$ . Nitrogen isotope based TP estimates are continuous rather than discrete because they incorporate omnivory (Fig. 1) and they have shown to correlate with organism size and accumulation of contaminants (e.g., Cabana and Rasmussen, 1996).

The accuracy of trophic position estimates depends on a number of factors: (1) the assumption that carbon and nitrogen flow through the food web similarly ( $\rho_{\text{C}} = \rho_{\text{N}}$ ; i.e. no concentration effect) and that isotopic enrichment of carbon during trophic transfer ( $\Delta_{\text{C}}$ ) is limited, (2) the accuracy of the isotopic enrichment per trophic transfer ( $\Delta_{\text{N}}$ ), and (3) accurate knowledge of carbon and nitrogen isotope signature of basal resources (Fry, 2006; Phillips, 2012). The variance in isotopic enrichment per trophic transfer or trophic fractionation ( $\Delta_{\text{N}}$ ) has received considerable attention. Nitrogen trophic fractionation appears to depend on the form of excretion, the diet, the tissue analyzed, the taxon and the environment (e.g. vanderZanden and Rasmussen, 2001; Post, 2002; Vanderklift and Ponsard, 2003; Caut et al., 2010; Auerwald et al., 2010; Bunn et al., 2013). The implications of this variance for trophic position estimates and food web reconstruction have been discussed in detail (van der Zanden and Rasmussen, 2001; Post, 2002) and progress has been made in understanding why  $^{15}\text{N}$  accumulates (del Rio et al. 2009; Wolf et al., 2009).

Less progress has been made on isotopic characterization of basal resources. While there is substantial documentation of isotopic heterogeneity among terrestrial plants and aquatic macrophytes (macroalgae, seagrasses; Raven, 1996; Fry, 2006), there

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is very little information on the isotopic variability of microbial resources (algae and bacteria). Direct  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  characterization of microbial basal resources is analytical challenging and most food-web studies therefore rely on indirect approaches: i.e. either on primary consumers (Cabana and Rasmussen, 1996; vanderZanden et al., 1999; Post, 2002) or proxies for (i.e. indirect measures of) isotope ratios of microscopic organisms (Marty and Planas, 2007). The former approach is most often used and has in the case of long-lived primary consumers (e.g. suspension feeding bivalves) the advantage that short-term and seasonal variability are integrated. However, putative primary consumers such as *Daphnia* spp. might as well consume allochthonous, locally-produced detrital organic matter or bacterially-derived material (van den Meersche et al., 2009; de Kluijver et al., 2012) and thus provide a biased picture of available resources. Moreover, this approach only allows reconstruction of food webs from primary consumers upwards, ignoring the microbial domain and flows of carbon and nitrogen via the microbial loop (Azam et al., 1983). The alternative approach to use bulk organic matter, physically separated size-classes or respired carbon dioxide as proxies for bacterial or algal isotope signatures has its problems as well: micro-organisms are often attached to particles, size-classes may overlap and the isotope signature of carbon respired may differ from that assimilated (e.g. Marty and Planas, 2007). Moreover, basal resources usually have a higher turnover than consumers and short-term and/or seasonal variability should be accounted for (van den Meersche et al., 2009).

### 3 Food-web reconstruction beyond $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

Food-web analysis based on carbon and nitrogen isotopes can in principle reveal the contribution of three (number of isotopes + 1) resources to the diet of consumers. However, in reality diet inferences and food-web relationships based on isotope mixing models are more complex because (1) there is substantial variability in the isotopic composition of resources and consumers, (2) there is uncertainty in isotopic enrichment per trophic transfer, (3) isotopic composition of resources are not independent or

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(4) complex geometry of mixing polygons (Phillips and Gregg, 2001; Moore and Semmens, 2008; Parnell et al., 2010; Phillips, 2012; Fry, 2013). Moreover, there are often more potential resources than the number of isotopes + 1. For these cases, the solution to isotope mixing problem is mathematically underdetermined (like fitting a straight line through one data point) and thus non-unique (van Oevelen et al., 2010). Isotope mixing models have been developed that can deal with variability in resources, consumers and trophic transfer fractionation, overlapping isotope signatures and multiple resources and that allow exploring the domain of plausible solutions using techniques such as linear programming or Bayesian inferences (Phillips and Gregg, 2003, Lubetkin and Simenstadt, 2004; Parnell et al., 2010; Bogalhu et al., 2008). Usually a frequency distribution of feasible solutions is provided and these should not be averaged (Phillips and Greg, 2003; Fry, 2013). These state-of-the-art mixing models primarily document the uncertainty of inferences (i.e. range of frequency distribution) and provide information of what is not feasible (i.e. what is outside the frequency distribution), but it is only with inclusion of additional, independent information, e.g. other isotopes, tracers or biomarkers, in the inverse analysis that better constrained and more robust results can be obtained (van Oevelen et al., 2010; Fry, 2013).

This problem of more resources than isotopes and overlapping resource isotope signature, inspired pioneers in isotope ecology to explore sulphur isotopes ( $\delta^{34}\text{S}$ ) in estuarine and marine systems (Peterson and Fry, 1997; Connolly et al., 2004). Sulfur isotopes fractionate strongly during sulfate reduction and sulfur disproportionation processes (Canfield, 2001) and reducing environments and oxic-anoxic interfaces are therefore characterized by distinct negative sulfur isotope values. Primary producers assimilating sulfur from the environment inherit these differences and benthic and pelagic primary producers therefore often have distinct sulfur isotope signatures. These differences in resources propagate through the food web because of limited isotope enrichment per trophic transfer for  $\delta^{34}\text{S}$  (Peterson and Fry, 1997).

Overlapping isotope signatures of potential resources is a problem in many systems, but in particular in rivers and lakes where  $\delta^{13}\text{C}$  of river plankton often overlaps with that



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of terrestrial organic matter. Hydrogen isotope analysis has shown to be a powerful for fresh-water food-web studies because of the strong separation in  $\delta^2\text{H}$  between aquatic and terrestrial primary production (Doucett et al., 2007; Cole et al., 2011; Solomon et al., 2011; Cole and Solomon, 2012; Karlsson et al., 2012). This difference is due to the high evaporative stress on emergent vegetations compared to aqueous organisms. Fractionation of hydrogen isotopes during trophic transfer is believed to be limited, but organisms acquire a variable part ( $0.25 \pm 0.1$ ) of their hydrogen from the environment (Solomon et al., 2009; Cole et al., 2011).

Natural abundance radiocarbon measurements have also been used to trace carbon flow through food webs and provide complementary information to carbon, nitrogen and hydrogen isotopes. Radiocarbon has been used to identify consumer use of non-contemporary (i.e. aged) organic matter by freshwater fish and birds (Schell, 1983), by estuarine bacteria (McCallister et al., 2004), river zooplankton (Caraco et al., 2010) and consumers in lakes (McCallister et al., 2012; Zigah et al., 2012).

Although each of these isotopes has its own resolution power to elucidate food webs ( $\delta\text{S}^{34}$ : benthic vs. pelagic,  $\delta^2\text{H}$  aquatic vs. terrestrial,  $\Delta^{14}\text{C}$  modern vs. aged resources), it is through their combination and the combination of multiple isotopes with additional information and numerical mixing models that the next step in resource utilization and food-web reconstruction can be made.

#### 4 Compound specific isotope analysis allows including microbial domain

Stable isotope food-web studies have traditionally been limited to larger-sized organisms that can be physically separated and isolated for stable isotope measurements. The smaller members at the base of the food web (Archaea, Bacteria and eukaryotic primary producers) were neglected because primary consumers were used to characterize the isotopic composition of basal resources. This is unfortunate because food web dynamics depends on interactions between species and between species and the environment. Many environmental factors affect in particular members of the lower part

of the food web (nutrient, light, temperature). Detailed understanding food-web functioning in a changing world thus requires consideration of the entire food web including the micro-organisms governing energy and nutrient flows in most ecosystems.

Many ecosystems are fuelled not only by primary producers within the system, but also by external carbon subsidies (Polis et al., 1997). Rivers, lakes and estuaries receive large quantities of organic matter in particulate and dissolved organic form from the watershed and upstream systems (Cole et al., 2007). This allochthonous organic matter is consumed directly or indirectly via bacteria by many consumers in aquatic ecosystems (Cole et al., 2006). Advection of dissolved organic matter in the ocean may subsidize higher trophic levels after processing in the microbial loop (Azam et al, 1983; deLaender et al., 2010). For tracing organic matter flows and food-web functioning it is therefore essential to resolve the isotopic composition of bacteria and detritus from that of phytoplankton or bulk sedimentary organic matter (van Oevelen et al., 2006a).

Recently, compound specific isotope analysis (CSIA) of biomarkers has enabled us to include the microbial compartment of food webs (Middelburg et al., 2000; Boschker and Middelburg, 2002; Amelung et al., 2008; Bouillon and Boschker, 2006). Biomarkers are molecules specific for certain (groups of) organisms (Boschker and Middelburg, 2002). Through CSIA of biomarkers it is now possible to isotopically distinguish between micro-organisms and detritus (dead organic matter), to detect isotopic heterogeneity among microbial producers (e.g. between diatoms and green algae) and consumers, and to study the transfer of carbon in end-to-end food webs (i.e. from microbes to large metazoans). This resolution was required to estimate the contribution of bacteria and phytoplankton to zooplankton diets (van den Meersche et al., 2009), to elucidate the relative contribution of diatoms and cyanobacteria to the diets of meiofauna and macrofauna in sandy permeable sediments (Evrard et al., 2010, 2012), to distinguish reliance of zooplankton species on green algae and cyanobacteria (Pel et al., 2004; Bontes et al., 2006) and to infer the carbon substrate used by bacteria in macrophyte dominated coastal systems (Bouillon and Boschker, 2006; Bouillon et al., 2011).

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Compound specific isotope analysis is not limited to  $\delta^{13}\text{C}$ , but may also involve radiocarbon (Cherrier et al., 1999; McCallister et al., 2004),  $\delta^2\text{H}$  (Sessions et al., 1999) and  $\delta^{15}\text{N}$ . These have not yet been explored much in a food web context, but for nitrogen isotope analysis of amino acids. CSIA of amino acids in zooplankton revealed that some amino acids (trophic ones such as alanine and glutamic acids) become richer in  $^{15}\text{N}$  per trophic transfer, whereas others (source amino acids such as phenylalanine) are not (McClelland and Montoya, 2002; Hannides et al., 2009; Chikaraishi et al., 2009). This allowed them to estimate trophic levels for zooplankton and other consumers, and to derive the  $\delta^{15}\text{N}$  of basal resources. However, the trophic enrichment between glutamic acid and phenylalanine appears to be different for herbivory (plant-animal; 7.6‰) and carnivory (animal-animal; 4.3‰), complicating inferences based on compound-specific  $\delta^{15}\text{N}$  analysis (Germain et al., 2013). Basal resources  $\delta^{15}\text{N}$  values are notorious difficult to obtain because of high temporal variability due to intense recycling and assimilation of dissolved inorganic nitrogen by heterotrophic bacteria (Kirchman, 1994, Veuger et al., 2005). A pilot study on the  $\delta^{15}\text{N}$  of amino acids revealed systematic differences among prokaryotic and eukaryotic algae (McCarthy et al., 2013) that may be helpful to characterize base level  $\delta^{15}\text{N}$ , in particular when combined with isotopic characterization of D- alanine because this biomarker occurs in heterotrophic and autotrophic bacteria (Veuger et al., 2005).

## 5 Stable isotopes as deliberate tracers in food-web studies

Although the combination of multiple stable isotopes and isotope mixing models is often sufficient to resolve the problem of overlapping isotope signature of resources, an alternative approach involving deliberate stable isotope addition at tracer level might be useful. Studies during the last decade have shown that it is possible to selectively label primary producers through addition of  $^{13}\text{C}$ -enriched bicarbonate/carbon dioxide or  $^{15}\text{N}$ -enriched ammonium or nitrate (Middelburg et al., 2000; van den Meersche et al., 2011; Pace et al., 2004, 2007; Evrard et al., 2010). Similarly isotopically labeled dissolved

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organic matter can be added to label bacteria (van Oevelen et al., 2006a; Guilini et al., 2009) and  $^{13}\text{C}$  and/or  $^{15}\text{N}$  enriched phytodetritus can be added to trace the flow of carbon and/or nitrogen from detritus to consumers (Blair et al., 1996; Moodley et al., 2002; Woulds et al., 2007). The isotopically labeled resources are utilized and then part of the added label is assimilated in the consumer and provides a direct measure of the involvement of the specific organism in carbon or nitrogen flow. This information is highly relevant for linking organism identity and activity (Boschker et al., 1998).

Deliberate tracer experiments can be executed in enclosures (e.g. mesocosms) or in situ. The in situ approach has been successfully applied to elucidate food webs in lakes (Pace et al., 2004, 2007), streams (Hall and Meyer, 1998), estuaries (Hughes et al., 2001), tidal marshes (Gribsholt et al., 2005, 2007), tidal flats (Middelburg et al., 2000; Rossi et al., 2009) and deep-sea sediments (Blair et al., 1996). Stable isotopes have also been added in situ to benthic chambers in coastal (Oakes et al., 2010, 2012) and deep-sea sediments (Moodley et al., 2002; Witte et al., 2003) and to cores implemented at the seafloor (Nomaki et al., 2011). These studies have revealed that large-sized organism have either direct access to basal resources or via short links (Middelburg et al., 2000; Evrard et al., 2010; Woulds et al., 2009; Moodley et al., 2005).

The addition of heavy isotopes as deliberate tracers is increasingly being combined with natural abundance approaches because each approach has its strengths and weaknesses (Table 1) and they provide complementary food-web information (Herman et al., 2000; Evrard et al., 2010; Van Oevelen et al., 2006b, 2010; Cole et al., 2011; Solomon et al., 2011). Deliberate tracer studies have the advantage of being more conclusive than natural abundance studies because differences in isotope ratios of potential food resources is under experimental control, but isotope addition experiments can only trace dependence on contemporary production, i.e. the very same material produced just before the experiment is not included. Moreover, use of complex substrates of unknown composition (e.g. detritus) cannot be traced directly and use of  $^{15}\text{N}$  as a tracer excludes its use as a trophic level proxy. However, deliberate tracers experiments, when properly designed, also allow accurate mass-budgets to be established.

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domain, will for some time involve a trade-off between on the one hand resolution in taxonomy and on the other hand accurate quantification of tracer assimilation and thus organism activity (Fig. 2). While biomarker approaches have provided pivotal knowledge on the carbon isotopic characteristics of microbes, there is very little knowledge for other isotopes (Zhang et al., 2009) and for protozoans that are too small to be physically isolated for isotope determination (e.g. ciliates), yet may govern energy flow from microbes to putative primary consumers considered in most stable isotope food web studies. New technological developments such as microcombustion and liquid chromatography isotope ratio mass spectrometry (Boschker et al., 2008) and nanoSIMS (secondary ion mass spectrometry at submicrometer scale resolution; Wagner, 2009) may be needed to close this gap in our food web studies.

### 6.2 End-to-end food webs

Understanding carbon and nutrient flows between and within ecosystems is essential to advance our knowledge on biogeochemical cycling and ecosystem functioning. Food webs are often described in terms of the herbivory or green pathway involving plant-herbivory-predator chains or the brown, detritus-microbial loop (detritus-bacteria-metazoan consumers), but these two pathways are just the end-members of a continuum of parallel, interacting food webs (Legendre and Rassoulzadegan 1995; Krumin et al., 2013). There is growing awareness that many pelagic ecosystems receive substantial organic matter subsidies from other ecosystems and that consumers utilize both green and brown energy (Cole et al., 2006; Pace et al. 2007; van den Meersche et al., 2009). Similarly, traditional benthic food web reconstruction are based on utilization of particulate detrital organic matter of variable quality and do not consider herbivory or grazing on local primary production, either by benthic algae (Gattuso et al. 2006) or by chemoautotrophs (Middelburg, 2011; Molari et al., 2013).

Stable isotope studies should in principle be able to resolve the green and brown pathways in food webs. However, most pelagic food web reconstruction using stable isotopes are based on the green pathway and use phytoplankton or a proxy for phyto-

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plankton (seston or primary consumer) as basal resource. Challenges are to account for differences in the turnover of isotopes among primary producers and large consumers, differences in lipid content and analytical limitations to resolve the nitrogen isotopic composition of primary producers. In real food webs where green and brown pathways interact, there are challenges in identifying trophic levels because bacteria living on locally produced or external organic matter often assimilate dissolved nitrogen substrate from the environment with consequences for their  $^{15}\text{N}$  signature (Caraco et al., 1998). For any consumer that receives a fraction of its carbon and nitrogen via the microbial loop, it will be very difficult if not impossible to infer its trophic level. Trophic level estimates based on  $^{15}\text{N}$  data to date ignore bacterial nitrogen assimilation and as such are subject to uncertainty in ecosystems involving substantial energy flow from detrital resources.

More than 20 yr ago Hochberg and Lawton (1990) expressed the need to study the competition between kingdoms of life because of its ecological and evolutionary implications. This has not been followed up much. However, we now have the tools. By deliberately adding heavy isotopes to food webs and subsequent tracing of added label in organisms of different domains of life, we can now directly investigate the competition between bacteria, foraminifera and metazoans for the resource (Middelburg et al., 2000; Moodley et al., 2002; Woulds et al., 2007). Van Nugteren et al. (2009) employed  $^{13}\text{C}$  as a tracer to study the competition between sediment bacteria and a facultative detritivorous worm and identified that spatial distribution of resources governed relative use. Since  $^{13}\text{C}$  can also be incorporated into archaeal biomarkers, it would in principle be possible to study the relative use of resources by archaea, bacteria, protozoa and metazoans. This is analytical challenging, but Nomaki et al. (2011) have documented the transfer of  $^{13}\text{C}$  from dissolved organic carbon and phytodetritus to Archaea, foraminifera and metazoans. This suggests that all three domains of life in a food web may have almost direct access to detrital resources.

Food-web studies often focus on the transfer of carbon (and nitrogen) from one living compartment to another and ignore the carbon dioxide respired and the nitrogen

excreted/mineralized. This is unfortunate because this excludes the use of stringent mass balance constraints. Through the use of stable isotope as deliberate tracers and measurement of inorganic (carbon dioxide, ammonium, nitrate) and dead and living organic pools (organisms and detritus) it is possible to close the mass balance. Multiple phytodetritus addition experiments have shown that respiration is the main carbon sink rather than assimilation by organisms (Moodley et al., 2005; Woulds et al., 2009).

## 7 Summary

Stable isotopes are increasingly being used to elucidate and quantify food-web functioning and to identify carbon and nitrogen flows through ecosystems. Stable isotope measurements can be performed on inorganic substrates, detrital and living organic matter. This provides the unique opportunity to use the same currency for single-celled and large organisms and for living and dead organic matter, circumventing the problems associated with traditional currencies of differences branches of ecology. Stable isotope can be used at natural abundance making use of the small, systematic differences in ratios among organisms, as well as deliberately added tracers to focus on specific processes or organisms. The combination of tracer and natural abundance studies is very powerful since both approaches have their pros and cons. The use of stable isotope as tracers also allows accurate quantification of ecosystem budgets since not only assimilation but also respiration can be estimated. Stable isotopes also allow direct linking of biodiversity (identify of organisms) and ecosystem functioning (incorporation of isotope in organisms) and as such may help to improve our predictive capabilities of biogeochemical cycles in a changing world. Deliberate tracer experiments have been pivotal to elucidate the ecosystem-level response of pelagic ecosystems to ocean acidification (de Kluijver et al., 2013) and benthic ecosystems to hypoxia and recovery from hypoxia (Woulds et al., 2007; Rossi et al., 2009).

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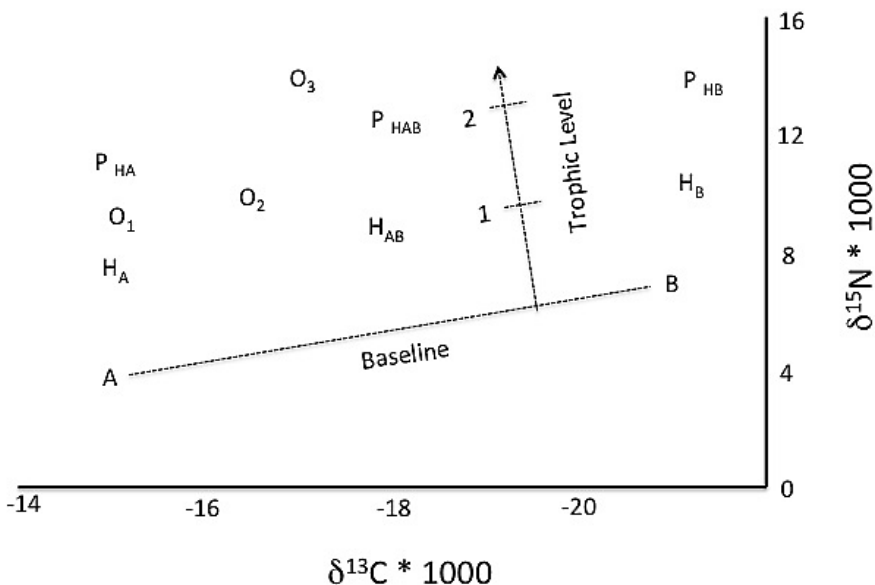
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**Table 1.** Strengths and weakness of deliberate tracer and natural abundance stable isotope studies.

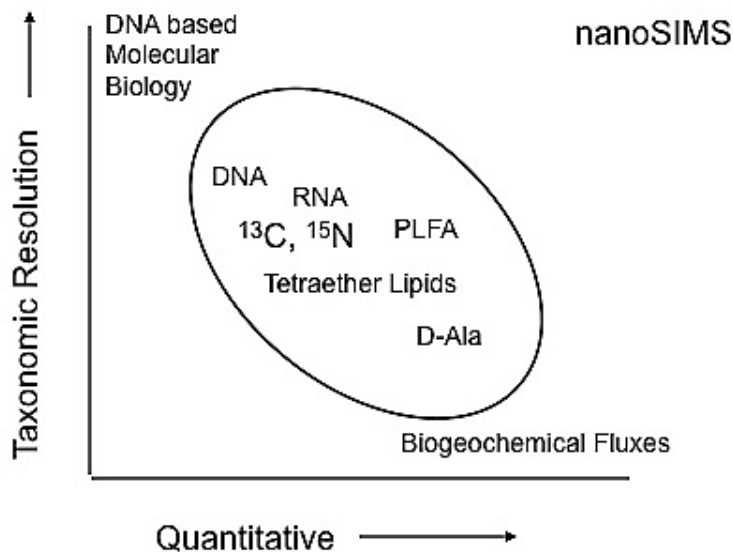
Natural Abundance	Deliberate Tracer
<p>Strengths:</p> <ul style="list-style-type: none"> <li>– Isotopes integrate over longer periods (weeks to months)</li> <li>– Samples collected in the field, no incubation or experimental manipulation required</li> <li>– Trophic level estimates</li> </ul>	<p>Weaknesses:</p> <ul style="list-style-type: none"> <li>– Traces only contemporary production in enriched area/volume</li> <li>– No trophic level estimates if <math>^{15}\text{N}</math> is added as tracer</li> <li>– Complex substrates (e.g. detritus) cannot be reproduced well</li> </ul>
<p>Weaknesses:</p> <ul style="list-style-type: none"> <li>– Isotopic differences among organisms or resources too small for <math>^{13}\text{C}</math> and <math>^{15}\text{N}</math></li> <li>– More sources than isotopes: underdetermined system</li> <li>– Trophic fractionation introduces some uncertainty</li> </ul>	<p>Strengths:</p> <ul style="list-style-type: none"> <li>– Conclusive (large differences among isotope ratios can be obtained)</li> <li>– Versatile: tracer can be added in different forms and different combination</li> <li>– Allows in situ experimentation</li> </ul>

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**Fig. 1.** Conceptual dual isotope plot. This hypothetical two isotope plot is based on two primary producers ( $A$ ,  $B$ ) that differ in both isotopes and are consumed by specialised herbivores ( $H_A$  and  $H_B$ ) and generalist herbivore ( $H_{AB}$  depending for 50% on both primary produces). These herbivores are consumed by predators ( $P_{HA}$ ,  $P_{HAB}$ ,  $P_{HB}$ ) as reflected in their increased  $^{15}\text{N}$  content (Y axis). Omnivores feeds multiple resources on multiple trophic levels ( $O_{1-3}$ ).



**Fig. 2.** Conceptual picture of trade-offs between identity of organism and accurate biogeochemical flux measurement using (microbial) biomarkers. Biogeochemical rates measurement (often based on isotope tracer techniques) provide accurate quantification but no information on the identity of the organisms involved. Molecular biology tools provide high quality information on the presence and potential capabilities of organisms but limited information on in situ rates. Compound-specific isotope characterization of biomarkers (DNA, RNA, PLFA, Tetraether lipids, D-alanine) in combination with tracer techniques provides rate as well as identity information but with low-to-moderate taxonomic resolution and intermediate accuracy. NanoSIMS can provide individual cell isotope ratio measurements and thus provides high accuracy and high taxonomic resolution, as well as spatial context.

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