REVIEW

Stable isotopes as tracers in aquatic ecosystems

Salvador Sánchez-Carrillo and Miguel Álvarez-Cobelas

Abstract: The addition of stable isotopes (SI) of ^{13}C and ^{15}N has been used to study several aquatic processes, thus avoiding environmental disturbance by the observer. This approach, employed for the last three decades, has contributed to expanding our knowledge of food-web ecology and nutrient dynamics in aquatic systems. Currently, SI addition is considered a powerful complementary tool for studying several ecological and biogeochemical processes at the whole-aquatic-ecosystem scale, which could not be addressed otherwise. However, their contributions have not been considered jointly nor have they been evaluated with a view to assessing the reliability and scope of their results from an ecosystem perspective. We intend to bridge this gap by providing a comprehensive review (78 scientific publications reporting in situ $^{13}C/^{15}N$ additions at the whole-aquatic-ecosystem scale) addressing the main results arising from their use as tracers. Specifically, we focus on: (*i*) reasons for SI additions at the whole-ecosystem scale to study ecological processes, (*ii*) the paradigms resulting from its use and the insights achieved, (*iii*) uncertainties and drawbacks arising from these SI addition experiments, and (*iv*) the potential of this approach for tackling new paradigms. SI tracer addition at the ecosystem scale has provided new functional insights into numerous ecological processes in aquatic sciences (importance of subsidies in lakes; heterotrophy dominance in benthic food webs in lakes, wetlands and estuaries; the decrease in N removal efficiency in most aquatic ecosystems due to anthropogenic alteration; the recognition of hyporheic zones and floodplains as hot spots for stream denitrification; and high rates of internal N recycling in tidal freshwater marshes). However, certain constraints such as the high cost of isotopes, the maintenance of the new isotopic steady state, and avoidance of biomass changes in any compartment or pool during tracer addition bear witness to the difficulties of applying this approach to all fields of aquatic ecology and ecosystems. The future development of this approach, rather than expanding to larger and complex aquatic ecosystems, should include other stable isotopes such as phosphorus ($P^{18}O_4$).

Key words: ^{13}C, ^{15}N, tracer addition experiments, food webs, biogeochemistry.

Résumé : L'ajout d'isotopes stables (IS) de ^{13}C et ^{15}N est utilisé pour étudier plusieurs processus aquatiques, évitant ainsi la perturbation environnementale par l'observateur. Cette approche, employée depuis les trois dernières décennies, a contribué à élargir nos connaissances de l'écologie des réseaux trophiques et de la dynamique des substances nutritives au niveau des systèmes aquatiques. Actuellement, l'ajout d'IS est considéré comme un outil complémentaire puissant permettant d'étudier plusieurs processus écologiques et biogéochimiques à l'échelle de l'écosystème aquatique entier, ce qui ne pourrait pas être abordé autrement. Cependant, leurs contributions n'ont pas été considérées conjointement, ni été évaluées en vue de déterminer la fiabilité et la portée de leurs résultats d'une perspective écosystémique. Nous avons l'intention de combler ces lacunes en présentant une revue complète (de 78 publications scientifiques rapportant des ajouts de $^{13}C/^{15}N$ in situ à l'échelle de l'écosystème aquatique entier) se penchant sur les principaux résultats découlant de leur utilisation comme traceurs. Spécifiquement, nous nous concentrons sur: (*i*) les raisons d'ajouter des IS à l'échelle de l'écosystème entier afin d'étudier des processus écologiques; (*ii*) les paradigmes issus de leur utilisation et les connaissances acquises; (*iii*) les incertitudes et les inconvénients découlant de ces expériences d'ajout d'IS et (*iv*) la possibilité de dépasser les nouveaux paradigmes par cette approche. L'ajout de traceur isotopiques stables à l'échelle écosystémique a fourni de nouvelles notions fonctionnelles au sujet des nombreux processus écologiques dans le domaine des sciences aquatiques (l'importance des contributions dans les lacs, la domination hétérotrophique des réseaux trophiques benthiques au niveau des lacs, des terres humides et des estuaires; la perte d'efficacité au niveau de l'élimination de l'azote dans la plupart des écosystèmes aquatiques en raison de changements anthropogéniques; la reconnaissance de zones hyporhéiques et de plaines inondables comme des points chauds susceptibles de dénitrification des cours d'eau et de hauts taux de recyclage interne de l'azote dans les marais d'eau douce à marée). Cependant, certaines contraintes, telles que le coût élevé des isotopes, le maintien du nouvel état stable isotopique et l'évitement de changements de biomasse dans tout compartiment ou bassin pendant l'ajout de traceur, témoignent des difficultés d'appliquer cette approche à tous les domaines d'écologie aquatique et à tous les écosystèmes. Le développement de cette approche à l'avenir, plutôt que de s'étendre aux écosystèmes aquatiques plus grands et complexes, devrait inclure d'autres isotopes stables, comme le phosphore ($P^{18}O^4$). [Traduit par la Rédaction]

Mots-clés : ^{13}C, ^{15}N, expériences d'ajout de traceurs, réseaux trophiques, biogéochimie.

S. Sánchez-Carrillo and M. Álvarez-Cobelas. Department of Biogeochemistry and Microbial Ecol., National Museum of Natural Sciences, Spanish National Research Council (MNCN-CSIC), Serrano 115 dpdo, E-28006-Madrid, Spain.

Corresponding author: Salvador Sánchez-Carrillo (email: sanchez.carrillo@mncn.csic.es).

Introduction

In aquatic science, the ecosystem scale approach is the ideal framework for the study of all ecological interactions among biotic and abiotic components and their processes within defined boundaries (Likens 1992). The main benefit of the whole-ecosystem scale approach is that it encompasses cross-habitat interactions (e.g., littoral–pelagic), which are critical for trophic interactions (Jeppesen et al. 1998). Another benefit of this approach is that nominal physical conditions are maintained (e.g., light, thermal structure, mixing) that affect growth, nutrient cycling, and succession (Carpenter et al. 2001). Although experimental manipulations of entire ecosystems entail certain constraints (for example, pseudoreplication), carefully designed experiments may be considered one of the most powerful approaches for studying process-level topics relative to ecosystems (Likens 1992). Several nutrient enrichment experiments (N and P fertilizations) at the ecosystem level have contributed to increase our knowledge of aquatic-ecosystem metabolism and trophic cascade effects, hence enabling recommendation of policies for eutrophication remediation (Falkowski et al. 2000; Elser et al. 2007; Schindler 2012; Rosemond et al. 2015; Gough et al. 2016). However, fertilization increases ecosystem disturbances—modifying process rates and fates and altering food-web structure through several feedbacks—and this can be good or bad depending on study goals (e.g., in oligotrophic systems when phytoplankton response is the goal and biomass needs to be increased or when studying some biogeochemical processes and rates are altered if the available substance is oversaturated).

The addition of stable isotopes (SI) of ^{13}C and ^{15}N has been used during the last three decades to study several aquatic processes, thereby avoiding disturbance. The temporal stability of SI and the processes governing isotopic fractionation make them useful as tracers, thus allowing measurement of simultaneous ecological and biogeochemical processes at the whole-ecosystem scale (Peterson and Fry, 1987; Schimel 1993; Fry 2006). Since Hershey et al. (1993) and Kling (1994) published their seminal papers using ^{13}C and ^{15}N additions at the whole-ecosystem scale, many other studies have been reported.

The whole-ecosystem isotope enrichment approach is a robust way to examine nutrient flows through multiple pools simultaneously while maintaining natural hydrologic and biogeochemical gradients (Tobias et al. 2003). In situ ^{13}C and ^{15}N additions at the whole-ecosystem scale have provided valuable results to advance (i) the study of carbon uptake, taking the relationships between terrestrial and lake biomes into account (e.g., Cole et al. 2002; Kritzberg et al. 2004; Pace et al. 2004; Carpenter et al. 2005; Taipale et al. 2008); (ii) the uptake, turnover, and retention processes of nitrogen (e.g., Tank et al. 2000a; Merriam et al. 2002; Hall et al. 2009b; Hadwen and Bunn 2005; Hughes et al. 2000; Gribsholt and Boschker 2005); and (iii) the trophic dynamics and food-web structure (e.g., Raikow and Hamilton 2001; Hamilton et al. 2004; Galván et al. 2012). Much of our current knowledge of freshwater ecology is grounded on the results provided by whole-ecosystem SI experimental additions. Now the time has come to evaluate the validity and scope of the results of these complex experiments to assess their impact on studies of aquatic ecology. However, the emerging success of SI addition experiments has not yet been viewed as a whole or evaluated to assess the reliability and scope of their results. This assessment is fundamental to decide the suitability of SI additions at the ecosystem scale for building in current and future paradigms in aquatic science. Here, we intend to bridge this gap by addressing the main subjects arising from the use of ^{13}C and ^{15}N as tracers at the whole-aquatic-ecosystem scale. Since the field of aquatic science is very broad we have focused on contributions to the knowledge of freshwater ecosystems, considering all those located in the continental context, including water bodies located in coastal areas with varying degrees of tidal influence (i.e., saltmarsh, tidal flats, and estuaries). Our approach is

Fig. 1. Number of papers published between 1990 and 2015 for in situ ^{13}C and ^{15}N tracer addition experiments carried out in aquatic continental ecosystems, splitting by system type (lentic vs lotic) and compound used (^{13}C vs ^{15}N). Lentic systems are lakes, intertidal (sand, mud) flats, and tidal salt and freshwater marshes; lotic systems are streams (including the hyporheic zone) and estuaries.
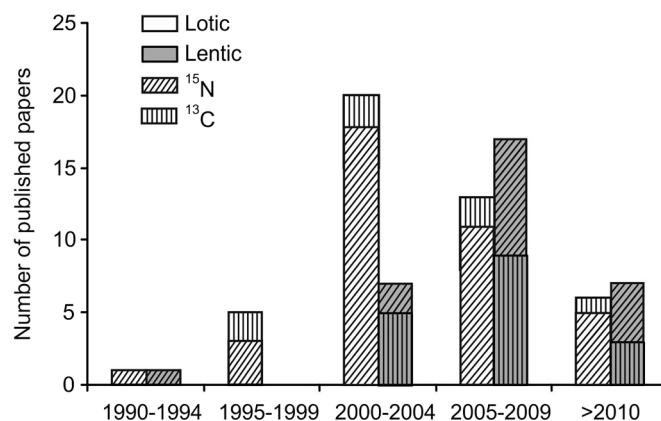
ecologically based to address the structure and functioning of ecosystems and, therefore, we have not addressed the study of groundwater, where studies also use SI as tracers together with their natural contents (e.g., Aravena and Robertson 1998; Smith et al. 2004). Specifically, we will focus on the following questions. (i) Why have SI additions been used at the whole-ecosystem scale in freshwaters to study ecological processes? (ii) To date, what paradigms have emerged at the experimental ecosystem scale using ^{13}C and ^{15}N additions and what insights have been achieved? (iii) What uncertainties and deficiencies have arisen from those SI addition experiments that should be considered when interpreting the scope of their results? (iv) Can (or should) this approach continue to be used to tackle new paradigms in the future? To answer these questions, we have reviewed 78 scientific publications reporting in situ $^{13}C/^{15}N$ additions to aquatic continental systems and their contributions have carefully been discussed.

SI addition experiments in freshwater: trends, compounds used, ecosystems studied, and geographic location

Figure 1 shows the trends in publications, from 1990, reporting whole-ecosystem experiments in aquatic systems using ^{13}C and ^{15}N additions. Most experimental additions were carried out from 2000 to 2009 with an increasing application to lentic systems. Although the use of both ^{13}C and ^{15}N additions displays similar trends, ^{15}N experimental additions are more frequent. This is because ^{15}N was used as a tracer of nitrogen dynamics in streams in two large-scale experiments (see below). The joint use of both SI is uncommon.

In situ ^{13}C enrichments have been performed in aquatic ecosystems through manipulation of the dissolved inorganic carbon pool (DI^{13}C; Carpenter et al. 2005), the dissolved organic carbon pool (DO^{13}C; Hall 1995) and the organic particulate pool (PO^{13}C; Bartels et al. 2012; Table 1). Enriched sodium bicarbonate (NaH$^{13}CO_3$ as 98 atom % ^{13}C) has been used extensively in lakes, tidal marshes, and estuaries to trace C transfer flows through food webs (Fig. 2; Cole et al. 2002, 2006; Pace et al. 2004; Carpenter et al. 2005; Kritzberg et al. 2004, 2006; Pace et al. 2007; Taipale et al. 2008). Labile organic compounds such as sodium acetate (CH$_3$^{13}COONa as 99 atom % ^{13}C), glucose ($^{13}C_6H^{12}O_6$ as 99 atom % ^{13}C), and compounds with a distinctive $\delta^{13}C$ stable isotope value, such as corn (a C4 plant) starch and dextrose, have also been added to study the microbial food web in streams (Hall 1995; Hall and Meyer 1998;

Table 1. Summary of ^{13}C tracer addition experiments conducted in aquatic continental ecosystems.

Isotope compound	Aquatic ecosystem type	Isotopic target level ($\Delta\delta$ ‰)	Addition method	Manipulated pool	References
NaH^{13}CO$_3$ (98 Atom % ^{13}C)	Lake (10)	15–40‰	Daily short pulses (hours) for a couple of weeks	DIC	Cole et al. (2002, 2006); Pace et al. (2004, 2007); Kritzberg et al. (2004, 2006); Carpenter et al. (2005); Solomon et al. (2008); Taipale et al. (2008); Weidel et al. (2008)
	Tidal marsh (3) and estuary (1)	*	Surface sediment spraying in instantaneous short pulses (minutes/hours)	DIC	Middelburg et al. (2000); Carman and Fry (2002); Galván et al. (2012); Lee et al. (2011)
CH$_3$^{13}COONa (99 Atom % ^{13}C)	Stream (5)	70–150‰	Continuous addition (3–4 weeks)	DOC	Hall (1995); Hall et al. (2000); Hall and Meyer (1998); Simon et al. (2003); Parkyn et al. (2005)
Corn (Corn starch or dextrose)	Lake (1) Stream (1)	13–18‰ 13–18‰	Single addition Long-term continuous addition (months)	POC DOC	Bartels et al. (2012); Wilcox et al. (2005)
^{13}C$_6$H$_{12}$O$_6$ (99 Atom % ^{13}C)	Tidal marsh (3)	20–520‰	Surface sediment spraying in discrete short pulses (hours)	DOC	van Oevelen et al. (2006a, 2006b); Veuger et al. (2006)

Note: Values in brackets in the aquatic ecosystem type column represent the number of stable isotope addition experiments conducted as reported in the literature. DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; POC, particulate organic carbon. Enrichments using starch or dextrose compounds are not true $\delta^{13}C$ enrichments, but they use the distinctive ^{13}C signature from the natural particulate organic matter and sediment.

*Data not found in the literature.

Fig. 2. (a) Percentage of total ^{13}C and ^{15}N tracer addition experiments in aquatic ecosystems related with the isotopic compound used (stream includes hyporheic zone experiments; salt marsh encompasses tidal flats, tidal salt, and freshwater marshes). (b) Aims of ^{13}C and ^{15}N tracer addition experiments in aquatic systems and the compounds used to achieve these goals. SI, stable isotope.
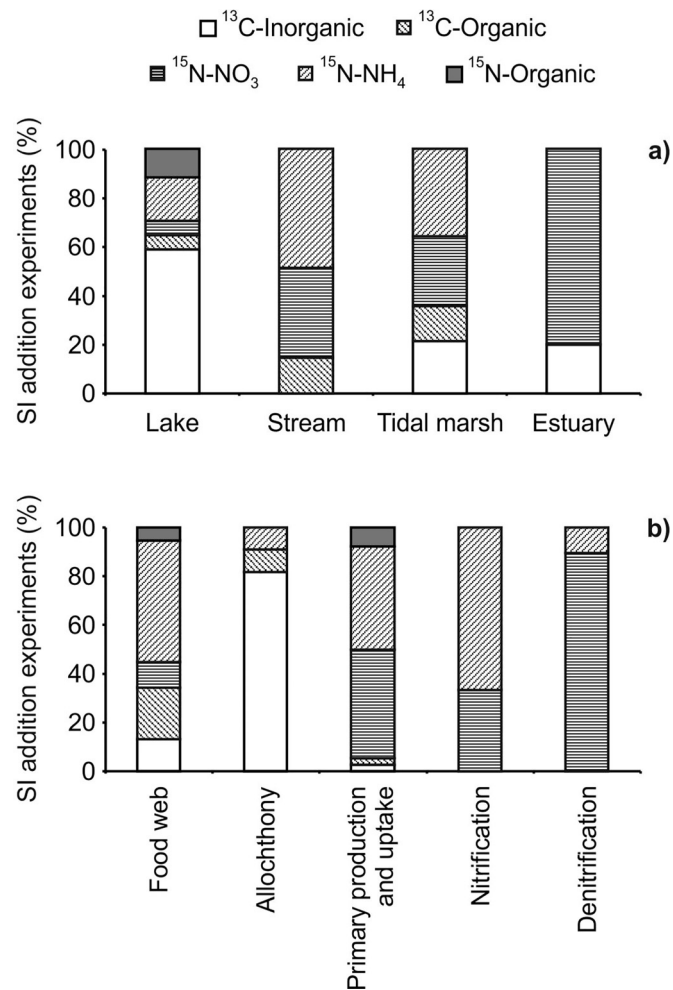
Simon et al. 2003), lakes (Bartels et al. 2012), and tidal flats (Van Oevelen et al. 2006a, 2006b; Veuger et al. 2006, Table 1 and Fig. 2).

In situ ^{15}N additions to aquatic ecosystems are performed to modify the dissolved inorganic nitrogen pool (DI^{15}N) via either nitrate (^{15}NO$_3$) or ammonium pools (^{15}NH$_4$). Modification of the nitrogen organic pool has seldom been undertaken and is based on ^{15}N labelling of algal compartment and subsequently used as enriched detritus (e.g., Li et al. 2010; Yu et al. 2013; Table 2 and Fig. 2). Potassium/sodium nitrate (K/Na^{15}NO$_3$ as 10–99 atom % ^{15}N), ammonium chloride (^{15}NH$_4$Cl as 10–99 atom % ^{15}N), and ammonium sulphate (^{15}NH$_4$SO$_4$ as 10.7 atom % ^{15}N) have been used in estuaries, lakes, tidal marshes, and streams (Fig. 2) to assess aquatic food-web structure (Hamilton et al. 2004; Galvan et al. 2008) and nitrogen-cycling (Peterson et al. 1997; Hall et al. 1998; Holmes et al. 2000; Sanzone et al. 2001; Gribsholt and Boschker 2005; Hall et al. 2009a; Fig. 2).

Geographically, experiments using ^{13}C and ^{15}N additions at the whole-ecosystem scale are clearly biased, with the vast majority (>95%) corresponding to the US, the remaining few in Europe (Belgium, The Netherlands, Finland, and Spain), Oceania (Australia), and Asia (China). Also most experiments were done in temperate and semi-arid climatic regions.

Table 2. Summary of ^{15}N tracer addition experiments conducted in aquatic continental ecosystems.

Aquatic ecosystem type	Isotope compound	Number of experiments	Isotopic target level ($\Delta\delta$ ‰)	Addition method	Manipulated pool	References
Stream	K/Na$^{15}NO_3$ (10–99 Atom % ^{15}N)	15	2000–10 000‰	Daily short pulses (2–24 hours) from one day to several weeks	DIN (NO_3)	Peterson et al. (2001); Böhlke et al. (2004); Mulholland et al. (2004, 2006, 2008, 2009); O'Brien et al. (2007, 2012); Bernot et al. (2006); Hamilton and Ostrom (2007); Hall et al. (2009a, 2009b); von Schiller et al. (2009); Hubbard et al. (2010); Sobota et al. (2012)
	$^{15}NH_4Cl$ (10–99 Atom % ^{15}N)	20	500–1100‰	Continuous pulses for several weeks	DIN (NH_4)	Hershey et al. (1993); Peterson et al. (1997); Hall and Meyer (1998); Wollheim et al. (1999); Tank et al. (2000a, 2000b); Mulholland et al. (2000a, 2000b); Raikow and Hamilton (2001); Sanzone et al. (2001, 2003); Hamilton et al. (2001, 2004); Merrian et al. (2002); Webster et al. (2003); Ashkenas et al. (2004); Parkyn et al. (2005); Morrall et al. (2006); Riis et al. (2012); Sánchez-Carrillo et al. (2017)
Tidal marsh	K/Na$^{15}NO_3$ (10–99 Atom % ^{15}N)	5	650–1000‰	Daily short pulses (hours) for a couple of weeks	DIN (NO_3)	Tobias et al. (2001); Galván et al. (2008, 2012); Drake et al. (2009)
	$^{15}NH_4Cl$ (10–99 Atom % ^{15}N)	2	*	Single short injection (hours)	DIN (NH_4)	Carman and Fry (2002); Gribsholt et al. (2009)
	($^{15}NH_4)_2SO_4$ (10–99 Atom % ^{15}N)	3	1900–4750‰	Single short injection (hours)	DIN (NH_4)	Gribsholt et al. (2005), (2007); Veuger et al. (2007)
Estuary	K/Na$^{15}NO_3$ (10–99 Atom % ^{15}N)	4	650–1000‰	Daily short pulses (hours) for a couple of weeks	DIN (NO_3)	Holmes et al. (2000); Hughes et al. (2000); Tobias et al. (2003)
Lake	K/Na$^{15}NO_3$ (10–99 Atom % ^{15}N)	1	*	Continuous addition for 10 days	DIN (NO_3)	Epstein et al. (2012)
	($^{15}NH_4)_2SO_4$ (10–99 Atom % ^{15}N)	2	500–*	Several short injections every 2 days for 10 days/Single short injection (hours)	DIN (NH_4)	Hadwen and Bunn (2005); Sánchez-Carrillo et al. (2017)
	$^{15}NH_4Cl$ (10–99 Atom % ^{15}N)	2	*	Continuous pulses for several weeks	DIN (NH_4)	Kling (1994); Armengol et al. (2012);
	^{15}N-organic (*Microcystis*)	2	1800‰	Single addition	PON	Li et al. (2010); Yu et al. (2013)
Hyporheic zone	K/Na$^{15}NO_3$ (10–99 Atom % ^{15}N)	2	10 000‰	Daily short pulses (hours) for a couple of weeks	DIN (NO_3)	Zarnetske et al. (2011a, 2011b)

Note: Values in brackets in the aquatic ecosystem type column represent the number of stable isotope addition experiments conducted as reported in the literature. DIN, dissolved inorganic nitrogen, PON, particulate organic nitrogen.

*Data not found in the literature.

What do SI tracer additions at the whole-ecosystem scale have that other approaches lack?

Isotopes as tracers have been used complementary to other studies to solve some of the main issues in freshwater ecology that could not be addressed otherwise. When isotopes are used at the whole-ecosystem scale, results are supported by all possible biotic and abiotic interactions occurring in the studied system. The addition of SI is most useful when employed to: (*i*) make parsimonious model choices (thus simplifying predictor variables), (*ii*) calibrate other more elegant and scalable approaches (like natural abundance SI interpretation; Galvan et al. 2012), and (*iii*) obtain in situ actual rates that cannot be obtained by either net mass balances and bench-top work or natural abundance measurements.

In the natural environment, fractionation can be a huge concern in SI studies. The overall goal of doing added tracer experiments is to discard ambiguity resulting from fractionation and source overlap using SI natural abundance. Due to kinetic isotope effects [KIE = $(k_I/k_H - 1) \times 1000$], which measures the ratio of reaction rate constants (k) of heavy (k_H) and light (k_I) isotopologues, expressed in units of per thousand (‰), the reaction product becomes enriched in the lighter isotope relative to the reactant. Furthermore, if the reactant pool is limited because it is in a closed system (e.g., anaerobic substrate compartments, along the groundwater flowpath in aquifers and riparian zones, and occasionally in river sediments; Kellman and Hillaire-Marcel 1998), the reactant becomes enriched in the heavier isotope. However, this could vary depending on the environmental conditions and whether the reaction is an equilibrium process (e.g., Mariotti et al. 1981; Mariotti et al. 1988; Fustec et al. 1991). In open-system experiments, the simultaneous addition of reactant may confound the natural isotopic signal of a given process. The reactant pool is changing because of the downstream transport of upstream substances into the study reach and the diffusion from the water column; neither process can be discriminated isotopically. For example, out of all N removal processes from rivers, only denitrification is accompanied by significant isotope fractionation enriching the remaining nitrate in ¹⁵N (Böttcher et al. 1990). However, the concomitant admixture of nitrate from other sources tends to increase riverine nitrate concentrations and this simultaneous addition of nitrate would readily mask any isotopic denitrification signal from SI natural abundance analyses (Mayer et al. 2002). Therefore, when adding SI as tracers, the target enrichment is great enough to clearly differentiate the pool of interest, making any potential fractionation trivial. There are two main situations where the contribution of SI tracer addition at the ecosystem scale has been crucial for incorporating new functional insights in aquatic ecology: food-web ecology and nutrient dynamics.

New approaches to the analysis of food webs

Stable isotopes used as tracers at the ecosystem scale have been considered beneficial because they add a functional dimension (food web) to the structural response (individual/biomass and species) commonly assayed in ecosystem experiments (Carman and Fry 2002). Food-web analysis based on SI natural abundances (most commonly using ¹³C and ¹⁵N, but also ³⁴S is often used) reveal the contribution of *n* (number of isotopes + 1) resources to diet. Natural abundances then assess all possible resource combinations to determine a range of possible source contributions (isotope mixing; Finlay and Kendall 2007). In reality, diet inferences and food-web relationships based on isotope mixing models are more complex: there are often more potential resources than the number of isotopes + 1. In this case, the solution to the isotope mixing problem is mathematically underdetermined and combined approaches involving deliberate stable isotope addition at tracer level with natural abundance are very useful because both perspectives provide complementary food-web information (Raikow and Hamilton 2001, Galvan et al. 2012). Tracer addition increases differences in isotope ratios and, when properly designed, experiments provide an accurate mass budget among food-web sinks and sources (Peterson et al. 1985). Moreover, natural abundance of carbon, nitrogen, and sulfur stable isotopes helps to distinguish organic matter sources, though often isotopic signal differences between autochthonous and allochthonous sources are not distinctive enough (Peterson et al. 1985, Sánchez-Carrillo et al. 2017). Labelling endogenous primary production with DI¹³C at the ecosystem scale provided direct measurements of the importance of terrestrial subsides supporting lake food webs (Pace et al. 2004, 2007). DI¹³C tracer addition identified basal resources supporting secondary production (Raikow and Hamilton 2001), even in multiple basal resource scenarios (e.g., Galván et al. 2012). Isotopically labelled ¹³C or ¹⁵N detritus has been used successfully to trace C and (or) N flows from detritus to consumers in lakes (Li et al. 2010; Yu et al. 2013). DO¹³C additions have resulted in new findings on the role of bacteria in food webs because this approach integrates several different processes such as DOC uptake by bacteria and invertebrates feeding on extracellular matter (Hall and Meyer 1998). Moreover, ¹⁵N addition in tracer experiments (combined with natural abundance) has benefitted from a more evident signal of trophic flow, revealing the quantitative importance of algae contribution to consumers in most aquatic ecosystems, whereas there was no difference with the contribution of heterotrophic microbes in streams (Hamilton et al. 2004; Hadwen and Bunn 2005; Galvan et al. 2008).

New approaches to the analysis of nutrient fluxes and transformations

The addition of SI as tracers in whole-ecosystem experiments has provided new insights into nutrient dynamics, particularly for the aquatic nitrogen cycle. Although traditional methods for the study of N-cycling (input–output budgets and experimental additions of ammonium or nitrate; Grimm 1987; Marti and Sabater 1996) have provided valuable information on N dynamics, they were found to have major shortcomings: (*i*) since processes of uptake, release, and downstream transport occur simultaneously in all ecosystem compartments, ambient rates of uptake, transformation, and retention could not be measured independently through mass balances, or were substantially altered by nutrient enrichment; (*ii*) the key role of specific biomass pools in N uptake and retention could only be indirectly inferred; and (*iii*) N turnover rates and the fate of N taken up could not be explicitly determined (Mulholland et al. 2000*a*; Drake et al. 2009). These shortcomings were overcome by using nutrient SI tracers as we will reveal below. Addition of ammonium or nitrate with high ¹⁵N-enrichment levels results in a negligible increase in ammonium or nitrate concentrations, avoiding the stimulatory effects of added nutrients. ¹⁵N additions have been used successfully to trace most N-cycle processes, allowing assessment of N uptake, turnover, and retention processes at whole-ecosystem scale in wetlands, lakes, and streams (Gribsholt and Boschker 2005; Mulholland et al. 2009; Sánchez-Carrillo et al. unpublished results) as well as in hydrodynamic complex systems such as estuaries, hyporheic zones, and floodplains (Holmes et al. 2000; Hall et al. 2009*b*; Hubbard et al. 2010; Zarnetske et al. 2011*a*, 2011*b*).

Main findings of ¹³C and ¹⁵N tracer additions at the whole-ecosystem scale advances aquatic science

Carbon subsidies supporting food webs

For a long time, ecologists viewed ecosystems as autotrophic or supported by energy derived from photosynthetic carbon fixation within the system. However, several facts revealed by metabolic studies (based upon bottle incubations, gas saturation, dissolved oxygen, and carbon dioxide diel changes) suggest that many lakes are net heterotrophic, meaning that system respiration (R) exceeds gross primary production (GPP; Jansson et al. 1999; J.L. Riera et al. 1999; Carignan et al. 2000; Cole et al. 2000). This net hetero-

Table 3. Summary of the main contributions of ^{13}C and ^{15}N tracer additions at the whole-ecosystem scale for the advancement of aquatic sciences.

Research area and topic	Ecosystem	Stable isotope	Finding	Earlier reference
Food webs:				
Allochthony	L	^{13}C	Ecosystem net heterotrophy is supported by allochthonous C	Cole et al. (2002)
Bacterial supports	S	^{13}C	Invertebrates derive a large C fraction from bacteria in headwater streams	Hall (1995)
	CW	^{13}C	Microphytobentos support meifauna but bacterial C is mainly a sink	Middleburg et al. (2000) and van Oevelen et al. (2006a)
Nutrient dynamics:				
N-cycling	S	^{15}N	NH_4 depends upon the physical environment	Mulholland et al. (2000a)
	S	^{15}N	NO_3 depends upon the chemical and biological environment	Böhlke et al. (2004)
	S	^{15}N	Denitrification depends upon physical, chemical and biological factors	Mulholland et al. (2009)
	S	^{15}N	Hotspots for denitrification mostly occur in transient storage zones (hyporheic zone and floodplain)	Zarnetske et al. (2011a)
	S, E, CW	^{15}N	N dynamics (cycling, uptake and sequestration) is inversely related with NO_3 concentration	Mulholland et al. (2008) and Drake et al. (2009)
	E, L	^{15}N	Long-term N retention occurs in the benthic habitat (epiphytes or macrophytes)	Gribsholt et al. (2005), Epstein et al. (2012) and Sánchez-Carrillo et al. (2017)

Note: L, lake; S, stream; CW, coastal wetland; E, estuary.

trophy was only maintained if R was supported by allochthonous organic matter. ^{13}C tracer additions at the whole-lake scale demonstrated that terrestrial (allochthonous) DOC is respired by bacteria and that this external carbon subsidizes food webs, being that this allochthony is inversely proportional to lake nutrient enrichment (Table 3; Cole et al. 2002, 2006; Kritzberg et al. 2004, 2006; Pace et al. 2004; Carpenter et al. 2005; Taipale et al. 2008). Moreover, experimental additions have shown that terrestrial carbon inputs subsidize benthic food webs and from here upward the pelagic habitats in oligotrophic lakes (Bartels et al. 2012), because benthic and pelagic habitats are more biologically coupled than thought previously (Vander Zanden et al. 2006). The question regarding the relative importance of internal primary production versus terrestrial organic matter in fuelling food webs has been answered by successful whole-lake ^{13}C tracer addition experiments.

The importance of bacteria supporting food webs in forest streams

In headwater forest streams, microbes can be more productive than primary producers, representing the trophic base of the ecosystem (net heterotrophic; Cummins 1974). In these systems, consumers feed on an assemblage of bacteria and detritus, and for years researchers have believed they rely mostly on detritus (plant debris) but were unaware of the trophic link between bacteria and larger consumers (meiofauna, macroinvertebrates, and protozoa; Hall 1995). For years, the fraction of invertebrate carbon derived from bacteria was a subject of controversy limited by the available methodology to determine bacterial consumption by grazers (visible bacterial tracers such as fluorescence or isotopic labelling of bacteria in laboratory assays during very short time periods; Hall et al. 1996). Additions of ^{13}C enriched in stream-scale (whole-system) experiments succeeded in labelling sediment bacteria, including biofilms and exopolymers, and were able to determine the fraction of invertebrate carbon derived from bacteria (Table 3). Studies using ^{13}C tracer additions have highlighted the importance of heterotrophic biofilms as the basic organic source supporting food webs in forest streams, suggesting that invertebrates derive between 10% and 100% of their carbon from bacteria (Hall and Meyer 1998; Simon et al. 2003; Parkyn et al. 2005; Wilcox et al. 2005), with exopolymers (exopolysacarides) reported as the basic organic source for both biofilm scrapers and predatory inverte-

brates (Hall 1995; Parkyn et al. 2005; Hall and Meyer 1998; Hall et al. 2000).

The old paradigm of the "detritus-based ecosystem" in coastal environments

Coastal food-web studies using the natural abundance of stable isotopes have brought into question the historical assumption regarding the dietary predominance of cordgrass (*Spartina*) in salt marshes, revealing the importance of benthic microalgae and phytoplankton in food webs (e.g., Haines and Montague 1979). Galvan et al. (2012) and P. Riera et al. (1999) measured natural abundance isotopes and performed experiments using ^{13}C additions in coastal water bodies, demonstrating that macrophyte detritus was less important as a food source for most infaunal species than algal sources. Microphytobenthos moderate carbon flow through benthic heterotrophs with a rapid, direct linkage between algae and bacteria via exudates (Table 3; Middleburg et al. 2000). In estuarine environments, studies tracing ^{13}C revealed the temporary strong coupling between bacterial and algal production during phytoplankton blooms, which influences the feeding strategies of mesozooplankton (van den Meersche et al. 2011). Bacterivory in coastal sediments was considered a marginal source for benthic heterotrophs (Hamels et al. 2001); however, the advent of assays using additions of enriched (^{13}C) organic dissolved compounds has enabled this assertion to be tested. Experiments performed by Van Oevelen et al. (2006a, 2006b) and Veuger et al. (2006) in tidal flats demonstrated that bacterial carbon is a primary sink of organic carbon in the food web, with recycling of carbon within the dissolved organic carbon–bacteria loop and bacterial respiration as the main loss process from this loop (Table 3).

Key factors controlling N-cycling in streams

Before the use of ^{15}N as tracer, our knowledge of the N-cycle in lotic systems was often incomplete. Streams were viewed as conduits for nitrogen from terrestrial to marine ecosystems (e.g., Howarth et al. 1996), but numerous studies clearly demonstrated the complexity and efficiency of the transformation and assimilation of nitrogen within stream ecosystems (e.g., Davis and Minshall 1999; Dent and Grimm 1999; Mulholland et al. 2000a; Peterson et al. 2001). Most information has been provided from LINX

projects (Lotic Intersite Nitrogen eXperiments; www.faculty.biol. vt.edu/webster/linx/), two large-scale ^{15}N addition experiments carried out in 15 headwater streams (LINX I using $^{15}NH_4^+$), and 72 streams (LINX II using $^{15}NO_3$) throughout US. The addition of ^{15}N at the stream scale elucidated how uptake and removal of NH_4^+ and NO_3^- depend on different variables (Table 3): the former on physical environment (stream size and hydrology, decreasing ammonium uptake and retention in large streams; Mulholland et al. 2000a; Tank et al. 2000b; Peterson et al. 2001; Wollheim et al. 2001; Dodds et al. 2002; Ashkenas et al. 2004; Hamilton et al. 2004; Morrall et al. 2006) and the latter on chemical and biological variables (nitrate concentration and autotrophic assimilation; Böhlke et al. 2004; Mulholland et al. 2008; Hall et al. 2009b; Mulholland et al. 2009). ^{15}N tracer additions revealed production of autochthonous dissolved organic nitrogen represents a substantial transformation of stream N (a similar magnitude to those of nitrification and denitrification), depending on ecosystem heterotrophy (Johnson et al. 2013). ^{15}N tracer experiments have demonstrated that a combination of hydrological (flow velocity × depth), chemical (NO_3^- and NH_4^+ concentrations), and biological (ecosystem respiration rate) factors are the most important controls of stream denitrification, with in situ denitrification from stream water accounting for a small fraction of nitrate removal (≈16%; Böhlke et al. 2004; Mulholland et al. 2009; Table 3) and with a neglected effect of DOC on nitrate stimulation (Johnson et al. 2012). Transient storage zones (including hyporheic zones) were recognized as hot spots for stream denitrification (Zarnetske et al. 2011a, 2011b). ^{15}N addition assays demonstrated that hyporheic zones and floodplains acted as nitrogen sinks, decreasing nitrogen removal capacity along with anthropogenic alteration of streams (Hall et al. 2009b; Hubbard et al. 2010). Other experimental additions of $^{15}NH_4^+$ have helped to confirm previous observations in relatively unexplored topics such as subsidies from aquatic to terrestrial habitats. In this respect, Sanzone et al. (2003) demonstrated that invertebrate insectivores (such as spiders and odonates) facilitate energy transfer from aquatic to terrestrial habitats by consuming emerging aquatic insects along the stream edge.

The role of the benthic zone in nutrient dynamics of lentic systems

Historically, research has neglected the role of the benthic zone (including the littoral) in nutrient dynamics of lakes. Vertical fluxes (sedimentation, eddy diffusion, fall turnover) have been considered largely responsible for controlling nutrient concentrations and therefore plankton production (Horne and Goldman 1994). However, the majority of lakes worldwide are relatively small and shallow, with extensive littoral zones, which may be important for nutrient uptake and recycling (Downing et al. 2006). ^{15}N additions in lakes are very scarce but experimental tracing has also shed light on nitrogen partitioning and transport; in oligotrophic lakes nitrogen enters the food web fastest via the seston (pelagic primary producers) but is retained for longer periods within the benthic zone (Epstein et al. 2012; Sánchez-Carrillo unpublished results; Table 3). Experiments undertaken in Lake Taihu (China) using detritus from cyanobacteria (Microcystis) cultures enriched with ^{15}N have demonstrated the key role of cyanobacterial detritus from blooms as a food source for both planktonic and benthic food webs, including quick assimilation by submerged and emergent macrophytes (Li et al. 2010; Zhang et al. 2010; Yu et al. 2013).

The $^{15}NO_3^-$ tracer addition technique has also improved our understanding of nitrogen-cycling in complex systems such as estuaries. Studies have revealed the key importance of benthos in the N-cycling of estuaries, which also supports phytoplankton demands (Holmes et al. 2000; Tobias et al. 2003). Further studies in estuaries demonstrate that nitrate loading plays a crucial role in N-dynamics: the efficiency in N-cycling, sediment sequestration, and efficiency of plant uptake in estuaries all decrease in conjunction with high NO_3^- concentrations (Drake et al. 2009). Also, ^{15}N

Fig. 3. Hierarchical distribution of the main topics under consideration in stable isotope (SI) tracer addition at the whole-ecosystem scale to trace ecological processes. The main issues that might currently limit a SI tracer addition experiment are marked with †; the main sources of uncertainty are noted by *. Changes in biomass of compartments have not been included; however, as the isotopic turnover rate varies with standing stocks of pools during the experiment, such changes must be monitored properly for inclusion in the model otherwise the experiment will be invalid.

tracer addition has revealed that the benthic microbial community is the most important mechanism for long-term nitrogen retention in tidal freshwater marshes (Gribsholt et al. 2005, 2006, 2007, 2009; Veuger et al. 2007; Table 3). A decline in denitrification efficiency as NO_3^- concentration increases has also been observed in coastal marsh ecosystems (Drake et al. 2009).

Constraints of ^{13}C and ^{15}N tracer additions at the whole-ecosystem scale

Target enrichment, dilution of added SI and exchange boundaries

The amount of ^{13}C or ^{15}N to be introduced into the system ($\Delta\delta_n$ = enriched δ – basal δ) must have a large enough target enrichment to be unambiguous and measurable (immediate, large, and transient increase in the isotopic signal), but simultaneously avoiding alterations in the ambient concentration by the added substance (Mulholland et al. 2000a; Tank et al. 2000b; Cole et al. 2002). Detectable amounts of SI added are dependent on pool sizes, pool concentrations, and the number of intermediate biogeochemical transformations before the target item is traced (Boschker and Middleburg 2002; Fig. 3). It is logical to think that since ^{13}C has lower fractionation than ^{15}N, lower enrichments are needed (Tables 1 and 2). However, it depends on the target, the compound used, and the system under study (Fig. 3); for example, if ^{13}C is added as inorganic carbon, there is a rapid loss to the atmosphere, and if the zooplankton community or planktivorous fish are the targets, large SI amounts should be added (Cole et al. 2002). In situ $^{15}NO_3^-$ tracer studies of denitrification are constrained by two main challenges: contamination of samples by air dinitrogen (N_2) and discrete increases in $\delta^{15}N_2$. The measurement of isotope-ratios of N_2 after $^{15}NO_3^-$ addition is subject to potential for contamination by N_2 in air. Dissolved gases need to be extracted into a headspace, and the headspace gas sample needs to be stored until analysis by isotope ratio mass spectrometry in the laboratory. Even seemingly slight contamination by air, either as bubbles in the water sample or from leakage during sample processing or storage, can impair the accuracy of analysis because the partial pressure of N_2 in the headspace sample is far less than that in the air. Moreover, dissolved N_2 in surface waters is continually exchanging with atmospheric N_2, and therefore background level
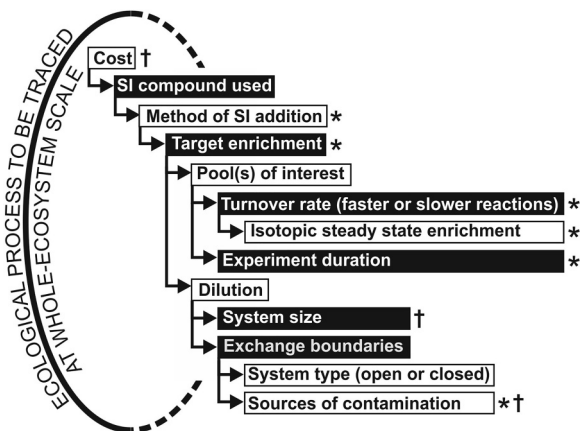
and dynamic turnover are high. Consequently, elevated ^{15}N enrichments are difficult to obtain, and in situ addition experiments commonly involve only discreet increases in $\delta^{15}N_2$ (Fig. 3). The amount of added tracer is constrained by the high cost of the isotope and the need to minimize the increase in NO_3 availability produced by tracer addition (not enhancing denitrification rates; Fig. 3). Thus, isotope addition experiments commonly produce only modest increases in the $\delta^{15}N$ of dissolved N_2, in which case measurement methods have to be optimized to quantify the tracer ^{15}N at enrichments that may be only a few ‰ above background (natural abundance; Hamilton and Ostrom 2007).

As previously stated, SI addition experiments make fractionation trivial. However, dilution of the added SI through exchange outside the control volume could be an important issue for in situ experiments (Fig. 3). In salt marshes $DI^{13}C$ additions can only be done at small scales or in some constrained environments because whole-ecosystem scale experiments face the dilution from seawater bicarbonates (Hardison 2009; Hardison et al. 2010). In larger streams and rivers, dilution continues to be an important constraint in ^{15}N addition experiments. Indeed, it is a simple fact that the amount of ^{15}N added is not enough to trace N dynamics. Clearly, the addition of a sufficient quantity of ^{15}N to be able to measure $^{15}N_2$ production and food-web relationships would become prohibitively expensive, but not impossible. Currently, it is incredibly difficult to label benthos sufficiently to determine the long-term fate of assimilated ^{15}N in streams (O'Brien et al. 2012).

Characterizing the exchange boundaries and fluxes outside the control volume is of vital importance in any ecosystem study and also in SI additions at the whole-ecosystem scale (Fig. 3). We have previously pointed out that in open systems the downstream transport of substances, such as nitrate carried from upstream into the study reach or diffusion from the water column, increases riverine nitrate concentration and changes the isotopic signal of denitrification. Sometimes concentrations and signatures can change considerably during the experiment due to the simultaneous addition of nitrate from sewage or manure (elevated $\delta^{15}N$) and this can worsen the accuracy of results and estimates of process rates (Fig. 3). Downstream transport of organic matter (algae and allochthonous organic matter) carried from upstream into the study reach can confound tracer labelling of consumers. Unlabelled material can be ingested by consumers that filter the water or feed on deposited organic matter, which does not reflect the isotopic enrichment of consumers, leading to overestimation of the importance of allochthonous sources (Hamilton et al. 2004).

Turnover rate and experiment duration

All SI addition experiments are designed for matching experiment duration to turnover times in pools of interest (Fig. 3); however, this is not always easy to achieve. The isotopic values of consumers may lag behind those of their food, with the lag period being a function of consumer turnover rates (Weidel et al. 2011). Although in-depth discussion on this topic falls outside the scope of this study (see Martínez del Río et al. 2009 for further information), some considerations are of interest to correctly assess SI additions at the whole-ecosystem scale. The question often arises as to the proper SI addition method based on both the pools under study and the turnover times (Table 3). The isotopic turnover rates and the factors that influence them must be considered to: (i) determine the time window through which pools of interest reflect the isotope change, and (ii) assume that variability in resource use by pools or tissues may be related with different turnover rates (Martínez del Río et al. 2009). Pulses with large SI enrichments are suitable for fast reactions like biogeochemical ones, provided that transport and dilution do not reduce them (e.g., Cole et al. 2006; Pace et al. 2007). For example, to study denitrification a groundwater pulse will work, but this will not be useful for surface water because of differences in gas exchange. In theory a pulse or prolonged SI tracer delivery will work for the food web as long as the

pulse is sufficiently enriched. Typically, for food-web studies the SI tracer is added for a sufficiently long timescale so that enrichment can be detected in the longest turnover pool of interest (Mulholland et al. 2000b). In practice, that is difficult but the general idea that emerges is that researchers can trade enrichment for duration.

One may expect that pools of interest are closer to the new isotopic steady-state enrichment during SI addition (Table 3). Therefore, an even distribution of SI tracer throughout the enrichment period is a prerequisite to trace the label properly and construct a reliable model for the whole ecosystem. However, numerous SI addition experiments have not reached this new steady-state enrichment for much of the assay duration. To what extent is it important to reach this new steady state? Most biological processes are generally unidirectional and kinetic isotope reaction rates are dependent on the ratios of isotopic masses and concentrations of products and reactants. Usually, in the course of experiments, researchers do not wait to perform sampling until the new steady state takes place, but they wait until they have data showing that the trend towards that new steady state is sufficient to fit a model. Although faster reaction steps (e.g., nitrification and denitrification) reach the new isotopic steady state enrichment early, to maintain it for a long time has proved to be more difficult than in slower reaction steps (e.g., food-web; Mulholland et al. 2009).

Changes in standing stocks

The assumption of turnover rate steady state would also be rejected if there was substantial biomass growth in any compartment during tracer addition (Hesslein et al. 1993). Standing stock measurements (i.e., changes in algal and microbial biomass) are recommended regularly, and they also have to be taken into account in the model of isotopic turnover (Dodds and Welch 2000).

Modelling results of SI additions at the whole-ecosystem scale

In the whole-ecosystem approach using ^{13}C and ^{15}N additions, box models (named compartmental models in some studies) are commonly used for data treatment. They have been implemented considering several approaches, according to the manipulated and target pools, as well as the process and the physical and biological features of each ecosystem (Carpenter et al. 2005). Due to the dynamic nature of SI addition experiments, steady-state models, like those used in studies of natural isotopic abundance, are considered inappropriate (Carpenter et al. 2005); however, when studying N-cycling, some authors have recently used this approximation to obtain variable daily inputs (e.g., Hall et al. 1998; Böhlke et al. 2004). Box dynamic models have been used extensively in SI addition at the whole-ecosystem scale to study C and N dynamics (Hall et al. 1998; Tank et al. 2000b; Cole et al. 2002; Carpenter et al. 2005; Cole et al. 2006; Pace et al. 2007; Solomon et al. 2008; Weidel et al. 2008). These models are essentially multipool finite difference dynamic models that assign functions (e.g., first order, zero order, or otherwise) to reactions (connecting the boxes), then a mass balance of each isotope is independently computed, and the enrichment at each time step is subsequently recalculated. Parameters are optimized to best fit the data. Models have been designed to simulate a high number of pools simultaneously and have been developed for each particular process to meet both the features of each ecosystem type (e.g., lotic versus lentic) and the aims of each assay (Cole et al. 2002). The first modelling in SI addition was developed by Hall et al. (1998) when studying N-cycling in streams, but they did not consider any transformation rates. The most comprehensive dynamic box model is the one created by Cole at al. (2006) studying C dynamics in four small northern US lakes. The model built was named dual-isotope flow modelling (DIF, as it simulates the flow of tot-C (^{12}C + ^{13}C) and ^{13}C, and was able to compute 12 carbon pools (DIC, pCO$_2$, DOC,

pelagic bacteria, phytoplankton, detrital POC, zooplankton, glass-worm (*Chaoborus*), periphyton and three fish compartments specific to the dominant fish species in each lake).

The study of N stream dynamics also requires complex box models based on exhaustive reaction rates, which include solute travel times, variable air–water gas exchanges affected by temperature and other gas fluxes, and reach-averaged rates of groundwater discharge, nitrification, and NO_3^- assimilation (Böhlke et al. 2004; Mulholland et al. 2004; Mulholland et al. 2008). Also more simple models based on a few assumptions have been developed to predict the response of one pool from one isotope manipulation at a time (e.g., the UNI model; Pace et al. 2004; Carpenter et al. 2005). Like parsimonious models, these overlook information on the dynamics of closely related time series and do not attempt to represent the specific ecological processes (Carpenter et al. 2005). Most problems appear when jointly modelling dynamics of slowly (benthos or fish) and rapidly (DIC) changing pools (Carpenter et al. 2005). An intermediate solution has also been created by incorporating some additional information such as the dynamics of closely related variables using a multivariate autoregression model (Ives et al. 2003). The main weaknesses of these dynamic box models (as in most models) are the assumptions of values and relationships which, whenever possible, are derived from their statistical fit to the data. Nonetheless, results from these models are usually very realistic, since they depend on a large number and diversity of measurements, many of which are based on in situ data; however, potentially complex errors can emerge as Carpenter et al. (2005) acknowledged. Evidently other model approaches could make an improvement but at this moment there is no evidence that they are more robust for interpreting SI data. Furthermore, some software has been developed based on dynamic box models such as Simulation Analysis and Modelling (WinSAAM, http://www.winsaam.org; Hamilton et al. 2004) to simulate and fit data and to model the transfer rate of tracers in a system, without requiring the system to have reached a steady state. Its use in SI additions at the ecosystem-scale is very recent (C flow in food webs; Lee et al. 2011), but results are promising given the implicit simplicity of the model.

Tracer addition assays for which experimental design includes circular statistics (Zar 1996) are becoming widely used to quantify the overall effects of SI addition on stable isotopes (Bartels et al. 2012) because they provide a quantitative understanding of complex isotopic changes at the community and food-web levels in time and (or) space (Schmidt et al. 2007). Stable isotope data have also been incorporated into ecological network analysis and simulation software to assess and identify system interactions and whole-ecosystem properties (Fath et al. 2007). The SI addition study of Lee et al. (2011) combined the software WinSAAM and EcoNet (http://eco.engr.uga.edu/index.html; Kazanci 2007) to assess food-web dynamics by providing system indices that quantify flows and food-web interactions. EcoNet uses network environmental analysis and deterministic and stochastic algorithms as well to quantify the actual relationship between compartments and environmental inputs and outputs (Tollner and Kazanci 2007; Ings et al. 2009). Results are interesting since this approach allows concurrent quantitative assessment of food-web structure and ecosystem functioning regardless of whether the system achieves the new isotopic steady state enrichment after SI tracer addition.

Future challenges of SI addition experiments to produce new paradigms in aquatic science

Since the first seminal in situ SI tracer addition experiment carried out by Kling (1994), aquatic science topics and paradigms have changed and studies have been modified to solve current environmental and ecological challenges. Today, one of the biggest challenges facing aquatic ecology and biogeochemistry concerns the role of microbial communities sustaining ecosystem

functioning (Hall and Meyer 1998). Metabolic rates and dynamics of nutrient transformations in Nature are not well known yet (Dumont and Murrell 2005; Jetten et al. 2009). Although ^{13}C and ^{15}N enrichment experiments are potentially able to solve most uncertainties in aquatic microbial ecology, a greater effort is needed to develop new methods and techniques to improve current results. In fact, SI tracer addition cannot be used everywhere. For example, we currently lack a good method for SI use in larger streams and rivers, which are able to ensure that SI addition is enough to label benthos sufficiently at an affordable cost and to determine the long-term fate of the assimilated ^{15}N (O'Brien et al. 2012). The use of stable isotope-labelled substrates in combination with biomarkers has allowed quantification of degradation rates and identification of organisms involved (Bull et al. 2000) and thus the co-experimentation of microbial molecular tools with in situ tracer experiments would be a valuable avenue to pursue. Only the effects of simple natural organic substrates such as POC have been simulated using ^{13}C additions in benthic food-web experiments, whereas the reproduction of complex substrates like detritus using SI will have to wait. This is complicated because it is not easy to establish a mixture of natural organic substances that show a clear signature contrast (e.g., corn starch –10.4‰ vs particulate organic matter –28.8‰; Bartels et al. 2012) with high insolubility, being a homogeneous material enough to quickly settling to be available to the benthic community alone. Finally, the importance of relatively stagnant N pools in some tissues should be investigated further in controlled laboratory studies, and methods for sampling fast-turnover tissues in small invertebrates should be developed for isotope addition studies.

Specifically, some processes, such as denitrification, still require further investigation as do other complex, recently described or identified processes like anammox, dissimilatory nitrate reduction to ammonia, and denitrification by sulfur-oxidizing bacteria (Mulholland et al. 2008, 2009; Burgin et al. 2012). Compound-specific isotope analysis as well as combining the SI addition approach with quantitative PCR of potential genes (e.g., Findlay et al. 2011; Vilar-Sanz et al. 2013) would provide a wide range of possibilities to study these complex microbial transformations in the aquatic environment (Boschker and Middelburg 2002; Johnson et al. 2012). Future assessment of other ecological assumptions that are still open to debate may be supported by results of experiments using ^{13}C and ^{15}N as tracers, for example: the role of allochthonous C inputs in lakes according to their environmental properties (Pace et al. 2007), the effects of increased N loads in lakes on nitrogen-cycling and food-web structure (Pace et al. 2007), the importance of wetlands and hyporheic zones in the nitrogen transformation at landscape scales (Duff and Triska 2000; Böhlke et al. 2009), the metabolic support of food webs (Pace et al. 2007), the N uptake rates and control variables in benthic regions (Wetzel 2001), the mechanisms of N transformation, storage and export in estuaries (Gribsholt and Boschker 2005), or the seasonal variability of food-web structure due to changes in biotic (basal resources, reproductive or stages of development) and abiotic (temperature, runoff, rainfall or evaporation) constraints (Layman et al. 2012).

In natural abundance studies, the dual-isotope approach (^{13}C and ^{15}N simultaneous study) is a common practice to assess trophic pathways in aquatic systems (Yoshii et al. 1999; Post 2002; García et al. 2007; Bratkič et al. 2012). In SI addition experiments, simultaneous ($^{13}C + ^{15}N$) enrichments are still seldom used. Some experiments at the ecosystem-scale have taken advantage of these simultaneous enrichments and their results are highly promising in terms of elucidating organic matter sources, ecosystem processes, food resources, and trophic patterns (Mulholland et al. 2000*b*; Carman and Fry 2002; Parkyn et al. 2005; Taipale et al. 2008; Hardison et al. 2010; van den Meersche et al. 2011; Galván et al. 2012). However, including a second tracer can drastically increase both the logis-

tics and expenses, which would currently limit where and when such techniques could be used.

So far the use of SI addition at the whole-ecosystem scale has exclusively relied on C and N stable isotopes. However, there are other isotopes that could be tested such as ^{18}O in the PO_4 molecule. This will enable researchers to deal with phosphorus dynamics for food webs and nutrient fluxes as well. Phosphorus has long been recognized as the main element limiting primary production in most freshwater environments (Vollenweider 1968). The detection and quantification of that isotopic signal started in soil studies some years ago (Tamburini et al. 2010). Recently, Goody et al. (2016) tested the usefulness of natural isotopic abundance for detecting eutrophication sources in UK streams. It is time to make attempts of in situ addition experiments with this isotope at the whole ecosystem scale.

Conclusions

Although with a regional bias, SI tracer addition at the ecosystem scale has been decisive for incorporating new functional insights in aquatic ecology. The functional dimension of SI as tracers at the ecosystem scale has proved successful in food-web ecology studies. In nutrient dynamics, the SI addition experiments have provided new insights, overcoming the several weaknesses found in traditional approaches to the study of N-cycling. Several ecological paradigms have emerged with the addition of ^{13}C and ^{15}N at the whole-ecosystem scale. Some of the main findings provided by SI tracer addition at the ecosystem scale are: the allochthony of lakes and the central role of heterotrophy dominance in benthic food webs in lakes, wetlands, and estuaries; the decrease in N removal efficiency in most aquatic ecosystems due to anthropogenic alteration; the recognition of hyporheic zones and floodplains as hot spots for stream denitrification; and the high rates of internal N recycling in tidal freshwater marshes. Despite these good scores, some constraints need to be taken into account when ^{13}C and ^{15}N tracer additions are performed at the whole-ecosystem scale. The amount of ^{13}C or ^{15}N to be introduced into the system under study (target enrichment) is often constrained by the high cost of the isotope and the need to minimize the increase in substance availability produced by the tracer addition. Discrete increases in $\delta^{13}C$ and $\delta^{15}N$ are important in the context of in situ experiments as they can compromise the accuracy of results and estimates of process rates. Said $\delta^{13}C$ and $\delta^{15}N$ increases may be affected by dilution, simultaneous addition and (or) contamination due to exchanges beyond the control volume. Moreover, an SI addition experiment is designed to match experiment duration to turnover times in pools of interest, which is not always easy to achieve. Usually, experiment duration is not conditioned by the time taken to reach the new steady state, but rather it is stopped when data exhibit a trend towards that new steady state, which is sufficient to fit a model. It is necessary to consider the choice of a proper SI addition method (pulses with large SI enrichments or prolonged additions with small SI enrichments) based on the pools under study (microbial process or large trophic interactions) as well as turnover times (faster or slower reaction steps). However, although faster reaction steps (e.g., nitrification and denitrification) reach the new isotopic steady-state enrichment early, maintaining this state for a long time may be more difficult than in slower reaction steps (e.g., food web). Finally, when substantial changes are observed in biomass in any compartment or pool during tracer addition, the experiment must be discarded as the assumption of steady state of turnover rate is then violated. Modelling is a key tool in the endeavor to validate results from SI addition experiments at the whole-ecosystem scale. Main concerns arise with potentially complex errors, which may emerge in box dynamic models based on assumptions and statistical fit of the data.

Most current uncertainties in aquatic microbial ecology can be solved using SI enrichment experiments at the whole-ecosystem scale; however, a greater effort is required to develop new methods and techniques to improve current results (e.g., combined with microbial molecular tools). Furthermore, methods must be improved to properly apply SI tracer addition to ecosystems where it has not been possible to date (large aquatic ecosystems). Concerning phosphorus, which is the main limiting factor of productivity in freshwaters, there is a clear need to undertake SI addition experiments at the whole-ecosystem scale, and although the methodology already exists it has only been used for quantifying natural isotopic contents so far. Finally, despite its high potential to jointly elucidate ecosystem process and trophic pattern, simultaneous ($^{13}C + ^{15}N + ^{18}(O)PO_4$) addition experiments are rarely used as we still lack an easy and cost-effective solution to their logistical and economic constraints.

Acknowledgements

References

Aravena, R., and Robertson, W.D. 1998. Use of multiple isotope tracers to evaluate denitrification in ground water: study of nitrate from a large-flux septic system plume. Groundwater, **36**: 975–982. doi:10.1111/j.1745-6584.1998.tb02104.x.

Armengol, X., Wurtsbaugh, W.A., Camacho, A., and Miracle, M.R. 2012. Pseudo-diel vertical migration in zooplankton: a whole-lake ^{15}N tracer experiment. J. Plank. Res. **34**: 976–986. doi:10.1093/plankt/fbs058.

Ashkenas, L.R., Johnson, S.L., Gregory, S.V., Tank, J.L., and Wollheim, W.M. 2004. A stable isotope tracer study of nitrogen uptake and transformation in an old–growth forest stream. Ecology, **85**: 1725–1739. doi:10.1890/03-0032.

Bartels, P., Cucherousset, J., Gudasz, C., Jansson, M., Karlsson, J., Persson, L., et al. 2012. Terrestrial subsidies to lake food webs: an experimental approach. Oecologia, **168**: 807–818. doi:10.1007/s00442-011-2141-7. PMID:21971586.

Bernot, M.J., Tank, J.L., Royer, T.V., and David, M.B. 2006. Nutrient uptake in streams draining agricultural catchments of the midwestern United States. Freshwater Biol. **51**: 499–509. doi:10.1111/j.1365-2427.2006.01508.x.

Böhlke, J.K., Harvey, J.W., and Voytek, M.A. 2004. Reach-scale isotope tracer experiment to quantify denitrification and related processes in a nitrate-rich stream, midcontinent United States. Limnol. Oceanogr. **49**: 821–838. doi:10.4319/lo.2004.49.3.0821.

Böhlke, J.K., Antweiler, R.C., Harvey, J.W., Laursen, A.E., Smith, L.K., Smith, R.L., and Voytek, M.A. 2009. Multi-scale measurements and modeling of denitrification in streams with varying flow and nitrate concentration in the upper Mississippi River basin, USA. Biogeochemistry, **93**: 117–141. doi:10.1007/s10533-008-9282-8.

Boschker, H.T., and Middelburg, J.J. 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol. Ecol. **40**: 85–95. doi:10.1111/j.1574-6941.2002.tb00940.x. PMID:19709215.

Böttcher, J., Strebel, O., Voerkelius, S., and Schmidt, H.-L. 1990. Using isotope fractionation of nitrate–nitrogen and nitrate–oxygen for evaluation of microbial denitrification in a sandy aquifer. J. Hydrol. **114**: 413–424. doi:10.1016/0022-1694(90)90068-9.

Bratkič., A., Šturm., M., Faganeli, J., and Ogrinc, N. 2012. Semi-annual carbon and nitrogen isotope variations in the water column of Lake Bled NW Slovenia. Biogeosciences, **9**: 1–11. doi:10.5194/bg-9-1-2012.

Bull, I.D., Parekh, N.R., Hall, G.H., Ineson, P., and Evershed, R.P. 2000. Detection and classification of atmospheric methane oxidizing bacteria in soil. Nature, **405**: 175–178. doi:10.1038/35012061. PMID:10821271.

Burgin, A.J., Hamilton, S.K., Jones, S.E., and Lennon, J.T. 2012. Denitrification by sulfur-oxidizing bacteria in a eutrophic lake. Aquat. Microb. Ecol. **66**: 283–293. doi:10.3354/ame01574.

Carignan, R., Planas, D., and Vis, C. 2000. Planktonic production and respiration in oligotrophic shield lakes. Limnol. Oceanogr. **45**: 189–199. doi:10.4319/lo.2000.45.1.0189.

Carman, K.R., and Fry, B. 2002. Small-sample methods for $\delta^{13}C$ and $\delta^{15}N$ analysis of the diets of marsh meiofaunal species using atural abundance and tracer-addition isotope techniques. Mar. Ecol. Prog. Ser. **240**: 85–92. doi:10.3354/meps240085.

Carpenter, S.R., Cole, J.J., Hodgson, J.R., Kitchell, J.F., Pace, M.L., Bade, D., et al. 2001. Trophic cascades, nutrients, and lake productivity: whole-lake experiments. Ecol. Monogr. **71**: 163–186. doi:10.1890/0012-9615(2001)071[0163:TCNALP]2.0.CO;2.

Carpenter, S.R., Cole, J.J., Pace, M.L., Van de Bogert, M., Bade, D.L., and Bastviken, D. 2005. Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. Ecology, **86**: 2737–2750. doi:10.1890/04-1282.

Cole, J.J., Pace, M.L., Carpenter, S.R., and Kitchell, J.F. 2000. Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. Limnol. Oceanogr. **45**: 1718–1730. doi:10.4319/lo.2000.45.8.1718.

Cole, J.J., Carpenter, S.R., Kitchell, J.F., and Pace, M.L. 2002. Pathways of organic carbon utilization in small lakes: results from a whole-lake 13C addition and couple model. Limnol. Oceanogr. **47**: 1664–1675. doi:10.4319/lo.2002.47.6.1664.

Cole, J.J., Carpenter, S.R., Pace, M.L., Van de Bogert, M.C., Kitchell, J.L., and Hodgson, J.R. 2006. Differential support of lake food webs by three types of terrestrial organic carbon. Ecol. Lett. **9**: 558–568. doi:10.1111/j.1461-0248.2006.00898.x. PMID:16643301.

Cummins, K.W. 1974. Structure and function of stream ecosystems. Bioscience, **24**: 631–641. doi:10.2307/1296676.

Davis, J.C., and Minshall, G.W. 1999. Nitrogen and phosphorus uptake in two Idaho (USA.) headwater wilderness streams. Oecologia, **119**: 247–255. doi:10.1007/s004420050783. PMID:28307975.

Dent, C.L., and Grimm, N.B. 1999. Spatial heterogeneity of stream water nutrient concentrations over successional time. Ecology, **80**: 2283–2298. doi:10.1890/0012-9658(1999)080[2283:SHOSWN]2.0.CO;2.

Dodds, W.K., and Welch, E. 2000. Establishing nutrient criteria in streams. J. N. Am. Benthol. Soc. **19**: 186–196. doi:10.2307/1468291.

Dodds, W.K., López, A.J., Bowden, W.B., Gregory, S., Grimm, N.B., Hamilton, S.K., et al. 2002. N uptake as a function of concentration in streams. J. N. Am. Benthol. Soc. **21**: 206–220. doi:10.2307/1468410.

Downing, J.A., Prairie, Y.T., Cole, J.J., Duarte, C.M., Tranvik, L.J., Striegl, R.G., et al. 2006. The global abundance and size distribution of lakes, ponds., and impoundments. Limnol. Oceanogr. **51**: 2388–2397. doi:10.4319/lo.2006.51.5.2388.

Drake, D.C., Peterson, B.J., Galván, K.A., Deegan, L.A., Hopkinson, C., Johnson, J.M., et al. 2009. Saltmarsh ecosystem biogeochemical responses to nutrient enrichment: a paired ^{15}N tracer study. Ecology, **90**: 2535–2546. doi:10.1890/08-1051.1. PMID:19769131.

Duff, J.H., and Triska, F.J. 2000. Nitrogen biogeochemistry and surface-subsurface exchange in streams. *In* Streams and ground waters. *Edited by* J.B. Jones and P.J. Mulholland. Academic Press, San Diego. pp. 197–220.

Dumont, M.G., and Murrell, J.C. 2005. Stable isotope probing- linking microbial identity to function. Nat. Rev. Microbiol. **3**: 499–504. doi:10.1038/nrmicro1162. PMID:15886694.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., et al. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine, and terrestrial ecosystems. Ecol. Lett. **10**: 1135–1142. doi:10.1111/j.1461-0248.2007.01113.x. PMID:17922835.

Epstein, D.M., Wurtsbaugh, W.A., and Baker, M.A. 2012. Nitrogen partitioning and transport through a subalpine lake measured with an isotope tracer. Limnol. Oceanogr. **57**: 1503–1516. doi:10.4319/lo.2012.57.5.1503.

Falkowski, P., Scholes, R.J., Boyle, E., Canadell, J., Canfield, D., Elser, J., et al. 2000. The global carbon cycle: a test of our knowledge of earth as a system. Science, **290**: 291–296. doi:10.1126/science.290.5490.291. PMID:11030643.

Fath, B.D., Scharler, U.M., Ulanowicz, R.E., and Hannon, B. 2007. Ecological network analysis: network construction. Ecol. Model. **208**: 49–55. doi:10.1016/j.ecolmodel.2007.04.029.

Findlay, S.E.G., Mulholland, P.J., Hamilton, S.K., Tank, J.L., Bernot, M.J., Burgin, A.J., et al. 2011. Cross-stream comparison of substrate-specific denitrification potential. Biogeochemistry, **104**: 381–392. doi:10.1007/s10533-010-9512-8.

Finlay, J.C. and Kendall, C. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, In: Stable Isotopes in Ecology and Environmental Science, Second Edition (eds R. Michener and K. Lajtha), Blackwell Publishing Ltd, Oxford, UK. doi:10.1002/9780470691854.ch10.

Fry, B. 2006. Stable isotope ecology. Springer, New York.

Fustec, E., Mariotti, A., Grillo, X., and Sajus, J. 1991. Nitrate removal by denitrification in alluvial ground water: role of a former channel. J. Hydrol. **123**: 337–354. doi:10.1016/0022-1694(91)90098-3.

Galván, K., Fleeger, J.W., and Fry, B. 2008. Stable isotope addition reveals dietary importance of phytoplankton and microphytobenthos to saltmarsh infauna. Mar. Ecol. Prog. Ser. **359**: 37–49. doi:10.3354/meps07321.

Galván, K., Fleeger, J.W., Peterson, B., Drake, D., Deegan, L.A., and Johnson, D.S. 2012. Natural abundance stable isotopes and dual isotope tracer additions help to resolve resources supporting a saltmarsh food web. J. Exp. Mar. Biol. Ecol. **410**: 1–11. doi:10.1016/j.jembe.2011.08.007.

Garcia, A.M., Hoeinghaus, D.J., Vieira, J.P., and Winemiller, K.O. 2007. Isotopic variation of fishes in freshwater and estuarine zones of a large subtropical coastal lagoon. Estuarine, Coastal Shelf Sci. **73**: 399–408. doi:10.1016/j.ecss.2007.02.003.

Gooddy, D.C., Lapworth, D.J., Bennett, S.A., Heaton, T.H.E., Williams, P.J., and Surridge, B.W.J. 2016. A multi-stable isotope framework to understand eutrophication in aquatic ecosystems. Water Res. **88**: 623–633. doi:10.1016/j.watres.2015.10.046. PMID:26562799.

Gough, L., Bettez, N.D., Slavik, K.A., Bowden, W.B., Giblin, A.E., Kling, G.W., et al. 2016. Effects of long-term nutrient additions on Arctic tundra, stream, and lake ecosystems: beyond NPP. Oecologia, **182**: 653–665. doi:10.1007/s00442-016-3716-0. PMID:27582122.

Gribsholt, B., Boschker, H.T.S., Struyf, E., Andersson, M., Tramper, A., De Brabandere, L., et al. 2005. Nitrogen processing in a tidal freshwater marsh: A whole-ecosystem ^{15}N labeling study. Limnol. Oceanogr. **50**: 1945–1959. doi:10.4319/lo.2005.50.6.1945.

Gribsholt, B., Struyf, E., Tramper, A., Andersson, M.G.I., Brion, N., De Brabandere, L., et al. 2006. Ammonium transformation in a nitrogen-rich tidal freshwater marsh. Biogeochemistry, **80**: 289–298. doi:10.1007/s10533-006-9024-8.

Gribsholt, B., Struyf, E., Tramper, A., De Brabandere, L., Brion, N., van Damme, S., et al. 2007. Nitrogen assimilation and short term retention in a nutrient-rich tidal freshwater marsh – a whole ecosystem ^{15}N enrichment study. Biogeosciences, **4**: 11–26. doi:10.5194/bg-4-11-2007.

Gribsholt, B., Veuger, B., Tramper, A., Middelburg, J.J., and Boschker, H.T.S. 2009. Long-term ^{15}N-nitrogen retention in tidal freshwater marsh sediment: elucidating the microbial contribution. Limnol. Oceanogr. **54**: 13–22. doi:10.4319/lo.2009.54.1.0013.

Grimm, N.B. 1987. Nitrogen dynamics during succession in a desert stream. Ecology, **68**: 1157–1170. doi:10.2307/1939200.

Hadwen, W.L., and Bunn, S.E. 2005. Food web responses to low-level nutrient and ^{15}N-tracer additions in the littoral zone of an oligotrophic dune lake. Limnol. Oceanogr. **50**: 1096–1105. doi:10.4319/lo.2005.50.4.1096.

Haines, E.B., and Montague, C.L. 1979. Food sources of estuarine invertebrates analyzed using ^{13}C/^{12}C ratios. Ecology, **60**: 48–56. doi:10.2307/1936467.

Hall, R.O. 1995. Use of a stable carbon isotope addition to trace bacterial carbon through a stream food web. J. N. Am. Benthol. Soc. **14**: 269–277. doi:10.2307/1467779.

Hall, R.O., and Meyer, J.L. 1998. The trophic significance of bacteria in a detritus-based stream food web. Ecology, **79**: 1995–2012. doi:10.1890/0012-9658(1998)079[1995:TTSOBI]2.0.CO;2.

Hall, R.O., Peredney, C.L., and Meyer, J.L. 1996. The effect of invertebrate consumption on bacterial transport in a mountain stream. Limnol. Oceanogr. **41**: 1180–1187. doi:10.4319/lo.1996.41.6.1180.

Hall, R.O., Peterson, B.J., and Meyer, J.L. 1998. Testing a nitrogen-cycling model of a forest stream by using a nitrogen-15 tracer addition. Ecosystems, **1**: 283–298. doi:10.1007/s100219900022.

Hall, R.O., Wallace, J.B., and Eggert, S.L. 2000. Organic matter flow in stream food webs with reduced detrital resource base. Ecology, **81**: 3445–3463. doi:10.1890/0012-9658(2000)081[3445:OMFISF]2.0.CO;2.

Hall, R.O., Baker, M.A., Arp, C.D., and Koch, B.J. 2009*a*. Hydrologic control of nitrogen removal, storage, and export in a mountain stream. Limnol. Oceanogr. **54**: 2128–2142. doi:10.4319/lo.2009.54.6.2128.

Hall, R.O., Tank, J.L., Sobota, D.J., et al. 2009*b*. Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: total uptake. Limnol. Oceanogr. **54**: 653–665. doi:10.4319/lo.2009.54.3.0653.

Hamels, I., Muylaert, K., Casteleyn, G., and Vyverman, W. 2001. Uncoupling of bacterial production and flagellate grazing in aquatic sediments: a case study from an intertidal flat. Aquat. Microb. Ecol. **25**: 31–42. doi:10.3354/ame025031.

Hamilton, S.K., and Ostrom, N.E. 2007. Measurement of the stable isotope ratio of dissolved N_2 in ^{15}N tracer experiments. Limnol. Oceanogr. Met. **5**: 233–240. doi:10.4319/lom.2007.5.233.

Hamilton, S.K., Tank, J.L., Raikow, D.F., Wolheim, W.M., Peterson, B.J., and Webster, J.R. 2001. Nitrogen uptake and transformation in a midwestern U.S. stream: a stable isotope enrichment study. Biogeochemistry, **54**: 297–340.

Hamilton, S.K., Tank, J.L., Raikow, D.F., Siler, E.R., Dorn, N.J., and Leonard, N.E. 2004. The role of instream vs allocthonous N in stream food webs: modeling the results of an isotope addition experiment. J. N. Am. Benthol. Soc. **23**: 429–448. doi:10.1899/0887-3593(2004)023<0429:TROIVA>2.0.CO;2.

Hardison, A.K. 2009. Interactions between macroalgae and the sediment microbial community: Nutrient cycling within shallow coastal bays. Ph.D. Dissertation, Faculty of the School of Marine Science, Virginia Institute of Marine Sciences.

Hardison, A.K., Canuel, E.A., Anderson, I.C., and Veuger, B. 2010. Fate of macroalgae in benthic systems: carbon and nitrogen cycling within the microbial community. Mar. Ecol. Prog. Ser. **414**: 41–55. doi:10.3354/meps08720.

Hershey, A.E., Pastor, J., Peterson, B.J., and Kling, G.W. 1993. Stable isotopes resolves the drift paradox for Baetis mayflies in an artic river. Ecology, **74**: 2315–2325. doi:10.2307/1939584.

Hesslein, R.H., Hallard, K.A., and Ramlal, P. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by δ^{34}S, δ^{13}C, and δ^{15}N. Can. J. Fish. Aquat. Sci. **50**: 2071–2076. doi:10.1139/f93-230.

Holmes, R.M., Peterson, B.J., Deegan, L.A., Hughes, J.E., and Fry, B. 2000. Nitrogen biogeochemistry in the oligohaline zone of a New England estuary. Ecology, **81**: 416–432. doi:10.1890/0012-9658(2000)081[0416:NBITOZ]2.0.CO;2.

Horne, A.J., and Goldman, C.R. 1994. Limnology. McGraw-Hill, New York.

Howarth, R.W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K., et al. 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. Biogeochemistry, **35**: 75–139. doi:10.1007/BF02179825.

Hubbard, K.A., Jr., Lautz, L.K., Mitchell, M.J., Mayer, B., and Hotchkiss, E.R. 2010. Evaluating nitrate uptake in a rocky mountain stream using labelled ^{15}N and ambient nitrate chemistry. Hydrol. Process. **24**: 3322–3336. doi:10.1002/hyp.7764.

Hughes, J.E., Deegan, L.A., Peterson, B.J., Holmes, R.M., and Fry, B. 2000. Nitrogen flow through the food web in the oligohaline zone of a New England Estuary. Ecology, **81**: 433–452. doi:10.1890/0012-9658(2000)081[0433:NFTTFW]2.0.CO;2.

Ings, T.C., Montoya, J.M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C.F., et al. 2009. Review: Ecological networks – beyond food webs. J. Anim. Ecol. **78**: 253–269. doi:10.1111/j.1365-2656.2008.01460.x. PMID:19120606.

Ives, A.R., Dennis, B., Cottingham, K.L., and Carpenter, S.R. 2003. Estimating community stability and ecological interactions from time-series data. Ecol. Monogr. **73**: 301–330. doi:10.1890/0012-9615(2003)073[0301:ECSAEI]2.0.CO;2.

Jansson, M., Bergström, A.-K., Blomqvist, P., Isaksson, A., and Jonsson, A. 1999. Impact of allochthonous organic carbon on microbial food web carbon dynamics and structure in Lake Örträsket. Fundam. Appl. Limnol. **144**: 409–428. doi:10.1127/archiv-hydrobiol/144/1999/409.

Jeppesen, E., Søndergaard, M., Søndergaard, M., and Christoffersen, K. 1998. The structuring role of submerged macrophytes in lakes. Springer-Verlag, Berlin, Germany.

Jetten, M.S., Van Niftrik, L., Strous, M., Kartal, B., Keltjens, J.T., and Op den Camp, H.J. 2009. Biochemistry and molecular biology of anammox bacteria. Crit. Rev. Biochem. Mol. **44**: 65–84. doi:10.1080/10409230902722783.

Johnson, L.T., Royer, T.V., Edgerton, J.M., and Leff, L.G. 2012. Manipulation of the dissolved organic carbon pool in an agricultural stream: responses in microbial community structure, denitrification, and assimilatory nitrogen uptake. Ecosystems, **15**: 1027–1038. doi:10.1007/s10021-012-9563-x.

Johnson, L.T., Tank, J.L., Hall, R.O., Mulholland, P.J., Hamilton, S.K., Valett, H.M., Webster, J.R., Bernot, M.J., McDowell, W.H., Peterson, B.J., and Thomas, S.M. 2013. Quantifying the production of dissolved organic nitrogen in headwater streams using 15N tracer additions. Limnol. Oceanogr. **58**: 1271–1285. doi:10.4319/lo.2013.58.4.1271.

Kazanci, C. 2007. EcoNet: A new software for ecological model simulation and network analysis. Ecol. Model. **208**: 3–8. doi:10.1016/j.ecolmodel.2007.04.031.

Kellman, L., and Hillaire-Marcel, C. 1998. Nitrate cycling in streams: using natural abundances of $NO_3 – \delta 15N$ to measure in situ denitrification. Biogeochemistry, **43**: 273–292. doi:10.1023/A:1006036706522.

Kling, G.W. 1994. Ecosystem scale experiments: the use of stable isotopes in freshwaters. *In* Environmental chemistry of lakes and reservoirs. *Edited by* L.A. Baker. American Chemical Society, Washington, D.C. pp. 91–120.

Kritzberg, E.S., Cole, J.J., Pace, M.L., Granéli, W., and Bade, D.L. 2004. Autochthonous versus allochthonous carbon sources of bacteria: results from whole-lake ^{13}C addition experiments. Limnol. Oceanogr. **49**: 588–596. doi:10.4319/lo.2004.49.2.0588.

Kritzberg, E.S., Cole, J.J., Pace, M.M., and Granéli, W. 2006. Bacterial growth on allochthonous carbon in humic and nutrient-enriched lakes: results from whole-lake ^{13}C addition experiments. Ecosystems, **9**: 489–499. doi:10.1007/s10021-005-0115-5.

Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., et al. 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol. Rev. **87**: 545–562. doi:10.1111/j.1469-185X.2011.00208.x. PMID:22051097.

Lee, K.M., Lee, S.Y., and Connolly, R.M. 2011. Combining stable isotope enrichment, compartmental modelling and ecological network analysis for quantitative measurement of food web dynamics. Methods Ecol. Evol. **2**: 56–65. doi:10.1111/j.2041-210X.2010.00045.x.

Li, K., Liu, Z., and Gu, B. 2010. The fate of cyanobacterial blooms in vegetated and unvegetated sediments of a shallow eutrophic lake: a stable isotope tracer study. Water Res. **4**: 1591–1597.

Likens, G.E. 1992. The ecosystem approach: its use and abuse. Ecology Institute, Oldendorf/Luhe, Germany. Available from http://www.int-res.com/articles/eebooks/eebook03.pdf.

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and Tardieux, P. 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. Plant Soil, **62**: 413–430. doi:10.1007/BF02374138.

Mariotti, A., Landreau, A., and Simon, B. 1988. ^{15}N isotope biogeochemistry and natural denitrification process in groundwater: application to the chalk aquifer of northern France. Geochim. Cosmochim. Ac. **52**: 1869–1878. doi:10.1016/0016-7037(88)90010-5.

Marti, E., and Sabater, F. 1996. High variability in temporal and spatial nutrient retention in Mediterranean streams. Ecology, **77**: 854–869. doi:10.2307/2265506.

Martínez del Río, C., Wolf, N., Carleton, S.A., and Gannes, L.Z. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol. Rev. **84**: 91–111. doi:10.1111/j.1469-185X.2008.00064.x. PMID:19046398.

Mayer, B., Boyer, E.W., Goodale, C., Jaworski, N.A., van Breemen, N., Howarth, R.W., et al 2002. Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: Isotopic constraints. Biogeochemistry, **57–58**: 171–198.

Merriam, J.L., Mcdowell, W.H., Tank, J.L., Wollheim, W.M., Crenshaw, C.L., and Johnson, S.L. 2002. Characterizing nitrogen dynamics, retention and transport in a tropical rainforest stream using an in situ ^{15}N addition. Freshwater Biol. **47**: 143–160. doi:10.1046/j.1365-2427.2002.00785.x.

Middelburg, J.J., Barranguet, C., Boschker, H.T.S., Herman, P.M.J., Moens, T., and Heip, C.H.R. 2000. The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study. Limnol. Oceanogr. **45**: 1224–1234. doi:10.4319/lo.2000.45.6.1224.

Morrall, D.D., Christman, S.C., Peterson, B.J., Wolheim, W.M., and Belanger, S.E. 2006. Utility of stable isotopes (^{13}C and ^{15}N) to demonstrate comparability between natural and experimental stream for environmental risk assessment. Ecotox. Environ. Safe. **65**: 22–35. doi:10.1016/j.ecoenv.2005.06.001.

Mulholland, P.J., Tank, J.L., Sanzone, D.W., Wolheim, W.M., Peterson, B.J., Webster, J.R., and Meyer, J.L. 2000*a*. Nitrogen cycling in a forest stream determined by a ^{15}N tracer addition. Ecol. Monogr. **70**: 471–493. doi:10.2307/2657212.

Mulholland, P.J., Tank, J.L., Sanzone, D.M., Wolheim, W.M., Peterson, B.J., Webster, J.R., and Meyer, J.L. 2000*b*. Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a ^{15}N tracer addition. J. N. Am. Benthol. Soc. **19**: 145–157. doi:10.2307/1468287.

Mulholland, P.J., Valett, H.M., Webster, J.R., Thomas, S.A., Cooper, L.W., Hamilton, S.K., and Peterson, B.J. 2004. Stream denitrification and total nitrate uptake rates measured using a field ^{15}N tracer addition approach. Limnol. Oceanogr. **49**: 809–820. doi:10.4319/lo.2004.49.3.0809.

Mulholland, P.J., Thomas, S.A., Valett, H.M., Webster, J.R., and Beaulieu, J. 2006. Effects of light on NO_3^- uptake in small forested streams: diurnal and day-to-day variations. J. N. Am. Benthol. Soc. **25**: 583–595. doi:10.1899/0887-3593(2006)25[583:EOLONU]2.0.CO;2.

Mulholland, P.J., Helton, A.M., Poole, G.C., Hall, R.O., Hamilton, S.K., Peterson, B.J., et al. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature, **452**: 202–205. doi:10.1038/nature06686. PMID:18337819.

Mulholland, P.J., Hall, R.O., Sobota, D.J., Dodds, W.K., Findlay, S.E., Grimm, N.B., et al. 2009. Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: denitrification. Limnol. Oceanogr. **54**: 666–680. doi:10.4319/lo.2009.54.3.0666.

O'Brien, J.M., Dodds, W.K., Wilson, K.C., Murdock, J.N., and Eichmiller, J. 2007. The saturation of N cycling in Central Plains streams: ^{15}N experiments across a broad gradient of nitrate concentrations. Biogeochemistry, **84**: 31–49. doi:10.1007/s10533-007-9073-7.

O'Brien, J.M., Hamilton, S.K., Podzikowski, L., and Ostrom, N. 2012. The fate of assimilated nitrogen in streams: an in situ benthic chamber study. Freshwater Biol. **57**: 1113–1125. doi:10.1111/j.1365-2427.2012.02770.x.

Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., Van de Bogert, M.C., et al. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature, **427**: 240–243. doi:10.1038/nature02227. PMID:14724637.

Pace, M.L., Carpenter, S.R., Cole, J.J., Coloso, J.J., Kitchell, J.F., Hodgson, J.R., et al. 2007. Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? Limnol. Oceanogr. **52**: 2177–2189. doi:10.4319/lo.2007.52.5.2177.

Parkyn, S.M., Quinn, J.M., Cox, T.J., and Broekhuizen, N. 2005. Pathways of N and C uptake and transfer in stream food webs: an isotope enrichment experiment. J. N. Am. Benthol. Soc. **24**: 955–975. doi:10.1899/04-082.1.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18**: 293–320. doi:10.1146/annurev.es.18.110187.001453.

Peterson, B.J., Howarth, R.W., and Garritt, R.H. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science, **227**: 1361–1363. doi:10.1126/science.227.4692.1361. PMID:17793771.

Peterson, B.J., Bahr, M., and Kling, G.W. 1997. A tracer investigation of nitrogen cycling in a pristine tundra river. Can. J. Fish. Aquat. Sci. **54**: 2361–2367. doi:10.1139/f97-142.

Peterson, B.J., Wollheim, W.M., Mulholland, P.J., Webster, J.R., Meyer, J.L., Tank, J.L., et al. 2001. Control of nitrogen export from watersheds by headwater streams. Science, **292**: 86–90. doi:10.1126/science.1056874. PMID:11292868.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology, **83**: 703–718. doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.

Raikow, D.F., and Hamilton, S.K. 2001. Bivalve diets in a midwestern U.S. stream: a stable isotope enrichment study. Limnol. Oceanogr. **46**: 514–522. doi:10.4319/lo.2001.46.3.0514.

Riera, J.L., Schindler, J.E., and Kratz, T.K. 1999. Seasonal dynamics of carbon dioxide and methane in two clear-water lakes and two bog lakes in northern Wisconsin, U.S.A. Can. J. Fish. Aquat. Sci. **56**: 265–274. doi:10.1139/f98-182.

Riera, P., Stal, L.J., Nieuwenhuize, J., Richard, P., Blanchard, G., and Gentil, F. 1999. Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen stable isotopes: importance of locally produced sources. Mar. Ecol. Prog. Ser. **187**: 301–307. doi:10.3354/meps187301.

Riis, T., Dodds, W.K., Kristensen, P.B., and Baisner, A.J. 2012. Nitrogen cycling and dynamics in a macrophyte-rich stream as determined by a ^{15}N-NH$_4$ release. Freshwater Biol. **57**: 1579–1591. doi:10.1111/j.1365-2427.2012.02819.x.

Rosemond, A.D., Benstead, J.P., Bumpers, P.M., Gulis, V., Kominoski, J.S., Manning, D.W.P., et al. 2015. Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. Science, **347**: 1142–1145. doi:10.1126/science.aaa1958. PMID:25745171.

Sánchez-Carrillo, S., Angeler, D.G., Álvarez-Cobelas, M., and Rojo, C. 2017. Abiotic drivers of consumer food-web structure in lakes. Fresh. Sci. [submitted].

Sanzone, D.M., Tank, J.L., Meyer, J.L., Mulholland, P.J., and Findlay, S.E.G. 2001. Microbial incorporation of nitrogen in stream detritus. Hydrobiologia, **464**: 27–35. doi:10.1023/A:1013930102876.

Sanzone, D.M., Meyer, J.L., Marti, E., Gardiner, E.P., Tank, J.L., and Grimm, N.B. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. Oecologia, **134**: 238–250. doi:10.1007/s00442-002-1113-3. PMID:12647165.

Schimel, D.S. 1993. Theory and application of tracers. Academic Press, San Diego.

Schindler, D.W. 2012. The dilemma of controlling cultural eutrophication of lakes. Proc. R. Soc. B, **279**: 4322–4333. doi:10.1098/rspb.2012.1032. PMID:22915669.

Schmidt, S.N., Olden, J.D., Solomon, C.T., and Vander Zanden, M.J. 2007. Quantitative approaches to the analysis of stable isotope food web data. Ecology, **88**: 2793–2802. doi:10.1890/07-0121.1. PMID:18051648.

Simon, K.S., Benfield, E.F., and Macko, S.A. 2003. Food web structure and the role of epilithic biofilms in cave streams. Ecology, **84**: 2395–2406. doi:10.1890/02-334.

Smith, R.L., Böhlke, J.K., Garabedian, S.P., Revesz, K.M., and Yoshinari, T. 2004. Assessing denitrification in groundwater using natural gradient tracer tests with ^{15}N: In situ measurement of a sequential multistep reaction. Water Resour. Res. **40**: W07101.

Sobota, D.J., Johnson, S.L., Gregory, S.V., and Ashkenas, L.R. 2012. A stable isotope tracer study of the influences of adjacent land use and riparian condition on fates of nitrate in streams. Ecosystems, **15**: 1–17. doi:10.1007/s10021-011-9489-8.

Solomon, C.T., Carpenter, S.R., Cole, J.J., and Pace, M.L. 2008. Support of benthic invertebrates by detrital resources and current autochthonous primary production: results from a whole-lake ^{13}C addition. Freshwater Biol. **53**: 42–54.

Taipale, S., Kankaala, P., Tiirola, M., and Jones, R.I. 2008. Whole-lake dissolved inorganic ^{13}C additions reveal seasonal shifts in zooplankton diet. Ecology, **89**: 463–474. doi:10.1890/07-0702.1. PMID:18409435.

Tamburini, F., Bernasconi, S.M., Angert, A., Weiner, T., and Frossard, E. 2010. A method for the analysis of the δ^{18}O of inorganic phosphate extracted from soils with HCl. Eur. J. Soil Sci. **61**: 1025–1032. doi:10.1111/j.1365-2389.2010.01290.x.

Tank, J.L., Mulholland, P.J., Meyer, J.L., Bowden, J.R., Webster, J.R., and Peterson, B.J. 2000a. Contrasting food web linkages for grazing pathway in three temperate forested stream using ^{15}N as a tracer. Verh. Internat. Verein Limnol. **27**: 2832–2835.

Tank, J.L., Meyer, J.L., Sanzone, D.M., Mulholland, P.J., Webster, J.R., Peterson, B.J., et al. 2000b. Analysis of nitrogen cycling in a forest stream during autumn using a ^{15}N tracer addition. Limnol. Oceanogr. **45**: 1013–1029. doi:10.4319/lo.2000.45.5.1013.

Tobias, C.R., Macko, S.A., Anderson, I.C., Canuel, E.A., and Harvey, J.W. 2001. Tracking the fate of a high concentration groundwater nitrate plume through a fringing marsh: a combined groundwater tracer and in situ isotope enrichment study. Limnol. Oceanogr. **46**: 1977–1989. doi:10.4319/lo.2001.46.8.1977.

Tobias, C.R., Cieri, M., Peterson, B.J., Deegan, L.A., Vallino, J., and Hughes, J. 2003. Processing watershed-derived nitrogen in a well-flushed New England Estuary. Limnol. Oceanogr. **48**: 1766–1778. doi:10.4319/lo.2003.48.5.1766.

Tollner, E.W., and Kazanci, C. 2007. Defining an ecological thermodynamics using discrete simulation approaches. Ecol. Model. **208**: 68–79. doi:10.1016/j.ecolmodel.2007.04.030.

van den Meersche, K., Soetaert, K., and Middelburg, J.J. 2011. Plankton dynamics in an estuarine plume: a mesocosm ^{13}C and ^{15}N tracer study. Mar. Ecol. Prog. Ser. **429**: 29–43. doi:10.3354/meps09097.

van Oevelen, D., Middelburg, J.J., Soetaert, K., and Moodley, L. 2006a. The fate of bacterial carbon in an intertidal sediment: modeling an in situ isotope tracer experiment. Limnol. Oceanogr. **51**: 1302–1314. doi:10.4319/lo.2006.51.3.1302.

van Oevelen, D., Moodley, L., Soetaert, K., and Middelburg, J.J. 2006b. The trophic significance of bacterial carbon in a marine intertidal sediment: results of an in situ stable isotope labeling study. Limnol. Oceanogr. **51**: 2349–2359. doi:10.4319/lo.2006.51.5.2349.

Vander Zanden, M.J., Chandra, S., Park, S.-Y., Vadeboncoeur, Y., and Goldman, C.R. 2006. Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. Can. J. Fish. Aquat. Sci. **63**: 2608–2620. doi:10.1139/f06-148.

Veuger, B., Van Oevelen, D., Boschker, H.T.S., and Middelburg, J.J. 2006. Fate of peptidoglycan in an intertidal sediment: An in situ ^{13}C-labeling. Limnol. Oceanogr. **51**: 1572–1580. doi:10.4319/lo.2006.51.4.1572.

Veuger, B., Eyre, B.D., Maher, D., and Middelburg, J.J. 2007. Nitrogen incorporation and retention by bacteria, algae, and fauna in a subtropical intertidal sediment: an in situ 15N-labeling study. Limnol. Oceanogr. **52**: 1930–1942. doi:10.4319/lo.2007.52.5.1930.

Vilar-Sanz, A., Puig, S., García-Lledó, A., Trias, R., Balaguer, M.D., Colprim, J., and Bañeras, L. 2013. Denitrifying bacterial communities affect current production and nitrous oxide accumulation in a microbial fuel cell. PLoS ONE, **8**: e63460. doi:10.1371/journal.pone.0063460.

Vollenweider, R.A. 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Technical Report DAS/CS 1/68.27. Organisation for Economic Co-operation and Development, Paris.

von Schiller, D., Martí, E., and Riera, J.L. 2009. Nitrate retention and removal in Mediterranean streams bordered by contrasting land uses: a ^{15}N tracer study. Biogeosciences, **6**: 181–196. doi:10.5194/bg-6-181-2009.

Webster, J.R., Mulholland, P.J., Tank, J.L., Valett, H.M., Dodds, W.K., Peterson, B.J., et al. 2003. Factors affecting ammonium uptake in streams – an inter-biome perspective. Freshwater Biol. **48**: 1329–1352. doi:10.1046/j.1365-2427.2003.01094.x.

Weidel, B., Carpenter, S., Cole, J., Hodgson, J., Kitchell, J., Pace, M., and Solomon, C. 2008. Carbon sources supporting fish growth in a north temperate lake. Aquat. Sci. **70**: 446–458. doi:10.1007/s00027-008-8113-2.

Weidel, B.C., Carpenter, S.R., Kitchell, J.F., and Vander Zanden, M.J. 2011. Rates and components of carbon turnover in fish muscle: insights from bioenergetics models and a whole-lake ^{13}C addition. Can. J. Fish. Aquat. Sci. **68**: 387–399. doi:10.1139/F10-158.

Wetzel, R. 2001. Limnology. Academic Press, San Diego.

Wilcox, H.S., Bruce Wallace, J., Meyer, J.L., and Benstead, J.P. 2005. Effects of labile carbon addition on a headwater stream food web. Limnol. Oceanogr. **50**: 1300–1312. doi:10.4319/lo.2005.50.4.1300.

Wollheim, W.M., Peterson, B.J., Deegan, L.A., Bahr, M., Hobbie, J.E., Jones, D., et al. 1999. A coupled field and modeling approach for the analysis of nitrogen cycling streams. J. N. Am. Benthol. Soc. **18**: 199–221. doi:10.2307/1468461.

Wollheim, W.M., Peterson, B.J., Deegan, LA, Hobbie, J.E., Hooker, B., Bowden, W.B., et al. 2001. Influence of stream size on ammonium and suspended particulate nitrogen processing. Limnol. Oceanogr. **46**: 1–13. doi:10.4319/lo.2001.46.1.0001.

Yoshii, K., Melnik, N.G., Timoshkin, O.A., Bondarenko, N.A., Anoshko, P.N., Yoshioka, T., and Wada, E. 1999. Stable isotope analyses of the pelagic food web in Lake Baikal. Limnol. Oceanogr. **44**: 502–511. doi:10.4319/lo.1999.44.3.0502.

Yu, J., Li, Y., Liu, X., Li, K., Chen, F., Gulati, R., and Liu, Z. 2013. The fate of cyanobacterial detritus in the food web of Lake Taihu: a mesocosm study using ^{13}C and ^{15}N labeling. Hydrobiologia, **710**: 39–46. doi:10.1007/s10750-012-1205-y.

Zar, J.H. 1996. Biostatistical analysis. Prentice Hall, Upper Saddle River.

Zarnetske, J.P., Haggerty, R., Wondzell, S.M., and Baker, M.A. 2011a. Dynamics of nitrate production and removal as a function of resident time in the hyporeic zone. J. Geophys. Res. **116**: G01025. doi:10.1029/2010JG001356.

Zarnetske, J.P., Haggerty, R., Wondzell, S.M., and Baker, M.A. 2011b. Labile dissolved organic carbon supply limits hyporheic denitrification. J. Geophys. Res. **116**: G04036. doi:10.1029/2011JG001730.

Zhang, L., Li, K., Liu, Z., and Middleburg, J.J. 2010. Sedimented cyanobacterial detritus as a source of nutrient for submerged macrophytes *Vallisneria spiralis* and *Elodea nuttallii*: an isotope labeling experiment using ^{15}N. Limnol. Oceanogr. **55**: 1912–1917. doi:10.4319/lo.2010.55.5.1912.