This manuscript is in press. It has been accepted for publication in *Transactions of the ASABE*. When the final, edited version is posted online this in-press version will be removed. Example citation: Authors. Year. Article title. *Trans. ASABE* (in press). DOI number.
The DOI for this manuscript is https://doi.org/10.13031/trans.-- . It will remain the same after publication.

IMPROVING IN-STREAM NUTRIENT ROUTINES IN WATER QUALITY MODELS USING STABLE ISOTOPE TRACERS: A REVIEW AND SYNTHESIS

Alexandria Jensen[1], William Ford[2], James Fox[3], Admin Husic[4]

[1]Graduate Assistant, Biosystems and Agricultural Engineering, University of Kentucky
[2]Assistant Professor, Biosystems and Agricultural Engineering, University of Kentucky (**corresponding author**)
[3] Professor, Civil Engineering Department, University of Kentucky.
[4] Graduate Assistant, Civil Engineering Department, University of Kentucky.

Corresponding author: William Ford, bill.ford@uky.edu, 859-218-4368

ABSTRACT

Water quality models serve as an economically feasible alternative to quantify fluxes of nutrient pollution and to simulate effective mitigation strategies; however, their applicability is often questioned due to broad uncertainties in model structure and parameterization leading to uncertain outputs. We argue that reduction of uncertainty is partially achieved by integrating stable isotope data streams within the fabric of water quality model architecture. This paper outlines the use of stable isotopes as a response variable within water quality models to improve model boundary conditions associated with nutrient source provenance, constrain model parameterization, and elucidate shortcomings in model structure. To assist researchers in future modeling efforts, we provide an overview of stable isotope theory; review isotopic signatures and applications for relevant carbon, nitrogen, and phosphorus pools; identify biotic and abiotic processes impacting isotope transfer between pools; review existing models that have incorporated stable isotope signatures; and highlight recommendations based on synthesis of existing knowledge. Broadly, we find existing applications that utilize isotopes have high efficacy for reducing water quality model uncertainty. We make recommendations towards the future use of sediment stable isotope signatures given their integrative capacity and practical analytical process. We also detail a method to incorporate stable isotopes as part of multi-objective modeling frameworks. Finally, we encourage watershed modelers to work closely with isotope geochemists to ensure proper integration of stable isotopes into in-stream nutrient fate and transport routines in water quality models.

Keywords. Isotopes, Water quality modeling, Nutrients, Uncertainty analysis, Watershed.

1 INTRODUCTION:

Deterministic water quality models provide an economically feasible approach to quantify fluxes and transformations of nutrients and for scenario analysis of dynamic management, land-use, and climate conditions. Nevertheless, the reliability of such models to assist with management decisions is questioned due to compounding uncertainties regarding in-stream transformation rates of contaminants (Beven, 2006; Rode et al., 2010; Robson, 2014; Yen et al., 2014; Wellen et al., 2015; Han and Zheng, 2016). It is the general sentiment in the hydrology and water quality community that researchers need to reduce uncertainty within water quality models. We work towards this goal by providing a review and synthesis of how stable isotope tracers can reduce uncertainty in these applications.

High uncertainty within water quality modeling is likely an artifact of historical development of water quality models and continued advancements in perceptual understanding of fluvial biogeochemistry. Following a historical period that saw the development of hydrologic and biogeochemical functions from data collected at the hillslope-plot and stream-reach scales in the early 1970's and 80's, watershed water quality modeling saw rapid advancement *via* computational capabilities in the 1990's and 2000's to address growing environmental issues related to nutrients (e.g., estuary seasonal hypoxia). Computational advancement allowed several modeling characteristics to take shape, including the ability to: inexpensively incorporate spatially explicit data; perform computations at a different resolution or environment than originally envisioned; and couple water, particulate, and dissolved phases within single numerical model formulations. However, computational advancement of water quality models has not necessarily translated into enhanced structural representation of in-stream physics and biogeochemistry. For example, conceptual models have been shown to be quite powerful for understanding fluxes from watersheds (Ford et al., 2017). However, new monitoring and measurement capabilities have shown researchers that coupled physio-biochemical processes may vary from the original hydrologic and biogeochemical functions in models. Furthermore, computational advancements have shifted parameterization of models away from inputs and parameters consistent with their original scale of observation and have produced numerous likely inputs and parameter sets within modeling frameworks (e.g., equifinality as described below). As a result, computational abilities have outweighed the modeler's ability to constrain input and parameter values and have promoted large posterior solution spaces resulting in high uncertainty. Such uncertainty should be accounted for when reporting and analyzing results of water quality models.

Given the need to constrain input and parameter values and prevent erroneous model parameterization, innovative data streams should be incorporated into water quality models. Integration of stable isotopes for carbon (C), nitrogen (N), and phosphorus (P) compounds within model architecture provides one such measurement tool to assist with model uncertainty reduction. This assertion comes following recent success of water isotope measurements to help parameterize model boundary conditions, reduce model uncertainty due to equifinality, and improve numerical representation of processes within hydrologic model structure (Seibert and McDonnell, 2002; McGuire and McDonnell, 2008; McDonnell and Beven, 2014; Windhorst et al. 2014; Soulsby et al. 2015; Yamanaka and Ma, 2016). In this light, this review article synthesizes the utility of stable isotopes within water quality models to reduce uncertainty contributed by overparameterization in numerical model estimates, given the ability of stable isotopes to be measured with relatively high precision and accuracy. Our focus is on in-stream biogeochemical modeling of macronutrients, namely C, N, and P, but at the same time it is well-realized that accurate representation of water and solids (i.e., sediment) within streams is a precursor to predicting C, N, and P fluxes and transformations.

We show recent literature evidence that coupling stable isotopes within watershed water quality modeling helps with improving data inputs associated with: (i) providing boundary conditions of the models, (ii) constraining model parameterization, and (iii) elucidating improvements needed within conceptual and numerical representation of processes, i.e., model structure. The efficacy of stable isotopes for this uncertainty reduction goal is noteworthy given that recent attention on watershed water quality modeling uncertainty have highlighted these same inaccuracies—problems with precision and accuracy of input and calibration measurements, uncertainty in parameter specification, and the problem of inaccurate model structure—as three major sources of uncertainty within models (Guzman et al., 2015).

(i) Boundary condition refers to the source contributions of C, N, and P phases that need to be considered within watershed water quality modeling. For example, within a nutrient focused model, the boundary condition inputs refer to the spectrum of potential nutrient inputs, such as N and P from agricultural and urban sources (Xue et al., 2009; Young et al, 2009; Kendall et al., 2010). As another example, within a sediment C focused model, the boundary condition inputs refer to the spectrum of potential sediment C inputs, such as inorganic C, terrestrial derived particulate C, and autochthonous derived particulate C (Fox and Ford, 2016; Husic et al., 2017a). The use of stable C, N, and P-bound isotopes to elucidate the boundary condition inputs within the fabric of watershed water quality modeling is perhaps the most obvious coupling of isotopes with the models given the widely-used data driven un-mixing analysis for apportioning source contributions of both dissolved and particulate phases. Source apportionment has existed as a standalone method and therefore coupling this method to assist within boundary conditions within water quality models seems natural. For these reasons, several studies have used stable isotopes to assist with boundary conditions within numerical models (Hong et al., 2014; Sebestyen et al., 2014; Xue et al., 2014; Fox and Martin, 2014; Ford and Fox, 2015; Husic et al., 2017b).

(ii) Constraining parameter uncertainty is another prominent problem with in-stream models, especially as the level of model complexity *via* coupling of processes and phases (i.e., dissolved, particulate, water) increases. For such models, the broad range of parameters leads to large posterior solution spaces for fluxes and transformations. Parameter specification uncertainty is robustly reflected through the concept of equifinality, which refers to the potential for a posterior solution space of acceptable calibrations to be met by multiple parameterizations, or realizations (Beven, 2006; Adiyanti et al., 2016). The Generalized Likelihood Uncertainty Estimation framework provides a means to quantify equifinality and is applied using Monte Carlo-based realizations of a global parameter space and evaluation of the subsequent solutions against measured data to create a posterior solution space (Beven and Binley, 1992; Dean et al., 2009; Jin et al., 2010; Gong et al., 2011; Shen et al., 2012; Ford and Fox, 2017). The acceptance into such a solution space depends on evaluation of measured and modeled data using statistical metrics such as Nash-Sutcliffe Efficiency, percent bias, and ratio of the root mean square error to standard deviation of measured data, e.g., Moriasi et al. (2007). While we commend the excellent work of researchers to quantify this uncertainty, it has been shown that stable isotopes may also be coupled with water quality models to further reduce such uncertainty (Adiyanti et al., 2016; Ford et al., 2017). In many ways, elucidation of parameterization *via* stable isotopes within watershed water quality is another highly conceivable method given the long history of stable isotopes to elucidate reactions (Sharp, 2007). Essentially, stables isotope mass balances that couple biogeochemical reactions within their structure may be added to the elemental mass balances of water quality models as described in Section 2. These added equations are often accompanied with few new unknowns or insensitive unknowns; therefore, a stable isotope data stream may assist with model parameterization. For these reasons, several studies have used stable isotopes to help with parameterizing water quality models (Tobias and Bohlke, 2011; Van Engeland et al., 2012; Hong et al., 2014; Fox and Martin, 2014; Ford and Fox, 2015; Adiyanti et al., 2016; Ford et al., 2017).

(iii) Elucidating improvements in model structure reflects a final opportunity where stable isotopes may assist with advancing research. As complexity of nutrient cycling continues to unravel through contemporary measurement techniques, it is recognized that numerical model error could be associated with epistemic uncertainties. Regarding epistemic uncertainty, model structure errors may stem from simplified conceptual models, the equations and algorithms used to reflect that conceptualization, and instabilities of the numerical scheme (Borah and Bera, 2003; Guzman et al., 2015). Recent critiques of water quality models have pointed to a need for improving in-stream biogeochemical simulations (Rode et al., 2010; Robson, 2014; Wellen et al., 2015). As an example, advanced deterministic models that reflect in-stream C and nutrient fate and transport (e.g., AQUATOX, QUAL2K, and WASP) conceptualize the benthos as a two layer, 1 mm aerobic and 10 cm anaerobic, system in which all particulate organic matter is contained in the anaerobic layer and is not subjected to erosion-deposition dynamics (DiToro, 2001; Wool et al., 2006; Chapra et al., 2008; Park et al., 2008). This conceptualization was well-validated for large, slow-moving waterbodies; however, for turbulent low-order and low-gradient streams, recent research has highlighted the importance of a dynamic 5-10 mm aerobic sediment layer – the surficial fine-grained laminae – that controls seasonality of benthic C and N dynamics (Droppo et al., 2001; Walling et al., 2006; Russo and Fox, 2012; Ford and Fox, 2014, 2015, 2017; Fox et al., 2014). As models become more robust, unique tools and approaches are needed that rigorously test our conceptualization of in-stream fate and transport. Stable isotopes coupled within water quality modeling may be used through iterations to enhance or test validity of model structure (Tobias and Bohlke, 2011; Hong et al., 2013; Sebestyen et al., 2014; Ford et al., 2017).

This review will explain to the reader the utility of stable isotopes to improve existing water quality model predictions and uncertainty reduction by improving in-stream nutrient fate and transport routines, specifically (i) boundary conditions of the models, (ii) constraining model parameterization, and (iii) elucidating improvements needed within model structure. In order that the reader might utilize stable isotopes within water quality models for such goals, we provide a sequential and comprehensive review of stable isotopes within the fabric of water quality models. First, we define and explain stable isotope theory for modelling-focused researchers whom have had minimal exposure to isotope signatures. Second, we describe pools, measurements, and applications of stable isotope signatures related to C, N, and P cycles in streams. Third, we describe the ability of the isotopes to elucidate sources and transformations so that the modeler might understand the breadth of possibilities of where the isotopes are applicable in the stream environment. Fourth, we review watershed water quality modeling studies that have coupled stable isotopes and show how these studies have used the isotopes to reduce uncertainty associated with (i) boundary conditions of the models, (ii) constraining model parameterization, and (iii) elucidating improvements needed within model structure. Fifth, we provide recommendations to the watershed water quality modelers as they couple isotopes into the fabric of the modeling architecture.

2 STABLE ISOTOPES OVERVIEW

Stable isotopes of a given element have identical chemical properties except for difference in atomic mass, which is caused by a variable number of neutrons in the nucleus. Carbon, nitrogen, oxygen, and hydrogen are elements that all have heavy and light stable isotopes in which relative abundance of the heavy isotope is measured with high precision using isotope ratio mass spectrometry. The relative abundance of heavy to light isotopes for different oxidation states of an element (e.g., ammonium, nitrate, nitrite) are defined utilizing the widely-used delta (δ) notation. In the determination of isotopic ratios, the relative differences between a sample and a reference standard may be ascertained with high precision. The delta notation (δ) was developed by McKinney et al. (1950) to report stable isotope data and is generically defined in Equations (1) and (2) as follows:

$$\delta = \left(\frac{R_{smpl} - R_{std}}{R_{std}}\right) * \ 1000, \quad (1)$$

where R is the ratio of the abundance of the heavy to light isotope, *smpl* is the sample, *std* is the reference standard that has a known isotope ratio. R is defined explicitly as:

$$R = \frac{[^{m}X]}{[^{n}X]}, \quad (2)$$

where ^{m}X is the heavy isotope, and ^{n}X is the light isotope.

The units of measurement for δ values are reported in per mil or parts per thousand, symbolized as ‰, which is reflective of the relatively low abundance of the heavy isotopes in the natural environment. A positive δ value would indicate that the ratio of heavy to light isotope is greater in the sample than the standard and vice versa for a negative δ value.

Stable isotopes are particularly effective in fingerprinting sources and quantifying rates of biogeochemical transformations due to preferential utilization of lighter isotopes in a process termed isotope fractionation. Fractionation is characterized by either equilibrium or kinetic isotope effects (Sharp, 2007). Equilibrium isotope-exchange reactions means that the forward and backward reaction rates of any single isotope are equal. Kinetic isotope effects cause isotope fractionation to happen when the system is not in isotopic equilibrium and the forward and backward reaction rates are not equal. In kinetic isotope fractionation, the reaction rates are factors of the isotope masses and their vibrational energy; bonds between the light isotopes break more easily than the heavy isotopes, which have stiffer bonds. This results in the preferential utilization of lighter isotopes during processes because of less energy required to break the lighter bonds. Fractionation processes that are not metabolically driven or kinetically controlled are associated with either an isotope fractionation factor, α, or an enrichment factor ε, which is determined either analytically or experimentally. These values are directly related to one another through Equations (3), (4), and (5) as:

$$\alpha_{A-B} = \frac{R_A}{R_B}, \tag{3}$$

where α_{A-B} is the partitioning of stable isotopes between two substances *A* and *B* (reflected in Figure 1) and *R* is the ratio of heavy to light isotope as described in Equation (1), calculated for each substance. This equation is expressed as:

$$\alpha_{A-B} = \frac{1000+\delta_A}{1000+\delta_B}, \tag{4}$$

where δ is the relative abundance calculated using Equation (2), calculated for each substance. The fractionation factor, α, is then related to the enrichment factor, ε, as:

$$\varepsilon = (\alpha - 1) * 1000 \tag{5}$$

Using the δ values and fractional contributions of known sources coupled with ε values and rates of reactions, the resulting value of a product is estimated. Namely, the famous Rayleigh formulation

(Kendall and Caldwell, 1998) shown in Equation (6) is used and coupled to isotope mass-balance considerations in separation processes where a product is removed from a reactant. The Rayleigh equation is used to describe isotopic fractionation processes under the following assumptions: (1) in a mixed system, material is continuously removed that contains molecules of at least two isotopic species (e.g., water with ^{18}O and ^{16}O), (2) the fractionation associated with the removal process at any instant may be described by the fractionation factor and the enrichment factor, and (3) that the fractionation factor and enrichment factor remain constant during the process (Kendall and Caldwell, 1998). The Rayleigh equation may be described as:

$$\delta^M X_B = \delta^M X_A - \varepsilon_{rxn} ln(f_{B-A}) \,, \tag{6}$$

where M is the atomic mass of the isotope, X is the isotope, A and B are the two substances, rxn is the reaction process or pathway of removal, and f is the fraction remaining after the process occurs. Application of this equation becomes invalid under transient kinetic fractionation, which occurs when the reactions leading to fractionation do not follow first-order kinetics (Maggi and Riley, 2009). In general, this limitation may be assumed to have minor impact for nutrient rich systems and would not be rate limiting in terms of lack of availability of the lighter isotope during removal.

Equation (6) is a suitable general definition of the enrichment process but may be further expanded to accurately represent dynamics of the system. Multiple inputs across a specified control volume will result in a mixing of sources, as is illustrated in Figure 1 (left box). To more accurately represent the upstream conditions, $\delta^M X_A$ may be broken into a summation incorporating the weighted average of each of the unique source inputs (e.g., the three-source mixing example depicted in Figure 1) as:

$$\delta^M X_A = \sum_{l=1}^{k} \delta^M X_l (W_l) \,, \tag{7}$$

where l represents the source identifier, k represents total number of sources, and W_l represents the fraction of element X from a source l. Furthering this concept of multiple factors influencing the overall $\delta^M X$ value, Figure 1 (right box) provides a generic definition sketch of the processes of isotope fractionation to impact stream isotope signatures in a generic stream reach with a generic isotope tracer. Prior to entering the stream at Input A, there is an abundance of the light isotope contrasted with the heavy isotope, as the substance flows through the stream channel, different biogeochemical processes (e.g., ε_1 and ε_2) occur that preferentially utilize the lighter isotope as opposed to the heavier isotope. These reactions impact the mass and isotope composition of the outputs depending on the magnitude of the process and the preference for the lighter isotope. As depicted in Output B of Figure 1, the size of the substances pool has decreased and the ratio of heavy to light isotope has increased relative to Input A because of the fractionating processes (ε_1 and ε_2). The influence of the different biogeochemical processes and fractionation factors may be reflected in the general expression of Equation (6) as:

$$\delta^M X_B = \delta^M X_A - \sum_{o=1}^{p} \varepsilon_o ln(f_o) \ , \tag{8}$$

where, o represents the enrichment factor identifier, and p represents total number of fractionation processes.

We may represent the isotope source mixing and fractionation processes dynamically by discretizing our systems spatially and temporally. Merging equations (7) and (8) and assuming constant enrichment factors through time and space we can use the following finite difference approximation for the stable isotope mass-balance:

$$\delta^M {X_B}_i^j = \sum_{l=1}^{k} \delta^M {X_{i,l}}^j ({W_{i,l}}^j) - \sum_{o=1}^{p} \varepsilon_o ln({f_{i,o}}^j) \ , \tag{9}$$

where, i is the timestep identifier, and j is the reach identifier. In this definition, the mass of an element remaining in a stream reach from a previous timestep is considered a source and is accounted for in the first summation term.

3 OVERVIEW OF C, N, AND P STABLE ISOTOPES IN FLUVIAL SYSTEMS

Isotope signatures have been utilized broadly by environmental and water resource engineers as well as aquatic biogeochemists to study C, N, and P dynamics in streams and rivers (Table 1). The following section will outline (i) the pools of C, N, and P species, (ii) the isotope signatures used to study C, N, and P dynamics in streams, and (iii) some overarching applications that isotopes have been commonly used for.

3.1 Carbon

Primary forms of C in fluvial ecosystems include dissolved organic C (DOC), particulate organic C (POC), and dissolved inorganic C (DIC) in the form of dissolved carbonates (see Hope et al., 1994). Briefly, DIC occurs as CO_3^{2-}, HCO_3^-, H_2CO_3, and dissolved CO_2, collectively form the carbonate system. POC and DOC are C from organic based compounds including terrestrial leaf litter and detritus, autochthonous biomass, and biota. POC is distinguished from DOC by size classification, i.e., the solid matter that is retained on a 0.45 μm filter. For the purposes of this paper, POC is further classified as fine POC (silt and clay sized particles, or $d < 53$ μm) or coarse POC (sand, cobble, or gravel sized particles, or $d > 53$ μm). DOC is primarily composed of fulvic and humic acids leached from upland soils and benthic organic matter.

Carbon exists in three isotopic forms with ^{12}C and ^{13}C as stable isotopes and ^{14}C as the radioactive isotope; here only the stable forms are considered. Carbon isotopic signatures are readily measured for all forms using well-accepted methods and are reported as the relative abundance of ^{13}C to ^{12}C for a sample as:

$$\delta^{13}C_{sample} = \left(\frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{VPDB}}{(^{13}C/^{12}C)_{VPDB}}\right) * \ 1000, \qquad (10)$$

where, VPDB is the reference standard Vienna Pee Dee Belemnite.

Well accepted methods exist to measure $\delta^{13}C$ of all three pools. $\delta^{13}C_{DIC}$ has been used as a tracer of C pathways, biotic uptake and regeneration, and atmospheric exchange rates (e.g., Doctor et al. 2008; Throckmorton et al. 2015). $\delta^{13}C_{DOC}$ has been used in a wide variety of applications including quantitative apportionment of allochthonous versus autochthonous organic matter (Grey et al., 2001; Zah et al., 2001; Kritzberg et al., 2004; Doi, 2008; Lau et al., 2009), providing information on trophic linkages (Rosenfeld et al., 1992; Zah et al., 2001; Doi, 2008; Lau et al., 2009), and characterizing nutrient sources and terrestrial inputs (Thornton and McManus, 1994; Palmer et al., 2001; Hood et al., 2005). $\delta^{13}C_{FPOC}$ has commonly been utilized as a fingerprint for sediment source apportionment (Papanicolaou et al., 2003; Fox and Papnicolaou, 2007; Fox, 2009; Jacinthe et al., 2009; Imberger et al., 2014), a metric to partition terrestrial versus allochthonous organic matter contributions in suspended loads (e.g., Kendall et al., 2001), and a metric to provide insight into organic matter quality (Ford et al., 2015; Fox and Ford, 2016; Lu et al., 2016).

3.2 Nitrogen

Prevailing pools of N in fluvial ecosystems include dissolved organic N (DON), dissolved inorganic N (DIN), and particulate organic N (PON). Distinctions between DOC and POC also apply for DON and PON. Regarding DIN, nitrate (NO_3^-) and ammonium (NH_4^+) are of the largest pools; however, nitrite (NO_2^-) may often also exist in measurable quantities in the water column. Nevertheless, NO_2^- is an intermediate step in the nitrification process and, in general, is rapidly converted to nitrate (Kendall, 1998).

Nitrogen has two stable isotopes (^{14}N and ^{15}N); hence stable isotope signatures reflect the relative abundance of $^{15}N/^{14}N$ as:

$$\delta^{15}\mathrm{N}_{\mathrm{sample}} = \left(\frac{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{sample}} - (^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{ref}}}{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{ref}}}\right) * 1000, \tag{11}$$

where, *ref* is derived from atmospheric N_2 or solid reference samples from NIST and IAEA (Sharp, 2007). In addition, dual isotope approaches are commonly used for nitrate source apportionment studies. Stable oxygen isotope signatures of nitrate reflect the relative abundance of 18-O to 16-O as:

$$\delta^{18}O_{NO3} = \left(\frac{(^{18}O/^{16}O)_{NO3} - (^{18}O/^{16}O)_{VSMOW}}{(^{18}O/^{16}O)_{VSMOW}}\right) * 1000, \tag{12}$$

where, VSMOW is the international standard Vienna Standard Mean Ocean Water (Tamburini et al., 2014).

As shown in Table 1, N isotope signatures are commonly measured for DIN, PON, and DON. Similar to C, δ^{15}N of PON and DON have been used to separate allochthonous and autochthonous pathways in trophic interactions (Rounick and Winterbourn, 1986), distinguish aquatic and terrestrial organic matter sources (Finlay, 2001; Kendall et al., 2001; England and Rosemond, 2004), denitrification and plant uptake rates (Clement et al. 2003), and perform sediment source apportionment (Fox and Papanicolaou, 2007; Fox, 2009). Measurements of DIN have included injection and ambient measures to assess sources and biogeochemical transformations. Enriched ^{15}N tracer applications of DIN have been widely used since the 1960s for investigations of monitoring of the specific product (^{15}N) input to the stream (Webster et al., 2003; Ashkenas et al., 2004; Bohlke et al., 2004) and have been useful in estimating biological uptake and regeneration rates in streams. Ambient measures of isotope signatures of ammonium, nitrate, and nitrite are commonly used in streams and rivers for source identification and assessing *in situ* rates of in-stream transformations. Ammonium isotope applications have incorporated $\delta^{15}N$ measurements to effectively indicate the amount of exchangeable ammonium in soils (Bremner and Keeney, 1966), algal assimilation of ammonium (Cifuentes et al., 1989), and determine the dissolved ammonium level at natural abundance conditions from estuarine waters (Velinsky et al., 1989). Ambient dual isotope approaches for nitrate are commonly employed and have been reviewed extensively elsewhere (Chang et al., 2002; Fukada et al., 2003; Wankel et al., 2006; Xue et al., 2009). The usage of ^{18}O of nitrate coupled with ^{15}N of nitrate is effective at linking the prior value to the entire N cycle that may typically be biased due to kinetic isotope fractionation or source mixing (Komor, 1997; Aravena and Robertson, 1998; Widory et al., 2004; Seiler, 2005).

3.3 Phosphorus

Analogous to C and N, primary pools of P include permutations of organic, inorganic, particulate, and dissolved phases and interactions between those phases (Figure 4a; Withers and Jarvie, 2008). Most commonly studied pools in stream ecosystems include dissolved inorganic (or reactive) P (DRP) and sediment exchangeable particulate inorganic P (PIP) that includes mineral precipitates and adsorption to sediment surfaces (Withers and Jarvie, 2008). These pools have likely received attention due to their relatively high abundance in urban and agroecosystems, and their ability to, independently, promote downstream eutrophication. Nevertheless, the fluvial P cycle is also affected by particulate organic P and dissolved organic P.

Isotope tracing of P source, fate, and transport is an emerging technique in freshwater ecosystems that has been successfully applied over the past decade to study dissolved inorganic P dynamics and microbial activity in soils and sediment. Phosphorus has three isotopes (^{31}P, ^{32}P, and ^{33}P), the heavier isotopes (^{32}P and ^{33}P) are radioactive, making direct stable isotope tracing of P impossible. Fortunately, oxygen is commonly bound to P as phosphate (PO_4^{3-}) and is resistant to equilibrium fractionation due to hydrolysis in natural environments; hence, oxygen may be used as a discriminator of P sources and an ambient indicator of P cycling (Young et al., 2009; Elsbury et al., 2009; Davies et al., 2014). The oxygen isotopic composition of phosphate is defined using standard delta notation as:

$$\delta^{18}O_{PO4} = \left(\frac{(^{18}O/^{16}O)_{PO4} - (^{18}O/^{16}O)_{VSMOW}}{(^{18}O/^{16}O)_{VSMOW}}\right) * \ 1000. \qquad (13)$$

Measurement of $\delta^{18}O$ values has been performed for DRP in streamwater and PIP in soils and sediments, which is in-line with most readily measured pools. Regarding DRP, the $\delta^{18}O_{DRP}$ signature has been found to be a potentially effective tracer for sources where variable rates of microbial processing is present (Young et al., 2009; Davies et al., 2014). For soils, $\delta^{18}O_{PO4}$ has been used as a source identifier to trace P movement through the environment, as an indicator of biological activity within soils, and to assess variability of $\delta^{18}O_{PO4}$ in plant-soil pools (Angert et al., 2012; Tamburini et al., 2012). We refer the reader to Tamburini et al. (2014) for a detailed review of relevant case-studies. Recently, extraction methods for benthic and transported sediment samples have been developed (Pistocchi et al., 2017). This approach provides high promise for tracking in-stream microbial processing of benthic sediment P and for integrating source signatures of upland DRP due to high affinity of sediments for phosphate adsorption (Pistocchi et al., 2017).

4 PROCESSES IMPACTING STREAM ISOTOPE COMPOSITIONS

In this section, we highlight the efficacy for isotope measurements to reflect water quality processes for C, N, and P cycles. Figures 2-4 highlight the biotic (2a, 3a, 4a) and abiotic (2b, 3b, 4b) processes that impact stream C (Ford and Fox, 2015), N (Peterson et al., 2001; Birgand et al., 2007; Ford et al., In Review), and P (Withers and Jarvie, 2008). We recognize that processes are often a mixture of biological, chemical, and physical mechanisms (e.g., biochemical reduction of nitrate to dinitrogen gas); therefore, for purposes of this study, we make the distinction between biotic (biological and biochemical) and abiotic (non-biological chemical and physical) processes. We highlight the impacts on atmospheric, water, biota, and sediment pools by showing isotope fractionations and flux contributions to and from each pool.

4.1 Carbon

Biotic uptake of autochthonous biomass and mineralization of organic matter by endogenous and heterotrophic respiration are the primary biotic mechanisms impacting fluvial organic C cycling (Figure 2a; Ford and Fox, 2014, 2017; Hotchkiss et al., 2015). Stabilization is a process in which DIC from the streamwater pool is assimilated in autochthonous biomass and then more complex organic C compounds are decomposed to fine sediments that have slower rates of decomposition, i.e., compounds are more recalcitrant to biotic mineralization (Lane et al., 2013). Autochthonous biota, including benthic algae, macrophytes, and phytoplankton, fix dissolved inorganic C into particulate organic C during photosynthesis. Regarding uptake, the C isotopic signature of stabilized autochthonous organic matter is typically low in ^{13}C relative to allochthonous matter due to ^{13}C of DIC that is depleted relative to atmospheric CO_2 and has a high isotope fractionation value (ε between 15 to 25‰) (Sharp, 2007; Tobias and Bohlke, 2011; Ford and Fox, 2015). Sediment decomposition and mineralization of organic C results in a loss from the sediment or biota pool and is added to the dissolved inorganic pool and may occur through either aerobic or anaerobic conditions. Sediment C regeneration through oxidation of organic matter to CO_2 imparts a small fractionation compared to the autochthonous fractionation on the DIC pool (ε < 2‰) (Jacinthe et al., 2009; Ford and Fox, 2015). Degradation of organic matter to methane under anaerobic conditions may be important in landscapes such as peat bogs resulting in fractionations of 5 to 10‰ (Galand et al., 2010).

Prominent abiotic processes impacting the fluvial C cycle include CO_2 flux across the air-water interface, mineral precipitation and dissolution, and hydrodynamic alterations to benthic sediment and biota pools (Figure 2b). CO_2 often evades the stream channel and acts as a source to the atmosphere due to the high rates of mineralization in soil water and benthic sediments that lead to excess partial pressures of CO_2 in stream water. Both equilibrium (ε = 1‰) and kinetic evasion (ε = 2‰) fractionations result from DIC exchange with the atmosphere. Precipitation of dissolved inorganic C is a prominent potential sink for DIC and is balanced by mineral dissolution. Results from Tobias and Bohlke (2011) highlighted carbonate precipitation to be an equally important sink to primary production in a low-order stream in an agroecosystem. While algal uptake exerts a strong kinetic isotopic fractionation on the dissolved inorganic pool, precipitation-dissolution imparts a small equilibrium fractionation (ε < 1‰) (Mook, 2006; Tobias and Bohlke, 2011). Erosion-deposition dynamics of sediment is well documented to impact benthic C isotopic signatures, which reflects sediment C quantity and quality (Ford et al., 2014). Newly deposited sediments are mixed with existing sediments through turbulent advection of the overlying streamwater into the benthos (Russo and Fox, 2012; Ford and Fox, 2014). The level of mixing is scale-dependent, but in low to mid order streams with high prominence of fine-cohesive sediments, sediment within surficial fine-grained laminae of the streambed surface is typically well-mixed (Droppo et al., 2000). Fluvial sloughing of algal biomass has the potential to impact sediment isotope compositions, especially in low DIC systems where fractionations due to autochthonous growth in response to biotic population disequilibrium will have a larger footprint on the DIC isotope pool (Ford and Fox, 2015). The dynamics for site-specific conditions are discussed further in Section 5.

4.2 Nitrogen

Practically all N fractionation takes place through biologically mediated pathways including the aforementioned autochthonous growth, heterotrophic and endogenous respiration (mineralization), nitrification, and denitrification (Figure 3a; Sharp 2007). Regarding autotrophic assimilation of N species, biotic algal uptake of N imparts a fractionation on its DIN source of 6 to 13‰ for NO_3 (Needoba et al., 2003; Kendall et al., 2007) and 0 to 27‰ for NH_4 (Fogel and Cifuentes, 1993; Kendall et al., 2007). However, fractionations for ammonium are likely small (ε < 4‰) in most aquatic systems with low ammonium concentrations (Fogel and Cifuentes, 1993; Kendall et al., 2007). Regarding N, remineralization of organic N to ammonium fractionations are typically negligible with $\varepsilon \pm$ 1‰ (Kendall et al., 2007).

The benefit of N isotopes to reflect in-stream biotic cycling is recognized from the high fractionations reported for N and O isotopes during dissolved inorganic transformation during nitrification and denitrification processes (Kendall et al., 2007). Nitrification is the two-step aerobic oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and then nitrate (NO_3^-). As previously mentioned, $\delta^{15}N$ and $\delta^{18}O$ of nitrate are measured using the dual-isotope approach. With regard to $\delta^{15}N$, researchers have found that the first step is (NH_4^+—NO_2^-) is often the rate-determining step in ammonium-rich systems and occurs very slowly resulting in large fractionations on the ammonium N pool with ε values ranging from 14 to 38‰ (Mariotti et al., 1981; Casciotti et al., 2003; Kendall et al., 2007). In ammonium limited systems, the fractionation of the N isotope is relatively small. Further, the second step (NO_2—NO_3^-) is rapid and typically does not result in a net fractionation. With regard to δ^{18}O of nitrate, oxygen isotope composition will generally reflect a mixture of the oxygen isotope signature of water and dissolved oxygen; however, the level of fractionation is not well understood (Kendall et al., 2007). For denitrification, or the anaerobic reduction of nitrate to N-based gaseous byproducts, enrichment factors of $\delta^{15}N_{NO3}$ ranges from 1 to 18 depending on where denitrification occurs (i.e., water column, benthos, riparian zone) (Brandes and Devol, 2002; Sebilo et al., 2003; Lehmann et al., 2004; Sigman et al., 2005; Kendall et al., 2007).

Abiotic processes controlling N cycling and isotope signatures in-stream not only include the aforementioned hydrodynamic and hydraulic factors (analogous to C) but also chemi-physical sorption of DIN to benthic sediments. Abiotic adsorption of ammonium is widely recognized as a transient N sink with reported apparent equilibrium fractionations ranging from 1 to 11‰ (Delwiche and Steyn, 1970; Karamanos and Rennie, 1978; Bernot and Dodds, 2005; Bohlke et al., 2006). Abiotic adsorption of nitrate in streams is not currently part of the perceptual model (Peterson et al., 2001; Birgand et al., 2007; Ford and Fox, 2017); however, evidence exists for the nitrate adsorption to variably charged sesquioxides in benthic sediments, analogous to processes reported in soils (Ford et al., 2015). Given the limited understanding of the magnitude and significance of this flux, the isotopic fractionation is not well understood; therefore, future work is needed to test the significance of the sorption mechanism and identify potential ranges of isotope fractionation under differing sediment and streamwater chemistry.

4.3 Phosphorus

Regarding biotic processes, the primary mechanism leading to changes in $\delta^{18}O_{PO4}$ is associated with microbial mediated recycling of orthophosphate. Enzymatic breaking of the P-O bond during microbial cycling of orthophosphate drives the phosphate signature towards a temperature dependent equilibrium fractionation value with $\delta^{18}O_{H2O}$ following regeneration to the water column (Young et al., 2009; Davies et al., 2014). Therefore, in areas where microbial P cycling is rapid (e.g., benthic biofilms), the $\delta^{18}O_{PO4}$ of DRP reflects a mixture of its source signature and rates of microbial P regeneration. Regarding sediment and biota P, we did not find information on fractionation associated with uptake or mineralization on the sediment or biota pools; however, techniques for measuring sediment PO_4 signatures are relatively new and do not explicitly distinguish between organic and inorganic P sources (Pistocchi et al., 2017).

Abiotic processes including erosion-deposition, precipitation-dissolution and sorption-desorption are more significant for fluvial P cycling than for C and N which stems from the high sorption capacity of cohesive soils. Soil P may be highly stratified in adsorbed inorganic P and hence erosion deposition dynamics is important in fluvial ecosystems (Jarvie et al., 2014). Authigenic production of orthophosphate occurs through co-precipitation with calcite, precipitation with iron and hydroxide in oxic porewaters, and precipitation as vivianite under anaerobic, eutrophic conditions (Withers and Jarvie, 2008 and references within). The mineral growth process is rapid and fractionation effects between mineral and dissolved phosphate is low; hence the $\delta^{18}O$ signature of authigenic P is commonly reflective of its phosphate source, and vice versa for dissolution (Joshi et al., 2015). P uptake through sorption is widely acknowledged within streams and may be a significantly higher sink of P as compared with algal assimilation (Withers and Jarvie, 2008). Further, P desorption may become a prominent source of legacy P under specific redox conditions in agroecosystems which tend to retain rich stores of P in benthic sediments (Jarvie et al., 2014; Joshi et al., 2015; Baker et al., 2017). Oxygen isotope signatures of phosphate are not subjected to equilibrium fractionations under abiotic processes; hence phosphate adsorped to sediment surfaces should be reflective of its inorganic P source and its regenerated product (Davies et al., 2014).

5 REVIEW OF STABLE ISOTOPES IN WATER QUALITY MODELING

Coupling of stable isotopes within water quality models is in its infancy within the water resources community, and there are likely many permutations of coupling that might be performed in future research and model development. Nevertheless, based on our review of previous research as well as their own research advancements in recent years, we highlight that three common themes, defined in the introduction, emerge in that stable isotopes are coupled with water quality models to (i) improve data inputs associated with boundary conditions of the models, (ii) constrain model parameterization associate with equifinality, and (iii) elucidate improvements needed within model structure. Table 2 highlights the relevant watershed water quality modeling papers in the literature. Specifically, we provide summaries of how each study addresses one or, in some cases, multiple themes. As will be shown, at least one of these uncertainty-associated components is overcome when the researchers coupled the stable isotopes within the watershed water quality modeling. These studies represent an exhaustive list of water quality model applications that incorporate stable isotopes of C and N to our knowledge. In this manner, current use of stable isotopes in water quality modeling has highlighted their utility for improving reliability and reducing equifinality in hydrologic and water quality simulations. We have separated this section into C and N isotope applications since no applications for P have been performed to date.

5.1 Carbon

5.1.1. Dissolved Carbon

Tobias and Bohlke (2011) quantified the relative amounts of biological and geochemical controls on DIC cycling and flux within a 1 km first-order agricultural stream reach using daily $\delta^{13}C$ of DIC and $\delta^{18}O$ of O_2 applied to a finite-differencing mass balance model. The usage of $\delta^{18}O_{O_2}$ was to aid in constraining interpretations of the $\delta^{13}C_{DIC}$ and DIC measurements. Their logic was that when the usage of chemical and isotope modeling is applied in combination with daily observations, there would be an improvement in the overall mechanistic understanding of the diel fluctuations and environmental factors that influence DIC fate and transport. Results of the model output contrasted the collected data in that model $\delta^{13}C_{DIC}$ estimates were too high and did not reproduce cation cycles. The values of input parameters needed to reproduce accurate output values were unrealistically high and the insensitivity of the $\delta^{13}C_{DIC}$ variation to carbonate reaction suggested that the indicator acted as a poor indicator of diel processes except for photosynthesis rates in highly productive systems.

Stable isotope signatures of DIC ($\delta^{13}C_{DIC}$) have recently been implemented in marine and estuarine environments to reduce equifinality. Van Engeland et al. (2012) investigated model uncertainty reduction through inclusion of $\delta^{13}C_{DIC}$ results for injected mesocosm experiments into a marine nitrogen-phytoplankton-zooplankton-detritus (NPZD) model. Equifinality was reduced by explicitly resolving stable isotope dynamics within the parametric modeling framework. The additions of the $\delta^{13}C_{DIC}$ tracers constrained uncertainty of biogeochemical transformations of the model predicted rates and fluxes associated with C mass balance. Evaluation of the NPZD model with and without isotope calibration data was performed. The authors found that calibrations using solely concentration data exhibited higher standard deviations of uncertain parameters, strong correlations between fitted parameters (suggesting parameter value dependence), and inaccurate estimates of zooplankton grazing and detritus sinking rates as compared with multi-objective calibration with concentration and stable isotope response variables. Quantitatively, the authors provide evidence of this through a higher multicollinearity index for the reduced (no isotope) dataset relative to the full model evaluation dataset (values of 3.43 and 1.64 respectively).

Adiyanti et al. (2016) collected high spatial resolution data in a sub-tropical estuary over five sampling campaigns and analyzed samples for dissolved inorganic, dissolved organic, and POC isotope signatures. The authors utilized a mixed 1-D, 3-D modeling approach that coupled hydrodynamics with C biogeochemistry for the estuary and utilized DIC and DOC isotope and concentration measures as model response variables. The authors highlight that the addition of the isotope response variables allowed better constraint for biogeochemical process parameters as compared to using Markov Chain Monte Carlo optimization without the isotopes. Parameter space constraint was observed because of sensitive fractionation effects on the isotope response variables that led to rejection of implausible model outputs. The authors highlight the utility of the approach for advancing C budgeting by using the model to describe spatial variability of trophic state within the estuary.

5.1.2 Particulate Carbon

Sediment particulate C isotope signatures ($\delta^{13}C_{FPOC}$) have been utilized to improve model calibration and parameterization for conservative and non-conservative tracer behavior. A study by Fox and Martin (2014) used stable sediment C and N isotopes of sediment to separate forest, reclaimed mine, and stream bank sources and to highlight the efficacy of coupling stable isotope fingerprinting with deterministic sediment yield modeling in mixed-use landscapes. Carbon isotopes were used in conjunction with N isotopes as a dual tracer approach to estimate time-varying sediment source contributions within the watersheds, subsequently acting as an additional response variable in sediment yield model evaluation. The authors utilized the added isotope-based response variable to calibrate the sediment transport capacity coefficient, sediment delivery ratio for reclaimed mining soils, and stream bank erosion parameters. The source uniqueness and time-varying nature of the forest source allowed the authors to elucidate the impact of reclamation practices on sediment yield with their model. The authors' study was found to be applicable for steep gradient watersheds with relatively conservative tracers (in-stream) due to low residence time.

For non-conservative systems (e.g., low-gradient agroecosystems with pronounced sediment storage), Ford et al. (2015) utilized stable C isotopes of transported sediments ($\delta^{13}C_{FPOC}$) to constrain a reach-scale C fate and transport model that considers benthic autochthonous and terrestrial C sources. A deterministic C mass-balance model for benthic sediment, algae, and DIC pools (ISOFLOC) was coupled to a sediment storage and transport model to assess the impact of algae on the fluvial C budget. Stable isotope mass-balances were simulated for each C pool and eight years of ambient concentrations of fine POC and C isotope data was utilized to evaluate the model. The isotope response variable was found to be highly sensitive to the critical shear stress of algae and the algal POC source (DIC) and it's time-varying isotope signature. As a result, calibration using the isotope sub model reduced uncertainty of sloughed algal fluxes by 80%. These highly dependent relationships between biogeochemical processes, physical processes and the ability of stable isotopes to reflect these processes highlight the importance of ambient isotope response variable to account for non-conservative contaminant behavior in complex fluvial systems.

Sediment stable isotopes of C have also been effectively used to establish boundary conditions of sediment C sources. Husic et al. (2017a, b) applied sediment C fingerprinting at the upstream monitoring station of a phreatic karst conduit in central Kentucky. The authors separated sediment C fractions from surface stream autochthonous detritus, labile terrestrial soil C, and relatively recalcitrant soil C sources. Given the variability of biological turnover rates of these C pools and the subsequent implications for water quality in perennial springs that serve as drinking water supplies, the authors highlight the potential utility of the approach. Further, the authors discuss the enhanced adoption of sediment fingerprinting within the hydrologic and water quality community, highlighting the natural linkage to the water quality modeling community.

5.2 Nitrogen

5.2.1 Dissolved Nitrogen

Xue et al. (2014) uses unmixed isotope inputs for a model that is unlike other studies reviewed here, because the model is not a physically-based mechanistic model; however, it did include isotopes as inputs for a decision tree model. The study used two years of monthly $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ data from a multitude of sampling locations as inputs for a mixing model (SIAR) to determine nitrate source apportionment, and the study also assessed the effectiveness of isotopic data as input in a decision tree model that used physicochemical data. In decision tree models, a critical component in their construction is called the split selection, which is the decision of choosing the best option to proceed with in the model. The decision tree model was simulated with and without isotope data, and in this study the isotope data did not improve the performance of the decision tree model. The authors speculate this could be due to complex land use of the study site that result in scattered nitrate isotope values. The authors do, however, posit that an opportunity is created to use $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ data to cultivate a dependable nitrate polluting activity classification.

Sebestyen et al. (2014) used a dual isotope method of nitrate ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) to study N cycling and source contributions during autumn in a forested stream ecosystem. The study ties together the interactions among biogeochemical processes, N source allocation, and flow paths to look at how these components affect N variation. Modeling consisted of streamwater and solute mass-balances and stable isotope mass balances with Rayleigh fractionations. Inclusion of the stable isotopes improved constraint of stream biochemical reactions and source contributions. Model estimates suggest that in-stream transformations retain 72% of the nitrate entering the stream channel. Further, through the isotope mass-balance approach, the study found higher inputs of unprocessed atmospheric nitrate than what is commonly acknowledged for non-snowmelt periods in forested landscapes.

5.2.2 Particulate Nitrogen

Fox et al. (2010) focused on modeling sediment transport and sediment source apportionment using N stable isotopes ($\delta^{15}N_{FPN}$). The study used N stable isotopes of sediment to aid in differentiation of the sediment sources and model the sediment transport because of $\delta^{15}N$*'s* effectiveness at separating sediment sources in watersheds that contain vegetation with like photosynthetic pathways. Nevertheless, the authors found $\delta^{15}N_{FPN}$ (and sediment N) to vary substantially due to physical and biogeochemical processes impacting the transient storage zones in sediments. While the paper did not examine robust uncertainty analysis for biogeochemical parameters, it did highlight the efficacy of the stable isotopes to be used to help establish inputs from upland and bank sediment sources and highlight the importance of the fate of the N isotope tracer in productive agroecosystems.

Building on Fox et al. (2010) and Ford and Fox (2015), Ford et al. (2017) developed a reach-scale N model to simulate in-stream N fate and transport in low-gradient agroecosystems. The N model that includes stable N isotope subroutines is known as *TRANSFER*, which refers to Technology for Removable Annual Nitrogen in Streams For Ecosystem Restoration. The authors coupled N mass balances for dissolved and particulate phases to the previously developed *ISOFLOC* model (see section 5.1) and included a N stable isotope mass-balance equation for each of the elemental mass-balances. During model evaluation of a case-study, the authors found that fine PN isotope signatures ($\delta^{15}N_{FPN}$) were sensitive to sediment sources and non-conservative in-stream sediment N generation from autochthonous material and organic N degradation (and hence isotopic signatures of DIN). As a result, the authors reduced equifinality of estimates of transient DIN removal via algal sloughing and permanent removal via denitrification. The authors show that reduction of uncertainty by combining sediment elemental and isotope calibration parameters to DIN concentrations resulted in a 67% reduction from the original parameter solution space for downstream DIN flux estimates. This is compared to a 44% reduction from the original parameter solution space when calibrating with DIN concentrations alone. The reduced equifinality elucidated the significance of the transient DIN store and the potential for over-estimation of denitrification during sensitive timeframes (e.g., late summer/early fall), when sloughed algal biomass may potentially fuel downstream HNABs. In addition, the case-study revealed disagreement in measured and modeled results for the isotope response variable during winter/spring potentially highlighting limitations in our existing perceptual models for in-stream N fate and transport, such as the lack of inclusion of abiotic mobilization/demobilization.

Hong et al. (2014) utilized $\delta^{15}N$ signatures in dolphins to determine the methyl mercury (CH_3Hg) dietary exposure in the Sarasota Bay. Utilizing the N stable isotope aided in identifying where mercury loading was present and how it was being discharged into the bay system. In the study, when one bioconcentration factor in lower trophic level organisms and one biomagnification rate were coupled with a predetermined $\delta^{15}N$, the mercury distributions in the ecosystem were successfully reproduced. This relationship enabled modeling of the fate, transport, and bioaccumulation of monomethyl mercury within the waterbody.

6 RECOMMENDATIONS FOR ISOTOPES IN WATER QUALITY MODELS:

Synthesis of the studies in Table 2 point to the ability of stable isotopes to constrain uncertainty of hydrologic and water quality models, improve perceptual understanding of in-stream contaminant fate, and establish boundary conditions for in-stream models. Consistent with the themes recognized in the literature review, we provide some recommendations and precautions for water quality modelers to integrate stable isotopes into existing and new models.

RECOMMENDATION #1: MODELLERS SHOULD USE ISOTOPES OF SEDIMENTS SINCE THEY INTEGRATE PROCESSES, REFLECT SOURCE CONTRIBUTIONS, AND ARE INEXPENSIVE TO MEASURE

We perceive high utility in integration of sediment stable isotopes into in-stream routines in water quality modeling frameworks given the following factors: integrative capacity of benthic sediments, the abundance of sediment stable isotope data from watershed sediment source apportionment, the utility of stable isotopes to improve water quality modeling structure and uncertainty reduction, and the now inexpensive costs associated with stable isotope analyses of solids. Sediment fingerprinting has been a popular method for sediment source apportionment over the past 20 years (Collins et al., 1998; Fox and Papanicolaou, 2007; Davis and Fox, 2009). Sediment source apportionment using stable C and N isotopes is limited by the fate of the organic matter in the system (Davis et al. 2009; Koiter et al. 2013). In part we find this non-conservative behavior reflects processes such as stabilization of algal biomass through algal decomposition to fine sediment and integration into the benthos, or sorption-desorption of N phases onto fine sediment aggregates (see Section 4). Therefore, sediment fingerprints not only reflect upland organic matter and sediment sources but also rates of processes, and fingerprints of dissolved inorganic nutrient species. For this reason, we foresee high utility in integrating these widespread measurements of C and N isotopes that have been collected across a broad range of landscapes to test and improve water quality models. Further, we recommend integration of sediment stable isotope measurements into routine water quality monitoring efforts because sampling equipment is easy to build using standard household items, reflect temporal and spatially integrated measures of in-stream transported sediment C and N signatures over the course of an event (Phillips et al., 2000), and are relatively inexpensive to process in the laboratory and analyze. We caution that the sediment stable isotope signatures should not be utilized as a replacement for concentration response variables in water quality modeling but instead a supplement. Yet, we foresee that the low cost and relatively low processing time for analysis makes this added response variable a plausible supplementary data collection effort in watershed-based monitoring and modeling programs.

RECOMMENDATION #2: MODELLERS SHOULD USE MULTI-OBJECTIVE CALIBRATION WHEN USING ISOTOPES

We highlight the importance of utilizing isotope response variables in multi-objective calibration frameworks to reduce issues with model equifinality. Multi-objective calibration refers to the process of using a set number of weighted numerical metrics that target specific aspects of goodness of fit between model results and measured data (van Griesven and Bauwens, 2003; Rode et al., 2007; Ford and Fox, 2015; Haas et al., 2016). A thrifty approach that has greatly extended the utility of existing concentration data is to utilize time-varying, multi-objective calibration whereby calibration statistics are calculated for specific periods to target calibrating parameters when they have heightened sensitivity, i.e., baseflow vs. event-flow, seasonal performance, rising vs. falling limb of chemograph (e.g., Haas et al., 2016). Such sensitivities may be identified using time-varying global sensitivity analysis approaches (Reusser et al., 2011; Muleta, 2012; Wang et al., 2013; Herman et al., 2013; Ford et al., 2015). Nevertheless, issues persist with using concentration-based measures since they may be insensitive to nutrient residence times, i.e., transient storage and discriminating rates of in-stream processes (e.g., Jarvie et al., 2014; Ford and Fox, 2017). In this light, integration of isotope response variables within the multi-objective framework may be highly valuable, especially given the widely recognized utility to reduce equifinality in Section 5.

RECOMMENDATION #3: WATER QUALITY MODELLERS SHOULD WORK COLLABORATIVELY WITH ISOTOPE GEOCHEMISTS

With continued advancements in isotope measurement techniques and technology, watershed modelers need to work closely with isotope geochemists to integrate stable isotope measurements in water quality modeling frameworks. From a management perspective, engineers need high resolution data, especially during storm fluxes to accurately characterize loadings and source contributions of nutrient fluxes at the watershed scale. Current measurement techniques for grab sample analysis for isotopic measurements of dissolved nutrients are rather expensive, labor intensive, and limit the economic feasibility of high resolution measurements. Nevertheless, we have seen a rise in in-situ technologies, and researchers now have the capability to obtain high-resolution measurements of $\delta^{18}O_{H2O}$ and $\delta^{13}C_{DIC}$. As these technologies continue to extend to other nutrients (e.g., nitrate) and become more affordable, it will be important for watershed modelers to understand the limitations and applicability of the high-resolution data-streams, which will require close collaboration with isotope geochemists. We foresee high utility for water quality model frameworks that use high resolution isotope sensing to inform practical watershed management decisions.

Further, the cutting-edge work that has been conducted on $\delta^{18}O_{PO4}$ DRP and PIP over the past decade and the lack of ambient tracers of P source fate and transport (Jarvie et al., 2014; Williams et al., 2016) suggests a need to assess the efficacy of $\delta^{18}O_{PO4}$ in water quality model frameworks. Several challenges exist that will require interdisciplinary collaboration to recognize the full potential of the oxygen isotope signature of phosphate as a tool for informing water quality models. Regarding dissolved inorganic phosphate, a current barrier is the large sample volume needed to precipitate an adequate mass of Ag_3PO_4 for isotope analysis given the low ambient DRP concentrations (McLaughlin et al., 2004; Young et al., 2009; Pistocchi et al., 2017). Also, based on existing datasets it is not clear that the approach may robustly distinguish between non-point pollution sources, which has led to suggestions of database expansion of P source characterization in freshwater ecosystems (Young et al., 2009; Davies et al., 2014). A second limitation is that most existing methods for soil and sediment extraction are not pool specific (see Haney et al., 2013 for the exception) and typically reflect adsorbed phosphate, dissolution of phosphate bearing precipitates, and mineralized organic matter (Tamburini et al., 2014; Davies et al., 2014; Pistocchi et al., 2017). Despite this limitation, the measurement provides valuable information of biological processing of P that is not otherwise measurable with existing methods (Pistocchi et al., 2017). We foresee $\delta^{18}O_{PO4}$ to hold great promise for numerical model advancement and we foresee that concurrent advancement of water quality modeling technology with the analytical techniques may lead to more robust management of P in the fluvial landscape.

CONCLUSIONS

While model uncertainty continues to be a major challenge facing scientists and engineers, stable isotopes are promising tools for improving in-stream nutrient fate and transport routines in water quality models. The authors feel that this is an exciting time for water quality modelers as new data streams such as stable isotopes offer the promise of constraining our uncertainty. This review highlighted the ability of stable isotopes to (i) improve estimates of boundary conditions, (ii) reduce model equifinality, and (iii) elucidate model improvement needs by identifying deficiencies in perceptual or numerical model frameworks. As a final note, regarding (ii), we highlight the importance for modelers to provide quantitative evidence of uncertainty reduction in future applications. Often this quantitative evidence is missing from recent studies given the emphasis of the studies on establishing new methodologies and showing their efficacy. This effort should be commended, but nevertheless future studies might report quantitative evidence so that researchers may start to understand when the extra data stream and modeling effort is most useful and when it is not. We foresee that such quantitative evidence will help provide practitioners with a metric to inform cost-benefit analysis associated with making model data collection decisions.

ACKNOWLEDGEMENTS

We thank the University of Kentucky Biosystems and Agricultural Engineering for partial funding of the graduate student while at UK. We gratefully acknowledge financial support of this research under National Science Foundation Award 163288 which provided partial support for the graduate student and corresponding author. This is publication No. 17-05-074 of the Kentucky Agricultural Experiment Station and is published with the approval of the Director. This work is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture. Finally, we thank the three anonymous reviewers whose input and suggestions greatly improved the quality of the manuscript.

REFERENCES

Adiyanti, S., Eyre, B. D., Maher, D. T., Santos, I., Golsby-Smith, L., Mangion, P., Hipsey, M. R. (2016). Stable isotopes reduce parameter uncertainty of an estuarine carbon cycling model. *Environmental Modelling & Software*, *79*, 233-255.

Angert, A., Weiner, T., Mazeh, S., Sternberg, M. (2012). Soil phosphate stable oxygen isotopes across rainfall and bedrock gradients. *Environmental Science & Technology*, *46*(4), 2156-2162.

Aravena, R., 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*(6), 975-982.

Ashkenas, L. R., Johnson, S. L., Gregory, S. V., Tank, J. L., Wollheim, W. M. (2004). A stable isotope tracer study of nitrogen uptake and transformation in an old< growth forest stream. *Ecology*, *85*(6), 1725-1739.

Baker, D. B., Johnson, L. T., Confesor, R. B., Crumrine, J. P. (2017). Vertical stratification of soil phosphorus as a concern for dissolved phosphorus runoff in the Lake Erie Basin. *Journal of Environmental Quality*, doi:10.2134/jeq2016.09.0337.

Bernot, M. J., Dodds, W. K. (2005). Nitrogen retention, removal, and saturation in lotic ecosystems. *Ecosystems*, *8*(4), 442-453.

Beven, K. (2006). A manifesto for the equifinality thesis. *Journal of Hydrology*, 320(1), 18-36.

Beven, K., Binley, A. (1992). The future of distributed models: model calibration and uncertainty prediction. *Hydrological processes*, *6*(3), 279-298.

Birgand, F., Skaggs, R. W., Chescheir, G. M., Gilliam, J. W. (2007). Nitrogen removal in streams of agricultural catchments—a literature review. *Critical Reviews in Environmental Science and Technology*, *37*(5), 381-487.

Böhlke, J. K., Harvey, J. W., Voytek, M. A. (2004). Reach< scale isotope tracer experiment to quantify denitrification and related processes in a nitrate< rich stream, midcontinent United States. *Limnology and Oceanography*, *49*(3), 821-838.

Böhlke, J. K., Smith, R. L., Miller, D. N. (2006). Ammonium transport and reaction in contaminated groundwater: Application of isotope tracers and isotope fractionation studies. *Water Resources Research*, *42*(5).

Borah, D. K., Bera, M. (2003). Watershed-scale hydrologic and nonpoint-source pollution models: Review of mathematical bases. *Transactions of the ASAE*, *46*(6), 1553.

Brandes, J. A., Devol, A. H. (2002). A global marine< fixed nitrogen isotopic budget: Implications for Holocene nitrogen cycling. *Global Biogeochemical Cycles*, 16(4).

Bremner, J. M., Keeney, D. R. (1966). Determination and isotope-ratio analysis of different forms of nitrogen in soils: 3. Exchangeable ammonium, nitrate, and nitrite by extraction-distillation methods. *Soil Science Society of America Journal*, *30*(5), 577-582.

Casciotti, K. L., Sigman, D. M., Ward, B. B. (2003). Linking diversity and stable isotope fractionation in ammonia-oxidizing bacteria. *Geomicrobiology Journal*, *20*(4), 335-353.

Chang, C. C., Kendall, C., Silva, S. R., Battaglin, W. A., Campbell, D. H. (2002). Nitrate stable isotopes: tools for determining nitrate sources among different land uses in the Mississippi River Basin. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(12), 1874-1885.

Chapra, S. C., Pelletier, G. J., Tao, H. (2008). QUAL2K: A Modeling Framework for Simulating River and Stream Water Quality, Version 2.11: Documentation and Users Manual. Civil andEnvironmental Engineering Dept., Tufts University, Medford, MA., Steven.Chapra@tufts.edu

Cifuentes, L. A., Fogel, M. L., Pennock, J. R., Sharp, J. H. (1989). Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. *Geochimica et Cosmochimica Acta*, *53*(10), 2713-2721.

Clément, J. C., Holmes, R. M., Peterson, B. J., Pinay, G. (2003). Isotopic investigation of denitrification in a riparian ecosystem in western France. *Journal of Applied Ecology*, *40*(6), 1035-1048.

Collins, A. L., Walling, D. E., Leeks, G. J. L. (1998). Use of composite fingerprints to determine the provenance of the contemporary suspended sediment load transported by rivers. *Earth Surface Processes and Landforms*, *23*(1), 31-52.

Davies, C. L., Surridge, B. W., Gooddy, D. C. (2014). Phosphate oxygen isotopes within aquatic ecosystems: Global data synthesis and future research priorities. *Science of the Total Environment*, *496*, 563-575.

Davis, C. M., Fox, J. F. (2009). Sediment fingerprinting: review of the method and future improvements for allocating nonpoint source pollution. *Journal of Environmental Engineering*, *135*(7), 490-504.

Dean, S., Freer, J., Beven, K., Wade, A. J., Butterfield, D. (2009). Uncertainty assessment of a process-based integrated catchment model of phosphorus. *Stochastic Environmental Research and Risk Assessment*, *23*(7), 991-1010.

Delwiche, C. C., Steyn, P. L. (1970). Nitrogen isotope fractionation in soils and microbial reactions. *Environmental Science & Technology*, *4*(11), 929-935.

Di Toro, D. M. (2001). *Sediment Flux Modeling* (Vol. 116). New York: Wiley-Interscience.

Doctor, D. H., Kendall, C., Sebestyen, S. D., Shanley, J. B., Ohte, N., Boyer, E. W. (2008). Carbon isotope fractionation of dissolved inorganic carbon (DIC) due to outgassing of carbon dioxide from a headwater stream. *Hydrological Processes*, *22*(14), 2410-2423.

Doi, H. (2009). Spatial patterns of autochthonous and allochthonous resources in aquatic food webs. *Population Ecology*, *51*(1), 57-64.

Droppo, I. G., Walling, D. E., Ongley, E. D. (2000). Influence of floc size, density and porosity on sediment and contaminant transport. *IAHS Publication (International Association of Hydrological Sciences)*, 263, 141-147.

Droppo, I. G., Lau, Y. L., Mitchell, C. (2001). The effect of depositional history on contaminated bed sediment stability. *Science of the Total Environment*, *266*(1), 7-13.

Elsbury, K. E., Paytan, A., Ostrom, N. E., Kendall, C., Young, M. B., McLaughlin, K., ... & Watson, S. (2009). Using oxygen isotopes of phosphate to trace phosphorus sources and cycling in Lake Erie. *Environmental Science & Technology*, *43*(9), 3108-3114.

England, L. E., Rosemond, A. D. (2004). Small reductions in forest cover weaken terrestrial< aquatic linkages in headwater streams. *Freshwater Biology*, *49*(6), 721-734.

Finlay, J. C. (2001). Stable< carbon< isotope ratios of river biota: implications for energy flow in lotic food webs. *Ecology*, *82*(4), 1052-1064.

Fogel, M. L., Cifuentes, L. A. (1993). Isotope fractionation during primary production. In *Organic Geochemistry* (pp. 73-98). Springer US.

Ford, W. I., Fox, J. F. (2014). Model of particulate organic carbon transport in an agriculturally impacted stream. *Hydrological Processes*, *28*(3), 662-675.

Ford, W. I., Fox, J. F. (2015). Isotope< based Fluvial Organic Carbon (ISOFLOC) Model: Model formulation, sensitivity, and evaluation. *Water Resources Research*, *51*(6), 4046-4064.

Ford, W. I., Fox, J. F. (2017). Stabilization of benthic algal biomass in a temperate stream draining agroecosystems. *Water Research*, *108*, 432-443.

Ford, W. I., Fox, J. F., Rowe, H. (2015). Impact of extreme hydrologic disturbance upon the sediment carbon quality in agriculturally impacted temperate streams. *Ecohydrology*, *8*(3), 438-449.

Ford, W. I., Fox, J. F., Pollock, E., Rowe, H., Chakraborty, S. (2015). Testing assumptions for nitrogen transformation in a low-gradient agricultural stream. *Journal of Hydrology*, *527*, 908-922.

Ford, W. I., Fox, J. F., Pollock, E. (2017). Reducing equifinality using isotopes in a process< based stream nitrogen model highlights the flux of algal nitrogen from agricultural streams. *Water Resources Research, 53*(8), 6539-6561.

Fox, J.F. (2009) Measurements of sediment transport processes in forested watersheds with surface coal mining disturbance using carbon and nitrogen isotopes. *Journal of the American Water Resources Association*, *45*(5): 1273-1289.

Fox, J. F., & Ford, W. I. (2016). Impact of landscape disturbance on the quality of terrestrial sediment carbon in temperate streams. *Journal of Hydrology*, *540*, 1030-1042.

Fox, J. F., Martin, D. K. (2014). Sediment fingerprinting for calibrating a soil erosion and sediment-yield model in mixed land-use watersheds. *Journal of Hydrologic Engineering*, *20*(6), C4014002.

Fox, J. F., Papanicolaou, A. N. (2007). The use of carbon and nitrogen isotopes to study watershed erosion processes. *JAWRA Journal of the American Water Resources Association*, *43*(4), 1047-1064.

Fox, J. F., Davis, C. M., Martin, D. K. (2010). Sediment source assessment in a lowland watershed using nitrogen stable isotopes. *JAWRA Journal of the American Water Resources Association*, *46*(6), 1192-1204.

Fox, J., Ford, W., Strom, K., Villarini, G., Meehan, M. (2014). Benthic control upon the morphology of transported fine sediments in a low< gradient stream. *Hydrological Processes*, *28*(11), 3776-3788.

Fukada, T., Hiscock, K. M., Dennis, P. F., Grischek, T. (2003). A dual isotope approach to identify denitrification in groundwater at a river-bank infiltration site. *Water Research*, *37*(13), 3070-3078.

Galand, P., Yrjälä, K., Conrad, R. (2010). Stable carbon isotope fractionation during methanogenesis in three boreal peatland ecosystems. *Biogeosciences, 7*, 3893-3900.

Gong, Y., Shen, Z., Hong, Q., Liu, R., Liao, Q. (2011). Parameter uncertainty analysis in watershed total phosphorus modeling using the GLUE methodology. *Agriculture, Ecosystems & Environment*, *142*(3), 246-255.

Grey, J., Jones, R. I., Sleep, D. (2001). Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnology and Oceanography*, 46(3), 505-513. Guzman, J. A., Shirmohammadi, A., Sadeghi, A., Wang, X., Chu, M. L., Jha, M. K., Parajuli, P. B., Harmel, R. D., Khare, Y., Hernandez, J. (2015). Uncertainty considerations in calibration and validation of hydrologic and water quality models. *Transactions of ASABE 58*(6):1745-1762.

Haas, M. B., Guse, B., Pfannerstill, M., Fohrer, N. (2016). A joined multi-metric calibration of river discharge and nitrate loads with different performance measures. *Journal of Hydrology*, *536*, 534-545.

Han, F., Zheng, Y. (2016). Multiple-response Bayesian calibration of watershed water quality models with significant input and model structure errors. *Advances in Water Resources*, *88*, 109-123.

Haney, R. L., White, M. J., Arnold, J. G., Haney, E. B., Harmel, R. D. (2013). Re-defining and Quantifying Inorganic Phosphate Pools in the Soil and Water Assessment Tool. *Journal of Soil Science and Environmental Management* *4*(8):155-162.

Herman, J. D., Reed, P. M., Wagener, T. (2013). Time< varying sensitivity analysis clarifies the effects of watershed model formulation on model behavior. *Water Resources Research*, *49*(3), 1400-1414.

Hong, Y. S., Rifkin, E., Bouwer, E. J. (2014). Modeling Mercury Distribution in the Sarasota Bay Ecosystem Using SERAFM and Stable Isotope Ratios of Nitrogen (δ15N) in Biota. *Environmental Engineering Science*, *31*(3), 135-147.

Hood, E., Williams, M. W., McKnight, D. M. (2005). Sources of dissolved organic matter (DOM) in a Rocky Mountain stream using chemical fractionation and stable isotopes. *Biogeochemistry*, *74*(2), 231-255.

Hope, D., Billett, M. F., Cresser, M. S. (1994). A review of the export of carbon in river water: fluxes and processes. *Environmental pollution*, *84*(3), 301-324.

Husic, A., Fox, J., Agouridis, C., Currens, J., Ford, W., Taylor, C. (2017a). Sediment carbon fate in phreatic karst (Part 1): Conceptual model development. *Journal of Hydrology*, *549*, 179-193.

Husic, A., Fox, J., Ford, W., Agouridis, C., Currens, J., Taylor, C. (2017b). Sediment carbon fate in phreatic karst (Part 2): Numerical model development and application. *Journal of Hydrology*, *549*, 208-219.

Imberger, S. J., Cook, P. L., Grace, M. R., Thompson, R. M. (2014). Tracing carbon sources in small urbanising streams: catchment< scale stormwater drainage overwhelms the effects of reach< scale riparian vegetation. *Freshwater Biology*, *59*(1), 168-186.

Jacinthe, P. A., Lal, R., Owens, L. B. (2009). Application of stable isotope analysis to quantify the retention of eroded carbon in grass filters at the North Appalachian experimental watersheds. *Geoderma*, *148*(3), 405-412.

Jarvie, H. P., Sharpley, A. N., Brahana, V., Simmons, T., Price, A., Neal, C., ... & Haggard, B. E. (2014). Phosphorus retention and remobilization along hydrological pathways in karst terrain. *Environmental Science & Technology*, *48*(9), 4860-4868.

Jin, X., Xu, C. Y., Zhang, Q., Singh, V. P. (2010). Parameter and modeling uncertainty simulated by GLUE and a formal Bayesian method for a conceptual hydrological model. *Journal of Hydrology*, *383*(3), 147-155.

Joshi, S. R., Kukkadapu, R. K., Burdige, D. J., Bowden, M. E., Sparks, D. L., Jaisi, D. P. (2015). Organic matter remineralization predominates phosphorus cycling in the mid-bay sediments in the Chesapeake Bay. *Environmental Science & Technology*, *49*(10), 5887-5896.

Karamanos, R. E., Rennie, D. A. (1978). Nitrogen isotope fractionation during ammonium exchange reactions with soil clay. *Canadian Journal of Soil Science*, *58*(1), 53-60.

Kendall, C., Caldwell, E. A. (1998). Fundamentals of isotope geochemistry. *Isotope Tracers in Catchment Hydrology*, 51-86.

Kendall, C., Silva, S. R., Kelly, V. J. (2001). Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrological Processes*, *15*(7), 1301-1346.

Kendall, C., Elliott, E. M., Wankel, S. D. (2007). Tracing anthropogenic inputs of nitrogen to ecosystems. *Stable Isotopes in Ecology and Environmental Science*, *2*, 375-449.

Kendall, C., Young, M. B., Silva, S. R. (2010). Applications of stable isotopes for regional to national-scale water quality and environmental monitoring programs. In *Isoscapes* (pp. 89-111). Springer Netherlands.

Koiter, A. J., Owens, P. N., Petticrew, E. L., Lobb, D. A. (2013). The behavioural characteristics of sediment properties and their implications for sediment fingerprinting as an approach for identifying sediment sources in river basins. *Earth-Science Reviews*, *125*, 24-42.

Komor, S. C. (1997). Boron contents and isotopic compositions of hog manure, selected fertilizers, and water in Minnestoa. *Journal of Environmental Quality*, *26*(5), 1212-1222.

Kritzberg, E. S., Cole, J. J., Pace, M. L., Granéli, W., Bade, D. L. (2004). Autochthonous versus allochthonous carbon sources of bacteria: Results from whole< lake 13C addition experiments. *Limnology and Oceanography*, *49*(2), 588-596.

Lane, C. S., Lyon, D. R., Ziegler, S. E. (2013). Cycling of two carbon substrates of contrasting lability by heterotrophic biofilms across a nutrient gradient of headwater streams. *Aquatic Sciences*, *75*(2), 235-250.

Lau, D. C., Leung, K. M., Dudgeon, D. (2009). What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong. *Freshwater Biology*, *54*(1), 127-141.

Lehmann, M. F., Bernasconi, S. M., Barbieri, A., Simona, M., McKenzie, J. A. (2004). Interannual variation of the isotopic composition of sedimenting organic carbon and nitrogen in Lake Lugano: A long< term sediment trap study. *Limnology and Oceanography*, *49*(3), 839-849.

Lu, L., Cheng, H., Pu, X., Wang, J., Cheng, Q., Liu, X. (2016). Identifying organic matter sources using isotopic ratios in a watershed impacted by intensive agricultural activities in Northeast China. *Agriculture, Ecosystems & Environment*, *222*, 48-59.

Maggi, F., Riley, W. J. (2009). Transient competitive complexation in biological kinetic isotope fractionation explains nonsteady isotopic effects: Theory and application to denitrification in soils. *Journal of Geophysical Research: Biogeosciences*, *114*(G4).

Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P. (1981). Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant and Soil*, *62*(3), 413-430.

McDonnell, J. J., Beven, K. (2014). Debates—The future of hydrological sciences: A (common) path forward? A call to action aimed at understanding velocities, celerities and residence time distributions of the headwater hydrograph. *Water Resources Research*, *50*(6), 5342-5350.

McGuire, K., McDonnell, J. (2007). Stable isotope tracers in watershed hydrology. *Stable Isotopes in Ecology and Environmental Science*, 334.

McKinney, C. R., McCrea, J. M., Epstein, S., Allen, H. A., Urey, H. C. (1950). Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. *Review of Scientific Instruments*, *21*(8), 724-730.

McLaughlin, K., Silva, S., Kendall, C., Stuart< Williams, H., Paytan, A. (2004). A precise method for the analysis of δ18O of dissolved inorganic phosphate in seawater. *Limnology and Oceanography: Methods*, *2*(7), 202-212.

Mook, W. G. (2006). *Introduction to Isotope Hydrology*. Taylor & Francis.

Moriasi, D. N., Arnold, J. G., Van Liew, M. W., Bingner, R. L., Harmel, R. D., Veith, T. L. (2007). Model evaluation guidelines for systematic quantification of accuracy in watershed simulations. *Transactions of the ASABE*, *50*(3), 885-900.

Muleta, M. K. (2012). Uncertainty Analysis and Calibration of SWMM Using a Formal Bayesian Methodology. In *World Environmental and Water Resources Congress 2012: Crossing Boundaries* (pp. 562-568).

Needoba, J. A., Waser, N. A., Harrison, P. J., Calvert, S. E. (2003). Nitrogen isotope fractionation in 12 species of marine phytoplankton during growth on nitrate. *Marine Ecology Progress Series*, *255*, 81-91.

Palmer, S. M., Hope, D., Billett, M. F., Dawson, J. J., Bryant, C. L. (2001). Sources of organic and inorganic carbon in a headwater stream: evidence from carbon isotope studies. *Biogeochemistry*, *52*(3), 321-338.

Papanicolaou, A. N., Fox, J. F., Marshall, J. (2003). Soil fingerprinting in the Palouse Basin, USA, using stable carbon and nitrogen isotopes. *International Journal of Sediment Research*, *18*(2), 278-284.

Park, R. A., Clough, J. S., Wellman, M. C. (2008). AQUATOX: Modeling environmental fate and ecological effects in aquatic ecosystems. *Ecological Modelling*, *213*(1), 1-15.

Peterson, B. J., Wollheim, W. M., Mulholland, P. J., Webster, J. R., Meyer, J. L., Tank, J. L., ... McDowell, W. H. (2001). Control of nitrogen export from watersheds by headwater streams. *Science*, *292*(5514), 86-90.

Phillips, J. M., Russell, M. A., Walling, D. E. (2000). Time< integrated sampling of fluvial suspended sediment: a simple methodology for small catchments. *Hydrological Processes*, *14*(14), 2589-2602.

Pistocchi, C., Tamburini, F., Gruau, G., Ferhi, A., Trevisan, D., Dorioz, J. M. (2017). Tracing the sources and cycling of phosphorus in river sediments using oxygen isotopes: Methodological adaptations and first results from a case study in France. *Water Research*, *111*, 346-356.

Reusser, D. E., Buytaert, W., Zehe, E. (2011). Temporal dynamics of model parameter sensitivity for computationally expensive models with the Fourier amplitude sensitivity test. *Water Resources Research*, *47*(7).

Robson, B. J. (2014). When do aquatic systems models provide useful predictions, what is changing, and what is next?. *Environmental Modelling & Software*, *61*, 287-296.

Rode, M., Suhr, U., Wriedt, G. (2007). Multi-objective calibration of a river water quality model—Information content of calibration data. *Ecological Modelling*, *204*(1), 129-142.

Rode, M., Arhonditsis, G., Balin, D., Kebede, T., Krysanova, V., Van Griensven, A., Van der Zee, S. E. (2010). New challenges in integrated water quality modelling. *Hydrological Processes*, *24*(24), 3447-3461.

Rosenfeld, J. S., Roff, J. C. (1992). Examination of the carbon base in southern Ontario streams using stable isotopes. *Journal of the North American Benthological Society*, *11*(1), 1-10.

Rounick, J. S., Winterbourn, M. J. (1986). Stable carbon isotopes and carbon flow in ecosystems. *BioScience*, *36*(3), 171-177.

Russo, J., Fox, J. (2012). The role of the surface fine-grained laminae in low-gradient streams: A model approach. *Geomorphology*, *171*, 127-138.

Sebestyen, S. D., Shanley, J. B., Boyer, E. W., Kendall, C., Doctor, D. H. (2014). Coupled hydrological and biogeochemical processes controlling variability of nitrogen species in streamflow during autumn in an upland forest. *Water Resources Research*, *50*(2), 1569-1591.

Sebilo, M., Billen, G., Grably, M., Mariotti, A. (2003). Isotopic composition of nitrate-nitrogen as a marker of riparian and benthic denitrification at the scale of the whole Seine River system. *Biogeochemistry*, *63*(1), 35-51.

Seiler, R. L. (2005). Combined use of ^{15}N and ^{18}O of nitrate and ^{11}B to evaluate nitrate contamination in groundwater. *Applied Geochemistry*, *20*(9), 1626-1636.

Sharp, Z. (2007). *Principles of Stable Isotope Geochemistry* (p. 344). Upper Saddle River, NJ: Pearson education.

Shen, Z. Y., Chen, L., Chen, T. (2012). Analysis of parameter uncertainty in hydrological and sediment modeling using GLUE method: a case study of SWAT model applied to Three Gorges Reservoir Region, China. *Hydrology and Earth System Sciences*, *16*(1), 121.

Seibert, J., McDonnell, J. J. (2002). On the dialog between experimentalist and modeler in catchment hydrology: Use of soft data for multicriteria model calibration. *Water Resources Research*, *38*(11).

Sigman, D. M., Granger, J., DiFiore, P. J., Lehmann, M. M., Ho, R., Cane, G., van Geen, A. (2005). Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin. *Global Biogeochemical Cycles*, *19*(4).

Soulsby, C., Birkel, C., Geris, J., Dick, J., Tunaley, C., & Tetzlaff, D. (2015). Stream water age distributions controlled by storage dynamics and nonlinear hydrologic connectivity: Modeling with high< resolution isotope data. *Water Resources Research*, *51*(9), 7759-7776.

Tamburini, F., Pfahler, V., Bunemann, E. K., Guelland, K., Bernasconi, S. M., Frossard, E. (2012). Oxygen isotopes unravel the role of microorganisms in phosphate cycling in soils. *Environ. Sci. Technol. 46*:5956–5962. doi:10.1021/es300311h

Tamburini, F., Pfahler, V., von Sperber, C., Frossard, E., Bernasconi, S. M. (2014). Oxygen isotopes for unraveling phosphorus transformations in the soil–plant system: A review. *Soil Science Society of America Journal*, *78*(1), 38-46.

Thornton, S. F., McManus, J. (1994). Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. *Estuarine, Coastal and Shelf Science*, *38*(3), 219-233.

Throckmorton, H. M., Heikoop, J. M., Newman, B. D., Altmann, G. L., Conrad, M. S., Muss, J. D., ... Wilson, C. J. (2015). Pathways and transformations of dissolved methane and dissolved inorganic carbon in Arctic tundra watersheds: Evidence from analysis of stable isotopes. *Global Biogeochemical Cycles*, *29*(11), 1893-1910.

Tobias, C., Böhlke, J. K. (2011). Biological and geochemical controls on diel dissolved inorganic carbon cycling in a low-order agricultural stream: Implications for reach scales and beyond. *Chemical Geology*, *283*(1), 18-30.

Engeland, T. V., Kluijver, A. D., Soetaert, K., Meysman, F. J. R., Middelburg, J. J. (2012). Isotope data improve the predictive capabilities of a marine biogeochemical model. *Biogeosciences Discussions*, *9*(7), 9453-9486.

Van Griensven, A., Bauwens, W. (2003). Multiobjective autocalibration for semidistributed water quality models. *Water Resources Research*, *39*(12).

Velinsky, D. J., Pennock, J. R., Sharp, J. H., Cifuentes, L. A., Fogel, M. L. (1989). Determination of the isotopic composition of ammonium-nitrogen at the natural abundance level from estuarine waters. *Marine Chemistry*, *26*(4), 351-361.

Walling, D. E., Collins, A. L., Jones, P. A., Leeks, G. J. L., Old, G. (2006). Establishing fine-grained sediment budgets for the Pang and Lambourn LOCAR catchments, UK. *Journal of Hydrology*, *330*(1), 126-141.

Wang, J., Li, X., Lu, L., Fang, F. (2013). Parameter sensitivity analysis of crop growth models based on the extended Fourier Amplitude Sensitivity Test method. *Environmental Modelling & Software*, *48*, 171-182.

Wankel, S. D., Kendall, C., Francis, C. A., Paytan, A. (2006). Nitrogen sources and cycling in the San Francisco Bay Estuary: A nitrate dual isotopic composition approach. *Limnology and Oceanography*, *51*(4), 1654-1664.

Webster, C. R., Heymsfield, A. J. (2003). Water isotope ratios D/H, 18O/16O, 17O/16O in and out of clouds map dehydration pathways. *Science*, *302*(5651), 1742-1745.

Wellen, C., Kamran-Disfani, A. R., Arhonditsis, G. B. (2015). Evaluation of the current state of distributed watershed nutrient water quality modeling. *Environmental Science & Technology*, *49*(6), 3278-3290.

White, W. M. (2015). *Isotope Geochemistry*. John Wiley & Sons.

Widory, D., Kloppmann, W., Chery, L., Bonnin, J., Rochdi, H., Guinamant, J. L. (2004). Nitrate in groundwater: an isotopic multi-tracer approach. *Journal of Contaminant Hydrology*, *72*(1), 165-188.

Williams, M. R., King, K. W., Ford, W., Buda, A. R., Kennedy, C. D. (2016). Effect of tillage on macropore flow and phosphorus transport to tile drains. *Water Resources Research*, *52*(4), 2868-2882.

Windhorst, D., Kraft, P., Timbe, E., Frede, H. G., Breuer, L. (2014). Stable water isotope tracing through hydrological models for disentangling runoff generation processes at the hillslope scale.

Withers, P. J. A., Jarvie, H. P. (2008). Delivery and cycling of phosphorus in rivers: a review. *Science of the Total Environment*, *400*(1), 379-395.

Wool, T. A., Ambrose, R. B., Martin, J. L., Comer, E. A., Tech, T. (2006). Water quality analysis simulation program (WASP). *User's Manual, Version*, *6*.

Xue, D., Botte, J., De Baets, B., Accoe, F., Nestler, A., Taylor, P., ... & Boeckx, P. (2009). Present limitations and future prospects of stable isotope methods for nitrate source identification in surface-and groundwater. *Water Research*, *43*(5), 1159-1170.

Xue, D., De Baets, B., Van Cleemput, O., Hennessy, C., Berglund, M., Boeckx, P. (2013). Classification of nitrate polluting activities through clustering of isotope mixing model outputs. *Journal of Environmental Quality*, *42*(5), 1486-1497.

Yamanaka, T., Ma, W. (2017). Runoff prediction in a poorly gauged basin using isotope-calibrated models. *Journal of Hydrology*, *544*, 567-574.

Yen, H., Wang, X., Fontane, D. G., Harmel, R. D., Arabi, M. (2014). A framework for propagation of uncertainty contributed by parameterization, input data, model structure, and calibration/validation data in watershed modeling. *Environmental Modelling & Software*, *54*, 211-221.

Young, M. B., McLaughlin, K., Kendall, C., Stringfellow, W., Rollog, M., Elsbury, K., ... Paytan, A. (2009). Characterizing the oxygen isotopic composition of phosphate sources to aquatic ecosystems. *Environmental Science & Technology*, *43*(14), 5190-5196.

Zah, R., Burgherr, P., Bernasconi, S. M., Uehlinger, U. (2001). Stable isotope analysis of macroinvertebrates and their food sources in a glacier stream. *Freshwater Biology*, *46*(7), 871-882.

FIGURES AND TABLES

Figure 1. Definition sketch of stable isotope signatures impacted by mass-balance mixing of source inputs (left box) and preferential utilization of lighter isotopes via Rayleigh fractionation during biochemical processes (right box). Element pool compositions are reflected by heavy and light isotope ratios in the pie chart and size of the pie chart reflects total mass of a substance (for instance substance A is larger than substance B). Mathematical expressions accounting for these processes are described using a Rayleigh-based mass balance formulation shown in Equation (8).

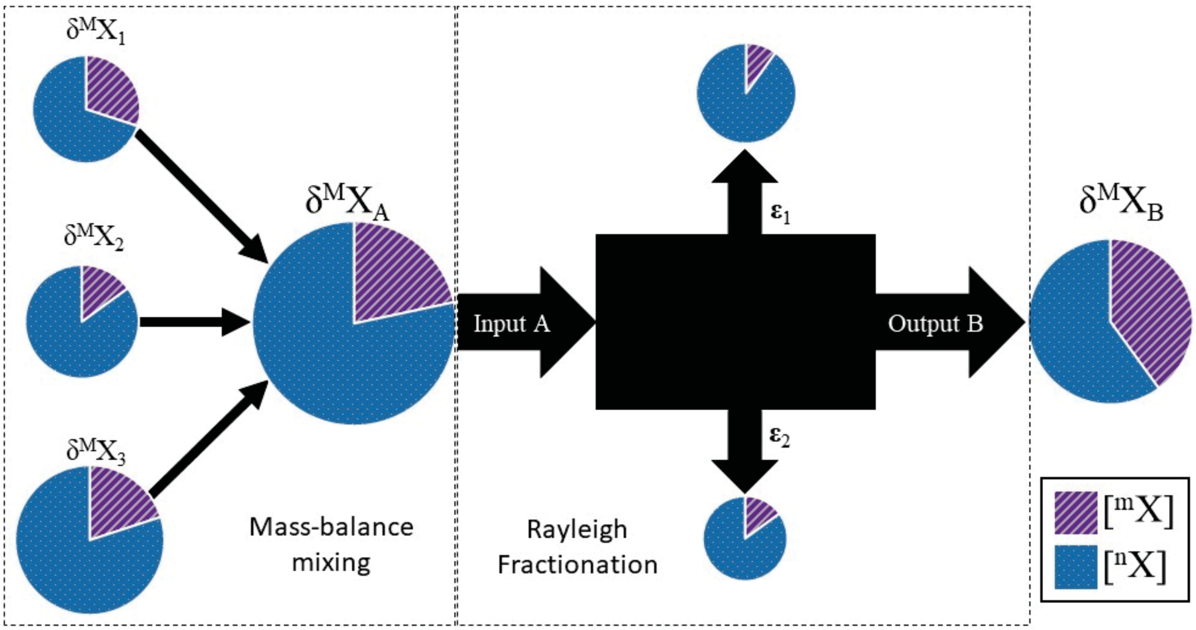

Figure 2. Depiction of a) biotic and b) abiotic processes impacting dissolved, biotic, and sediment C isotope pools. Where applicable, processes include a range of typical fractionation factors observed in the literature. Mass balance Rayleigh-like equations (extending Equation 9) are shown for environmentally relevant pools often considered in water quality models.

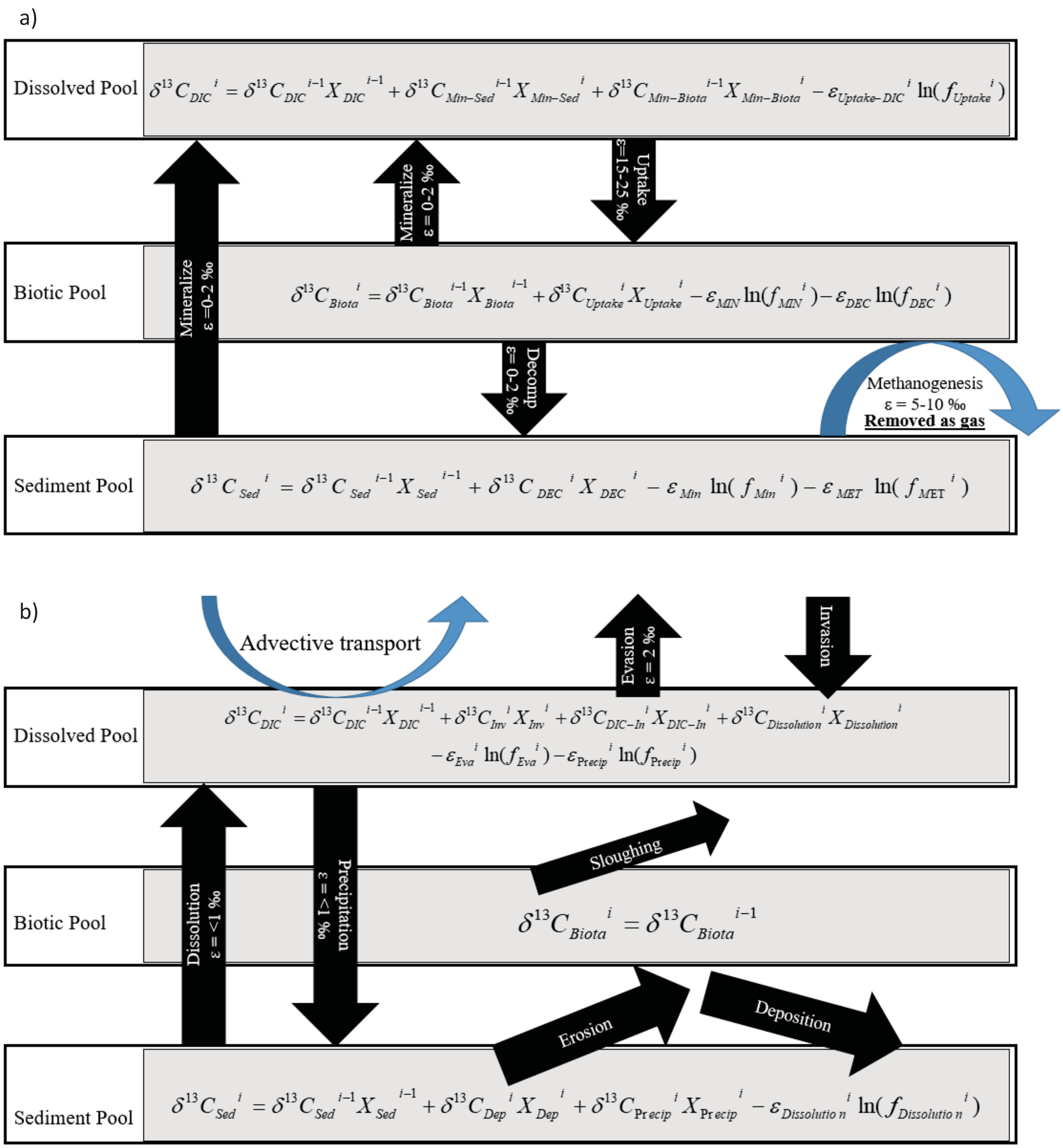

Figure 3. Depiction of a) biotic and b) abiotic processes impacting dissolved, biotic, and sediment N isotope pools. Where applicable, processes include a range of typical fractionation factors observed in the literature. Mass balance Rayleigh-like equations (extending Equation 9) are shown for environmentally relevant pools often considered in water quality models.

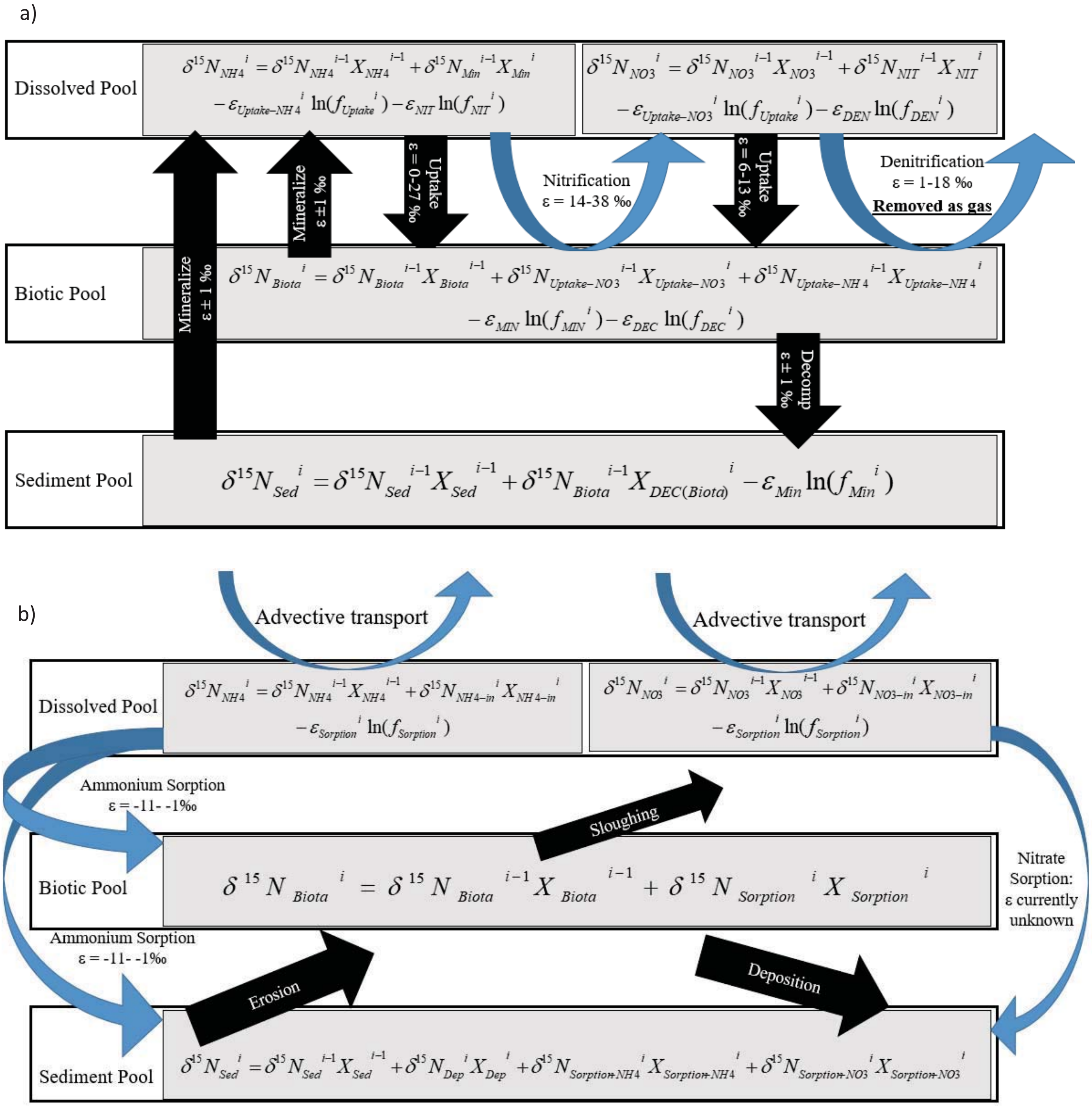

Figure 4. Depiction of a) biotic and b) abiotic processes impacting dissolved, biotic, and sediment P isotope pools. Where applicable, processes include a range of typical fractionation factors observed in the literature. Mass balance Rayleigh-like equations (extending Equation 9) are shown for environmentally relevant pools often considered in water quality models.

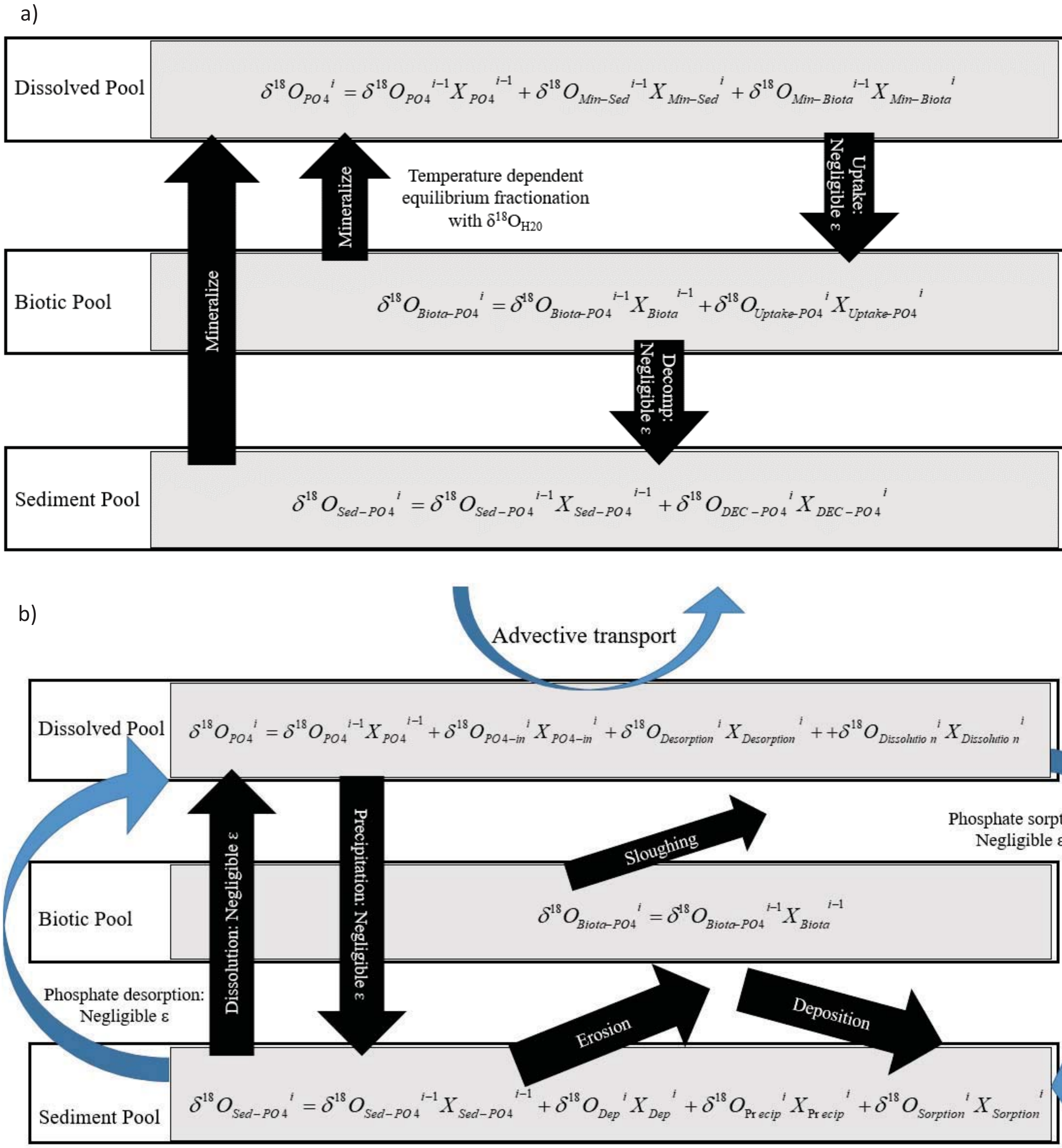

Table 1. Matrix summary denoting measurable isotope signatures of C, N, and O isotope signatures and their relevance to significant phases of C, N, and P in stream and riverine environments.

	Pool	Carbon ($\delta^{13}C$)	Nitrogen ($\delta^{15}N$)	Oxygen ($\delta^{18}O$)	References
Carbon	Dissolved inorganic carbon (H_2CO_3; HCO_3; CO_3; CO_2)	X			Doctor et al., 2008; Gammons et al., 2011; Rounick et al., 1982;
	Particulate Organic Carbon (POC)	X			Zah et al., 2001; Kendall et al., 2001; Kao and Liu, 2000;
	Dissolved Organic Carbon (DOC)	X			Palmer et al., 2001; Schiff et al., 1990; Raymond et al., 2007;
Nitrogen	Nitrate (NO_3^-) and Nitrite (NO_2^-)		X	X	Fukada et al., 2003; Pardo et al., 2004; Chang et al., 2002; Kaown, 2009;
	Ammonium (NH_4^+)		X		Webster et al., 2003;Hamilton et al., 2001;Ashkenas et al., 2004
	Particulate Organic Nitrogen (PON)		X		Kendall et al., 2001; Angradi et al., 1994; Sara et al., 2003; Stelzer et al., 2003;
Phosphorus	Dissolved reactive phosphate (PO_4^{3-})			X	Young et al., 2009; Elsbury et al., 2009; Davies et al., 2014;
	Soil and sediment extractable phosphate			X	Tamburini et al., 2010, 2014; Pistocchi et al., 2017

Table 2. Review table of watershed water quality modeling studies using stable isotopes of nutrients and sediment to improve boundary condition estimates, improve perceptual understanding of C, N and P pathways and model structure, and constrain uncertainty.

Citation	Isotope Parameters Used	Watershed Water Quality Modeling Application	Benefits of Using the Isotopes		
			Establish boundary conditions	**Constraining uncertainty of biogeochemical cycling**	**Improving perceptual understanding of C, N or P pathways and model structure**
Fox et al., 2010	$\delta^{15}N_{sediment}$	To model sediment transport (incl. temporarily stored streambed sediments) and separate sediment source contributions at the outlet of a lowland watershed	Streambank and surface soils separated through the use of $\delta^{15}N$ and C:N signatures from collected pasture and surface soils	--	Showed fate of the total N and $\delta^{15}N$ signature of the temporarily stored streambed sediments
Tobias and Bohlke, 2011	$\delta^{13}C_{DIC}$, $\delta^{18}O_{O2}$	To quantify rates of photosynthesis, respiration, groundwater discharge, air-water exchange of CO_2, and carbonate precipitation/ dissolution	--	Use of $\delta^{18}O_{O2}$ helps to constrain the interpretations of the $\delta^{13}C_{DIC}$ measurements and DIC data; C isotopes useful for confirming appropriate photosynthesis and respiration rates on which the DIC budget was framed.	Chemical and isotope modeling used with diel observations aids in mechanistic understanding of reactions and environmental factors that contribute to patterns of DIC fate and transport;
Van Engeland et al., 2012	$\delta^{13}C_{DIC}$	To predict carbon cycling under differing carbon dioxide systems within a controlled environment to study ocean acidification effects	--	Labelled $\delta^{13}C_{DIC}$ injections into mesocosm experiments helped expand the data set used for calibration which resulted in independent parameter values leading to a more constrained model output	--
Hong et al., 2014	$\delta^{15}N_{biota}$	To model the fate, transport, and bioaccumulation of $CH3Hg^+$ and look at mercury distributions to assess health risks to humans and biota surrounding/within the waterbody	Modeling showed where mercury loading was occurring and how it was being discharged into waterbody	Uncertainty of biogeochemical processes in calculating mercury levels in fish tissues reduced from relationship between mercury and $\delta^{15}N$ concentrations	By modeling a linear relationship between logarithmic mercury concentrations and $\delta^{15}N$, the fate, transport, and bioaccumulation of $CH3Hg^+$ was shown
Sebestyen et al., 2014	$\delta^{15}N_{NO3-}$, $\delta^{18}O_{NO3-}$	To study timing, length, and magnitude of stream nitrate changes, DON, and NH_3, to study changes in nitrate sources and cycling, and to study source areas heavily influencing N dynamics	Isotopes assist in estimating source contributions of nitrate to the stream channel	--	Higher inputs of unprocessed atmospheric nitrate were found relative to what is commonly acknowledged for non-snowmelt periods in forested landscapes
Xue et al., 2014	$\delta^{15}N_{NO3-}$, $\delta^{18}O_{NO3-}$, $\delta^{11}B$	Apportionment of nitrate sources in surface water from five potential sources	Major sources of nitrates were identified, and their proportional input quantified	--	--
Fox and Martin, 2014	$\delta^{13}C_{sediment}$, $\delta^{15}N_{sediment}$	Estimating yield of sediment source end member contribution from different land uses in a watershed	Isotopes separated forest, reclaimed mine, and streambank sources in watersheds	Further calibration of the transport capacity coefficient, sediment delivery ratio, and stream bank erosion parameters was found through the usage of sediment fingerprinting	--
Ford and Fox, 2015	$\delta^{13}C_{DIC}$, $\delta^{13}C_{sediment}$	Estimation of the fluvial organic carbon budget of streams with benthic autochthonous carbon	Input parameterization of allochthonous sediment sources and DIC pool.	80% reduction in uncertainty of algal C fluxes due to the sensitivity of the isotope response variable to algal sloughing.	--
Adiyanti et al., 2016	$\delta^{13}C_{DIC}$, $\delta^{13}C_{DOC}$	To quantify C cycling in an estuary	--	Reduce equifinality of the model through addition of direct constraints on matter and energy transfer between pools	--
Ford et al., 2017	$\delta^{15}N_{NO3}$, $\delta^{15}N_{sediment}$	To quantify the significance of transient and permanent removal pathways	--	Reduce model uncertainty from erroneous parameterization of a fluvial N cycle by applying sediment N fingerprints	Discrepancy in isotope measurements and model simulations at event-based scales highlight limited understanding of mobilization and demobilization through biotic and abiotic pathways.

Husic et al., 2017b	$\delta^{13}C_{sediment}$	To model time distributed processes that control the fate of sediment carbon in phreatic karst	Fingerprinting was used to unmix soil, algal, and litter contributions from urban and agricultural tributaries to a karst conduit	--	--

**** Note that studies conducting isotope mass-balance un-mixing are not included, nevertheless they support the concept of boundary condition establishment.**