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Application of Isotope Techniques for Assessing Nutrient Dynamics in River Basins

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THE OXYGEN ISOTOPIC COMPOSITION OF PHOSPHATE: A TRACER FOR PHOSPHATE SOURCES AND CYCLING

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Abstract

Phosphorus (P) is a limiting macro-nutrient for primary productivity and anthropogenic P-loading to aquatic ecosystems is one of the leading causes of eutrophication in many ecosystems throughout the world. Because P has only one stable isotope, traditional isotope techniques are not possible for tracing sources and cycling of P in aquatic systems. However, much of the P in nature is bonded to four oxygen (O) atoms as orthophosphate (PO_4^{3-}). The P–O bonds in orthophosphate are strongly resistant to inorganic hydrolysis and do not exchange oxygen with water without biological mediation (enzyme-mediated recycling). Thus, the oxygen isotopic composition of dissolved inorganic phosphate ($\delta^{18}O_p$) may be used as a tracer for phosphate sources and cycling in aquatic ecosystems. Recently, several studies have been conducted utilizing $\delta^{18}O_p$ as a tracer for phosphate sources and cycling in various aquatic environments. Specifically, work to date indicates that $\delta^{18}O_p$ is useful for determining sources of phosphate to aquatic systems if these sources have unique isotopic signatures and phosphate cycling within the system is limited compared to input fluxes. In addition, because various processes imprint specific fractionation effects, the $\delta^{18}O_p$ tracer can be utilized to determine the degree of phosphorous cycling and processing through the biomass. This chapter reviews several of these studies and discusses the potential to utilize the $\delta^{18}O_p$ of phosphate in rivers and streams.

1. INTRODUCTION

Agricultural expansion is expected to be accompanied by a 2.4 to 2.7 fold increase in nitrogen (N)- and phosphorus (P)-driven eutrophication of terrestrial, freshwater and near shore marine environments [1]. Much of the N and P from fertilizers and animal waste enters surface waters and groundwater [2] and these nutrient loads can stimulate large scale macroalgal and/or phytoplankton blooms in receiving waters [3, 4]. Nutrient enrichment in aquatic systems can cause diverse problems such as harmful algal blooms, anoxia, fish kills, loss of habitat and biodiversity, as well as other problem [1, 2]. Thus, identifying and understanding nutrient inputs and their effects on aquatic ecosystems are of critical importance to management and restoration efforts.

Phosphorus is a required element for life; consequently, its availability may impact primary production rates as well as species distribution and ecosystem structure [5–7]. Phosphorus may limit primary productivity in some aquatic systems [8–10], and may be co-limiting in others [11, 12]. Because P has only one stable isotope, P natural abundance stable isotope ratios cannot be used for studies of nutrient sources, cycling and utilization (as is the case for nitrogen

and carbon). Radioactive P isotopes (^{32}P, ^{33}P) have been used for investigation of P transformations in aquatic systems [13–15]; however, there are many complications involved with this procedure. The use of natural stable isotope signatures has advantages because this approach does not perturb the system (for example, by adding phosphate) and integrates processes over longer time scales. While P has only one stable isotope, P in most organic and inorganic P forms is strongly bonded to oxygen (O), which has three stable isotopes, providing a system to track phosphorus cycling and transformations using the stable isotopes of O in phosphate ($\delta^{18}O_p$). The oxygen isotopic composition of phosphate is reported in standard delta notation ($\delta^{18}O$) in per mil units (‰), and is calculated using the following equation:

$$\delta^{18}O(‰) = \left[\frac{R_{sample}}{R_{VSMOW}} - 1\right] \times 1000 \quad (1)$$

where R_{sample} is the ratio of $^{18}O/^{16}O$ in a sample and R_{VSMOW} is the ratio of $^{18}O/^{16}O$ in the isotopic standard for O, Vienna Standard Mean Ocean Water (VSMOW).

The P–O bond in phosphate is resistant to inorganic hydrolysis and, at the temperature and pH of most natural systems, phosphate does not exchange O with water without biological mediation [16–18]. Thus, observed variability in the $\delta^{18}O_p$ will reflect mixing of isotopically distinct sources of phosphate, the alteration of the phosphate $\delta^{18}O$ as the result of O exchange during cycling of phosphate, or a combination of these processes. In the latter case, isotope fractionations associated with reactions and transformations operating in the P cycle have been determined in controlled laboratory experiments (Table 1) and this information provides the basis for interpretation of isotope data ($\delta^{18}O_p$) obtained from phosphate in the natural environment.

In the absence of biological activity at ambient temperatures, pH, and pressure, isotope exchange between phosphate oxygen and water (or other solutions) is slow and can be considered negligible for the time scales of concern of most environmental applications [16, 18–20]. The expected equilibrium $\delta^{18}O_p$ for precipitation of mineral phosphate (apatite) from water can be calculated from the temperature and the $\delta^{18}O$ of the environmental water ($\delta^{18}O_w$) using the empirically derived fractionation equation between phosphate and water developed by [19]:

$$T(°C) = 111.4 - 4.3\ (\delta^{18}O_p - \delta^{18}O_w) \quad (2)$$

where T is the environmental temperature, $\delta^{18}O_p$ is the isotopic composition of the phosphate, and $\delta^{18}O_w$ is the isotopic composition of the environmental water.

Studies of precipitation and dissolution of various P bearing minerals and studies of P adsorption and desorption onto/from mineral surfaces indicate that the fractionation associated with these processes (given equilibration time of more than a few hours) is small — in the range of 1‰ [21–23]. Typically the heavier isotopes in these reactions are associated with the mineral phase while the solution retains phosphate with lighter isotopes. Precipitation or dissolution of apatite minerals (inorganically) will be accompanied by a small oxygen isotope fractionation in the range of +0.7‰ to +1‰ [20]. Similarly, adsorption or precipitation with sesquioxides and hydroxides imprints a small positive isotope effect [23].

In contrast to inorganic reactions, enzyme mediated biological activity can break the P–O bond in processes that involve large isotopic fractionation. Intracellular as well as extracellular en-

TABLE 1. ISOTOPIC FRACTIONATION EFFECTS ASSOCIATED WITH VARIOUS BIOGEOCHEMICAL PROCESSES

Process	Fractionation (Δ or ε)	Reference
Precipitation/dissolution of P minerals (apatite)	+0.7‰ to +1‰ heavy isotope in mineral phase	[17]
Adsorption/desorption of P to/from mineral surfaces	~ +1‰ heavy isotope in mineral phase	[22]
Precipitation with sesquioxides and hydroxides	~ +1‰ heavy isotope in mineral phase	[23]
Abiotic hydrolysis of polyphosphate (O:P = 3.33), pyrophosphate (O:P = 3.5), phosphonates (O:P = 3.0), monoesters (O:P = 3.0) and diesters (O:P = 2.0)	No fractionation or temperature effect, however incorporation of oxygen from water during formation of PO_4 (O:P =4) occurs	[24]
Transport by water or air	No fractionation or temperature effect	[25]
Assimilation by phytoplankton	Light isotopes preferentially utilized, enrichment of the residual solution (ε = –3‰)	[25]
Intracellular processing such as inorganic pyrophosphatase (PPase) catalysis	Equilibrium isotopic exchange T and $\delta^{18}O_w$ impact (equation 3)	[25]
Alkaline phosphatase (APase) hydrolization of phosphomonoesterase (extracellular) kinetic isotope effects	ε = –30‰ effecting only the newly incorporated oxygen	[27]
5′-nucleotidase hydrolization (extracellular) kinetic isotope effects	ε = –10‰ effecting only the newly incorporated oxygen	[27]
First step of DNAse hydrolization kinetic isotope effects	ε = –20‰ effecting only the newly incorporated oxygen	[27]
First step of RNAse hydrolization kinetic isotope effects	ε = +20‰ effecting only the newly incorporated oxygen	[27]

zymes are expressed by various organisms for the uptake and utilization of P and may play a role in determining the oxygen isotopic composition of phosphate in aquatic systems. Different enzymatic processes induce different isotopic fractionations (Table 1). The dominant enzymatic process controlling $\delta^{18}O_p$ in the environment is the intracellular activity of pyrophosphatase (PPase) [26], which involves equilibrium isotopic exchange. Blake et al. [26] found that this enzymatic activity results in isotopic equilibrium of oxygen in phosphate similar to that described in Ref. [19]. The equation for phosphate extracted from microbial cultures was described in Ref. [20]:

$$T(°C) = 155.8 - 6.4\,(\delta^{18}O_p - \delta^{18}O_w) \quad (3)$$

These equilibrium relations have been observed in the tissues of a variety of organisms, including fish, mammals [28], bacteria and algae [17, 29, 26]. Results of an algae culture experiment indicate that intracellular oxygen isotope exchange between phosphorus compounds and water

is very rapid [29]. These processes are expected to occur in aquatic systems by all organisms and release of this phosphate from cells to the environment will impact dissolved phosphate $\delta^{18}O_p$. Extracellular remineralization and hydrolization of organic P (P_o) compounds by phosphohydrolase enzymes such as alkaline phosphatase (APase) and 5′- nucleotidase, involves incorporation of one or more oxygen atoms from the ambient water with an isotope fractionation of –30‰ and –10‰, respectively [27]. The resulting phosphate from such processes will reflect this fractionation and would typically shift $\delta^{18}O_p$ towards values that are lower than equilibrium. Work by several groups is currently ongoing to determine the isotope fractionation associated with additional enzymes, and will enable better interpretation of field data. Uptake and utilization (assimilation) of phosphate by aquatic plants, algae, and microorganisms is also associated with isotope fractionation. The phosphate with lighter O isotopes is preferentially utilized, a process that could enrich the residual solution with phosphate that has heavy O isotopes [26].

Oxygen isotope tracer studies of dissolved phosphate and particulate phosphate in natural environments are limited. However, recent field studies have demonstrated the utility of $\delta^{18}O_p$ of DIP as a tracer for the mixing of phosphate sources in lakes, estuaries and the coastal ocean ecosystems [30–33] and for tracing phosphate sources in sediments and soils in an estuarine watershed [24]. These applications are based on the assumption that extensive recycling and turnover will lead to isotopic equilibrium while deviation from equilibrium may reflect source signatures or other processes that do not result in isotopic equilibrium, such as expression of extracellular enzymes or phosphate uptake. Furthermore, other studies have suggested that different sources may be isotopically distinct [34, 35].

2. METHOD FOR ASSESSING THE OXYGEN ISOTOPIC COMPOSITION OF PHOSPHATE

The oxygen isotopic composition of phosphate can be measured in both dissolved and particulate phases. McLaughlin et al. [36] outlines the protocol for extraction of dissolved inorganic phosphate from water samples for isotopic analysis. McLaughlin et al. [37] presents a method for isotopic analysis of phosphate oxygen in particulate inorganic and organic matter samples. Each of these methods is described briefly below. Other methods [16, 25, 26, 28, 38–42]which are slightly different than those discussed below have also been published.

2.1. Dissolved inorganic phosphate

Water samples are collected in acid washed high density polyethylene bottles and filtered through 0.45 μm filters to remove particulates. The amount of water required for each sample depends on the dissolved inorganic phosphate (DIP) concentration, and typically ranges from 1 L for waste water treatment plant effluent to more than 40 L for some lakes and oligotrophic areas of the ocean. For low salinity samples, magnesium chloride (ACS grade, with low phosphate content) is added as needed to raise the magnesium content to that of seawater. Samples are then processed through an extraction and purification protocol according to McLaughlin et al. [36]. Phosphate is initially co-precipitated with magnesium hydroxide, the pellet collected and subsequently dissolved in nitric acid. This step reduces the volume of the sample. Cerium phosphate is then precipitated from the solution in order to separate phosphate from competing anions. This precipitate is washed and centrifuged several times, before being dissolved in a small amount of 4 M nitric acid. The solution is passed through a cation exchange resin to

remove the cerium ions (to prevent interference during the final precipitation), and phosphate is ultimately precipitated as silver phosphate for isotope ratio analysis. The silver phosphate is thermally decomposed in the presence of carbon to form carbon monoxide, which is then analysed by isotope ratio mass spectrometry (IR–MS) for masses 28, 29 and 30. Results are calibrated and precision monitored using internal standards with known isotopic composition. All oxygen isotopic measurements are reported in the standard delta notation in per mil units (‰) with respect to Vienna Standard Mean Ocean Water (VSMOW); the precision of $\delta^{18}O_p$ is approximately ±0.3‰. Currently no international silver phosphate reference standards exist for phosphate oxygen isotopic analysis and most laboratories use internal standards.

Problems with the precipitation of cerium phosphate and silver phosphate have been experienced when working with water samples containing very high concentrations of dissolved organic matter. Several promising approaches for addressing this problem have been explored, including UV radiation of the water sample [43], passing the sample through phosphate free activated carbon [34], and the use of resins (such as DAX–8) to remove organics while leaving the DIP in solution [44]. Treatment of the silver phosphate with 15% H_2O_2 at room temperature was also suggested [45].

2.2. Particulate phosphate

Particulate phosphatic compounds must be converted to silver phosphate prior to isotopic analysis, a process that involves digestion of particulate matter in acid. This digestion will hydrolyze some of the phosphatic compounds such that oxygen from the acid solution could be incorporated into the sample as these phosphatic compounds are converted to orthophosphate (PO'^{-}). As described in Ref. [37], duplicate samples of particulate organic matter samples (periphyton, sediment, soils, plant material, etc.) are weighed into separate 50 ml polyethylene depressed cap centrifuge tubes. Ten millilitres of 10 M nitric acid is added to one of these and ten millilitres of 10 M nitric acid that had been amended with $H_2{}^{18}O$ (Isotec T88–70022 batch # EQ0820) is added to the remaining sample. All samples are then heated on a hot plate held at 50 ºC. The acid is neutralized with 8 M potassium hydroxide (Fisher Scientific, ACS grade) and the solution is then buffered with 1 M potassium acetate (Fisher Scientific, ACS grade). Purified silver phosphate is ultimately precipitated and analysed for $\delta^{18}O_p$ according to McLaughlin et al. [36] or other published procedures. Results from this study indicate that there is no isotopic fractionation associated with acid digestion at 50ºC and that reagent oxygen incorporation is a function of the oxygen to phosphorus ratio (O:P) of the digested compound whereby the percentage of reagent oxygen incorporated into the sample is the same as that which is required to convert all of the P-compounds into orthophosphate [37]. Thus, the isotopic composition of organic matter samples can be calculated by solving a system of mass balance equations, one for the spiked reagent and one for the unspiked:

$$\delta^{18}O_{\text{spiked measured}} = X\,\delta^{18}O_{\text{sample}} + (1-X)\,\delta^{18}O_{\text{spiked reagent}} \quad (4)$$

$$\delta^{18}O_{\text{unspiked measured}} = X\,\delta^{18}O_{\text{sample}} + (1-X)\,\delta^{18}O_{\text{unspiked reagent}}$$

where X is the proportion of oxygen from the sample, $(1–X)$ is the proportion of oxygen from the reagent, $\delta^{18}O_{\text{spiked reagent}}$ is the isotopic composition of the ^{18}O amended acid, $\delta^{18}O_{\text{unspiked reagent}}$ is the isotopic composition of the unspiked acid solution, $\delta^{18}O_{\text{sample}}$ is the 'true' isotopic composition for the sample, $\delta^{18}O_{\text{spiked measured}}$ is the oxygen isotopic composition of the sample measured after digestion of the sample in the spiked nitric acid reagent, and $\delta^{18}O_{\text{unspiked measured}}$

is the isotopic composition of the sample after digestion in the unspiked nitric acid reagent. Solving these two equations for the $\delta^{18}O_{sample}$ yields:

$$\delta^{18}O_{sample} = \frac{(\delta^{18}O_{spiked\ reagent} \times \delta^{18}O_{unspiked\ measured}) - (\delta^{18}O_{spiked\ measured} \times \delta^{18}O_{unspiked\ reagent})}{(\delta^{18}O_{unspiked\ measured} - \delta^{18}O_{spiked\ measured} + \delta^{18}O_{spiked\ reagent} - \delta^{18}O_{unspiked\ reagent})} \quad (5)$$

3. END-MEMBER VALUES FOR PHOSPHATE SOURCES

Identifying point and non-point nutrient sources is critical to understanding ecosystem health, and has important implications for management practices, including industry regulation and allocation of funding and research efforts. P sources can be separated into point sources, such as sewage discharge sites, and non-point sources like soil leaching and agricultural run-off. One of the most significant limitations for the application of this tracer is that very little data exists regarding the $\delta^{18}O_p$ of various natural and anthropogenic phosphate sources. Recent research has shown that the $\delta^{18}O_p$ of various primary anthropogenic phosphate sources to aquatic ecosystems spans a wide range of $\delta^{18}O_p$ values, indicating that this can be a useful tool for distinguishing phosphate sources in certain systems.

Young et al. [35] analysed an initial set of potential P sources for $\delta^{18}O_p$, including wastewater treatment plant effluent, chemical and organic fertilizers, semi-processed phosphorite ore (used to make chemical fertilizers), dish washing detergents, toothpaste, soil leachates, and aerosol (dust) samples (Fig. 1). A considerable range of $\delta^{18}O_p$ values was measured in the various P sources (samples ranged from 6 to 27‰), and the differences were much larger than the analytical precision (± 0.3‰) associated with this technique. Although there is considerable overlap in $\delta^{18}O_p$ measured in the various groups of samples, these results indicate that in specific geographic regions, different P source types may span a narrower range and have distinct signatures. In these cases, the $\delta^{18}O_p$ could be useful for identifying the contribution of the different sources. For example, while the entire range of reported $\delta^{18}O_p$ values for wastewater treatment plant (WTP) effluent (3 WTPs; France, Connecticut, California) overlaps with the values measured for multiple types of detergents, organic fertilizers, and chemical fertilizers, all $\delta^{18}O_p$ values for the Palo Alto Regional Water Quality Control Plant were significantly lower than any of the measured fertilizers and detergents [35]. Thus, if phosphate is not heavily cycled within an ecosystem, such that the source signature is reset, $\delta^{18}O_p$ can be used to identify isotopically distinct phosphate sources and/or the extent of phosphate cycling in aquatic systems (such as the deviation from the isotopic composition of the source from the expected equilibrium value).

Wastewater treatment effluent is a significant source of phosphate in many areas, and studies are currently underway looking at how the $\delta^{18}O_p$ of wastewater treatment plant effluent changes over time and throughout treatment processes. It is currently not known if the $\delta^{18}O_p$ of wastewater treatment plant effluent is a reflection of the P source signature, biological equilibration with the $^{18}O_w$ of water within the sewer system and/or treatment plant, or a combination of both. Samples were collected at each stage of treatment at the Palo Alto Water Quality Control Plant, and no significant differences were found throughout the treatment cycle, indicating that the particular treatment processes used at this plant does not change the $\delta^{18}O_p$ signature. However, there was a significant change in the $\delta^{18}O_p$ of the effluent between October 2005 and January 2006, which also corresponded to a similar change in the $^{18}O_w$ of the incoming water. It is likely that the $\delta^{18}O_p$ of the effluent is controlled by a combination of $\delta^{18}O_p$ sources and

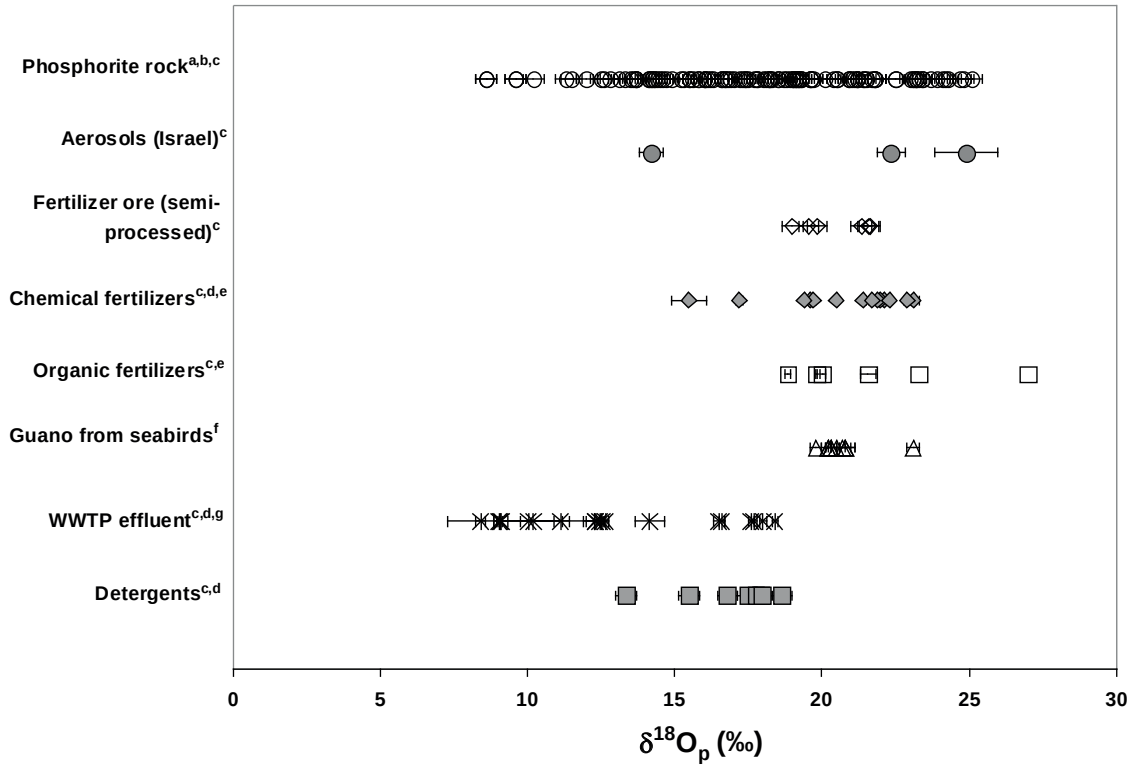

FIG. 1. Range of potential end-member values for natural and anthropogenic phosphate sources to aquatic systems.

biological equilibration prior to entering the treatment plant, but more sampling is required to in order to understand these processes and potential variations, depending on treatment types and plant locations.

4. PHOSPHATE SOURCES AND CYCLING IN ESTUARINE AND COASTAL ENVIRONMENTS

To date, there are relatively few studies assessing the oxygen isotopic composition of DIP in natural aquatic systems. Pioneering work by Longinelli et al. [16] found no variation in the $\delta^{18}O_p$ of DIP in seawater or of marine organism soft tissue with either depth or latitude in the Atlantic and Pacific Oceans, although there was a significant difference between the two ocean basins. The $\delta^{18}O_p$ values were thought to reflect kinetic-biological isotopic fractionation. However, Longinelli et al. [16] extracted P from seawater without pre-filtration and used Fe-coated fibres, which absorb both inorganic and organic P and such complications may confound interpretation of their results. More recently, Colman [30] concluded that the large deviations in $\delta^{18}O_p$ between riverine and coastal waters in the Long Island Sound reflected equilibration with local water and indicated that rapid microbial cycling overprints source $\delta^{18}O_p$ values on a timescale of weeks. In contrast, phosphate in the San Francisco Bay estuary is typically not equilibrated with environmental water and reflects two end-members mixing between oceanic phosphate and riverine phosphate with seasonally important additional inputs along this flow path [32]. In California coastal waters (Monterey Bay), phosphate oxygen isotope ratios tracked seasonal changes in phosphate cycling through the biomass (for example, phos-

phate utilization rates) with the greatest phosphate oxygen isotope exchange occurring during the upwelling season [31]. The $\delta^{18}O_p$ in open ocean waters is a function of DIP transport and biological turnover in both the Atlantic and the Pacific Oceans and highlights the importance of cell lysis in the regeneration of DIP in the euphotic zone [46]. Furthermore, at depth, $\delta^{18}O_p$ values are near temperature dependent equilibrium, suggestive of bacterial turnover of DIP in seawater [46]. These data indicate that the $\delta^{18}O_p$ can be used as a powerful tool for identifying and quantifying the contribution of non-point sources of phosphate pollution into some aquatic systems and that it could be used to determine relative rates of P cycling and utilization in marine systems.

The next few sections briefly discuss the utilization of the oxygen isotopic composition of phosphate as a tool for identifying phosphate sources and cycling in estuarine environments and describe the potential of expanding usage into riverine environments.

4.1. San Francisco Bay, California, USA: Use of $\delta^{18}O$ of dissolved inorganic phosphate as a tracer for phosphate sources

In a study of North San Francisco Bay, McLaughlin et al. [32] used $\delta^{18}O_p$ to assess mixing of dissolved inorganic phosphate (DIP) sources along an estuarine flowpath. The $\delta^{18}O$ of DIP ($\delta^{18}O_p$) will largely be determined by the isotopic composition ($\delta^{18}O_w$) and temperature of the water. Because the $\delta^{18}O_w$ of water in rivers and oceans is significantly different, the $\delta^{18}O$ of phosphate recycled in these waters will also be different as a result of equilibrium fractionation. Thus, phosphate $\delta^{18}O_p$ may be used to either characterize mixing between oceanic and riverine

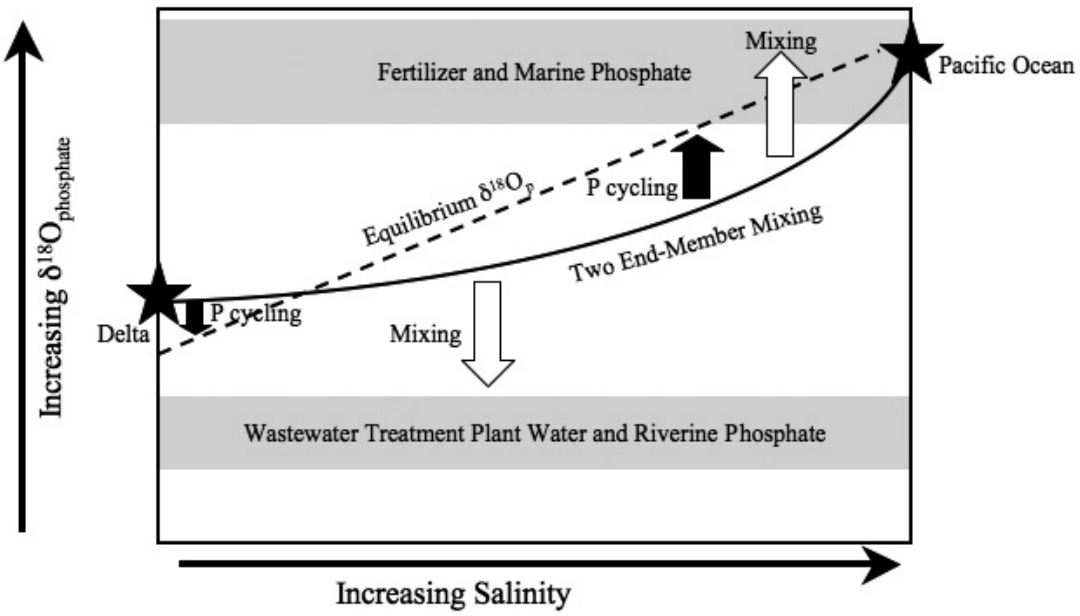

FIG. 2. Diagram indicating two end-member mixing and the expected equilibrium mixing line. Deviations below both the two end-member mixing (white down facing arrow) indicate mixing with either a riverine or wastewater treatment plant effluent. Deviations which move off the two end-member mixing line in the direction of equilibrium mixing may be indicative of phosphate cycling, though they may also represent mixing with fertilizer phosphate. Deviations which fall off the two-end member mixing line in the direction of equilibrium, but in excess of equilibrium are indicative of mixing with fertilizer phosphate or treatment plant effluent, depending on where along the salinity gradient the deviation occurs.

phosphate or to determine the extent of phosphate cycling. North San Francisco Bay can be characterized by a two end-member mixing model between Pacific Ocean waters and the freshwaters of the San Joaquin and Sacramento Rivers based on salinity and $\delta^{18}O_w$ [32–47]. This mixing model can be adapted to represent an expected mixing line for $\delta^{18}O_p$, and the expected equilibrium value at each site can be calculated (Figs 2 and 3). Deviations from the $\delta^{18}O_p$ mixing line that are not consistent with equilibrium are most likely the result of the contribution

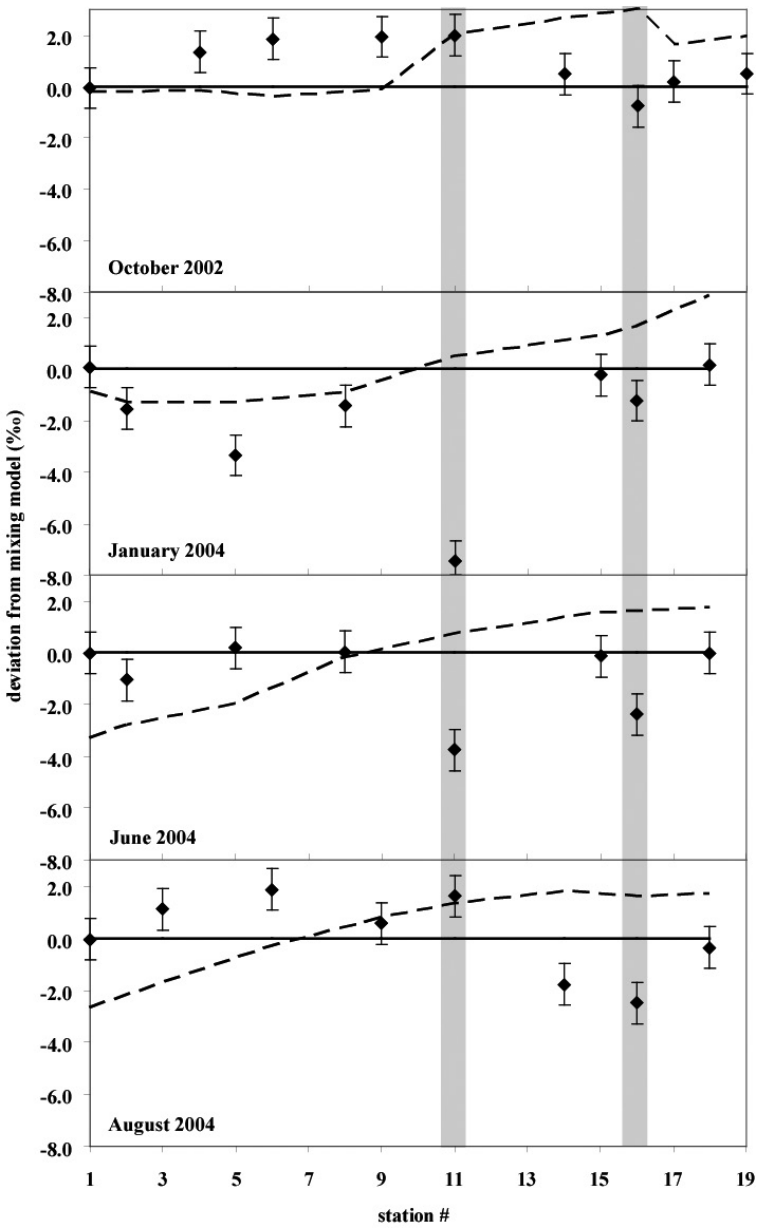

FIG. 3. $\delta^{18}O_p$ deviations from those expected of two end-member mixing models (solid line) for each station and month sampled. Deviations from the mixing line were calculated by taking the difference between the expected $\delta^{18}O_p$ calculated for each salinity and the measured $\delta^{18}O_p$. Deviations of the equilibrium $\delta^{18}O_p$ from the two end-member mixing models are represented by the dashed line. The mixing model value in each of the above plots is represented by 0‰. Vertical highlighting shows the largest deviations observed at Station 11 in January 2004 and June 2004 and the most persistent deviations at Station 16 in all months. Error bars indicate the error propagated through the mixing models.

of phosphate with unique $\delta^{18}O_p$ signatures at various point and non-point locations, such as the discharge points of tributaries (e.g. the Napa River) and wastewater treatment plants.

The general lack of isotopic equilibrium in the DIP throughout the Bay indicates that phosphate cycling is not rapid compared to phosphate input (low utilization rate, short residence time), and that source phosphate $\delta^{18}O$ contributes to the observed $\delta^{18}O_p$ at most, if not all, stations. The deviations from the $\delta^{18}O_p$ mixing model represent inputs of phosphate from local phosphate sources within the North Bay (Fig. 3). In this study, we demonstrated that it is possible to use $\delta^{18}O_p$ to identify point and non-point source phosphate inputs to aquatic systems and suggest that this may be applied in other impacted systems to identify specific anthropogenic sources, such as fertilizer and sewage phosphate, or natural sources of phosphate. This information is crucial for mitigation of pollution impacts and successful restoration of estuaries and other aquatic systems.

4.2. Carneros Creek Watershed, California, USA: Use of $\delta^{18}O$ of particulate phosphate as a tracer for phosphate sources

The Carneros Creek watershed drains into Elkhorn Slough, a small seasonal estuary in central California. This watershed has been subjected to increased nutrient loading from agricultural and other non-point sources. However, because nutrients do not behave conservatively and multiple sources may be present, tracing nutrient sources and the relative contribution of those sources in ecosystems like Elkhorn Slough and the Carneros Creek Watershed has been difficult to do using nutrient concentrations alone. Because of the high concentrations of phosphate within the watershed, P-demand is low relative to input and phosphate may not be heavily cycled within the ecosystem. Thus, the $\delta^{18}O$ of phosphate will reflect the isotopic composition of phosphate sources to the system. McLaughlin et al. [24] utilized the $\delta^{18}O$ of reactive phosphate from water, sediment, and soil samples collected within the watershed to understand phosphate sources and cycling.

The variability in $\delta^{18}O_p$ observed in soils collected throughout the watershed and in sediments from Carneros Creek was indicative of the wide range of land uses and soil types in the watershed. Soil samples had higher total and reactive P concentrations than Carneros Creek sediments. However, both the creek sediments and soil samples spanned the same range of $\delta^{18}O_p$, which indicates that P sources to the creek sediments and to the soils originated from sources with similar isotopic signatures, most likely fertilizers. Samples from a single farm within the watershed (compost, soil and sediment pond samples) provided an example of how phosphate oxygen isotopes may be fractionated within a system. The $\delta^{18}O_p$ of reactive phosphate extracted from the compost was low (18.8‰) relative to the $\delta^{18}O_p$ of the nitric-acid-extractable phosphate of that compost (23.3‰), implying that phosphate with low $\delta^{18}O_p$ is more readily mobilized. Furthermore, the $\delta^{18}O_p$ of reactive phosphate in the compost (18.8‰) was higher than the farm soil sample (17.7‰), which in turn was higher than the sediment pond (15.5‰). Thus, low $\delta^{18}O_p$ phosphate was preferentially moved from the compost to the soils and from there into the sediment pond. This was the first time that such an isotope effect has been reported and this observation should be confirmed with more data from other sites.

Sediment samples from within Carneros Creek bed indicated the prevalence of fertilizer use within the watershed: all samples analysed had high $\delta^{18}O_p$ values (18.5‰, 22.5‰, 21.6‰), similar to the fertilizers measured. In all cases, the $\delta^{18}O_p$ of creek sediments was higher than that of soils sampled nearby, suggesting that most of the phosphate in the creek water and sedi-

ments came directly from the fertilizer rather than through leaching of phosphate from soils. Alternatively, the $\delta^{18}O_p$ of creek sediments could have a higher isotopic signature than the soil P due to preferential removal of low $\delta^{18}O_p$ phosphate from the sediment into creek waters. Indeed, creek water sampled in the lower reaches of the creek had considerably lower $\delta^{18}O_p$ values than sediment samples upstream. The variability observed within this limited set of samples was very promising and indicates that $\delta^{18}O_p$ could be a powerful tool for identifying non-point source P pollution in watersheds and aquatic systems.

5. PHOSPHATE SOURCES AND CYCLING IN FRESHWATER SYSTEMS

The range of potential $\delta^{18}O_p$ values for DIP in lakes and rivers is much greater than the range expected for open ocean and coastal waters due to the wider range of temperatures, $\delta^{18}O$ water values, and phosphate sources found in riverine systems. Furthermore, land use patterns are thought to have a significant impact on nutrient stoichiometry and concentrations in freshwater environments [48–50], thus, differences in land use could provide unique $\delta^{18}O_p$ signatures with which to trace the relative influence of specific sources to receiving waters. However, research on $\delta^{18}O_p$ in freshwater systems is relatively sparse, and measured $\delta^{18}O_p$ values are only available for a few locations. Many rivers may have phosphate inputs that are different from those found in open ocean or near shore environments. Common phosphate sources for rivers include wastewater treatment effluent, agricultural and urban runoff, manure, leaking septic systems, and natural rock and soil weathering. In addition, river discharge can be viewed as a source of phosphate in relation to other systems; for example, tributaries entering larger rivers, lakes, estuaries, or coastal waters. Although the $\delta^{18}O_p$ of river water is usually controlled by a complex combination of source inputs and biogeochemical cycling, if the $\delta^{18}O_p$ of the discharging water is known, it can be used to trace river phosphate as it enters a different environment or moves down a river's flow path.

Use of the oxygen isotopic composition of phosphate as a tracer for biological cycling within a river system is complicated by several factors (Fig. 4). Phosphorus bioavailability in lakes, rivers, and streams can be altered by adsorption and desorption onto fluvial suspended particles

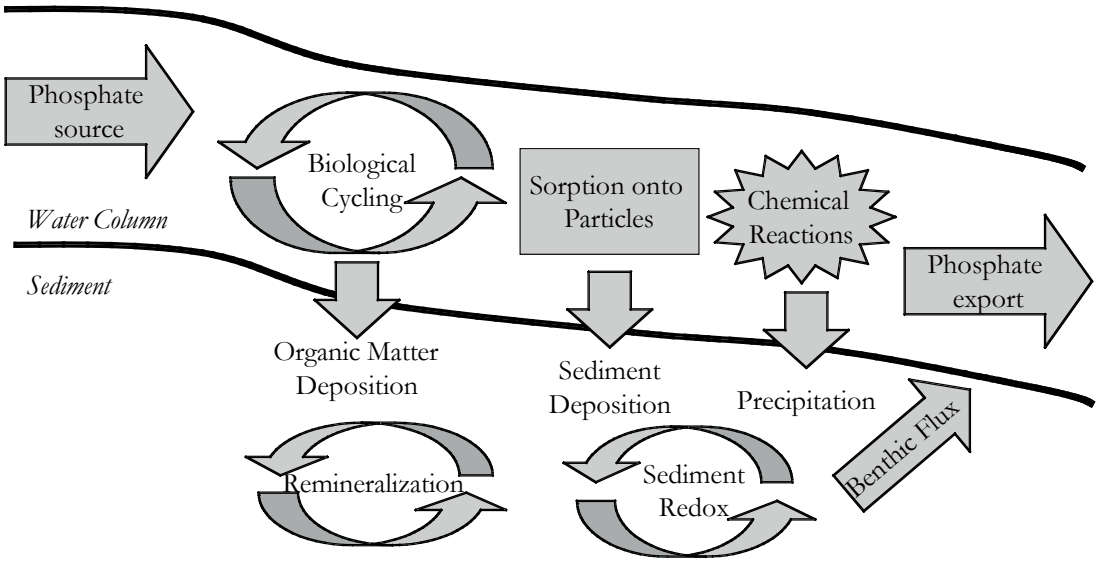

FIG. 4. Factors potentially affecting the $\delta^{18}O_p$ in riverine environments.

[51], formation of colloidal compounds with ferric hydroxides [52], and co-precipitation with minerals such as calcite [53]. At this stage it is not known whether adsorption or co-precipitation will result in an isotopic fractionation although such an effect is expected to be small. Phosphate can be deposited in sediments and may eventually be effluxed from sediments at a later date [52, 54]. Thus, measurements of $\delta^{18}O_p$ will reflect a combination of source signatures, enzyme mediated biological cycling, and physio-chemical reactions, each of which may impart an isotopic fractionation. Furthermore, these isotopic fractionations are not well understood in freshwater environments and more research is needed to fully characterize them.

5.1. San Joaquin River, California, USA

Water samples were collected from the San Joaquin River (SJR), a hypereutrophic river in the major agricultural region of the California Central Valley, in order to assess whether or not the $\delta^{18}O_p$ of the DIP was in isotopic equilibrium with the river water and if measurable differences in $\delta^{18}O_p$ existed throughout the river, tributaries, and drains [35]. Samples were collected at seven sites along the main stem of the SJR, and from nine separate drains and tributaries. Several of the same sites were sampled one week apart in order to see if short term changes could also be detected.

The range of $\delta^{18}O_p$ measured in the SJR and tributaries was much larger than analytical error, and only one of the samples fell along the expected equilibrium line. Interestingly, the samples did not show any consistent offset from equilibrium, further indicating that the $\delta^{18}O_p$ at least partially reflected inputs of phosphate sources with different $\delta^{18}O_p$ signatures, rather than full biological cycling and complete oxygen exchange with water (Fig. 5). The highest $\delta^{18}O_p$ values were measured at several sites that drain large wetland areas upstream of the lower San Joaquin River, while the lowest values were measured in the Merced River and in Harding Drain. This data set is preliminary and is just documented here to show the potential for using phosphate $\delta^{18}O_p$ to assess distinct sources in freshwater systems. Further research is required to fully interpret this data set.

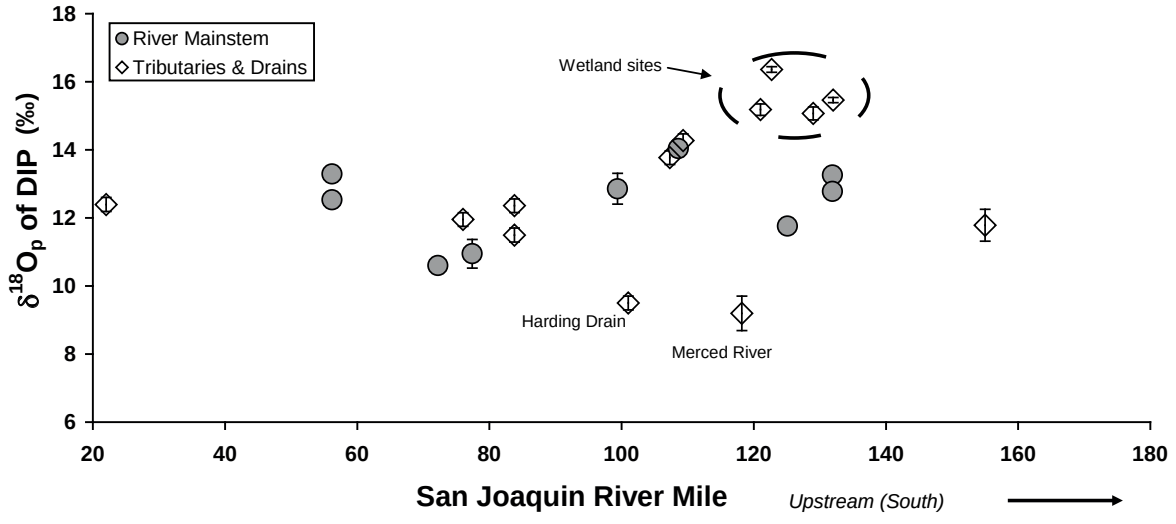

FIG. 5. The $\delta^{18}O_p$ of dissolved inorganic phosphate in the San Joaquin River and tributaries as a tracer for phosphate sources.

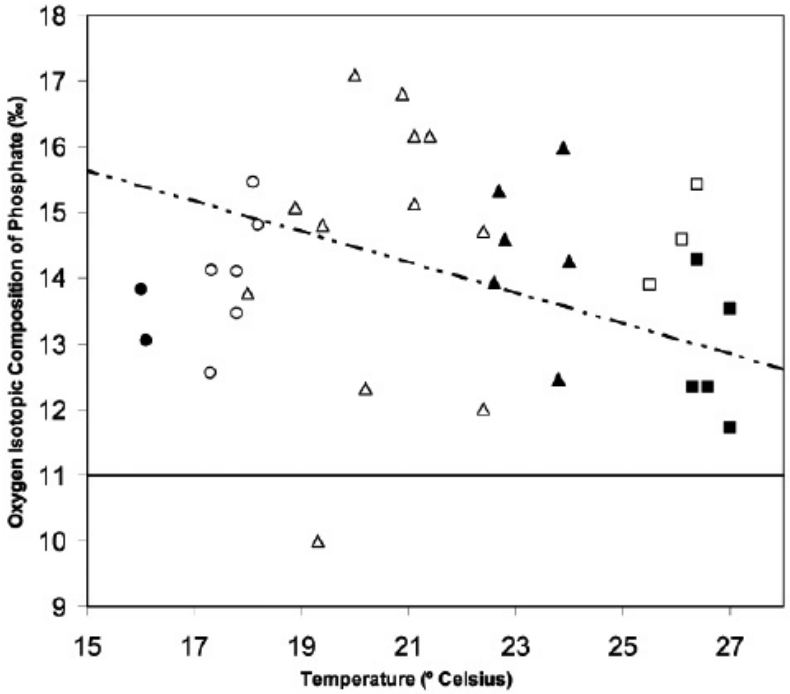

FIG. 6. Oxygen isotopic composition of phosphate ($\delta^{18}O_p$) of Lake Erie surface water. Symbols refer to different sampling trips. The line at +11‰ represents the weighted average riverine $\delta^{18}O_p$. The dashed line represents the expected $\delta^{18}O_p$ value calculated based on the temperature for each sample and at the average lake surface $\delta^{18}O_w$ of –6.78‰ (standard deviation 0.3‰). Samples plotting between the river and equilibrium lines could be explained by P cycling, a process that would tend to erase source signature and bring $\delta^{18}O_p$ values towards equilibrium. Lines above the equilibrium line suggest a source with $\delta^{18}O_p$ higher than 17‰.

5.2. Lake Erie, USA

Elsbury et al. [55] recorded the distribution of $\delta^{18}O_p$ in water samples from the western and central basins of Lake Erie along with several potential sources (rivers, waste water treatment plants, atmospheric deposition). The $\delta^{18}O_p$ of lake water was largely out of equilibrium with ambient conditions, indicating that source signatures may be discerned. The $\delta^{18}O_p$ values in the lake ranged from +10‰ to +17‰, whereas equilibrium value was expected to be around +14‰ and riverine weighted average $\delta^{18}O_p$ value was +11‰ (Fig. 6). It was concluded that some of the lake $\delta^{18}O_p$ values could not be explained by any known source or process. This indicated that there must be one or more as yet uncharacterized source(s) of phosphate with a high $\delta^{18}O_p$ value. In this study, the authors speculate that a likely source may be the release of phosphate from sediments under reducing conditions created during anoxic events in the hypolimnion of the central basin of Lake Erie.

5.3. Research needs

Several gaps in our understanding of how phosphate oxygen is fractionated in freshwater systems must be addressed before this tracer can be fully utilized in lakes, rivers and streams. As noted in the introduction above, there is a distinct difference between the isotopic fractionation of phosphate oxygen associated with intracellular phosphate cycling versus extracellular phosphate cycling [26]. In estuarine environments, which are typically nitrogen limited

rather than phosphorus limited, extracellular phosphate cycling and its associated kinetic isotope fractionation will most likely play a less important role relative to intracellular biological cycling and its associated equilibrium fractionation. However, many freshwater systems are phosphorus limited [7, 56], and thus extracellular regeneration of dissolved organic carbon compounds may play a larger role in lakes and rivers than in estuaries. Therefore, the relative importance of extracellular versus intracellular biological phosphate cycling in these systems must be more fully understood before phosphate $\delta^{18}O_p$ can be fully utilized. Furthermore, there is a dearth of data on the fractionation associated with freshwater periphyton (soft algae and diatoms) and freshwater heterotrophic bacteria. Research has suggested that bacteria are superior competitors for phosphate in aquatic systems compared to phytoplankton [57]; however, differences in isotopic fractionation associated with bacterial cycling of phosphorus compared to algal cycling have not been fully defined. More research is needed to understand how these organisms fractionate phosphate oxygen under a variety of temperature and phosphorus concentration regimes.

Isotopic fractionation associated with sorption onto particulate matter and in association with co-precipitation of phosphate must also be further explored. Phosphate interactions with sediments and co-precipitates in lakes and streams have been found to be an important factor in controlling the dissolved phosphate pool [52, 53]. Immobilization of phosphate in sediment has been found to differ substantially between freshwater and salt water systems. In oxic freshwater environments, phosphorus is strongly immobilized in the sediment; whereas in salt water environments phosphorus is released from sediments in association with benthic decomposition [58]. If precipitation reactions or sorption onto particles have a fractionation associated with them, it could contribute significantly to the measured $\delta^{18}O_p$. Precipitation of phosphate minerals (for example, apatite) in freshwater systems should impart an equilibrium isotopic fractionation [19], which would make such reactions indistinguishable from intracellular biological cycling. However, fractionations associated with sorption and co-precipitation with minerals like calcite has not been characterized. Such effects are assumed to be negligible in most systems but could potentially play a role in hardwater systems where co-precipitation of phosphate can result in the removal of up to 30% of the dissolved P pool [53].

6. FUTURE DIRECTIONS

The oxygen isotopic composition of DIP and particulate phosphatic compounds can be utilized for management and restoration efforts as a tracer for phosphate sources. This tracer will be most effective in eutrophic systems in which phosphate concentrations are high and the source signatures minimally altered. When used in this manner, $\delta^{18}O_p$ can identify both point sources of phosphate (such as wastewater treatment plant outfall) or non-point sources (like agricultural or urban runoff). Indeed, $\delta^{18}O_p$ has already proven to be an excellent tracer for source tracking in estuarine environments [24, 32].

The $\delta^{18}O_p$ could potentially be effective as a tracer for phosphate uptake within phosphorus limited streams. Spiking a stream reach with an ^{18}O-enriched phosphate source and following the change in isotopic signature would be a direct measure of such uptake rates. Understanding phosphate uptake in such streams is encumbered by low phosphate concentrations; however, addition of a phosphate spike to the system and subsequent determination of the isotopic alteration of the spike downstream could potentially provide information on the uptake of phosphate

within the reach (H.M. Valett personal communication). This tracer is currently being developed by H.M. Valett (Virginia Institute of Technology) and others for watersheds in the USA.

7. SUMMARY AND CONCLUSION

The $\delta^{18}O_p$ stable isotope tracer has been successful in identifying sources and cycling of phosphate in coastal environments. It is particularly useful in estuarine systems due to the mixing of freshwaters (with low $\delta^{18}O_w$ and thus, low $\delta^{18}O_p$) and seawater (with higher $\delta^{18}O_w$ and thus, higher $\delta^{18}O_p$). Sources of phosphate with unique isotopic signatures can be identified as deviations of the simple two-end member mixing line in such systems. The $\delta^{18}O_p$ tracer can be utilized in a similar fashion in nutrient rich streams to identify specific sources of phosphate that are causing eutrophic conditions in such systems. In oligotrophic phosphate limited rivers and streams, a number of other factors may confound the $\delta^{18}O_p$. Use of phosphate oxygen isotopic spikes may help understand phosphate uptake in P-limited streams. More research must be conducted before the $\delta^{18}O_p$ stable isotope tracer can be fully utilized in such environments.

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