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Evaluating the Stable Isotopic Composition of Phosphate Oxygen as a Tracer of Phosphorus from Waste Water Treatment Works

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Abstract

Eutrophication is a globally significant challenge facing freshwater ecosystems and is closely associated with anthropogenic enrichment of phosphorus (P) in the aquatic environment. Phosphorus inputs to rivers are usually dominated by diffuse sources related to farming activities and point sources such as waste water treatment works (WwTW). The limited availability of inherent labels for different P sources has constrained understanding of these triggers for eutrophication in natural systems. There have been substantial recent advances in the use of phosphate oxygen isotopes ($\delta^{18}O_{PO4}$) as a way of understanding phosphate sources and processing. Results from all previous studies of the $\delta^{18}O_{PO4}$ composition of WwTW effluent and septic tanks are combined together with significant new data from the UK to assess $\delta^{18}O_{PO4}$ compositions in waste water sources. The overall average $\delta^{18}O_{PO4}$ value is 13.9‰, ranging from 8.4 to 19.7‰. Values measured in the USA are much lower than those measured in Europe. A strong positive correlation exists between $\delta^{18}O_{PO4}$ and $\delta^{18}O_{H2O}$, suggesting biologically-mediated exchange between the water molecules and the phosphate ions. A comparison of $\delta^{18}O_{PO4}$ and the offset from isotopic equilibrium showed a strong positive linear correlation (ρ = 0.94) for the data from Europe but no relationship for the historic USA data which may be due to recent advances in the extraction procedure or to a relative paucity of data. This offset is most strongly controlled by the $\delta^{18}O_{H2O}$ rather than temperature, with greater offsets occurring with lower $\delta^{18}O_{H2O}$. Time series data collected over 8-24 hours for three sites showed that, although there were significant changes in the phosphate concentration, for a given WwTW the $\delta^{18}O_{PO4}$ stayed relatively constant. Two new studies that considered instream processing of $\delta^{18}O_{PO4}$ downstream of WwTWs showed mixing of the upstream source with effluent water but no evidence of biological cycling 3 km downstream. It is suggested that $\delta^{18}O_{PO4}$ can be an effective tool to trace P from WwTWs provided the source of the effluent is known and samples are collected within a day.

Keywords: Eutrophication; Phosphate oxygen isotopes; biogeochemical cycling; Waste water; Rivers

1. INTRODUCTION

Phosphorus (P) is the rate-limiting factor for microbial and primary producer communities in many freshwater systems including rivers and streams (Smith, 1984). It is well established that high human population densities and intensive agriculture can lead to the oversupply of P to freshwaters, resulting in eutrophication and a variety of other problems for the environment and human society (Schindler, 2012). Phosphorus inputs to rivers in agricultural catchments are usually dominated by diffuse sources related to various farming activities (Carpenter et al., 1998), but in more heavily populated catchments, point sources such as waste water treatment works (WwTWs) are of great importance (Howarth et al., 1996; Jordan et al., 1997; Jarvie et al., 2006; Palmer-Felgate et al., 2010). One response to eutrophication has been the introduction of P consents on WwTWs leading to capital and operating investment in P removal through tertiary treatment (Pretty et al., 2003) and other technologies. The treatment of waste water is a billion pound industry in the UK (Bailey, 2003) and some of these costs are directed towards the removal of an estimated 44,000 tonnes of P entering its WwTWs annually (Comber et al., 2013).

Whilst there is evidence that P removal at WwTW can significantly reduce the concentration of P in receiving waters (Kinniburgh and Barnett, 2010), there remains uncertainty over whether changes in WwTW-derived P loads directly control the composition or functioning of river biota (Neal et al., 2006). This is important since legislative drivers (e.g Water Framework Directive, 2000) for future investment in P removal at WwTWs go beyond assessments solely of river chemical status to also consider the biological status of receiving waters. Uncertainty regarding the relationship between WwTW-derived P and river biological status can be explained by four key factors:

i) Despite P-removal through tertiary treatment at specific WwTWs, elevated P concentrations may continue to be driven by the cumulative effect of upstream point sources (Jarvie et al., 2006);

ii) Diffuse sources of P may be sufficiently large that elevated P concentrations are maintained despite the investment in P removal at WwTWs (Gooddy et al, 2017);

iii) The structure and functioning of river biota may only be indirectly controlled by WwTW-derived P, mediated by additional physical chemical or biological processes within a river (Hilton et al., 2006).

iv) Despite huge reductions in P concentration, the P concentrations are still in excess for algal growth, and so aquatic ecology is unaffected by these P reductions (Bowes et al., 2012)

These issues reflect limitations in understanding the contribution of different sources, the degree of biogeochemical cycling and biological impact of P within the environment. Traditional approaches to quantifying the importance of different sources of P include those based on in-river assessments, usually mass flux budgets or spatial and temporal analysis of P concentration (Bowes et al., 2014), those based on export coefficient approaches (Johnes et al., 1996), and those based on indirect tracers of source such as boron (Vengosh et al., 1994). However, none of these approaches provides an inherent label for specific sources of P. As a result, none offers a direct means of tracing sources and in-river transformations of P, and none can be used to assess cause-effect relationships between P sources and the response of river biota.

The past few years have seen substantial advances in the use of phosphate $^{18}O/^{16}O$ analysis ($\delta^{18}O_{PO4}$) as a way of identifying sources of phosphate pollution and examining its biological and abiological processing (Young et al., 2009; Jaisi et al., 2010; McLaughlin et al., 2013; Gooddy et al., 2015; Gooddy et al., 2016; Granger et al., 2017). The basis to the use of $\delta^{18}O_{PO4}$ in aquatic ecosystems has recently been reviewed by Davies et al. (2014).

Briefly, because the P-O bonds in inorganic phosphate (P_i) are resistant to inorganic hydrolysis under typical temperature, pressure and pH conditions in the Earth's surface water and groundwater ecosystems (O'Neil et al., 2003). Under these conditions, $\delta^{18}O_{PO4}$ may reflect the isotope composition of P sources within an ecosystem. In contrast, enzyme-catalysed reactions cleave P-O bonds leading to exchange between the isotopes of O in P_i and O in a surrounding fluid, either within a cell or within the extracellular environment (Blake et al., 2005). Intracellular metabolism of P involving the inorganic pyrophosphatase enzyme results in rapid, temperature-dependent equilibrium fractionation between O in P_i and O within the intracellular fluid. The latter is expected to be identical in O-isotope composition to water-O in the extracellular environment. Given sufficient intracellular-extracellular exchange of P_i to maintain non-lethal intracellular P_i concentrations, a temperature-dependent equilibrium will be established between $\delta^{18}O_{PO4}$ and water-O in the extracellular environment. Negligible O isotope exchange occurs between P_i and water within ecosystems without biological mediation (Tudge, 1960; Blake et al., 1997). The equilibrium oxygen isotope fractionation between dissolved P_i and water ($\alpha_{PO4\text{-}H2O}$) at surface temperatures has recently been determined (Chang and Blake, 2015), using laboratory solutions catalyzed by the inorganic pyrophosphatase enzyme. These authors derived the equation:

$$10^3 ln\alpha_{PO4-H2O} = 14.43 \times (10^3/T) - 26.54 \qquad (1)$$

where T is in degrees Kelvin. Since:

$$\alpha_{PO4-H2O} = (\delta^{18}O_{PO4} + 1000)/(\delta^{18}O_{H2O} + 1000) \qquad (2)$$

by combining 1 and 2 above, expected equilibrium $\delta^{18}O_{PO4}$ values may be calculated from:

$$\delta^{18}O_{PO4} = \left(\delta^{18}O_{H2O} + 1000\right) \times e^{[14.43\times(10^3/T)-26.54]/1000} - 1000 \qquad (3)$$

In this paper the currently limited existing global literature for WwTW final effluent source values is synthesized and augmented with additional data collected as part of this study. This larger data set is then evaluated to provide greater understanding of the major factors controlling on $\delta^{18}O_{PO4}$ values in waste water relative to equilibrium calculations in order to determine whether there has been any biological processing of P_i. Seasonal and daily variation in the source term is assessed by combining published data with new data collected at a sub-daily time interval from WwTW's. Instream transects, up to 3 km downstream of a WwTW, are compared to equilibrium values to determine whether the source term is preserved or processed rapidly by in-stream microbial cycling. Lastly we summarise the current state of $\delta^{18}O_{PO4}$ knowledge with respect to WwTW effluents and highlight key areas of future work.

2. METHODS

2.1 Spot Sampling of WwTW

Effluent samples were collected at the final outfall from 16 sites spanning a range of WwTW person equivalents, as well as those which employ a range of secondary, tertiary and other treatment technologies (Table 1). Sufficient sample (1- 10 L) was collected to enable the generation of ~1 mg of silver phosphate (Ag_3PO_4) which is required for isotopic analysis. Samples were filtered on site using high volume 0.45µm filter cartridges and water temperature was measured immediately following collection. A 5mL sub-sample from each site was also taken for analysis of $\delta^{18}O_{H2O}$.

2.2 Sub-Daily Sampling of WwTW

The composition of WwTW effluent may vary significantly over a daily cycle reflecting both the nature and volume of waste water that is treated. To understand better this variation and the impact it may have on the P_i concentration and $\delta^{18}O_{PO4}$, studies were undertaken on the final effluent of three different WwTW. All of the treatment works studied were in relatively rural settings and so it is assumed there were minimal industrial effluents entering the WwTW and indeed the influent was relatively similar between the three, being situated within 10km^2 of each other in Oxfordshire, UK. By contrast, each of the sites had different treatment processes (See Table 1, sites 14, 15 and 16).

At site 14 an automated sampler was used to collect 400mL samples at hourly intervals starting at 9am and finishing at 6am the following day. These were then bulked into 3 hour blocks to provide sufficient mass for extraction and analysis of P_i. A data logger was also used at this site to provide a continuous measure of the effluent temperature. At site 15, 8 samples were collected manually every hour from 9am until 4pm. At site 16, difficulties with filtration meant that sufficient sample volumes were only obtained for three samples collected at 9am, 1pm and 4pm.

2.3 Sampling Upstream and Downstream Transects

Two WwTW (sites 1 and 18) and their associated river systems were selected to evaluate whether downstream changes in $\delta^{18}O_{PO4}$ occurred, and if so, to provide insight into the fate of WwTW-derived P within the river. Samples were collected in September over a period of an hour from the thalweg of the river at one sample point upstream of the effluent outfall and at several points downstream, up to 3 km at site 1 and 0.75 km at site 18. The transect at site 18 was much shorter due to the intersection of another tributary 0.8 km downstream of the WwTW. Stream samples were processed in the same manner as the WwTW detailed above.

2.4 Sample preparation and isotope analysis

The method used to isolate P_i from water samples and precipitate Ag_3PO_4 for isotope analysis has already been presented in Gooddy et al. (2016) and is described in detail in Lapworth et al. (2014). Samples were processed within 24 h of collection and were stored in the dark at 4 °C prior to processing. In brief, the majority of dissolved organic matter is first removed by passing the sample through an organic exchange resin and P_i is then isolated from the remaining matrix using an anion exchange resin. Phosphate is eluted from the anion exchange resin and chromatographically separated from competing anions using 0.3 M KCl. Eluted fractions containing phosphate are then processed using a modified McLaughlin et al. (2004) method to produce a final Ag_3PO_4 precipitate for $\delta^{18}O_{PO4}$ analysis. Any residual organic matter is removed by treating the Ag_3PO_4 with a solution of 15% hydrogen peroxide prior to analysis. Combined with the organic exchange resin, this is a significant advance over methods that use a repeated $CePO_4$ precipitation (e.g. Li et al., 2011).

$^{18}O/^{16}O$ ratios of Ag_3PO_4 were analysed by thermal conversion to CO gas at 1400 °C in a TC–EA on-line to a Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany). $\delta^{18}O$ values versus Vienna Standard Mean Ocean Water (VSMOW) were calculated by comparison with an internally run laboratory standard (Alfa Aesar silver phosphate 99%). In the absence of an international Ag_3PO_4 reference material, we derived the $\delta^{18}O$ value of the laboratory standard by comparison with the Ag_3PO_4 standard 'B2207' (supplied by Elemental Microanalysis Ltd, Okehampton, England), measured in an inter-laboratory comparison to have a certified $\delta^{18}O$ value of +21.7‰ versus VSMOW. CO yields of the Ag_3PO_4 samples were always within ±10% of those of the laboratory standard, and any organic contamination was deemed negligible on the basis that samples contained <0.2% carbon (based on separate elemental analysis). Precision on the measurement are consistently <0.2‰ and all less than 0.3‰. On this basis, we consider any difference of >0.3‰ as a reasonable indicator that any two samples have a different isotopic composition for a reason other than analytical error.

3. RESULTS AND DISCUSSION

3.1 Comparison of Global Data

A compilation of inorganic phosphate and $\delta^{18}O_{PO4}$ values for all currently published data for effluent from WwTW is presented in Table 2. Previous studies have been undertaken in France, Sweden, the UK and the USA. The manuscript reports additional data from 16 new sites all from the UK. The data contained within Table 2 is a mixture of spot samples taken once, seasonal samples taken during spring, summer, autumn and winter, and a mean of time series data taken over the period of a day. Four Swedish sites were from septic tank outflows and included in this study as they are considered to be comparable as sources of human waste water. All other samples were from WwTW outflow effluents. Most of these effluents underwent primary and secondary treatments but only a few underwent any form of tertiary P treatment, where the intention is to achieve PO_4 concentrations in the final effluent <1 mg/L. Phosphate concentrations in the effluent ranged from 0.16 to 25.2 mg PO_4/L, with a mean concentration of 9.5 mg PO_4 /L (σ 7.0, median 9.0 mg PO_4 /L).

These WwTWs serve between ~500-40,000 person equivalents (PE), and these data show no relationship between PE and PO_4 concentration or $\delta^{18}O_{PO4}$. Limited data are available for levels of effluent treatment in published studies, although from the new work presented here there appears to be no clear relationship between $\delta^{18}O_{PO4}$ and secondary or tertiary treatment processes.

For all the data, the average $\delta^{18}O_{PO4}$ value was 13.9‰ (σ 3.2‰, median 14.2‰), and a range of 8.4-19.7‰. Figure 1 shows box-plots of the measured $\delta^{18}O_{PO4}$ grouped by country. There do appear to be some clear geographical differences, particularly between measured values in the USA and those in Europe, although the sample size is still relatively small. Based on our calculations equilibrium values ranged from 3.4 to 20.0‰ with a mean of 15.8‰ (σ 4.2‰, median 17.3‰) (Table 2). Where temperature and $\delta^{18}O_{H2O}$ were available and calculations could be made, 78% of samples had measured values lower than the equilibrium value. All measured values from the USA were higher than the equilibrium calculated values. This may reflect higher $\delta^{18}O_{H2O}$ values (values ~-2‰, McLaughlin et al., 2006) as well as higher temperatures and greater evaporation of water during the treatment process (Young et al., 2009).

If no microbial cycling of P_i occurs during the treatment process then the isotopic composition of the outflow effluent should simply reflect the mixture of $\delta^{18}O_{PO4}$ values of P_i sources received from wastewater network. Pyrophosphatase mediated intracellular microbial cycling will shift the $\delta^{18}O_{PO4}$ towards the calculated isotopic equilibrium which will be determined by the $\delta^{18}O_{H2O}$ and temperature of the water in the WwTW. Figure 2 shows the positive correlation ($\rho = 0.72$) between $\delta^{18}O_{PO4}$ and $\delta^{18}O_{H2O}$, suggesting that there is biologically-mediated exchange occurring between the water molecules and the PO_4 ions. This is consistent with the use of secondary biological treatment at each of the WwTWs presented, in which the transformation of organic P compounds to inorganic P through enzyme hydrolysis, alongside the uptake and intracellular cycling of P, results in exchange of oxygen atoms between water and PO_4. However, in all cases $\delta^{18}O_{PO4}$ is not at the expected pyrophosphatase equilibrium value which indicates incomplete intracellular cycling, and so is consistent with the high concentrations of P in WwTW effluents.

3.2 Seasonal and Temperature Influences

Microbial reactions are commonly controlled by temperature with greater reaction rates occurring at higher temperatures. For biological processing of $\delta^{18}O_{PO4}$ it might be expected therefore that higher temperatures are associated with $\delta^{18}O_{PO4}$ values closer to equilibrium. Similarly therefore, for a given site, samples taken in the summer may be closer to equilibrium than those taken in winter.

Samples 18 to 21 (Table 2) have all been sampled in at least two seasons and so provide a comparison to test the hypothesis that there is a relationship between P processing and season/temperature. Site 18 (WwTP in the River Beult catchment, UK) showed consistent $\delta^{18}O_{PO4}$ values of around 16.2 ‰ from January 2014 to September 2015 although is ~1.5‰ lower in September 2013. Between September 2013 and March 2015 the final effluent remained a fairly constant -1.2‰ away from equilibrium, but this fell to -0.6‰ in September 2015. Samples from WwTW sites 19 and 20 (in the River Taw catchment, UK) sampled at the same time showed markedly different values between the WwTW of around 3‰. In terms of seasonal variation, site 19 was furthest from equilibrium in June but closer to calculated equilibrium in December. By contrast, site 20 was furthest from equilibrium in December but closer to calculated equilibrium in September. Samples taken from site 21 (California Bay, USA) showed a difference of ~3‰ between sampling in October and January, although the sample taken in January was closer to the calculated equilibrium value by more than 1‰.

In all of the cases from the UK there was some ~10°C variation in the temperatures observed during sampling (site 18 a range of 8.8 to 20.3°C; site 19 a range of 9.5 to 18.0°C; and site 20 a range of 10.5 to 19.8 °C). For $\delta^{18}O_{H2O,}$ seasonal differences of around 0.6-0.9‰ were also observed at each of the sites (site 18 a range of -7.32 to -6.84‰; site 19 a range of -5.57 to -6.35‰; and site 20 a range of -5.62 to -6.31‰).

As temperature and $\delta^{18}O_{H2O}$ are the two variables in the equilibrium calculation, a comparison was been made across the full data set to see if any relationship can be discerned between measured and equilibrium $\delta^{18}O_{PO4}$ values. Figure 3 shows the relationships between measured $\delta^{18}O_{PO4}$ and the offset from isotopic equilibrium of $\delta^{18}O_{PO4}$ in terms of a) sample temperature and b) sample $\delta^{18}O_{H2O}$. For data from Europe the goodness of fit for the difference between measured and offset from equilibrium calculated $\delta^{18}O_{PO4}$ is very strong ($\rho = 0.94$) suggesting there is a relationship between the source term and the equilibrium. Data from the US however does not seem to show any clear relationship ($\rho = 0.41$) which possibly reflects the use of an older analytical method for P_i extraction that is less effective at removing organic carbon (see McLaughlin et al., 2006 and Gooddy et al., 2015).

For the samples from Europe, Figure 3a shows no convincing trend for a relationship between sample temperature and the distance from equilibrium i.e. temperature of the effluent water alone is not a good indicator that the $\delta^{18}O_{PO4}$ will be closer or further from equilibrium. Figure 3b however does show some quite strong clustering of the data, whereby samples with the more negative $\delta^{18}O_{H2O}$ are further from equilibrium while the samples with more positive $\delta^{18}O_{H2O}$ fall closer to equilibrium. This implies that, for this limited data set, $\delta^{18}O_{H2O}$ is a better predictor of how close the measured $\delta^{18}O_{PO4}$ will be to the calculated equilibrium. Since rainfall values of $\delta^{18}O_{H2O}$ are quite predictable due to weather patterns and orography (Darling et al. 2003) this suggests the geographical location of the WwTW could determine how close the measured $\delta^{18}O_{PO4}$ is to the equilibrium value.

3.3 Diurnal changes in phosphate, $\delta^{18}O_{PO4}$ and $E\delta^{18}O_{PO4}$

Figure 4 shows time series data for three WwTWs from SE England. As reflected by the varying degrees of treatment, and the presence or absence of chemical PO_4 stripping (Table 1), concentrations varied from 2.35 mg PO_4 /L at site 14, to 0.35 mg PO_4 /L at Site 15 and 0.15 mg PO_4 /L at Site 16. Each of the WwTWs showed a significant change in P_i concentrations over the sampling interval. Over 24 hours at site 14 there was a 20% increase in P_i, at site 15 there is a 25% decrease over 7 hours and at site 16 there was a 12% decrease also over 7 hours.

In contrast to the P_i concentration, $\delta^{18}O_{PO4}$ values stayed relative constant over the sampling intervals. For site 14 the mean $\delta^{18}O_{PO4}$ was 14.5‰ (σ 0.3 ‰), at site 15 the mean was 16.5‰ (σ 0.2 ‰), and at site 16 the mean was 11.5‰ (σ 0.3 ‰). Similarly, for equilibrium values at site 14 the mean $\delta^{18}O_{PO4}$ was 16.9‰ (σ 0.05 ‰), at site 15 the mean was 16.5‰ (σ 0.1 ‰), and at site 16 the mean was 17.3‰ (σ 0.05 ‰).

One implication of this finding is that the treatment type appears to have an impact on the $\delta^{18}O_{PO4}$ value, for example where chemical PO_4 stripping was employed (Site 16) $\delta^{18}O_{PO4}$ was much lower. If this was sorption of P to $FeCl_2$ or Al_2SO_4, higher $\delta^{18}O_{PO4}$ values would be expected in this system as the isotopically lighter $P^{16}O_4$ is preferentially incorporated into sorbed/solid phases (Jaisi et al., 2010). Clearly the kinetics of the reaction are important and require further investigation to fully understand. However, it is also worth noting that for the UK sites in Table 1 there was no clear relationship between treatment type and $\delta^{18}O_{PO4}$ values, i.e. with chemical PO_4 stripping, lower $\delta^{18}O_{PO4}$ values were not necessarily observed. This strongly suggests that the P outflow from a WwTW is highly dependent on the treatment technologies and conditions at the plant, although over a relatively short period, the $\delta^{18}O_{PO4}$ values remain constant even though the PO_4 concentrations change. This is in contrast to the seasonal data where changes are seen, possibly due to a change in dominant P source throughout the year. In terms of understanding WwTW as a source this is a positive finding as it suggests over a normal daily sampling interval there is unlikely to be a significant change in the $\delta^{18}O_{PO4}$ value. The data suggest that WwTW effluent needs to be characterised locally for any particular study (i.e. a global/regional mean cannot be assumed) and that at least seasonal temporal characterisation is required.

3.4 Instream Processing of WwTW Source

Having established that over an 8-24 hour period that the $\delta^{18}O_{PO4}$ value of final effluent from a WwTW does not vary significantly, additional work was undertaken to determine whether or not this source term is preserved downstream of the effluent input to the river. Figure 5 shows 2 transects taken from different sites, including samples taken upstream and downstream of WwTWs. In both cases it can be observed that there is a significant spike in P_i concentrations resulting from the input of effluent from the WwTW although it is also important to note that both streams have significant upstream concentrations of P_i (~0.4-0.5 mg PO_4/L) suggesting the river is not P limited.

At site 1, P_i had an upstream concentration of 0.38 mg PO_4/L. Effluent from the WwTW entered at 7.65 mg PO_4/L and this then decreased to 0.59 ± 0.04 mg PO_4/L in the downstream reach. There was clearly significant dilution of the P_i emanating for the WwTW by the upstream river water. The calculated equilibrium value 18.4‰ changes very little from upstream to downstream. The Effluent water had a higher temperature (~2.5°C) and a higher $\delta^{18}O_{H2O}$ (0.3‰) which when combined produced a similar equilibrium value to the rest of the stream. Upstream, $\delta^{18}O_{PO4}$ was 14.0‰ (-4.4‰ from equilibrium) which decreased to 11.1‰ (-7.2‰ from equilibrium) when the effluent water entered the stream. As the effluent moved downstream the $\delta^{18}O_{PO4}$ value increased slightly to an average of 12.3 ± 0.3‰ (-6.2 ± 0.3‰ from equilibrium) but with no observed shift towards equilibrium 3 km downstream of the WwTW.

At site 18, P_i had an upstream concentration of 0.47 mg PO_4/L. Effluent from the WwTW entered at 1.80 mg PO_4/L and this then stayed fairly constant at to 1.77 ± 0.01 mg PO_4/L in the downstream reach. The small decrease in concentration reflected the large volume of the WwTW effluent relative to the upstream flow. The calculated equilibrium value for upstream was 16.5‰. The calculated equilibrium value for the effluent was 16.8‰ and further downstream this increased slightly to 17.0‰ The effluent water had a lower temperature (~2.9°C) and a higher $\delta^{18}O_{H2O}$ (0.2‰). The slightly higher equilibrium value further downstream resulted from a lower water temperature. Upstream, $\delta^{18}O_{PO4}$

was 17.0‰ (+0.6‰ from equilibrium) which decreased to 16.1‰ (-0.6‰ from equilibrium) when the effluent water entered the stream. As the effluent moved downstream the $\delta^{18}O_{PO4}$ value increased very slightly to an average of 16.2 ± 0.1‰ (-0.6 ± 0.1‰ from equilibrium) but with no shift towards equilibrium 0.75 km downstream of the WwTW.

In both cases river water $\delta^{18}O_{PO4}$ samples were not at equilibrium. The $\delta^{18}O_{PO4}$ values upstream also strongly suggested there is a different source of P_i to the WwTW at the point of measurement. Importantly, downstream of the WwTWs there was no significant shift towards the equilibrium value. This indicates that there was no rapid or significant microbial processing of the P and the source term, allowing for mechanical mixing, was therefore retained. This enabled tracing of the effluent down to distances of 3 km, and quite possibly further depending on the river network pattern and flow, microbial activity, as well as downstream inputs of additional sources of P. These data are consistent with the conclusion that the concentration of P_i upstream of the WwTWs was already in excess of metabolic requirements, meaning that WwTW-derived P was not closely coupled to the metabolism of in-river biota. However, recent experimental work (Chang et al., 2015) suggests microbial reaction $t_{1/2}$ times are of the order of several hours depending on temperature which would be equivalent to more than 10 km downstream from a WwTW discharging into a moderately flowing river. Hence little or no metabolism of P would be observed.

4 CONCLUSIONS

This study has shown there can be considerable variability between the $\delta^{18}O_{PO4}$ values in the effluent of different WwTWs. This does not appear to be related to treatment type or population equivalents. There are also significant differences in $\delta^{18}O_{PO4}$ observed for a given WwTW between seasons, although this study suggests the dominant control on difference between measured $\delta^{18}O_{PO4}$ and isotopic equilibrium is the $\delta^{18}O_{H2O}$ rather than temperature although more data is need to confirm this.

Studies examining diurnal cycles suggested that, although the P_i concentration of the effluent water changed markedly, there was no comparable variation in the $\delta^{18}O_{PO4}$ values. As such the $\delta^{18}O_{PO4}$ value from an effluent recorded in the morning could be assumed to be the same as the value recorded throughout the day. When $\delta^{18}O_{PO4}$ is monitored downstream of a WwTW, there is no evidence of processing of P up to a distance of at least 3 km and the measured value is a reflection of the upstream P source mixing with the effluent. This is due to the system having an excess of P (i.e. not P limited) but also due to the reaction times required for biotic cycling.

This study builds significantly on the earlier work of Gruau et al. (2005), who cast doubt on the use of $\delta^{18}O_{PO4}$ as a tracer as a means to differentiate between fertilisers and effluents due to the overlap in isotopic source values. Based on this study and using a much improved extraction method for Ag_3PO_4, which is better suited to processing effluents compared with the previous studies of both Gruau et al (2005) and Young et (2009), it is considered that $\delta^{18}O_{PO4}$ can be used to both trace WwTW effluents and to also understand the metabolism of WwTW-derived P in waters. For this, you simply need a difference between effluent $\delta^{18}O_{PO4}$ and river $\delta^{18}O_{PO4}$ immediately upstream. From the work presented here, it is recommended that the effluent sampling and tracing downstream are carried out on the same day due to both seasonal variations and the range of values obtained from different WwTWs.

However, based on the variations observed both between and within countries, further work is required to determine if variation in final effluent $\delta^{18}O_{PO4}$ reflects differences in sources and loads of P to individual WwTWs, and hence differences in $\delta^{18}O_{PO4}$ within influent P sources. Differences in antecedent weather conditions and hence WwTW residence time is also an important consideration that needs to be addressed to improve understanding. Lastly, further fundamental work is required to understand the isotopic fractionation that is potentially imposed by different treatments within a WwTW and whether $\delta^{18}O_{PO4}$ can be used to optimise these processes.

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