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A multi-stable isotope framework to understand eutrophication in aquatic ecosystems

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Abstract

Eutrophication is a globally significant challenge facing aquatic ecosystems, associated with human induced enrichment of these ecosystems with nitrogen (N) and phosphorus (P). However, the limited availability of inherent labels for P and N has constrained understanding of the triggers for eutrophication in natural ecosystems and appropriate targeting of management responses. This paper proposes and evaluates a new multi-stable isotope framework that offers inherent labels to track biogeochemical reactions governing both P and N in natural ecosystems. The framework couples highly novel analysis of the oxygen isotope composition of phosphate ($\delta^{18}O_{PO4}$) with dual isotope analysis of oxygen and N within nitrate ($\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$) and with stable N isotope analysis in ammonium ($\delta^{15}N_{NH4}$). The River Beult in England is used as an exemplar system for initial evaluation of this framework. Our data demonstrate the potential to use stable isotope labels to track the input and downstream fate of nutrients from point sources, on the basis of isotopic differentiation for both P and N between river water and waste water treatment work effluent (mean difference = +1.7‰ for $\delta^{18}O_{PO4}$; +15.5‰ for $\delta^{15}N_{NH4}$ (under high flow); +7.3‰ for $\delta^{18}O_{NO3}$ and +4.4‰ for $\delta^{15}N_{NO3}$). Stable isotope data reveal nutrient inputs to the river upstream of the waste water treatment works that are consistent with partially denitrified sewage or livestock sources of nitrate ($\delta^{15}N_{NO3}$ range = +11.5 to +13.1‰) and with agricultural sources of phosphate ($\delta^{18}O_{PO4}$ range = +16.6 to +19.0‰). The

importance of abiotic and metabolic processes for the in-river fate of N and P are also explored through the stable isotope framework. Microbial uptake of ammonium to meet metabolic demand for N is suggested by substantial enrichment of $\delta^{15}N_{NH4}$ (by 10.2‰ over a 100m reach) under summer low flow conditions. Whilst the concentration of both nitrate and phosphate decreased substantially along the same reach, the stable isotope composition of these ions did not vary significantly, indicating that concentration changes are likely driven by abiotic processes of dilution or sorption. The in-river stable isotope composition and the concentration of P and N were also largely constant downstream of the waste water treatment works, indicating that effluent-derived nutrients were not strongly coupled to metabolism along this in-river transect. Combined with in-situ and laboratory hydrochemical data, we believe that a multi-stable isotope framework presents a powerful approach for understanding and managing eutrophication in natural aquatic ecosystems.

1. Introduction

Perhaps the most significant challenge facing aquatic ecosystems globally is cultural eutrophication (Schindler, 2012), the process of ecosystem change triggered by human induced enrichment of ecosystems with phosphorus (P) and nitrogen (N). Given the adverse ecological, social and economic impacts associated with eutrophication (Dodds et al., 2009; Pretty et al., 2003), significant research efforts have been directed towards understanding the causes of this process and targeting mitigation strategies. In the context of aquatic ecosystems, two long-standing paradigms suggest that primary production is limited by an individual nutrient element which thereby represents the trigger for eutrophication. In freshwaters the focus has been on limitation by the availability of P (e.g. Likens, 1972; Schindler, 1977), whilst within estuarine and coastal marine ecosystems the focus has been on N limitation (e.g. Ryther and Dunstan, 1971; Howarth, 1988). However, these paradigms have been subject to growing debate, stimulated by evidence of N limitation in freshwaters (e.g. Mischler et al., 2014; James et al., 2003), N/P co-limitation in freshwaters (e.g. Xu et al., 2010; Conley et al., 2009), or P limitation in marine/estuarine waters (e.g. Blomqvist et al, 2004).

This debate reflects uncertainty regarding a number of the fundamental questions that surround eutrophication and appropriate responses to eutrophication (Smith and Schindler, 2009). An important source of this uncertainty is reliance on bioassays and mesocosms as the experimental basis for understanding nutrient limitation and eutrophication in aquatic ecosystems. These experimental approaches may not accurately reflect the large-scale, long-term processes that govern eutrophication in natural ecosystems, resulting in a bias towards identification of proximate rather than ultimate limiting nutrients (Vitousek et al., 2010) and data that do not scale successfully to natural ecosystems (Schindler, 2012). Past reliance on bioassays and mesocosms partly reflects the lack of inherent tracers that can be used to understand the sources and the reaction pathways which control P and N biogeochemistry in natural ecosystems (Karl, 2000). In this paper, we propose and evaluate a new multi-stable isotope framework that offers inherent tracers for N and P within aquatic ecosystems. Whilst multi-stable isotope approaches are increasingly used in other research fields, for example employing the stable isotopes of nitrate and sulphate in combination (e.g. Mayer, 2005; Kaown et al., 2009; Urresti-Estalaa et al., 2015) or combining stable isotope analyses in boron and nitrate (e.g. Briand et al., 2013), similar frameworks are yet to be developed in the context of P and N biogeochemistry within aquatic ecosystems.

Dual-isotope approaches for nitrate ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) and stable ammonium isotope analyses ($\delta^{15}N_{NH4}$) have been used to understand sources and reaction mechanisms for these ions in both groundwater and surface water (e.g. Böttcher et al., 1990; Böhlke and Denver 1995; Wassenaar 1995; Kendall 1998; Silva et al., 2000; Heaton et al., 2012; Gooddy et al., 2014). Biogeochemical cycling of P in aquatic ecosystems has previously been examined using the radioactive isotopes ^{32}P and ^{33}P (e.g. Benitez-Nelson, 2000; Benitez-Nelson and Karl, 2002). However, the use of radioisotopes is constrained by short isotope half-lives, perturbation of experimental systems associated with labelling, or the use of incubations which omit irregular events in natural ecosystems, such as seasonal algal blooms (Levine et al., 1986; Thingstad et al., 1993; Benitez-Nelson, 2000). Stable isotope analyses cannot be conducted on the P atom in P-containing compounds, because ^{31}P is the only stable P isotope. However, because P is often bound strongly to oxygen (O) in the dissolved inorganic

phosphate ion (Blake et al., 1997), hereafter P_i, attention has recently focussed on whether the stable isotope composition of O in P_i ($\delta^{18}O_{PO4}$) can provide new insights into sources and biogeochemical cycling of P in the environment (e.g. Young et al., 2009; Tamburini et al., 2014; Gooddy et al., 2015). 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 P_i are resistant to inorganic hydrolysis under typical temperature and pressure conditions in the Earth's surface water and groundwater ecosystems (O'Neil et al., 2003), negligible O isotope exchange occurs between P_i and water within these ecosystems without biological mediation (Tudge, 1960; Blake et al., 1997). Under such abiotic conditions, $\delta^{18}O_{PO4}$ may therefore reflect the isotope composition of P sources to an ecosystem. In contrast, enzyme-catalysed reactions cleave P-O bonds leading to fractionation 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 to maintain non-lethal intracellular P concentrations, a temperature-dependent equilibrium will be established between $\delta^{18}O_{PO4}$ and water-O in the extracellular environment. The equilibrium oxygen isotope fractionation between dissolved inorganic phosphate 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:

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

where T is in degrees Kelvin. Since:

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

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

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

However, only limited research has explored the use of $\delta^{18}O_{PO4}$ in aquatic ecosystems, particularly within freshwater ecosystems. We are not aware of any research to date that has evaluated whether a multi-stable isotope approach has the potential to provide new insights into the controls on P and N biogeochemistry within natural ecosystems. Therefore, the objectives of our research were to: i) develop and apply a multi-stable isotope approach for N and P in freshwater ecosystems; and ii) evaluate the insights into the sources and reaction mechanisms controlling P and N biogeochemistry in freshwater ecosystems that can be provided through a multi-stable isotope approach.

2. Materials and methods

2.1 Study area

The River Beult which rises near Ashford in Kent, UK was used as an exemplar system to evaluate the multi-stable isotope framework. The Beult is the largest tributary of the River Medway and the only riverine Site of Special Scientific Interest (SSSI) in the county. Landuse within the Beult catchment is predominantly rural, with scattered settlements and an urban land coverage of <1% of the total catchment area. The catchment is predominantly underlain by a thick clay formation (Weald Clay), largely excluding exchange between groundwater and river water. However, there is evidence that some groundwater discharge to surface waters may occur in the catchment, either where small areas of limestone outcrop or where the Weald Clay is discontinuous (Lapworth et al., 2009). Elevated concentrations of P are found widely within the catchment. For example in a survey conducted in 2008, 75% of surface waters were found to exceed 100 µg PO_4-P/L (Lapworth et al., 2013). Elevated P concentrations place the SSSI in an "unfavourable condition" and exceed target water quality standards under the European Water Framework Directive (WFD, 2000). Elevated

133 nitrate (NO_3) concentrations are also of concern, with many sites exceeding 30 mg NO_3/L and
134 therefore exceeding the surface water drinking directive limit of 25 mg NO_3/L (Council Directive
135 75/440/EEC) and the mean annual concentration for the European Environment Agency's river basin
136 district (RBD) classification (Class 5 for the study RBD). Given the predominant landuse within the
137 catchment, agricultural sources coupled with effluent from rural waste water treatment works
138 (WwTWs) are hypothesised to dominate N and P loads delivered to surface waters in the Beult
139 catchment (Lapworth et al. 2013). However, the roles of these nutrient sources in controlling
140 productivity and eutrophication risk in the catchment remain uncertain, as they do within many
141 aquatic ecosystems globally.

142 **2.2 Sites and sampling**

143 A c.200m reach along the River Beult to the south east of the town of Sutton Valence was sampled
144 during this research (Fig. 1). A total of seven sampling sites (SV1-SV7) were established along an in-
145 river transect that ran both upstream and downstream of Sutton Valence WwTW (Table 1). Samples
146 were collected from these sites twice in a six month period, to provide a seasonal contrast between
147 low flow (September 2013) and high flow (January 2014) conditions. River water samples were
148 collected from the centre of the flowing water course at each site using a submersible pump, ensuring
149 that the inlet of the pump did not disturb river bed sediments during sampling. On-site parameters
150 (dissolved oxygen (DO), pH, temperature and specific electrical conductance (SEC)) were measured
151 and, where appropriate, were allowed to stabilise prior to sampling. DO, SEC and pH were measured
152 in a flow-through cell to obtain representative field values. Samples for analysis of chloride, N
153 species, soluble reactive P (SRP) and total dissolved P (TDP) were 0.45 µm filtered in the field and
154 collected in 30 mL plastic bottles. Samples for total P (TP) were not filtered and also collected in 30
155 mL plastic bottles. All samples for isotope analysis were also filtered in the field at 0.45 µm using
156 high capacity filters. Samples for $\delta^{15}N_{NO3}$, $\delta^{15}N_{NH4}$ and $\delta^{18}O_{NO3}$ determination were filtered into 1 L
157 plastic bottles; the samples for $\delta^{15}N_{NH4}$ determination were acidified in the field with concentrated
158 HCl to pH 2-4. Samples for $\delta^{18}O_{PO4}$ determination were filtered into 10 L plastic bottles. Samples for

159 water-oxygen isotope analysis ($\delta^{18}O_{H2O}$) were collected in 10 mL glass bottles with rubber sealing
160 caps.

161 ***[Fig. 1. Location of the Beult catchment in England, UK (a) and schematic map of sample***
162 ***locations along a section of the river (b)]***

163 **2.3 Hydrochemical analyses**

164 Soluble reactive P concentration, a measure of the inorganic monomeric and easily-hydrolysable P in
165 a sample, was determined colorimetrically using the method of Murphy and Riley (1962) as modified
166 by Neal et al. (2000). Total phosphorus concentration, the combination of TDP and particulate P
167 concentrations, was determined by the method of Eisenreich et al. (1975) on unfiltered samples,
168 whilst TDP concentration was determined using the same method but on filtered aliquots. Samples
169 were analysed for the concentrations of Cl, NO_3 and nitrite (NO_2) using ion chromatography (IC), and
170 for ammonium (NH_4) concentration by flow colorimetery.

171 **2.4 Sample preparation for isotope analysis**

172 Nitrate was separated from the sample matrix using anion exchange resins and prepared as silver
173 nitrate using a method based on Chang et al. (1999). Ammonium was converted to ammonium
174 sulphate on acidified quartz filter papers using a static ammonia diffusion technique (adapted from
175 Sigman et al., 1997).

176 We developed and applied a new method to isolate P_i from water samples and precipitate silver
177 phosphate (Ag_3PO_4) for isotope analysis, shown in Fig. 2 and described in detail in Lapworth et al.
178 (2014). Samples were processed within 24 h of collection and were stored in the dark at 4 °C prior to
179 processing. In brief, the majority of dissolved organic matter in a sample is first removed using an
180 organic exchange resin and P_i was then isolated from the remaining matrix using an anion exchange
181 resin. Phosphate was eluted from the anion exchange resin and chromatographically separated from
182 competing anions using 0.3 M KCl. Eluted fractions containing phosphate are then processed using a
183 modified McLaughlin et al. (2004) method to produce a final Ag_3PO_4 precipitate for $\delta^{18}O_{PO4}$ analysis.

Any residual organic matter remaining on the Ag_3PO_4 precipitate is removed by treatment with 15% hydrogen peroxide. We believe that the method shown in Fig.2 represents an advance over alternative sample preparation protocols (e.g. repeated $CePO_4$ precipitation, Li et al. (2011)), in that it successfully prevents contamination of the final Ag_3PO_4 precipitate with organic compounds (see section 2.5 below) whilst also maintaining the final Ag_3PO_4 yield. The method reported in Fig. 2 is a multi-stage process (c. 14 days in total) and was carried out in batches of eight samples.

[Fig. 2. Schematic of the modified McLaughlin et al. (2004) protocol used to process water samples for $\delta^{18}O_{PO4}$ analysis].

2.5 Mass spectrometry

The ratio $^{15}N/^{14}N$ in NH_4 and NO_3 was analysed by combustion in a Flash 1112 EA on-line to a Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany), with $\delta^{15}N$ values versus atmospheric N_2 calculated by comparison with standards IAEA N-1 and N-2 assuming these had $\delta^{15}N$ values of +0.4‰ and +20.3‰, respectively. Analytical precision (1 SD) was typically <0.8‰, from repeat analysis of a sample. $^{18}O/^{16}O$ ratios of NO_3 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), with $\delta^{18}O$ values versus VSMOW calculated by comparison with standard IAEA-NO_3 assuming it had a $\delta^{18}O$ value of +25.6‰. Analytical precision (1 SD) was typically <1.2‰.

$^{18}O/^{16}O$ 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).The $\delta^{18}O_{PO4}$ value versus VSMOW was 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), which has a certified $\delta^{18}O$ value of +21.7‰ versus VSMOW. Any organic contamination of the Ag_3PO_4 produced using the protocol in Fig 2. was deemed to be negligible, based on CO yields of the Ag_3PO_4 samples always being within ±10% of those of a laboratory Ag_3PO_4 standard, coupled with Ag_3PO_4 samples containing <0.2% carbon (based

on separate elemental analysis). Full replicates were processed through each stage of the extraction protocol reported in Fig. 2 on three occasions and on each occasion gave $\delta^{18}O$ values within a range of ±0.1‰ (see Table 2). Analytical precision (1sd) was consistently <0.2‰ and always less than 0.3‰ (Table 2). On this basis, we consider a difference in $\delta^{18}O_{PO4}$ of ≥0.3‰ to be a reasonable indicator that two samples differ in isotopic composition for reasons other than analytical error.

3. Results

3.1 Inorganic chemistry

Table 1 reports on-site and laboratory hydrochemical data for low flow (September 2013) and high flow (January 2014) sampling events. Temperature data reflect the climatic difference between seasons, with average water temperature >11 °C warmer in September 2013 compared to January 2014. Dissolved oxygen concentration at sites SV1 and SV2 was elevated under high flow compared to low flow conditions, consistent with temperature-related control on oxygen solubility. However, DO concentration was particularly low at SV1 under low flow conditions, suggesting either an input of strongly anoxic water to the River Beult at this site, or that there was significant consumption of oxygen upstream of SV1. Sutton Valence WwTW (SV4) delivered effluent with elevated Cl concentrations to the River Beult under both high and low flow conditions. Consistent with the very low DO concentration, Cl concentrations at SV1 under low flow conditions were elevated compared to SV2 and compared to SV1 and SV2 under high flow conditions.

Concentrations of NO_3 and SRP at river sampling sites were generally elevated under low flow compared to high flow conditions, likely driven by reduced dilution of point sources given lower discharge (Jarvie et al., 2006). Under low flow conditions, the concentrations of SRP and other P fractions were particularly high at SV1 and SV2, whilst the concentration of NO_3 at SV1 was elevated compared to that at SV2. Ammonium and NO_2 concentrations were generally low at all sampling sites during both sampling events, apart from SV1 and SV2 where high NH_4 and NO_2 concentrations were observed under low flow conditions and, to a much reduced extent and for NH_4 only, under high flow conditions. Fig. 3 reports the relationship between NO_3 and Cl concentration for all sites, under both

low and high flow conditions (r=+1.00, p <0.01 and r=+0.929, p <0.01 respectively). For samples collected under low flow, a clustering of sites with high NO_3 and high Cl concentrations is revealed, associated with the WwTW effluent (SV3 and SV4) and river sites downstream of the WwTW (SV5-SV7). Sites SV1 and SV2 were characterised by relatively low NO_3 and Cl concentrations during this sampling event. In contrast, three clusters of sites are revealed under high flow conditions, with SV5-SV7 occupying an intermediate position between upstream sites (SV1 and SV2) and sites associated with the WwTW (SV3 and SV4). A similar clustering of sites and flow-dependency to the clustering is revealed in the relationships between SRP and Cl concentrations (Fig. 4), where r=-0.901, p <0.01 under low flow and r=+0.901, p<0.01 under high flow.

[Fig. 3. Nitrate concentration against chloride concentration for low and high flow sampling events.]

[Fig. 4. Soluble reactive phosphorus (SRP) concentration against chloride concentration for low and high flow sampling events (note change in scale of SRP concentration between low and flows).]

3.2 Stable isotope data

Table 2 summarises the stable isotope dataset from the River Beult. The overall range for $\delta^{15}N_{NO3}$ was +4.7 to +13.1‰, whilst for $\delta^{18}O_{NO3}$ the range was -0.8 to +8.6‰. Both $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ at SV1 and SV2 were enriched compared to all other sites and this pattern was consistent under both high and low flow conditions. Values of $\delta^{15}N_{NO3}$ for SV3 and SV4 were reduced, by 3.2-3.9 ‰, during high flow compared to low flow conditions. Upstream river site SV2 was isotopically enriched compared to the final outflow from the WwTW (SV4), both for $\delta^{15}N_{NO3}$ (by 2.9‰ under low flow conditions and 5.9‰ under high flow conditions) and for $\delta^{18}O_{NO3}$ (by 7.6‰ under low flow conditions and 7.2‰ under high flow conditions). Sites downstream of the WwTW (SV5-7) had $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values

261 that were either dominated by those of the final effluent from the WwTW (low flow conditions), or
262 were intermediate between values for SV4 and for SV1 and SV2 (high flow conditions). The
263 relationship between $\delta^{15}N_{NO3}$ and NO_3 concentration under low and high flow conditions is reported
264 in Fig. 5 (r=-0.901, p <0.1 and r=-0.901, p <0.01 respectively). A trend of decreasing $\delta^{15}N_{NO3}$ with
265 increasing NO_3 concentration was observed for both sampling events, with lowest NO_3 concentrations
266 and highest $\delta^{15}N_{NO3}$ values occurring upstream of the WwTW. Similar trends exist for $\delta^{18}O_{NO3}$,
267 although for brevity these data are not reported in a separate figure.

268 ***[Fig. 5. Nitrate-nitrogen isotope composition ($\delta^{15}N_{NO3}$) composition against nitrate concentration***
269 ***for high and low flow sampling events. Vertical bars show standard deviation on $\delta^{15}N_{NO3}$ (note***
270 ***change in scale for nitrate concentration between high and low flow events).]***

271 A range of +10.0 to +33.7‰ was observed for $\delta^{15}N_{NH4}$. Under low flow conditions, substantial
272 enrichment of $\delta^{15}N_{NH4}$ was observed between SV1 and SV2, increasing by 12.2‰ over an in-river
273 length of approximately 100 m. Unfortunately, the concentration of NH_4 at all other sites during this
274 sampling event was too low to enable analysis of $\delta^{15}N_{NH4}$. A smaller enrichment in $\delta^{15}N_{NH4}$ (3.1‰)
275 was observed between SV1 and SV2 under high flow conditions. Under high flow conditions, $\delta^{15}N_{NH4}$
276 was enriched by 15.5‰ in the WwTW outflow (SV3) compared to upstream river site SV2, although
277 insufficient $\delta^{15}N_{NH4}$ data were available to make this comparison under low flow conditions. At SV5-
278 SV7 under high flow conditions, $\delta^{15}N_{NH4}$ was intermediate between that of sites SV2 and SV3, whilst
279 both the concentration of NH_4 and $\delta^{15}N_{NH4}$ remained relatively constant in the river downstream of
280 SV4 during this sampling event.

281 The stable isotope composition of P_i varied between +15.1 and +19.0‰ across the samples. Under
282 low flow conditions, $\delta^{18}O_{PO4}$ was relatively constant between SV1 and SV2. Although the absolute
283 value of $\delta^{18}O_{PO4}$ was constant between these sites, the departure from the theoretical equilibrium value
284 changed from +0.1‰ to -1.5‰ as a result of shifts in water temperature and $\delta^{18}O_{H2O}$ between SV1
285 and SV2 and therefore in the theoretical equilibrium of $\delta^{18}O_{PO4}$ (Table 3). In contrast, under high flow
286 conditions, the absolute value of $\delta^{18}O_{PO4}$ increased by 0.4‰ from site SV1 to SV2, whilst the

theoretical equilibrium value remained unchanged. Values of $\delta^{18}O_{PO4}$ at SV2 were 1.2‰ and 2.2‰ enriched compared to the final effluent from the WwTW (SV4) under low and high flow conditions respectively. Under high flow conditions, $\delta^{18}O_{PO4}$ at sites downstream of the WwTW outflow (SV5-SV7) remained relatively constant at an average of +17.6‰ ±0.1. Under low flow conditions, there was some evidence of decreasing $\delta^{18}O_{PO4}$ with distance downstream of the WwTW, although $\delta^{18}O_{PO4}$ only decreased by 0.3‰ over the 35 m river reach and these samples remained between -0.9‰ and -1.2‰ depleted compared to the theoretical equilibrium $\delta^{18}O_{PO4}$ (Table 3). Marked differences in $\delta^{18}O_{PO4}$ were observed across all sites between low and high flow conditions, with samples taken in January 2014 an average of 2‰ higher than samples taken in September 2013. The relationship between $\delta^{18}O_{PO4}$ and SRP concentration under low and high flow conditions is shown in Fig. 6. Under low flow conditions, there was a trend of increasing $\delta^{18}O_{PO4}$ with increasing SRP concentration ($r=+0.90$, $p<0.01$), with the highest SRP concentration and $\delta^{18}O_{PO4}$ occurring at sites SV1 and SV2. In contrast, under high flow conditions the trend was reversed ($r=-0.811$, $p<0.05$ – note lower confidence interval), with lowest SRP concentration and highest $\delta^{18}O_{PO4}$ values occurring in samples from these same sites.

[Fig. 6. Stable phosphate oxygen composition ($\delta^{18}O_{PO4}$) composition against soluble reactive phosphorus (SRP) concentration for low and high flow sampling events. Vertical bars show standard deviation on $\delta^{18}O_{PO4}$ (note change in scale for SRP concentration between low and high flow)].

4. Discussion

4.1 Hydrochemical insights into controls on nutrient biogeochemistry

The temporal and spatial variation in N and P concentrations reported in Table 1 may be interpreted through a mixing relationship between effluent from the WwTW and water in the River Beult upstream of the effluent discharge point. Under high flow conditions, WwTW effluent represents an

313 end-member with elevated NO_3 and SRP concentration and is diluted on entering the river. Dilution
314 produces downstream concentrations of NO_3 and SRP in the River Beult that are intermediate
315 between the composition of the two end-members (Figs 3 and 4). Under low flow conditions, this
316 mixing pattern is repeated for NO_3 although with reduced dilution of the WwTW effluent. However,
317 for both SRP and NH_4 under low flow conditions, the final WwTW effluent effectively dilutes
318 enriched upstream river water, to such an extent that downstream river concentrations of NH_4 and
319 SRP predominantly reflect effluent quality (Jarvie et al., 2010; Macdonald et al., 1995). Dissolved
320 oxygen, Cl, NO_3, NH_4 and P concentration data suggest a particular source of nutrient-enriched water
321 influenced SV1 under low flow conditions, although these hydrochemical parameters suggest that the
322 impact of this water source appeared to be absent, or at least significantly reduced, under high flow
323 conditions.

324 Whilst the existence of the effluent and upstream end members, alongside flow-dependent variation in
325 the mixing relationship between these end-members, is revealed by on-site and laboratory
326 hydrochemical data, these data do not offer direct insights into two key questions related to
327 understanding of the eutrophication process in aquatic ecosystems. Firstly, whilst the WwTW effluent
328 appears to be an important source of N and P to the River Beult, the source of other nutrient inputs to
329 the river remain uncertain, particularly at SV1 and SV2 under low flow conditions. This reflects the
330 broader challenge of identifying the original source of nutrients, alongside the relative importance of
331 different sources, in aquatic ecosystems (Jarvie et al., 2006). Secondly, concentration data alone do
332 not provide direct insight into the biogeochemical mechanisms that govern the fate of nutrient
333 elements during downstream transport within river ecosystems. Whilst indirect methods of source
334 assessment have been developed, including the use of boron as a chemically conservative marker for
335 WwTW effluent input to rivers (e.g. Neal et al., 2000) and microbial source tracking to identify
336 human versus agricultural sources of faecal contamination (e.g. Scott et al., 2002), these methods do
337 not offer an inherent label for either P or N. As a result, they lack a direct and specific means of
338 tracing in-river transformations of these nutrients. For example, both upstream and downstream of the
339 WwTW, concentration changes may be driven by physical mixing of water sources, by abiotic

geochemical mechanisms, or by metabolic processes. Discriminating between these individual processes is important if effective responses to eutrophication in aquatic ecosystems are to be developed. For example, understanding whether nutrients derived from WwTWs are strongly coupled to in-river metabolism is critical if capital and operating expenditure on nutrient removal technology at WwTWs is to be prioritised. Therefore, the extent to which a multi-stable isotope approach can provide insight into the key questions of source and in-river fate of nutrient elements is considered below.

4.2 Differentiating sources of P and N on the basis of stable isotope composition

Stable isotope data can provide insight into the original sources of N and P that contribute to aquatic ecosystems. Stable isotope analyses indicate that $\delta^{15}N_{NO3}$ at SV1 is enriched compared to the typical composition of NO_3 derived from nitrification of NH_4 within soils (Kendall, 1998; Heaton et al., 2012). Instead, the $\delta^{15}N_{NO3}$ composition is similar to that reported for NO_3 derived from sewage or from livestock slurry or manure which has undergone partial denitrification, resulting in $\delta^{15}N_{NO3}$ between +10 and +14‰ (Anisfield et al., 2007; Kendall et al., 2007). Denitrification of these sources of N upstream of SV1 may have been responsible for the $\delta^{15}N_{NO3}$ composition at this site. However, whilst the dual isotopes of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ provide a powerful basis for differentiating atmospheric and inorganic fertiliser sources of NO_3 from each other, and from livestock or sewage sources, this dual isotope approach cannot distinguish between sewage and livestock sources, which requires the use of additional source markers such as boron isotopes (e.g. Briand et al., 2013). Enriched $\delta^{15}N_{NH4}$ at SV1 (>10‰) is also consistent with a livestock manure or slurry source that may have become isotopically enriched following volatilisation of ammonia (e.g. Widory et al., 2004). Although the existing global dataset remains relatively small, $\delta^{18}O_{PO4}$ at SV1is broadly consistent with the stable isotope composition of P derived from inorganic fertiliser or livestock excreta, sources that are characterised by $\delta^{18}O_{PO4}$ between approximately +16 and +20‰ (see Davies et al., 2014).

Under low flow conditions, Cl, DO, NO_3, NH_4 and SRP concentrations are consistent with isotopic evidence for a distinct source of N and P that influences the River Beult at SV1, likely associated with agricultural activity or unsewered households in the upstream catchment. Under high flow conditions, significant changes in these hydrochemical parameters were observed at SV1, although it is not clear whether these changes in concentration are consistent with alternative sources of N and P influencing the river under high flow compared to low flow conditions. However, stable isotope data indicate that SV1 was dominated by similar sources of N and P under both low and high flow conditions, despite substantial changes in nutrient concentration between the two sampling events. Enrichment of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$, depletion of $\delta^{15}N_{NH4}$ and enrichment of $\delta^{18}O_{PO4}$ is observed at SV1 compared to downstream river sites and compared to the effluent from the WwTW, under both high and low flow conditions. This, coupled with relatively constant $\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$, and $\delta^{15}N_{NH4}$ values at SV1 across both sampling events, suggests that a common nutrient source influenced SV1 in September 2013 and January 2014. The 1.9‰ shift in $\delta^{18}O_{PO4}$ at SV1 between low and high flow sampling events could indicate changes in the dominant source of P to the River Beult across these events. However, $\delta^{18}O_{PO4}$ was consistent with the theoretical equilibrium value on both occasions, suggesting the 1.9‰ shift resulted from changes in equilibrium fractionation driven by changes in water temperature and $\delta^{18}O_{H2O}$ between sampling events, rather than a change in P source. Fig. 7 shows this effect for all samples, by considering theoretical equilibrium values for a range of water temperatures.

[Fig. 7. A comparison of $\delta^{18}O_{PO4}$ and $\delta^{18}O_{H2O}$ for samples collected in low flow (filled symbols) and high flow (open symbols). Vertical and horizontal hashed areas represent range of measured $\delta^{18}O_{H2O}$ during low and high flows respectively. Diagonal dashed lines represent the $\delta^{18}O_{PO4}$ equilibrium values for ambient water for the range of temperatures at low and high flows calculated using the equation of Chang and Blake (2015).]

Under both high and low flow conditions, the stable isotope composition of NO_3, NH_4 and SRP in final effluent samples differed substantially from that in river water upstream of the WwTW. Differentiation of nutrient sources on the basis of their stable isotope composition is the fundamental pre-requisite for using subsequent isotope fractionation to trace metabolism of nutrients derived from

individual sources during in-river transport. Whilst previous work has examined the stable isotope composition of NO_3 and NH_4 in river water and in final effluent samples from WwTWs (e.g. Sebilo et al. 2006; Hood et al., 2014), our research represents some of the first data to demonstrate differentiation between effluent and upstream river water samples in terms of $\delta^{18}O_{PO4}$ (although see also Gruau et al., 2005; McLaughlin et al., 2006; Young et al., 2009) The 1.4‰ shift in $\delta^{18}O_{PO4}$ in WwTW effluent between low and high flow conditions may indicate differences in the composition of waste water arriving at the WwTW, water residence time, or extent of metabolism within the works, and emphasises the need for more intensive characterisation and explanation of variation in $\delta^{18}O_{PO4}$ within sources of P, such as WwTW effluent (see also Gruau et al., 2005). At SV3 under both high and low flow conditions, $\delta^{18}O_{PO4}$ remained -1.1‰ from theoretical equilibrium. This observation suggests that P is in excess of metabolic requirements within the WwTW, and is consistent with either an isotopically depleted source of SRP entering the works and passing conservatively through the treatment processes, or with kinetic isotope fractionation during the hydrolysis of organic P compounds within the WwTW that shifts $\delta^{18}O_{PO4}$ towards isotopically depleted values (Blake et al., 2005).

4.3 Stable isotope evidence for the in-river fate of N and P

Under low flow conditions, the concentration of NH_4 decreased by two orders of magnitude between SV1 and SV2, suggesting potential nitrification within the stream network. The decrease in the concentration of NO_3 between these same sites could be interpreted as evidence for in-stream denitrification or biological uptake of NO_3 occurring alongside nitrification. For example, stream bed sediments have been shown to be potentially important locations for denitrification in river ecosystems (e.g. Seitzinger, 1988). Coupling stable isotope data for NH_4 and NO_3 enables the roles of nitrification and denitrification to be explored. Substantial increases in $\delta^{15}N_{NH4}$ were observed between SV1 and SV2, consistent with nitrification and a kinetic isotope effect in which isotopically lighter NH_4 ions are preferentially nitrified, resulting in isotopic enrichment of the remaining NH_4 in

418 the extracellular environment (Middelburg and Nieuwenhuize, 2001). However, $\delta^{15}N_{NO3}$ did not
419 decrease consistently between SV1 and SV2 across both sampling events, as would be expected
420 following generation of NO_3 through nitrification (Sebilo et al., 2006). In addition, substantial
421 decreases in NO_3 concentration were observed between SV1 and SV2 under both high and low flow
422 conditions, which is not consistent with nitrification. Whilst denitrification or biological uptake of
423 NO_3 may have been responsible for decreases in NO_3 concentration, no clear evidence was observed
424 for enrichment in $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ that would be expected if denitrification or biological uptake
425 were responsible for the decrease in NO_3 concentration between SV1 and SV2 (Heaton et al., 2012).
426 Sebilo et al. (2003) demonstrated that denitrification in stream bed sediments was not associated with
427 a large fractionation of $\delta^{15}N_{NO3}$, because diffusion of NO_3 from the water column into reduced
428 sediments is the rate-limiting, but non-fractionating, step for denitrification in these environments.
429 However, given the three-fold decrease in NO_3 concentration between SV1 and SV2 under low flow
430 conditions, the fact that $\delta^{15}N_{NO3}$ actually decreased between these sites on the River Beult is not
431 consistent with denitrification exerting a significant control on the fate of N. There does not appear to
432 be strong isotopic evidence for denitrification within this upstream reach of the river.

433 Instead, stable isotope data suggest NH_4 uptake and incorporation into biomass may have been
434 responsible for the decreases in NH_4 concentration observed between SV1 and SV2. Biological
435 uptake under eutrophic conditions is associated with a kinetic isotope effect in which isotopically
436 lighter ions are preferentially taken up and incorporated into biomass, resulting in isotopic enrichment
437 of the remaining extracellular NH_4 (Cifuentes et al., 1989). Whilst ammonia volatilisation may also
438 increase $\delta^{15}N_{NH4}$ in any remaining NH_4, stream temperature and pH were relatively consistent
439 between SV1 and SV2 during both sampling events, meaning that volatilisation is unlikely to have
440 been responsible for the observed decrease in NH_4 concentration between these sites.

441 The lack of any substantial change in $\delta^{15}N_{NO3}$ or $\delta^{18}O_{NO3}$ between SV1 and SV2 suggests decreases in
442 NO_3 concentration were unlikely to be due to metabolism, but were instead caused by an abiotic
443 mechanism. Given the low affinity of NO_3 for sediment sorption sites, the decrease in NO_3
444 concentration alongside relatively constant $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ suggests dilution may have been

445 responsible for the decreases in NO_3 concentration between SV1 and SV2. The existence of an
446 additional input of water to the River Beult is also supported by an increase in $\delta^{18}O_{H2O}$ between SV1
447 and SV2, although this was only observed under low flow conditions suggesting that groundwater
448 discharge to the river may have been responsible for dilution of NO_3 between these sites. However,
449 $\delta^{18}O_{H2O}$ at SV2 under low flow conditions was outside the isotopic range for groundwater in this area
450 of the UK, which lies between +6 and +7 ‰ (Darling et al. 2003). Instead, $\delta^{18}O_{H2O}$ suggests a water
451 source characterised by partially evaporated surface water influenced the river at SV2, likely from
452 drainage systems associated with the farming activity between SV1 and SV2. Whilst dilution may
453 also have contributed to the decrease in NH_4 concentration between these sites under low flow
454 conditions, our data indicate that changes in NH_4 concentration were also associated with substantial
455 increases in $\delta^{15}N_{NH4}$. This is indicative of metabolic processes influencing the in-river fate of NH_4 but
456 not NO_3, offering insights into differences in the in-river fate of these ions as governed by their role
457 in meeting metabolic demand for N.

458 In contrast to $\delta^{15}N_{NH4}$, the value of $\delta^{18}O_{PO4}$ for SV1 and SV2 under low flow conditions did not
459 change substantially, despite a decrease of almost 50% in the concentration of SRP between these two
460 sites. Consistent with NO_3, the lack of substantial changes in stable isotope composition, coupled with
461 a substantial decrease in concentration, suggests that an abiotic rather than metabolic mechanism
462 controlled the concentration of SRP in this upstream reach of the river. Whilst dilution may also have
463 been responsible for the change in SRP concentration, adsorption of P_i to stream bed sediments can be
464 significant (Jarvie et al., 2012). Some research has suggested that the initial stages of some abiotic
465 reactions, such as sorption, are associated with kinetic isotope effects in which isotopically lighter P_i
466 ions are preferentially removed from solution (e.g. Jaisi et al., 2010). However, we observed no
467 evidence for this in $\delta^{18}O_{PO4}$ data at SV1 and SV2 under low flow conditions. Further, whilst the
468 absolute value of $\delta^{18}O_{PO4}$ did not change between SV1 and SV2, the deviation from expected
469 equilibrium $\delta^{18}O_{PO4}$ changed from 0.1 to -1.5‰ as a result of differences in water temperature and
470 $\delta^{18}O_{H2O}$ between SV1 and SV2 and therefore the theoretical equilibrium value of $\delta^{18}O_{PO4}$. The
471 increased deviation between observed and equilibrium $\delta^{18}O_{PO4}$values suggests a lack of intracellular

472 metabolism of SRP in this upstream reach of the River Beult. In turn, this is consistent with molar N:P
473 and $\delta^{15}N_{NH4}$ in this reach that suggest N (and specifically NH_4) rather than P is likely to limit
474 metabolic activity. Under high flow conditions, SRP concentration and $\delta^{18}O_{PO4}$ increased from SV1 to
475 SV2. These data indicate that either an external source of P, enriched in $\delta^{18}O_{PO4}$, entered the river
476 between SV1 and SV2, or that SRP was re-generated from organic P compounds with partial
477 inheritance of an isotopically enriched $\delta^{18}O_{PO4}$ composition from the source organic P compound
478 (Blake et al., 1997; Colman et al., 2005). Molar N:P between SV1 and SV2 suggest an increased
479 probability of P limitation or N/P co-limitation, meaning that regeneration of SRP from organic P
480 compounds between these sites may have been promoted.

481 Examination of stable isotope data from SV5-SV7 also enables the potential links between N and P
482 from WwTW effluent and in-stream metabolism to be examined. This is only possible because of the
483 difference between the stable isotope composition of N and P in the effluent and in the river
484 immediately upstream of the WwTW. With respect to $\delta^{18}O_{PO4}$, strong coupling between effluent-
485 derived P and in-river metabolism would be expected to rapidly imprint an equilibrium fractionation
486 on SRP downstream of the WwTW, due to extensive uptake of SRP-intracellular equilibrium
487 fractionation-release of SRP (Blake et al., 2005). Given reduced molar N:P at SV5-SV7 under low
488 flow compared to high flow conditions, these samples are most likely to reveal isotopic evidence for
489 in-stream metabolism of SRP. However, $\delta^{18}O_{PO4}$ remained approximately 1‰ away from the
490 theoretical equilibrium along the 35 m transect downstream of the WwTW under low flow conditions,
491 indicating little evidence for significant in-stream metabolism of effluent derived SRP in this reach.
492 Under high flow conditions, molar N:P suggests increased potential for N limitation or N/P co-
493 limitation at SV5-SV7. Values of $\delta^{18}O_{PO4}$ remained relatively constant across these sites and, on
494 average, 0.6‰ away from the theoretical equilibrium. However, there was also little isotopic evidence
495 for in-stream metabolism of NO_3 between SV5-SV7 under either high or low flow conditions. It is
496 likely that the constrained transect length and associated residence time provided only limited
497 opportunity for intracellular cycling and release of SRP to imprint an equilibrium isotope fractionation
498 on $\delta^{18}O_{PO4}$, or for metabolic processes to generate fractionation in the stable isotope composition of

499 either NH_4 or NO_3. Longer downstream transects should be considered in order to fully evaluate the
500 potential links between WwTW-derived nutreints and in-stream metabolism within streams and rivers
501 using stable isotope approaches.

502

503 **5. Conclusions**

504 In-situ and laboratory hydrochemical data collected from the River Beult indicate that flow-dependent
505 changes in mixing between upstream river water and final effluent from a WwTW strongly influence
506 downstream river nutrient concentrations. However, these hydrochemical data alone provide no
507 insight into the original sources of nutrients that influence eutrophication risk within the river, nor into
508 the biogeochemical processes that govern the downstream fate of these nutrients. Our research
509 demonstrates how a multi-stable isotope framework can provide additional insights into such
510 questions that are fundamental to understanding the eutrophication process within freshwater
511 ecosystems.

512 Stable isotope data suggest that nutrient input to the upstream reaches of the River Beult is dominated
513 by sewage or agricultural sources under both high and low flow conditions, despite substantial
514 changes in nutrient concentration across these different flow conditions. Stable isotope data support
515 the need for measures to reduce diffuse water pollution from agriculture in order to address nutrient
516 enrichment and eutrophication risk in the upstream reaches of the River Beult. In-river changes in
517 stable isotope composition suggest an important role for microbial uptake of NH_4 to meet metabolic
518 demands for N, particularly under low flow conditions. These data suggest that measures which target
519 reductions in NH_4 concentration within the River Beult should be prioritised in order to drive changes
520 in autotrophic production within upstream river reaches. In contrast, changes in the concentration of
521 NO_3 and SRP, interpreted through the stable isotope data, indicate that abiotic mechanisms control the
522 fate of these ions in the upstream reach. Stable isotope data also suggest that N and P derived from a
523 WwTW are not strongly coupled to metabolism within the river immediately downstream of the
524 effluent discharge point, confirming the importance of addressing upstream sources of these nutrients.

However, further sampling along a more extensive downstream transect would be required to determine the ultimate fate of WwTW-derived nutrients within rivers using changes in stable isotope composition.

Three priorities for further research should be addressed in order to fully realise the potential of the multi-stable isotope framework proposed here. Firstly, the degree to which individual sources of nutrients can be reliably distinguished on the basis of their stable isotope composition requires further evaluation, particularly for $\delta^{18}O_{PO4}$. Secondly, stable isotope data at higher temporal frequency are required in order to evaluate short-term changes in the isotope composition of sources, for example diurnal changes in final effluent from smaller WwTWs with short residence times. Finally, more extensive and higher-intensity spatial sampling is required to assess the in-river fate of nutrients derived from a range of sources, on the basis of changes in stable isotope composition. It is hoped that the initial evaluation of a multi-stable isotope framework for P and N reported in this paper will help stimulate future research to address these challenges.

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Table 1

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Table 1. On site parameters and concentration of selected nutrients and anions in the River Beult under low and high flow conditions.

Site		Temp.	pH	DO	Cl	NO_3	NO_2	NH_4	SRP	TDP	TP	N:P
		°C	-	mg/L	mg/L	mg NO_3/L	mg NO_2/L	mg NH_4/L	µg P/L	µg P/L	µg P/L	molar
Low Flow (Sep 2013)												
SV1	DS Poultry and soft fruit farm	21.0	7.80	<0.5	60.1	33.4	3.11	34.0	2090	2135	2440	30
SV2	50 m ds of Livestock Farm	17.2	7.77	3.2	38.8	10.6	0.251	0.324	1296	1322	1955	3.0
SV3	WWTW outflow	20.3	7.76	6.5	93.8	131	0.126	0.043	888	918	954	68
SV4	Reed bed outflow	18.6	7.54	5.5	93.3	130	0.064	0.008	948	942	952	68
SV5	5 m ds of outflow	18.3	7.80	5.4	86.9	119	0.056	0.012	964	974	978	61
SV6	10 m ds of outflow	18.4	7.62	5.5	92.8	126	0.063	0.010	956	938	1002	63
SV7	35 m ds of outflow	18.8	7.97	7.5	91.3	123	0.024	0.028	948	956	1016	60
High Flow (Jan 2014)												
SV1	DS Poultry and soft fruit farm	7.4	7.71	4.5	37.7	34.5	<0.005	0.352	181	195	297	60
SV2	50 m ds of Livestock Farm	7.1	7.85	10.1	32.7	23.8	<0.005	0.332	301	322	512	24
SV3	WWTW outflow	9.6	7.60	7.9	84.2	75.1	0.232	0.105	1020	1096	1264	29
SV4	Reed bed outflow	9.8	7.20	7.4	86.1	72.1	0.014	0.025	1036	1060	1152	31
SV5	5 m ds of outflow	7.3	7.57	4.1	47.9	37.1	<0.005	0.123	516	552	706	27
SV6	10 m ds of outflow	7.4	7.51	4.2	48.6	36.9	0.100	0.134	524	559	742	25
SV7	35 m ds of outflow	6.8	7.41	4.2	46.8	35.6	0.457	0.142	524	598	768	23

Table 2
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Table 2. Stable isotope data for nitrate, ammonium, phosphate and water sampled under high and low flow conditions on the River Beult. Average value reported for duplicates ([a]15.1 and 15.02; [b]16.6 and 16.65; [c]16.2 and 16.1). * indicates insufficient sample to calculate standard deviation.

Site	$\delta^{15}N\text{-}NO_3$		$\delta^{18}O\text{-}NO_3$		$\delta^{15}N\text{-}NH_4$		$H_2O\text{-}\delta^{18}O$	$\delta^{18}O\text{-}PO4$	
	‰	±	‰	±	‰	±		‰	±
Low Flow (Sep 2013)									
SV1	13.1	0.3	7.8	0.5	10.0	0.1	-6.08	16.7	0.2
SV2	12.2	0.4	8.6	0.4	20.2	0.7	-5.22	16.6[b]	0.1
SV3	8.6	0.2	-0.2	0.2	-		-6.84	14.7	0.1
SV4	9.3	0.2	1.0	0.2	-		-6.83	15.4	0.1
SV5	9.4	0.3	0.2	0.2	-		-6.77	15.4	0.2
SV6	9.4	0.2	-0.1	0.0	-		-6.78	15.2	0.1
SV7	9.5	0.4	0.2	0.3	-		-6.73	15.1[a]	0.2
High Flow (Jan 2014)									
SV1	11.5	0.1	7.0	0.6	15.1	0.1	-6.50	18.6	0.1
SV2	12.0	0.1	7.4	0.1	18.2	0.4	-6.60	19.0	0.2
SV3	4.7	0.1	-0.8	0.6	33.7	*	-7.32	16.2[c]	0.2
SV4	6.1	0.1	0.2	0.1	-		-7.34	16.8	0.1
SV5	8.9	0.1	2.1	0.2	20.2	*	-6.82	17.5	0.1
SV6	8.6	0.1	2.9	0.2	21.4	*	-6.90	17.7	0.2
SV7	8.6	0.1	3.2	0.3	19.2	*	-6.87	17.6	0.1

Table 3

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Table 3. Measured temperature, $\delta^{18}O_{PO4}$ and $\delta^{18}O_{H2O}$ for water samples collected under low and high flow events on the River Beult; and the theoretical equilibrium $\delta^{18}O_{PO4}$ calculated using Equation 3.

	Measured $\delta^{18}O_{PO4}$ (‰)	Measured $\delta^{18}O_{H2O}$ (‰)	Measured temperature (°C)	Theoretical equilibrium $\delta^{18}O_{PO4}$ (‰)	Difference (measured-theoretical) $\delta^{18}O_{PO4}$ (‰)
Low flow (Sep 2013)					
SV1	16.7	-6.08	21.0	16.6	0.1
SV2	16.6	-5.22	17.2	18.1	-1.5
SV3	14.7	-6.84	20.3	15.8	-1.1
SV4	15.4	-6.83	18.6	16.2	-0.8
SV5	15.4	-6.77	18.3	16.3	-0.9
SV6	15.2	-6.78	18.4	16.3	-1.1
SV7	15.1	-6.73	18.8	16.3	-1.2
High flow (Jan 2014)					
SV1	18.6	-6.50	7.4	18.5	0.1
SV2	19.0	-6.60	7.1	18.5	0.5
SV3	16.2	-7.32	9.6	17.3	-1.1
SV4	16.8	-7.34	9.8	17.2	-0.4
SV5	17.5	-6.82	7.3	18.2	-0.7
SV6	17.7	-6.90	7.4	18.2	-0.5
SV7	17.6	-6.87	6.8	18.3	-0.7

Figure Captions

Figure 1. Location of the Beult catchment in England, UK (a) and schematic map of sample locations along a section of the river (b).

Figure 2. Schematic of the modified McLaughlin et al. (2004) protocol used to process water samples for $\delta^{18}O_{PO4}$ analysis.

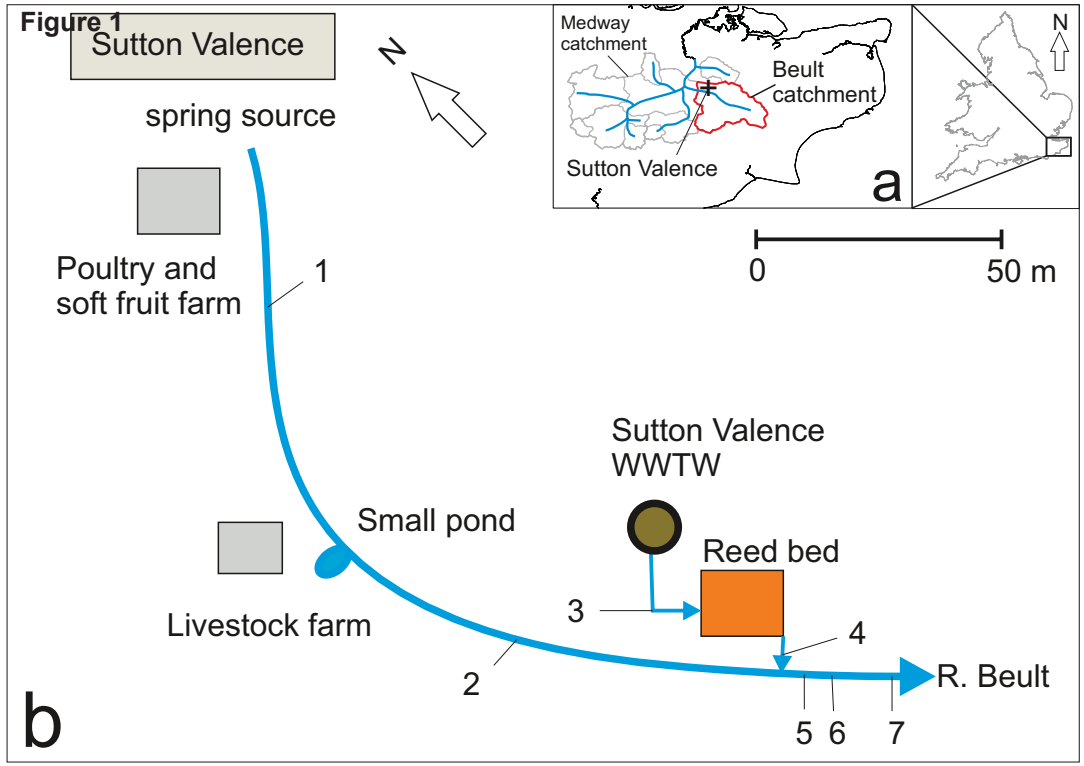
Figure 3. Nitrate concentration against chloride concentration for low and high flow sampling events.

Figure 4. Soluble reactive phosphorus (SRP) concentration against chloride concentration for low and high flow sampling events (note change in scale of SRP concentration between low and flows).

Figure 5. Nitrate-nitrogen isotope composition ($\delta^{15}N_{NO3}$) composition against nitrate concentration for high and low flow sampling events. Vertical bars show standard deviation on $\delta^{15}N_{NO3}$ (note change in scale for nitrate concentration between high and low flow events).

Figure 6. Stable phosphate oxygen composition ($\delta^{18}O_{PO4}$) composition against soluble reactive phosphorus (SRP) concentration for low and high flow sampling events. Vertical bars show standard deviation on $\delta^{18}O_{PO4}$ (note change in scale for SRP concentration between low and high flow).

Figure 7. A comparison of $\delta^{18}O_{PO4}$ and $\delta^{18}O_{H2O}$ for samples collected in low flow (filled symbols) and high flow (open symbols). Vertical and horizontal hashed areas represent range of measured $\delta^{18}O_{H2O}$ during low and high flows respectively. Diagonal dashed lines represent the $\delta^{18}O_{PO4}$ equilibrium values for ambient water for the range of temperatures at low and high flows calculated using the equation of Chang and Blake (2015).



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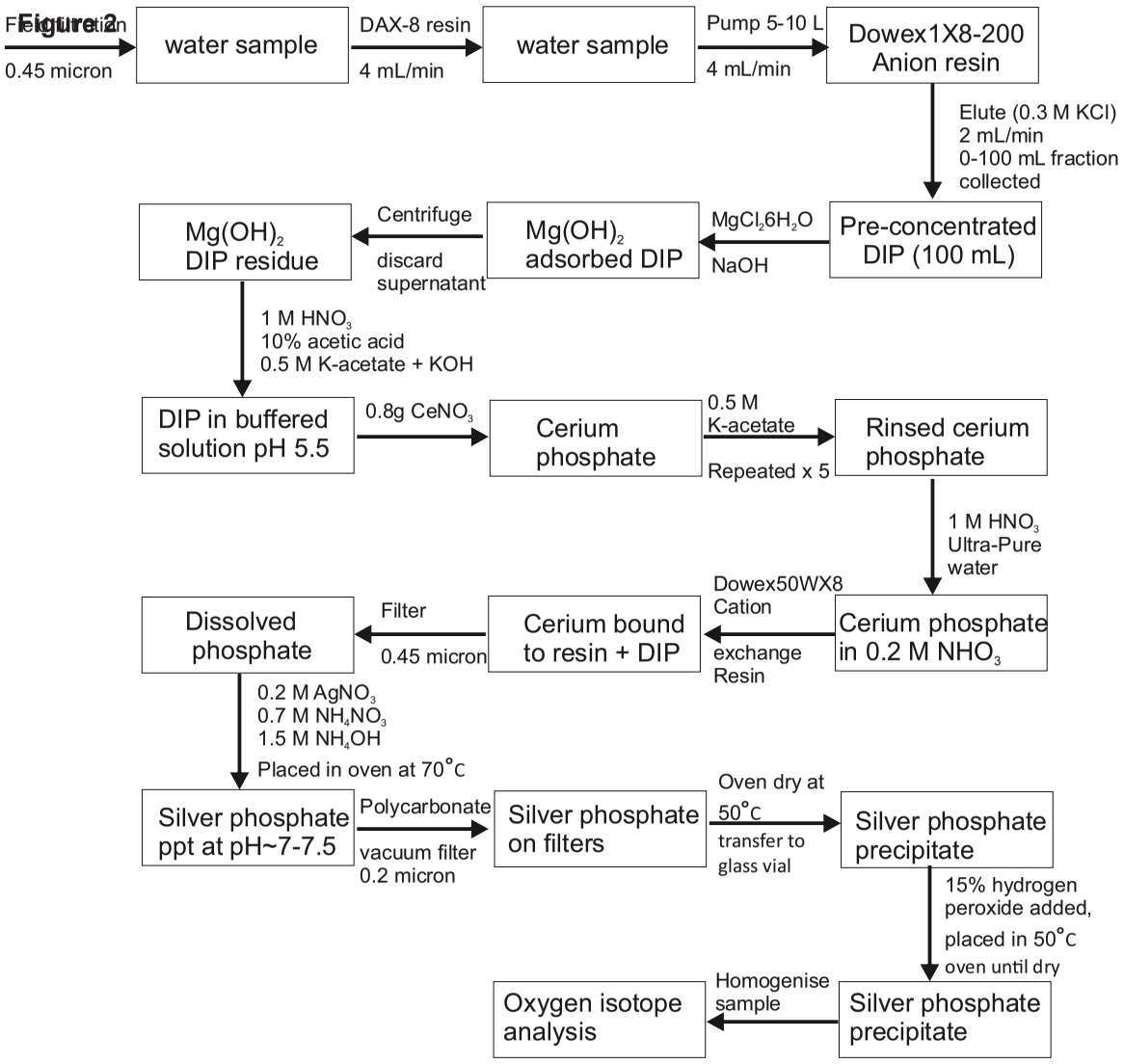

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Figure 3

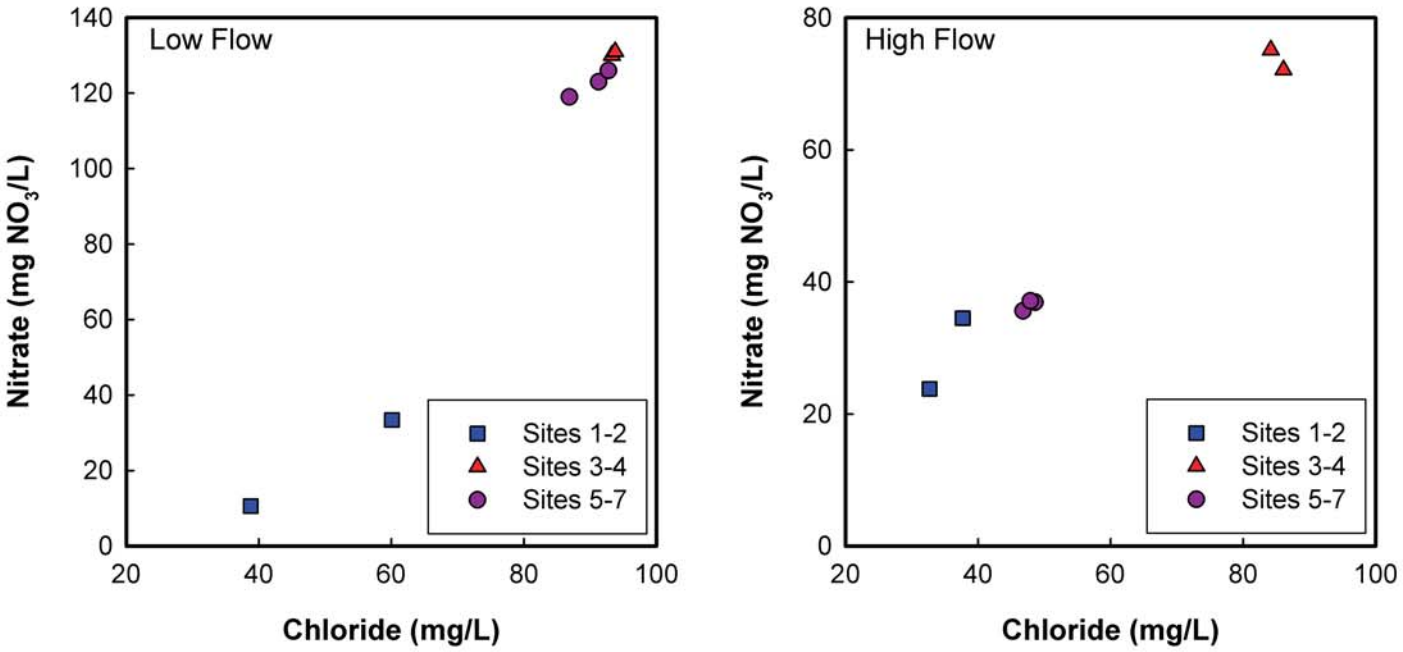

Figure 3. Nitrate concentration against chloride concentration for low and high flow sampling events.

Figure 4

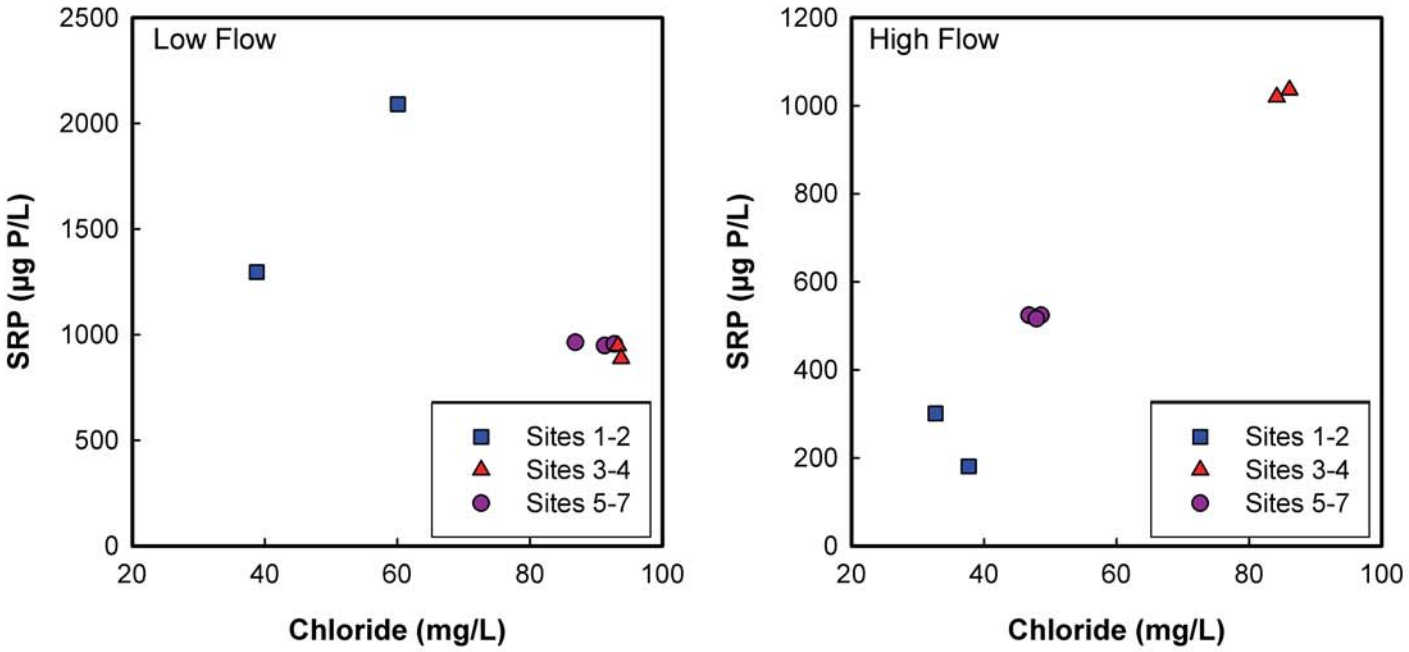

Figure 4. Soluble reactive phosphorus (SRP) concentration against chloride concentration for low and high flow sampling events (note change in scale of SRP concentration between low and flows).

Figure 5

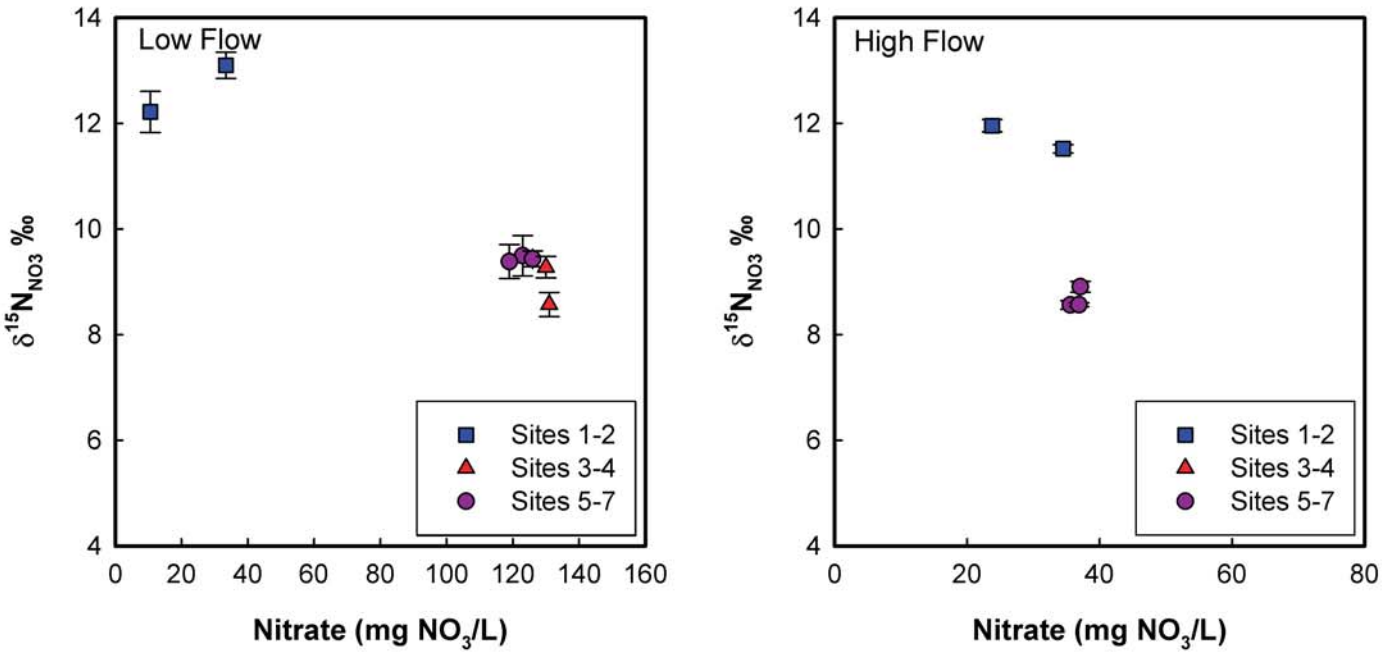

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Figure 6

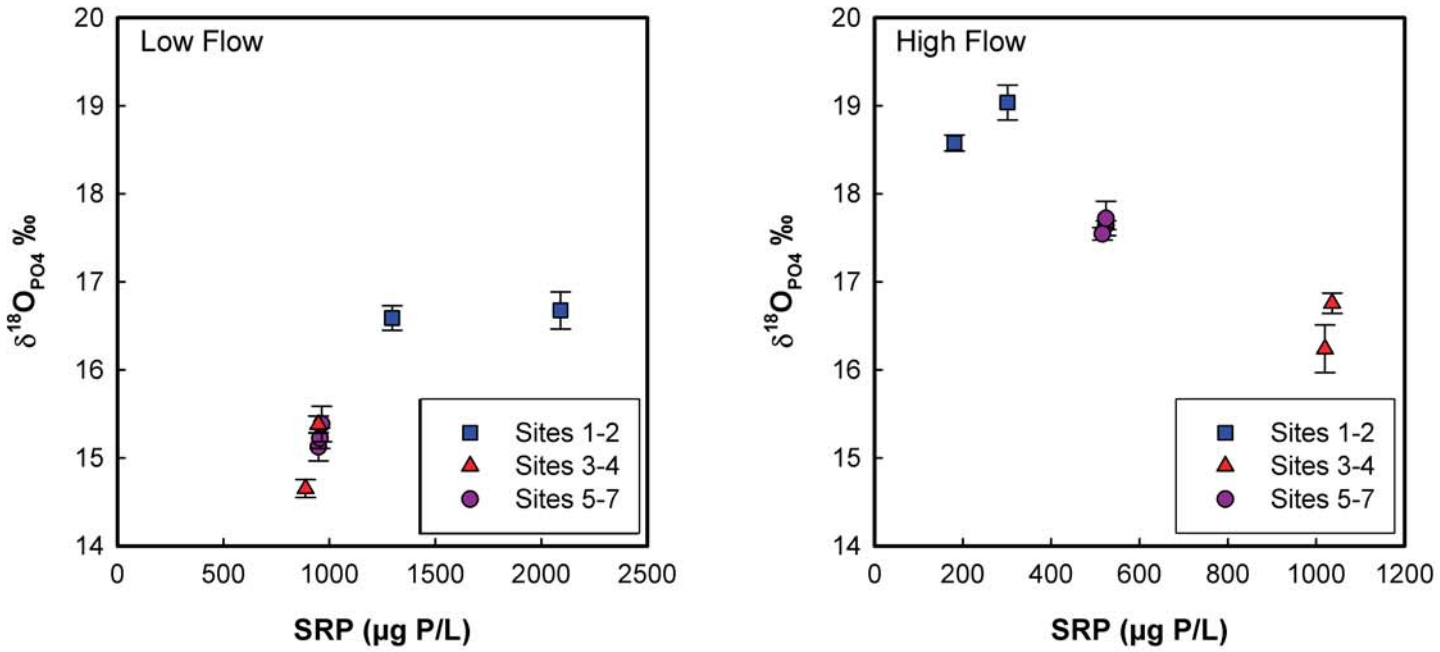

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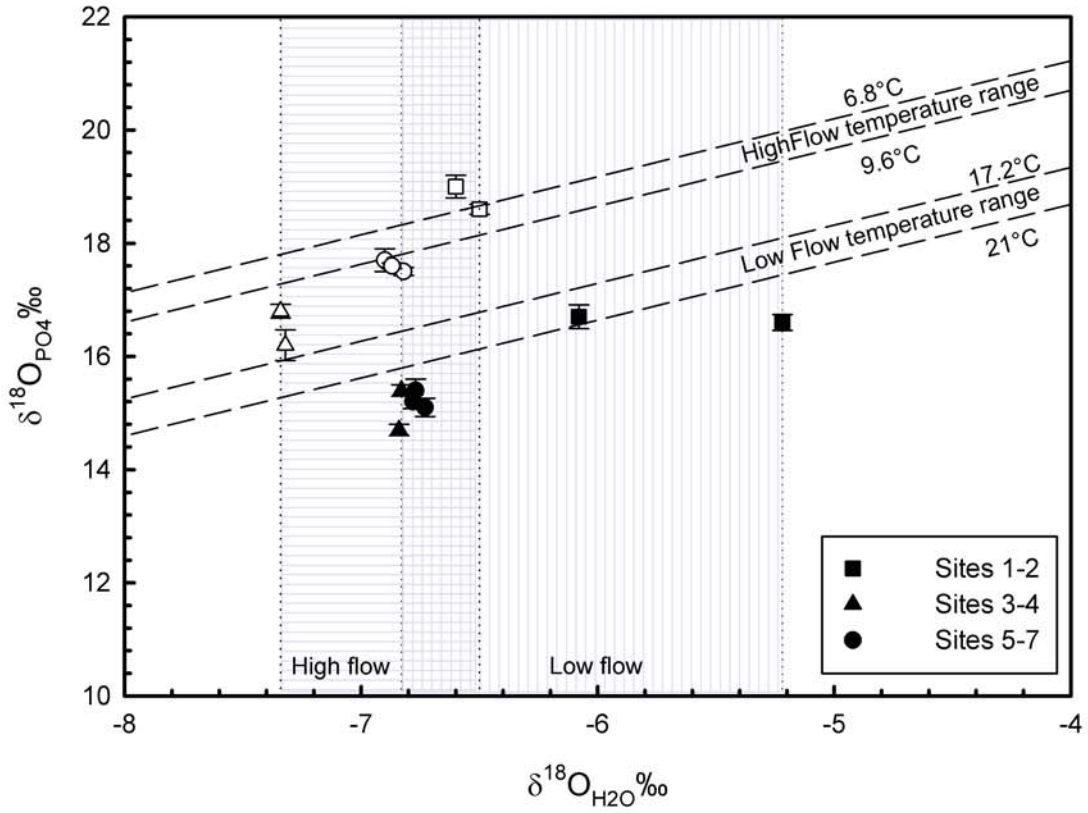
Figure 7



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