

# 1 A new technique to determine the phosphate oxygen isotope composition of freshwater 2 samples at low ambient phosphate concentration

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12

## 13 ABSTRACT

14 The oxygen isotope composition of dissolved inorganic phosphate ( $\delta^{18}\text{O}_p$ ) offers new  
15 opportunities to understand the sources and the fate of phosphorus (P) in freshwater  
16 ecosystems. However, current analytical protocols for determining  $\delta^{18}\text{O}_p$  are unable to  
17 generate reliable data for samples in which ambient P concentrations are extremely low,  
18 precisely the systems in which  $\delta^{18}\text{O}_p$  may provide new and important insights into the  
19 biogeochemistry of P. In this paper, we report the development, testing and initial application  
20 of a new technique that enables  $\delta^{18}\text{O}_p$  analysis to be extended into such ecosystems. The  
21 Twist Spinning Mode (TSM) protocol described here enables >1000 L of sample with a P  
22 concentration <0.016 mg P L<sup>-1</sup> to be initially processed within the field in approximately 24  
23 hours. Combined with a new freeze-drying method to maximise the yield and minimise the  
24 contamination of silver phosphate generated for isotope ratio mass spectrometry, the TSM  
25 protocol is able to generate accurate and precise  $\delta^{18}\text{O}_p$  data. We evaluated the TSM protocol  
26 using synthetic test solutions and subsequently applied the protocol to samples from locations  
27 around the Saint-Lawrence River in Montreal, Canada. We believe that the novel technique  
28 reported here offers the methodological basis for researchers to extend the application of  
29  $\delta^{18}\text{O}_p$  into a much wider range of freshwater ecosystems than has been possible to date.

30

## 31 INTRODUCTION

32 Phosphorus (P) is integral to the structure and function of many key biomolecules, including  
33 DNA, RNA, adenosine triphosphate and phospholipids, meaning that this element is essential  
34 to all life [1](#). Human society has dramatically increased the input of reactive P to the  
35 biosphere [2](#), often resulting in undesirable ecosystem changes that lead to significant, adverse  
36 economic and social impacts [3-5](#). In this context, understanding the sources and the biological  
37 impacts of P within ecosystems represent key challenges in many parts of the world.

38 Stable isotope analysis offers a potentially powerful tool through which to address these  
39 challenges. However, P has only one stable isotope (<sup>31</sup>P), preventing the application of

40 traditional stable isotope approaches that rely on an element possessing more than one  
41 isotope. Within natural environments, P is only strongly bound to oxygen (O) in the  
42 phosphate molecule. The P-O bonds within phosphate are resistant to inorganic hydrolysis  
43 under typical temperature and pressure conditions experienced within the Earth's surface  
44 environments [6](#). Cleavage of P-O bonds relies on metabolic processes catalysed by the action  
45 of intracellular or extracellular enzymes, which impart a range of equilibrium or kinetic  
46 isotope fractionations on the O atoms within resulting phosphate molecules [7-9](#). In addition,  
47 initial evidence suggests that the biological uptake of phosphate molecules from solution can  
48 be associated with isotope effects under certain conditions [9](#). Analysis of the O isotope  
49 composition of dissolved inorganic phosphate ( $\delta^{18}\text{O}_p$ ), followed by interpretation of  $\delta^{18}\text{O}_p$   
50 data through the isotopic effects ascribed to a range of metabolic processes, offers the  
51 potential for new insights into the sources and the cycling of P within natural ecosystems [10-](#)  
52 [17](#).

53 Accurate determination of  $\delta^{18}\text{O}_p$  relies on extraction of phosphate from an environmental  
54 sample, purification and finally measurement by isotope ratio mass spectrometry. Substantial  
55 methodological research has been undertaken within a range of environmental matrices to  
56 address these analytical challenges [18-19](#). Within aquatic ecosystems, initial work was  
57 undertaken within marine environments with large volumes of water usually requiring  
58 processing to generate reliable  $\delta^{18}\text{O}_p$  data [20](#). However, transfer of the methodologies  
59 developed for marine matrices to freshwaters can be problematic. For example, the presence  
60 of additional sources of O including dissolved organic carbon, nitrate and sulphate within  
61 freshwaters can bias the  $\delta^{18}\text{O}_p$  values. Further, in our experience, freshwater matrices can  
62 interfere with certain stages of the protocols developed for  $\delta^{18}\text{O}_p$  analysis within marine  
63 matrices, because they often present relatively low  $\text{PO}_4 / (\text{NO}_3, \text{SO}_4 \text{ or total organic carbon})$   
64 abundance ratios. More recent work has developed protocols for analysis of  $\delta^{18}\text{O}_p$  that seek to  
65 address the particular challenges of freshwater matrices [13](#). However, when P concentration  
66 in a sample is particularly low and the volume of sample required for  $\delta^{18}\text{O}_p$  analysis high,  
67 even the methodologies developed specifically for freshwaters may prove impracticable or  
68 may fail to generate reliable  $\delta^{18}\text{O}_p$  data. This represents a significant barrier to application of  
69  $\delta^{18}\text{O}_p$  analyses within freshwater ecosystems at low ambient P concentrations, precisely the  
70 environments in which the importance of bioavailable P is likely to be maximised and in  
71 which  $\delta^{18}\text{O}_p$  analyses are of particular interest.

72 In this paper, we report the development, testing and initial application of a new method  
73 designed to extract phosphate for  $\delta^{18}\text{O}_p$  analysis from large volumes of freshwater (c.1000 L),  
74 over relatively short timescales (<24 hours) and using techniques that avoid potential biases  
75 in  $\delta^{18}\text{O}_p$  values from additional sources of O. We believe that the novel approach we report  
76 provides researchers with the methodological basis on which to extend  $\delta^{18}\text{O}_p$  analyses to a  
77 wide range of freshwater ecosystems in which this technique has proved impossible to date.  
78 Moreover, the new approach we present combined with a new freeze-drying method avoids  
79 the use of a MagIC precipitation in the field, thereby reducing the use of toxic reactants such  
80 as NaOH and  $\text{HNO}_3$  in the field, lowers costs and minimizes the risk of incorporating organic

81 phosphate and organic carbon compounds, known to bias  $\delta^{18}\text{O}_p$  values with additional sources  
82 of O.

83

## 84 **MATERIALS AND METHODS**

85 Previously, we have developed and reported a protocol based on the use of chloride-form  
86 anion exchange resin in column mode to separate phosphate from competing oxyanions  
87 within freshwater samples [13](#), hereafter termed the Modified McLaughlin Method (MMM).  
88 However, pumping large volumes of sample ( $> 20$  L) through these columns can prove  
89 problematic, for example due to swelling of the resin bed. Pre-concentrating phosphate, for  
90 example using co-precipitation with brucite through the MagIC protocol [21](#), offers one  
91 potential solution. However, brucite precipitation requires the use of large masses of NaOH  
92 and  $\text{MgCl}_2$ , which incurs significant cost and which may bias  $\delta^{18}\text{O}_p$  in samples containing  
93 low P concentration, due to the trace amounts of P within reagents. Further, our previous  
94 research has demonstrated that brucite is not specific to the inorganic phosphate ion, with  
95 additional organic P compounds and oxyanions including nitrate and sulphate being co-  
96 precipitated, which may confound accurate determination of  $\delta^{18}\text{O}_p$  [8](#).

97 Here, we report a new approach that enables extraction of phosphate from large volumes of  
98 freshwater, termed the Twist Spinning Mode (TSM) protocol. The TSM protocol involves  
99 pumping a sample into a large (120 L) plastic barrel at a flow rate of  $800 \text{ mL min}^{-1}$  and  
100 subsequently through a column (96 cm tall, internal diameter of 10.5 cm) containing 50 mL  
101 of Dowex 1X8-200 chloride-form anion exchange resin with an exchange capacity of 1.2  
102 meq/ml (Figure 1). The inlet to the column is angled at  $30^\circ$ , causing the sample to rotate  
103 during passage through the column. This allows the resin to be permanently suspended in a  
104 continuous flow of sample, rather than pumping a sample through a static resin bed in column  
105 mode. The maximum flow rate is limited to avoid resin spilling into the plastic barrel.  
106 However, if a small amount of resin is lost from the column it is collected from the plastic  
107 barrel and returned to the inlet of the column.

108 Phosphate and other oxyanions bind to the anion exchange resin as a sample passes through  
109 the column. After sampling is complete, the anion exchange resin is recovered from the base  
110 of the cylinder by decanting the overlying water, and then packed into a separate column. In  
111 the laboratory, the recovered resin column is eluted with 0.3 M KCl and eluent fractions  
112 containing phosphate are collected for further processing, following the protocol described in  
113 the MMM. The physical components that underpin the TSM protocol are designed to be  
114 inexpensive and simple, allowing for field deployment, rather than requiring the transport of  
115 large volumes of sample to a laboratory for processing. Using this new approach, 1000 L of  
116 sample can be processed within 24 h without having to attend to the sampling apparatus.  
117 Given this, it is assumed here that the  $\delta^{18}\text{O}_p$  value obtained from the TSM protocol represents  
118 a daily mean of  $\delta^{18}\text{O}_p$  within a sampling location such as a river or lake.

119

## 120 **Laboratory Testing of the TSM protocol**

121 The TSM protocol was initially tested using synthetic laboratory solutions. Three, 1000 L  
 122 solutions containing varying but environmentally relevant concentrations of NO<sub>3</sub>, SO<sub>4</sub> and  
 123 humic acid as dissolved organic carbon (DOC) (Table 1) were prepared from KNO<sub>3</sub>, K<sub>2</sub>SO<sub>4</sub>  
 124 and commercially-available humic acid (H16752-Humic acid sodium salt, technical grade,  
 125 Sigma-Aldrich). Competing oxyanions and DOC were included in these solutions to evaluate  
 126 the ability of the TSM protocol to generate accurate δ<sup>18</sup>O<sub>p</sub> data given the presence of  
 127 contaminant sources of O within a sample. It is acknowledged that environmental DOC can  
 128 include both humic and fulvic acids which may behave slightly differently to our test  
 129 solutions. To each of these solutions, either KH<sub>2</sub>PO<sub>4</sub> (to yield a final P concentration of  
 130 16.3 µg P L<sup>-1</sup>) or a 20% P<sub>2</sub>O<sub>5</sub> fertiliser (to yield a final P concentration of 19.5 µg P L<sup>-1</sup>) was  
 131 added, generating six synthetic test solutions in total. The two sources of phosphate were  
 132 chosen to provide distinctly different δ<sup>18</sup>O<sub>p</sub> compositions: KH<sub>2</sub>PO<sub>4</sub> = 9.0 (±0.3)‰ vs  
 133 VSMOW (Vienna Standard Mean Ocean Water) and the 20% P<sub>2</sub>O<sub>5</sub> fertiliser = 16.6 (±0.3)‰  
 134 vs VSMOW, as determined by the MMM following dissolution of KH<sub>2</sub>PO<sub>4</sub> or fertiliser in  
 135 milliQ water.

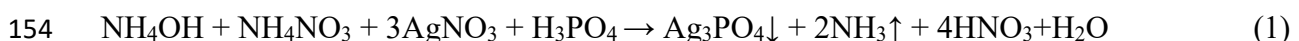
### 136 **Field Testing of the TSM protocol**

137 Further testing of the TSM protocol was subsequently undertaken in the field, based on a  
 138 study area located in and around the Saint-Lawrence River in Montreal, Canada. Samples  
 139 were collected in winter 2017 from two locations on tributaries of the Saint-Lawrence, the  
 140 Assomption river (Pa) and Achigan river (Pe), situated in predominately agricultural  
 141 catchments, as well as from the outflow of a waste water treatment works (Pw) downstream  
 142 of the city of Montreal (Figure 2). Samples from the tributaries and from the waste water  
 143 treatment works were processed using both the TSM protocol (1000 L of sample) and using  
 144 the MMM (20 L of sample), in order to compare δ<sup>18</sup>O<sub>p</sub> generated using the two protocols.

### 145 **Freeze-drying of silver phosphate precipitates**

146 The protocols reported in both McLaughlin et al.[20](#) and Gooddy et al. [13](#) require a slow  
 147 precipitation (SP) step in order to generate silver phosphate (Ag<sub>3</sub>PO<sub>4</sub>), which is subsequently  
 148 analysed by EA-IRMS (Elemental Analysis Isotope Ratio Mass Spectrometry) to determine  
 149 δ<sup>18</sup>O<sub>p</sub>. This commonly involves the addition of an ammoniacal-silver nitrate solution to the  
 150 purified phosphate solution (Equation 1) which is heated to 70°C. This solution is strongly  
 151 basic and can promote the precipitation of silver oxide [5](#) (Equations 2 and 3), a potential  
 152 source of contaminant-O in the determination of δ<sup>18</sup>O<sub>p</sub>.

153



157 In an attempt to mitigate this, a freeze drying (FD) step in the generation of  $\text{Ag}_3\text{PO}_4$  was  
158 tested in the research we report here (see Figure 3 for the full, revised protocol). This low-  
159 temperature process is performed at a near neutral pH, meaning that it is less favourable for  
160 silver oxide formation compared to SP.

161 A comparison of the performance of FD and SP approaches was undertaken. A 1 L synthetic  
162 test solution was prepared using  $\text{KH}_2\text{PO}_4$  to achieve a final concentration of  $19.5 \text{ mg P L}^{-1}$ .  
163 Precipitation of brucite from the 1 L solution using the MagIC approach was followed by  
164 stages 6-12 inclusive in the protocol reported in Figure 3. The purified phosphate solution  
165 achieved after step 12 in Figure 3 was divided into 10 equal aliquots. Ammoniacal silver  
166 nitrate solution was added to each aliquot. Five aliquots were subjected to precipitation of  
167  $\text{Ag}_3\text{PO}_4$  by the FD method. By freeze drying under vacuum, water and ammonia in the  
168 sample were sublimated and silver phosphate precipitated. For a sample aliquot of 30 mL  
169 volume, this takes approximately 24 h. The process of formation of  $\text{Ag}_3\text{PO}_4$  crystals occurs  
170 gradually. After precipitation of  $\text{Ag}_3\text{PO}_4$ , the crystals are washed with deionized water on a  
171  $0.45 \mu\text{m}$  acetate filter and placed in the freeze dryer again. Silver phosphate from the  
172 remaining five aliquots was generated using the SP approach described within the MMM.  
173 The  $\text{Ag}_3\text{PO}_4$  obtained by both SP and FD methods was analyzed by Scanning Electron  
174 Microscopy (SEM) and XRD to assess the purity of the crystals.

### 175 **Isotopic analysis of silver phosphate**

176 A minimum of  $200 \mu\text{g}$  of  $\text{Ag}_3\text{PO}_4$  was encased in silver capsules with the same mass of  
177 carbon black, with special care taken to minimize the inclusion of air [22](#). Samples were  
178 analyzed using an Elementar<sup>TM</sup> Vario PyroCube<sup>TM</sup> elemental analyzer via a heated  
179 autosampler ( $80^\circ\text{C}$ ), where  $\text{Ag}_3\text{PO}_4$  is carbothermally reduced in a glassy carbon reactor at  
180  $1450^\circ\text{C}$  for complete oxygen sample conversion into CO. The sample gas is carried by a  
181 continuous flow of helium, passes through a water trap filled with hygroscopic magnesium  
182 perchlorate and is collected in a CO trap. The carbon monoxide is then released and carried  
183 through a fresh helium flow to the TCD for oxygen content analysis and then to the isotope  
184 ratio mass spectrometer (IRMS Isoprime VisION<sup>TM</sup>) for isotopic measurement. The CO  
185 sample is measured after two monitoring CO pulses. Raw  $\delta^{18}\text{O}_p$  values are normalised on the  
186 VSMOW scale by a two-point linear calibration using IAEA-601 (+24.1‰) & USGS-35  
187 (+56.8‰) and verified with IAEA-C3 (+31.9).

188

## 189 **RESULTS AND DISCUSSION**

### 190 **Evaluation of the TSM protocol**

191 The initial mass of P contained in each 1000 L synthetic laboratory solution, alongside the  
192 mass of P eluted from the anion exchange resin using  $0.3 \text{ M KCl}$ , are reported in Table 2. For  
193 the synthetic solutions containing  $\text{KH}_2\text{PO}_4$ , the mass of P eluted from the resin decreased  
194 consistently as the mass of competing ions in solution increased (Solutions 1-3, Table 1). The  
195 P yields derived from the anion exchange resin ranged from 6% (Solution 1) to 1.5%

196 (Solution 3) of the total mass originally contained within each solution. For solutions that  
197 included the inorganic phosphate fertiliser, which contained a slightly higher initial mass of  
198 P, yields of P as a proportion of the original mass within the solutions were similar to those  
199 observed for solutions containing  $\text{KH}_2\text{PO}_4$ , ranging from a maximum of 5.7% in Solution 2 to  
200 a minimum of 2.7% in Solution 3.

201 In absolute terms, these P yields are clearly low. Excluding the humic acid added to the  
202 synthetic solutions, the anion content of Solution 1 equates to approximately 14 meq, which  
203 is substantially below the capacity of the anion exchange resin used with the TSM protocol  
204 (60 meq). Despite this, P yields for Solution 1 only reached a maximum of 6%. For Solutions  
205 2 and 3, the anion content equates to approximately 640 and 1360 meq respectively, and  
206 considerably in excess of the capacity of the anion exchange resin. Whilst saturation of the  
207 capacity of the anion exchange resin and competition for binding sites between phosphate  
208 and other anions in solution may explain low yields of P in Solutions 2 and 3, this is not the  
209 case for Solution 1 where there was an excess of available binding sites on the anion  
210 exchange resin. Instead, we believe that the low yields of P are associated with the relatively  
211 high flow rate of the solution through the column containing the anion exchange resin in the  
212 TSM protocol. Processing c.1000 L of solution within 24 h requires a flow rate of  
213 approximately  $800 \text{ mL min}^{-1}$ . Our data suggest that this flow rate is too high for a significant  
214 proportion of the phosphate within the test solutions to be bound to the anion exchange resin.  
215 Increasing the mass of anion exchange resin within the column in the TSM protocol may  
216 enhance P yields. Alternatively, solutions could be recirculated through the column, although  
217 this would effectively increase the total volume of solution to be processed through a column  
218 and therefore the time required to complete sampling using the TSM protocol. However,  
219 because the TSM protocol allowed c.1000 L of solution volume to be processed, sufficient P  
220 was extracted from solution by the anion exchange resin to ultimately enable a sufficient  
221 mass of  $\text{Ag}_3\text{PO}_4$  to be generated for  $\delta^{18}\text{O}_\text{p}$  determination. Importantly, the protocol reported  
222 by Gooddy et al. (2015) would have been unlikely to generate a sufficient mass of  $\text{Ag}_3\text{PO}_4$   
223 for analysis, based on processing a maximum solution volume of 50 L which would have  
224 taken in excess of eight days to load through the anion resin column at  $4 \text{ mL min}^{-1}$ .

225 Beyond a limit on the mass of  $\text{Ag}_3\text{PO}_4$  that can ultimately be generated, low recovery of P  
226 from solution using the TSM protocol would be problematic if isotopic fractionation was  
227 associated with low P recovery from a solution. However, our data do not indicate significant  
228 isotopic fractionation during sample processing using the TSM protocol. Table 3 reports  
229  $\delta^{18}\text{O}_\text{p}$  and O yield data for test Solutions 1-3 following the addition of either  $\text{KH}_2\text{PO}_4$  or  
230 inorganic fertiliser as sole sources of P. Based on the MMM,  $\text{Ag}_3\text{PO}_4$  synthesised from a pure  
231  $\text{KH}_2\text{PO}_4$  solution had a  $\delta^{18}\text{O}_\text{p}$  value of  $9.0\text{‰} (\pm 0.3)$ , whilst from a pure solution of inorganic  
232 fertiliser  $\delta^{18}\text{O}_\text{p} = 16.6\text{‰} (\pm 0.3)$ . For synthetic test solutions containing  $\text{KH}_2\text{PO}_4$ ,  $\delta^{18}\text{O}_\text{p}$  based  
233 on the TSM protocol ranged from 8.6‰ in Solution 2 to 9.5‰ in Solution 3 with a mean of  
234 9.1‰. For solutions containing inorganic fertiliser,  $\delta^{18}\text{O}_\text{p}$  ranged from 16.3‰ in Solution 1 to  
235 16.4‰ in Solutions 2 and 3, with a mean of 16.4‰ based on the TSM protocol. Further, O  
236 yield data ranged from 15.6% to 16.2% with a mean of 15.9%. This compares to an expected  
237 O yield for stoichiometrically-pure  $\text{Ag}_3\text{PO}_4$  of 15.3%, supporting the purity of the  $\text{Ag}_3\text{PO}_4$



238 produced through the TSM protocol (also see below). Therefore, despite low P yields from  
239 the original solutions, the TSM protocol appears able to generate accurate  $\delta^{18}\text{O}_p$  data from  
240 solutions in which low P concentrations would preclude analysis using alternative protocols.

#### 241 **Freeze Drying versus Slow Precipitation of silver phosphate**

242 The results of the comparison between FD and SP approaches to the generation of  $\text{Ag}_3\text{PO}_4$   
243 are reported in Table 4. Both methods generated very similar  $\delta^{18}\text{O}_p$  (averaging 8.7‰ ( $\pm 0.2$ )  
244 and 8.6 ( $\pm 0.3$ ) for FD and SP respectively) and oxygen yield (averaging 15.0% ( $\pm 0.3$ ) and  
245 14.7 ( $\pm 0.4$ ) for FD and SP respectively) data. Importantly, the yield of  $\text{Ag}_3\text{PO}_4$  appears to be  
246 somewhat higher and less variable for the FD approach compared to the SP approach. Figure  
247 4 reports example SEM spectra for  $\text{Ag}_3\text{PO}_4$  precipitated with SP and FD methods. Both  
248 approaches appear to produce relatively pure  $\text{Ag}_3\text{PO}_4$  crystals.

#### 249 **Application of TSM and MMM to samples from the Saint Lawrence River catchment**

250 The MMM protocol was unable to produce sufficient  $\text{Ag}_3\text{PO}_4$  for analysis from either the  
251 Assumption (Pa) or Achigan (Pe) river samples (Table 5). However,  $\delta^{18}\text{O}_p$  data were  
252 successfully generated from the wastewater treatment work effluent (Pw) using the MMM  
253 approach, giving a value of 17.4‰ compared to 18.3‰ from the TSM protocol. Whilst  
254 sufficient for determination of  $\delta^{18}\text{O}_p$ , yields of  $\text{Ag}_3\text{PO}_4$  for site Pw using the TSM protocol  
255 were low. We ascribe this observation to the fact that 1000 L of sample with high ionic  
256 concentration was passed through the resin column, likely leading to competition for binding  
257 sites on the resin and low P recoveries leading, ultimately, to low  $\text{Ag}_3\text{PO}_4$  yields. Although  
258 the present dataset is limited, it is interesting to observe that the  $\delta^{18}\text{O}_p$  values generated by  
259 both TSM and MMM protocols are broadly consistent with the global range of  $\delta^{18}\text{O}_p$  reported  
260 previously for municipal wastewaters, although these values do appear to exceed the range  
261 reported specifically for other sites in North America by at least 3.8‰ [11](#), [14](#), [17](#). Discussions  
262 on the actual sources of phosphate within the wastewater is beyond the scope of this paper  
263 and further data would be required in order to establish whether this observation reflects  
264 isotopically enriched P within the influent to sample location Pw, compared to other  
265 wastewater treatment works that have been analysed in North America, or isotopic  
266 enrichment associated with treatment processes within the specific works we sampled in the  
267 research reported here. In contrast to the MMM approach, the TSM protocol produced good  
268 yields of  $\text{Ag}_3\text{PO}_4$  for both of the tributary river sites. Values of  $\delta^{18}\text{O}_p$  for the Assumption and  
269 Achigan rivers were  $>5\%$  enriched compared to expected equilibrium values. This  
270 observation may indicate the presence of an isotopically-enriched source of P in the  
271 catchments of these two tributaries, in excess of the  $\delta^{18}\text{O}_p$  composition of the inorganic  
272 fertiliser used in the laboratory tests reported in this paper. Further tracking of in-river  
273 changes in  $\delta^{18}\text{O}_p$  using the TSM protocol we report here would enable the importance of  
274 multiple P sources and in-river P cycling to be explored within these tributaries of the Saint  
275 Lawrence river. In Canadian ecosystems, nutrient fluxes are very low, human activities bring  
276 discrete changes to nutrient budgets that lead rapidly to drastic changes in ecosystem  
277 dynamics. We hope that, by applying this new technique more broadly to such ecosystems,  
278 that it will be possible to better understand P sources, assimilation and re-cycling.

279 **Future Use of TSM to determine  $\delta^{18}\text{O}_p$  in the Context of Samples with Low P**  
280 **Concentration**

281 Despite relatively low recovery of P from synthetic test solutions, the TSM protocol we  
282 report offers new opportunities to determine  $\delta^{18}\text{O}_p$  for freshwaters in which P concentrations  
283 are below the threshold suitable for processing using existing protocols. Given the mass of P  
284 retained on the anion exchange resin in proportion to that in the original test solutions, the  
285 final  $\text{Ag}_3\text{PO}_4$  yields from the TSM protocol are relatively high and enable robust mass  
286 spectrometry to determine  $\delta^{18}\text{O}_p$ . Our SEM analyses coupled with quantification of O-yields  
287 indicate that the  $\text{Ag}_3\text{PO}_4$  we produced was pure. Critically, no significant fractionation of  
288  $\delta^{18}\text{O}_p$  was revealed following the TSM protocol, despite the low P yields described above,  
289 meaning that the TSM protocol, in combination with FD, appears able to generate accurate  
290  $\delta^{18}\text{O}_p$  data in samples with low ambient P concentrations ( $<0.016 \text{ mg P L}^{-1}$ ). Further  
291 optimisation of the TSM protocol may be achieved through increases in the mass of anion  
292 exchange resin used to bind phosphate from large volumes of solution processed at relatively  
293 high flow rates. However, this will also necessitate changes to the subsequent elution of anion  
294 exchange resin using KCl, in order to maintain chromatographic separation of phosphate  
295 from competing anions, which is dependent on KCl molarity but also on resin bed depth.  
296 Despite opportunities for further development, we believe that the TSM protocol reported  
297 here provides new opportunities to apply  $\delta^{18}\text{O}_p$  analyses to questions surrounding P  
298 biogeochemistry in freshwaters that have not been feasible sites for phosphate oxygen isotope  
299 analyses to date.

300

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308

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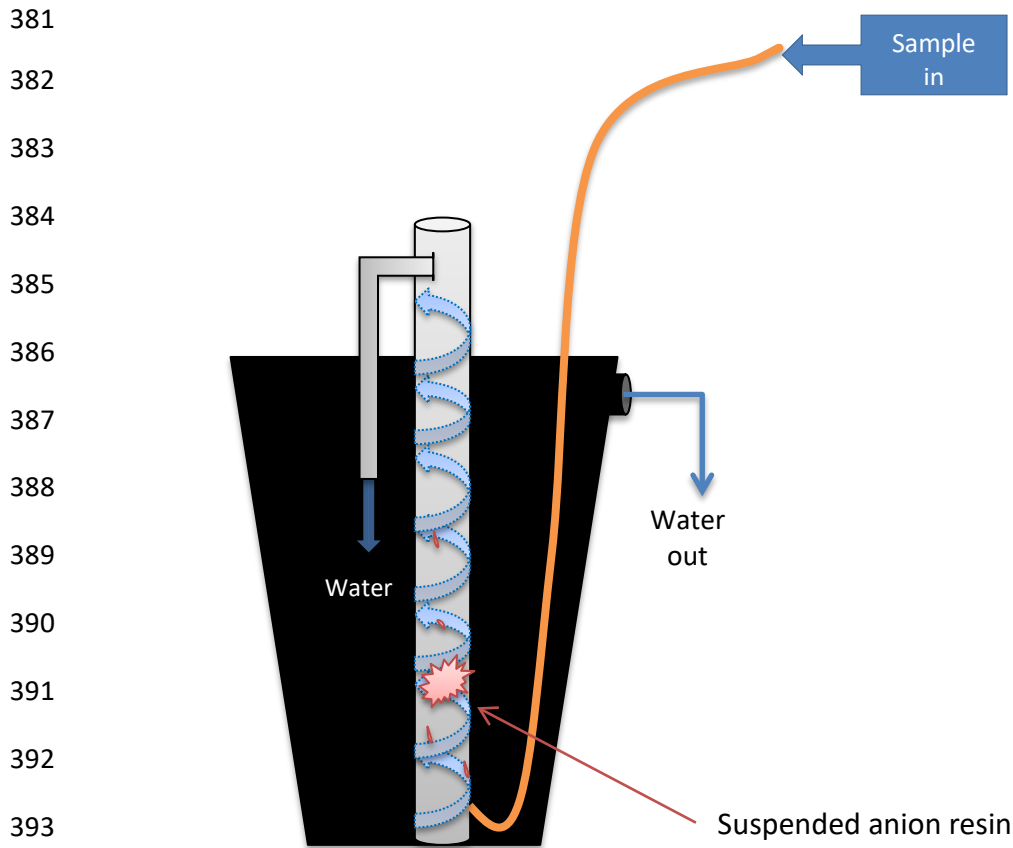
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396 Figure 1. Schematic diagram of Twist Spin Method (TSM) experimental setup. The sample  
397 container is made of plastic and has a volume of 120 litres. The internal column containing  
398 the anion exchange resin is 96 cm high and has an internal diameter of 10.5cm. The water  
399 inlet to the column is fixed at an angle of 30° at the base to induce spinning of the water  
400 column as it twists through the anion resin. The flow is adjusted to maintain the resin  
401 suspended in the internal column but also to minimise overspillage in the larger container.

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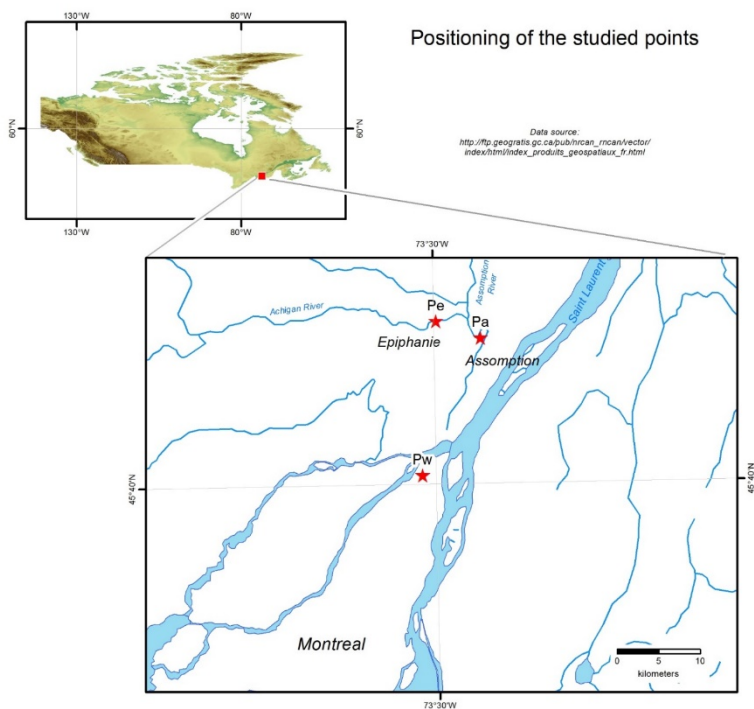
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415 Figure 2. Location of sample sites within the Montréal region used for initial application of  
416 TSM protocol.

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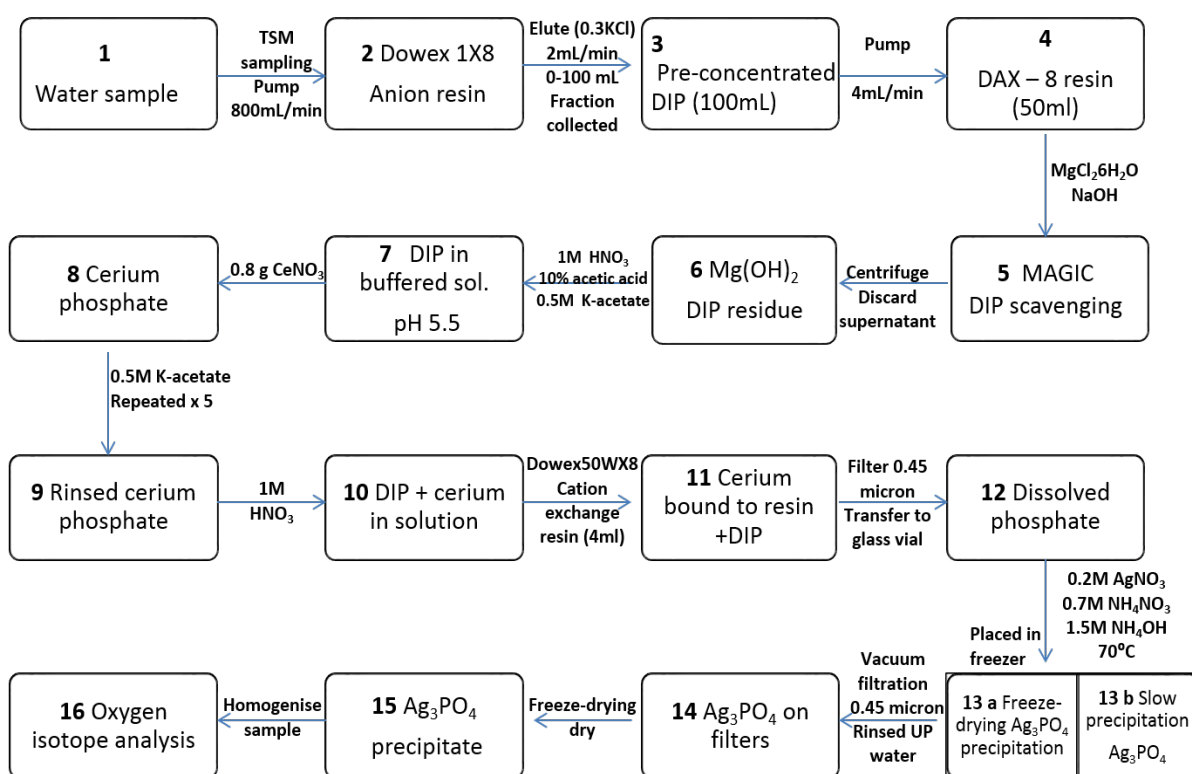
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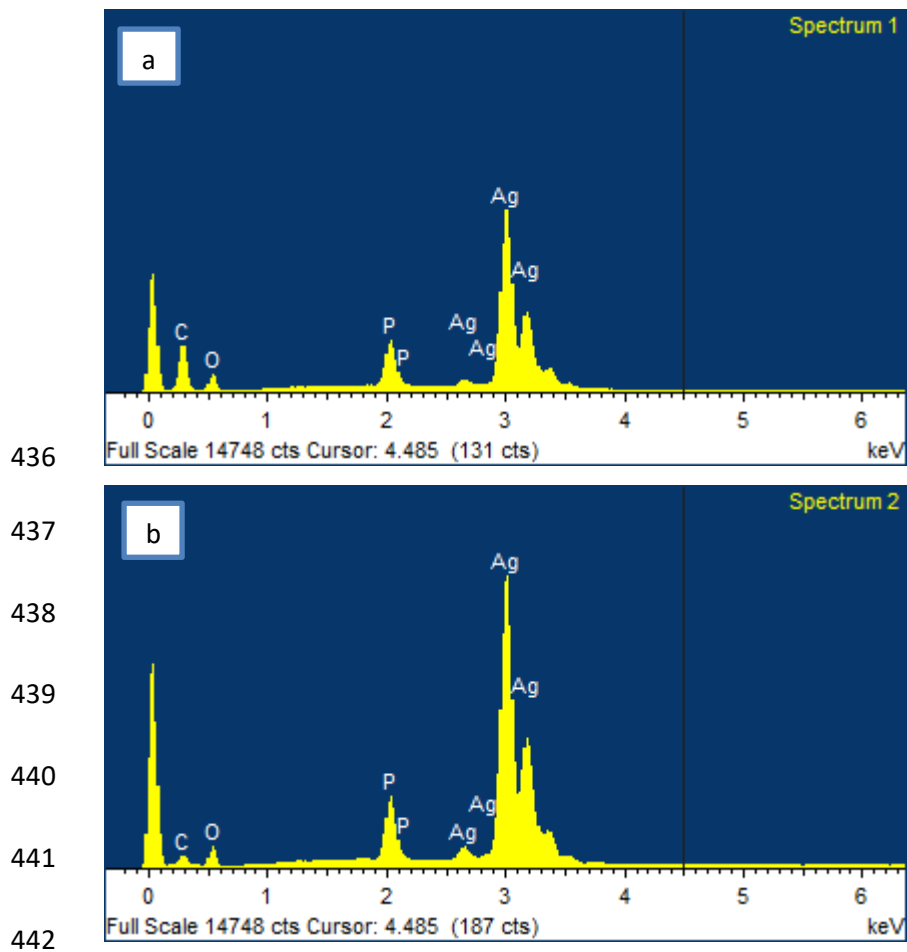
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433 Figure 3. Summary of Twist Spinning Mode (TSM) protocol for determination of <sup>18</sup>Op

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444 Figure 4. SEM spectra for  $\text{Ag}_3\text{PO}_4$  synthesized from DIP and precipitated by a) the slow precipitation  
 445 method and b) the Freeze-Drying method.

449 Table 1. Properties of synthetic solutions used in laboratory evaluations of TSM protocol. Pure humic  
 450 acid was used as source of DOC for this synthetic solution.

	<b>Solution 1</b>	<b>Solution 2</b>	<b>Solution 3</b>
$\text{NO}_3$ (mg/L)	0.5	5	11
$\text{SO}_4$ (mg/L)	0.2	27	57
Humic Acid (mg/L)	0.5	5	10
Conductivity ( $\mu\text{S}/\text{cm}$ )	15	130	200

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453 Table 2. Initial mass of P in synthetic solutions and final mass of P eluted from anion resins during  
 454 during laboratory evaluations of TSM protocol.

	<b>Solution 1</b>	<b>Solution 2</b>	<b>Solution 3</b>
<b>KH<sub>2</sub>PO<sub>4</sub></b>			
Total Mass P in (mg)	16.30	16.30	16.30
Mass P eluted (mg)	0.98	0.95	0.25
% Yield	6.0	5.8	1.5
<b>Fertiliser 20%P<sub>2</sub>O<sub>5</sub></b>			
Total Mass P in solution (mg)	19.56	19.56	19.56
Mass P eluted mg	0.78	1.08	0.52
% Yield	4.0	5.7	2.7

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457 Table 3. Measured  $\delta^{18}\text{O}_p$  for each synthetic test solution containing  $\text{KH}_2\text{PO}_4$  or a 20% inorganic  
 458 fertiliser after the TSM extraction and precipitation protocol. The measured  $\delta^{18}\text{O}_p$  for  $\text{KH}_2\text{PO}_4$   
 459 synthesised from solution is 9.0‰ and for inorganic fertilizer is 16.6‰. The oxygen yield expected  
 460 for stoichiometrically-pure  $\text{Ag}_3\text{PO}_4$  is 15.3%. The P concentrations are those originally in 1000 L  
 461 synthetic solutions used in these laboratory tests.

	<b>Source Phosphate</b>	<b>P (<math>\mu\text{gL}^{-1}</math>)</b>	<b><math>\delta^{18}\text{O}_{\text{PO}_4}</math> (<math>\pm 0.3</math> ‰ vs VSMOW)</b>	<b><math>\text{O}_{\text{Ag}_3\text{PO}_4}</math> (% weight, <math>\pm 0.2</math> ‰)</b>
<b>Solution 1</b>	$\text{KH}_2\text{PO}_4$	16.3	9.3	16.2
	Fertilisers 20%	19.5	16.3	15.8
<b>Solution 2</b>	$\text{KH}_2\text{PO}_4$	16.3	8.6	16.1
	Fertilisers 20%	19.5	16.4	16.0
<b>Solution 3</b>	$\text{KH}_2\text{PO}_4$	16.3	9.5	15.8
	Fertilisers 20%	19.5	16.4	15.6

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464 Table 4. Silver phosphate yields, measured  $\delta^{18}\text{O}_p$  and oxygen content of  $\text{Ag}_3\text{PO}_4$  for samples  
 465 prepared by freeze drying (FD) or slow precipitation (SP) techniques.

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467	<b>Precipitation method</b>	<b>Sample</b>	<b>Yield (%)</b>	$\delta^{18}\text{O}_p$ <b>(‰)</b>	$\text{O}_{\text{Ag}_3\text{PO}_4}$ <b>(%)</b>
468	FD	1	86.7	8.7	15.4
469		2	89.3	8.9	14.7
470		3	90.3	8.5	14.9
471		4	93.0	8.6	14.9
472		5	103.1	9.0	15.2
473	<b>Mean</b>		<b>92.5</b>	<b>8.7</b>	<b>15.0</b>
474	SP	1	88.4	8.5	14.7
475		2	81.7	8.5	14.9
476		3	74.6	8.6	14.1
477		4	64.2	8.3	15.1
478		5	104.2	9.0	14.9
479	<b>Mean</b>		<b>82.6</b>	<b>8.6</b>	<b>14.7</b>

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480 Table 5. Data from application of TSM and MMM protocols to determine phosphate oxygen isotope  
481 composition of samples from the Saint Lawrence river system. Equilibrium value is calculated using  
482 the approach reported by Chang and Blake (2015).

483

<b>Sample</b>	<b>P</b>	<b>SEC</b>	<b>T</b>	<b><math>\delta^{18}\text{O}_{\text{H}_2\text{O}}</math></b>	<b><math>\text{Ag}_3\text{PO}_4</math></b>	<b><math>\text{Ag}_3\text{PO}_4</math></b>	<b><math>\delta^{18}\text{O}_\text{P}</math></b>	<b><math>\delta^{18}\text{O}_\text{P}</math></b>	<b><math>\delta^{18}\text{O}_\text{P}</math></b>
	( $\mu\text{g L}^{-1}$ )	( $\mu\text{s cm}^{-1}$ )	( $^\circ\text{C}$ )	( $\text{‰}$ )	(MMM) (mg)	(TSM) (mg)	(MMM) ( $\text{‰}$ )	(TSM) ( $\text{‰}$ )	Equilibrium ( $\text{‰}$ )
Pw	500	1472	6.2	-9.34	21.0	0.2	17.4	18.3	15.9
Pa	9.1	196	0.4	-10.47	0.1	34.4	-	23.9	15.8
Pe	9.8	298	0.4	-9.99	0.2	30.9	-	21.4	16.3

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