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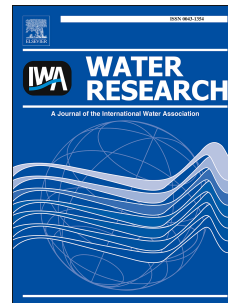
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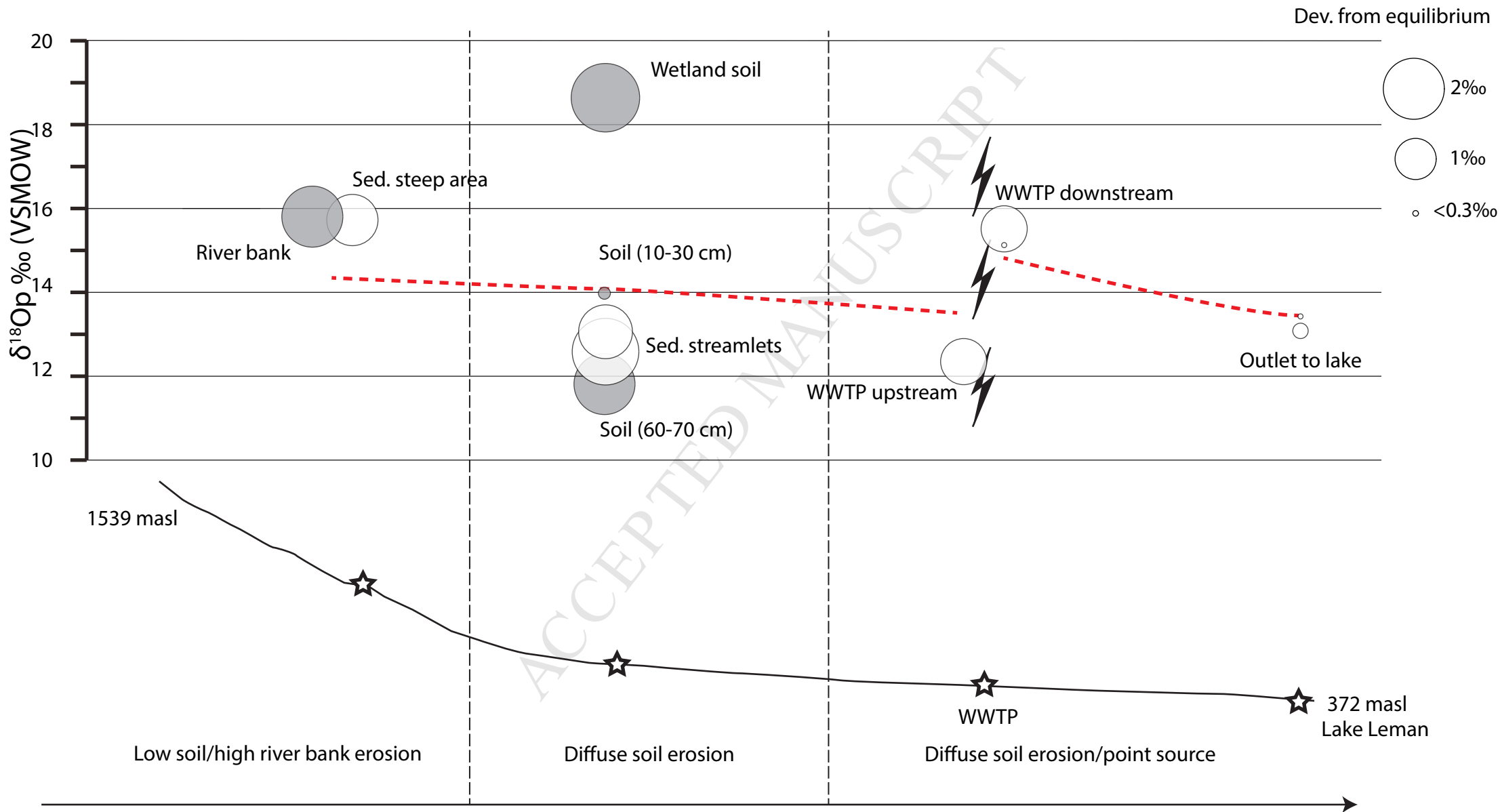
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1 **Tracing the sources and cycling of phosphorus in river sediments using oxygen isotopes:**  
2 **methodological adaptations and first results from a case study in France**

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11

12 **Abstract**

13 An essential aspect of eutrophication studies is to trace the ultimate origin of phosphate ions (P-  
14 PO<sub>4</sub>) associated with the solid phase of river sediments, as certain processes can make these  
15 ions available for algae. However, this is not a straightforward task because of the diversity of  
16 allochthonous and autochthonous sources that can supply P-PO<sub>4</sub> to river sediments as well as  
17 the existence of in-stream processes that can change the speciation of these inputs and obscure  
18 the original sources. Here, we present the results of a study designed to explore the potentials,  
19 limitations and conditions for the use of the oxygen isotope composition of phosphate ( $\delta^{18}\text{O}_\text{p}$ )  
20 extracted from river sediments for this type of tracing. We first tested if the method commonly  
21 applied to soils to purify P-PO<sub>4</sub> and to measure their  $\delta^{18}\text{O}_\text{p}$  concentrations could be adapted to  
22 sediments. We then applied this method to a set of sediments collected in a river along a  
23 gradient of anthropogenic pressure and compared their isotopic signatures with those from

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24 samples that are representative of the potential P-PO<sub>4</sub> inputs to the river system (soils and  
25 riverbank material).  
26 The results showed that following some adaptations, the purification method could be  
27 successfully transposed to river sediments with a high level of P-PO<sub>4</sub> purification (> 97%) and  
28 high  $\delta^{18}\text{O}_p$  measurement repeatability and accuracy (< 0.4‰). The values for the potential  
29 allochthonous sources varied from 11.8 to 18.3‰, while the  $\delta^{18}\text{O}_p$  value for the river sediments  
30 ranged from 12.2 to 15.8‰. Moreover, a sharp increase (> 3‰) in the sediment  $\delta^{18}\text{O}_p$  value  
31 immediately downstream from the discharge point revealed the strong impact of municipal  
32 wastewater. The calculation of the theoretical equilibrium  $\delta^{18}\text{O}_p$  values using the river water  
33 temperature and  $\delta^{18}\text{O}_w$  showed that the downstream sediments were in equilibrium, which was  
34 not the case for the upstream sediments. This difference could be related to the contrast  
35 between the short residence time of the transfer system in the catchment head, which can  
36 preserve the isotopic variability of the source materials, and the longer residence times and  
37 higher P bioavailability in the lower catchment, possibly fostering the recycling of P-PO<sub>4</sub> by  
38 the biota and the equilibration of the oxygen isotope signature in P-PO<sub>4</sub>. These results  
39 demonstrate the potential of the isotopic approach to assess the sources and in-stream turnover  
40 of sedimentary P in river systems.

41  
42 **KEYWORDS:** Phosphorus transfer, Agriculture, In-stream processes, Eutrophication,  
43 Phosphate oxygen isotopes

44  
45 **1. Introduction**  
46  
47 Phosphorus (P) associated with the solid phase and designated as “particulate-P” often accounts  
48 for the major part of phosphorus transferred from terrestrial to aquatic ecosystems (Kleinman et

49 al., 2011; Kronvang et al., 2007; Sharpley et al., 1999). The impact of particulate-P on the  
50 trophic state and eutrophication of the receiving aquatic ecosystems, especially lentic  
51 ecosystems, depends on the size of its potentially bioavailable fraction. The amount of P that  
52 occurs as inorganic phosphate ions (P-PO<sub>4</sub> pools), either in solution (dissolved P-PO<sub>4</sub>), sorbed  
53 onto, or precipitated within stream and river sediments, is thought to represent the most readily  
54 bioavailable P pool (Moorleggem et al., 2013). Therefore, an essential aspect of studies  
55 dedicated to the restoration of eutrophicated aquatic ecosystems is to quantify P-PO<sub>4</sub> pools in  
56 river water and sediments and to trace the ultimate origin of these pools (Kleinman et al., 2011;  
57 Mellander et al., 2015).

58 However, this quantification is not a straightforward task because of the diversity of the  
59 allochthonous and autochthonous sources that may supply P-PO<sub>4</sub> to river sediments. Several  
60 allochthonous compartments in the catchment can supply particulate-P to river sediments,  
61 including pristine and weathered basement rocks, forested or agricultural soils, riverbank  
62 sediments, etc. For example, the autochthonous addition of P-PO<sub>4</sub> to the sediments can occur  
63 through sorption onto the sediments of dissolved P-PO<sub>4</sub> ions supplied by sewage effluents or  
64 the in-stream mineralization of sewage organic matter (Poulenard et al., 2008). The in-stream  
65 retention of dissolved P-PO<sub>4</sub> by the sediments primarily occurs during low-flow periods when  
66 the retention time of the sediment is long enough to allow the necessary physical-chemical  
67 reactions to develop (Dorioz et al., 1998a, 1989; Ensign et al., 2006; Pistocchi et al., 2012) and  
68 is particularly intense immediately downstream of the point source pollution inputs.

69 Autochthonous P-PO<sub>4</sub> pools can also come from the recycling of allochthonous particulate-P by  
70 aquatic flora or by microorganisms in the sediment. The biological uptake by biofilm is another  
71 important seasonal process that is involved in the retention of P-PO<sub>4</sub> ions by the sediment  
72 (Wang et al., 1999). These in-stream recycling and retention processes, which help the P to turn  
73 over within the river ecosystem, are expected to be triggered by the bioavailability of

74 allochthonous P and to regulate the internal productivity of the river system. Their  
75 quantification can be used to assess the efficiency of a river system to recycle and transform  
76 any particulate P into more bioavailable P-PO<sub>4</sub>, but an efficient recycling can eventually  
77 obscure the ultimate origin of the P-PO<sub>4</sub> exported at the outlet.

78 It is often impossible to quantify and trace the different sources of particulate-P that are  
79 potentially involved in eutrophication using only the variability in P speciation as determined  
80 by classical chemical extraction methods (Burrus et al., 1989; Pacini and Gächter, 1999;  
81 Poulenard et al., 2009; Walling et al., 2008). As a result, there a need to develop tools or/and  
82 strategies that can be reliably used to trace this important P compartment, particularly its  
83 dominant inorganic P-PO<sub>4</sub> fraction, and to understand the extent and rate at which particulate-P  
84 is biologically recycled and modified within streams and rivers.

85 Oxygen isotopes could provide one such possible tool. The oxygen isotopic composition of  
86 phosphate ( $\delta^{18}\text{O}_\text{p}$ ) has already been successfully used to trace P sources and study the P-PO<sub>4</sub>  
87 cycle in soils, freshwater and marine sediments (Davies et al., 2014; Goldhammer et al., 2011;  
88 Goody et al., 2016; Gruau et al., 2005; Melby et al., 2013; Tamburini et al., 2014). This  
89 approach is based on: i) the existence of measurable differences in the abundance ratio for <sup>18</sup>O  
90 and <sup>16</sup>O atoms in the phosphate group of potential P-PO<sub>4</sub> sources (e.g. primary P-rich minerals,  
91 chemical fertilizers, chemical detergents, phosphate from wastewater, etc.); and ii) the fact that  
92 biologically mediated processes have the possibility to modify  $\delta^{18}\text{O}_\text{p}$  in a predictable way  
93 (Blake et al., 2005). The first property allows the oxygen isotope composition of P-PO<sub>4</sub> to be  
94 potentially used as a source tracer in environmental systems, whereas the second property  
95 allows it to be used to quantify the rate of biological recycling of P-PO<sub>4</sub>. Two enzymatic  
96 processes are mainly responsible for changes in the  $\delta^{18}\text{O}_\text{p}$  value. One is the isotope  
97 equilibration between oxygen in P-PO<sub>4</sub> and oxygen in ambient water. This process, which is  
98 caused by reversible phosphorylation/dephosphorylation reactions that are catalyzed by the

99 inorganic intracellular pyrophosphatase enzyme (Blake et al., 2005), is temperature dependent  
100 and has been described by an empirical equation (Longinelli and Nuti, 1973) that was recently  
101 revised by Chang and Blake (2015). The second is the kinetic isotope fractionation produced by  
102 the enzymatic hydrolysis of phosphoesters (Liang and Blake, 2006; von Sperber et al., 2015,  
103 2014), the magnitude of which depends on the type of enzyme involved (e.g. acid or alkaline  
104 phosphatases) and the final effect of which on the isotopic signature of the recycled P-PO<sub>4</sub> also  
105 depends on the  $\delta^{18}\text{O}_p$  value for the original substrate, i.e. inheritance effect (Liang and Blake,  
106 2006). Small kinetic isotope effects are associated with the initial stages of the abiotic reaction,  
107 such as the sorption of P-PO<sub>4</sub> onto the solid phases (Jaisi et al., 2010; Melby et al., 2013b) or  
108 biological uptake (Blake et al., 2005).

109 In freshwater ecosystem studies, the use of  $\delta^{18}\text{O}_p$  is at an early stage (Davies et al., 2014). For  
110 river sediments, the lack of a standardized method to extract, purify and analyze  $\delta^{18}\text{O}_p$  is a  
111 hinderance. In this study, we first adapted the method that is commonly used for soils to  
112 sediments and validated the capability of this method to provide precise and accurate results.  
113 We then applied this method to riverbed sediments from a river in the French Alps with the  
114 following objectives: i) to characterize the variability of the  $\delta^{18}\text{O}_p$  signatures in the riverbed  
115 sediments and the potential external sediment sources (soils, riverbank materials) in an  
116 exemplar river system; ii) to explore the potential, limitations and conditions of using the  
117 isotopic tool to assess the respective role of external inputs and internal recycling processes to  
118 build up the P-PO<sub>4</sub> budget of the riverbed sediments in this river.

119

## 120 **2. Materials and methods**

121

### 122 **2.1 Site description and sampling**



123 The Redon River is a small alpine torrential tributary (i.e. a shallow high-gradient, high-energy  
124 stream with an annual average discharge of  $0.5 \text{ m}^3/\text{s}$  at the outlet) of Lake Geneva (France).  
125 The sediment dynamic of this river has been already intensively studied (Al-Charideh, 1988;  
126 Dorioz et al., 1998b; Feuillade and Dorioz, 1992; Hamid et al., 1989; Poulenard et al., 2008)  
127 making it a particularly appropriate site for the present exploratory study. The total catchment  
128 surface area is 3,300 ha with a land use that consists mainly of forests (1,100 ha) in its upland  
129 part and grasslands (1,200 ha) and cultivated farmlands (300 ha) in its lowland part. Several  
130 wetlands (20 ha) are present, while urbanized areas are concentrated near the outlet, close to the  
131 shore of Lake Geneva. The Redon River is impacted by both point and diffuse sources of P.  
132 The main point source of P is located in the lower part of the river and consists of inputs of  
133 predominantly dissolved P from a wastewater treatment plant (WWTP), whereas the main  
134 diffuse sources occur in the upper part of the catchment, consisting mainly of particulate-P  
135 from agricultural and forested lands (Fig. 1).  
136 The soils are mainly Cambisols, formed from the weathering products of quaternary moraine  
137 material consisting of a large range of mainly calcareous sedimentary, metamorphic and  
138 igneous rocks from the Rhône glacier (Nicoud et al., 1993). The soil mineralogy is comprised  
139 of a mixture of minerals inherited from the weathering of this heterogeneous parent material  
140 and their secondary weathering products. Previous studies (Dorioz et al., 1998a, 1998b) have  
141 shown that the Redon River sediments are  $\text{CaCO}_3$ -rich (10 to 20%  $\text{CaCO}_3$ ,  $\text{pH} > 7$ ). Their fine  
142 ( $< 200 \mu$ ) fraction is dominated by loamy and sandy materials, with an organic matter content  
143 between 2 to 4.5%. The clay fractions (7-11%) consist mostly of soil-derived illite, biotite and  
144 chlorite (Poulenard et al., 2008). The water in the Redon River is bicarbonated-calcic.

145

146 [Figure 1]

147

## 148 2.2 Sampling strategy and sample preparation

149 Details regarding the sampling sites and sampling conditions are given in Tables 1 and 2. Three  
150 types of samples were collected: i) eight riverbed sediments collected at six locations (A, B, C,  
151 D, E and F in Fig. 1) along a downstream gradient of land use that changes from forested to  
152 mixed agricultural/urban areas, thus representing a gradient of increasing anthropogenic  
153 pressure; this includes two samples collected in streamlets draining a small agricultural sub-  
154 catchment (locations A and B in Fig. 1) that is representative of the type of agriculture  
155 practiced in the Redon River catchment (Dorioz and Ferhi, 1994); ii) four samples  
156 representative of the potential sources of river sediments, including cultivated topsoil and deep  
157 horizon, riverbank material, and soil from a 2 ha wetland, hydraulically connected to the river;  
158 and iii) two samples from sediments from Lake Geneva representing an average reference for  
159 the local superficial lacustrine sediments (collected in 2012 and provided by the EDYTEM  
160 laboratory).

161 Given that it was difficult to access the river bed, it was not possible to visit all six riverbed  
162 sediment sampling locations at the same time. Three locations were first sampled at the end of  
163 March 2012 (B, C and F), and five (A, B, D, E and F) were sampled (again) at the beginning of  
164 September 2012. Both periods were low-flow periods, where the September period corresponds  
165 to the lowest weekly discharge period of the year. Samples were taken in areas of preferential  
166 accumulation of fine sediments. At each location, single samples of the entire sediment matrix  
167 were taken manually at 3-5 different points and then mixed to obtain an average sample (0.5-1  
168 kg dry weight). Simultaneously, the electric conductivity (EC) was measured and a water  
169 sample was collected to determine the SRP (soluble reactive phosphorus) concentration and  
170 water oxygen isotope composition ( $\delta^{18}\text{O}_w$ ). Hourly water temperatures were obtained from  
171 automatic recorders at the outlet. The riverbank sample was collected in the upstream, forested  
172 part of the catchment (see Fig. 1). The cultivated soil samples were collected in the same small

173 sub-catchment used to sample the streamlet sediments (Fig. 1), as this sub-catchment is known  
174 to represent zones of maximum soil erosion risk in the Redon catchment (Dorioz and Ferhi,  
175 1994). In addition to taking a topsoil sample, a deeper soil sample was taken at a depth of  
176 approximately -70 cm to account for the possible erosion of deep soil materials along ditches  
177 or/and soil embankments. These two samples were obtained by pooling five subsamples (200-  
178 300 g) collected in different locations within a single field or its embankment. The pooled  
179 samples were then air-dried and sieved at 200  $\mu\text{m}$  before analysis. No river water samples were  
180 collected for the analysis of the  $\delta^{18}\text{O}_\text{p}$  values for the dissolved P- $\text{PO}_4$ , although these could  
181 have been useful in the interpretation of the riverbed sediment data. The decision not to sample  
182 the river water was due to the very low concentrations of dissolved P- $\text{PO}_4$  recorded in this river  
183 (Table 1), which makes it extremely difficult to analyze the  $\delta^{18}\text{O}_\text{p}$  in the current state of the  
184 analytical protocols.

185  
186 [Table 1]

187  
188 [Table 2]

189

## 190 **2.2 Sediment and soil P chemical analyses**

191

192 The total phosphorus (Pt) and Olsen P ( $\text{P}_{\text{Olsen}}$ ) concentrations were determined in all of the  
193 samples.  $\text{P}_{\text{Olsen}}$  (Olsen et al., 1954), which is considered as being representative of the  
194 bioavailable fraction of P in soils, has been shown to correlate linearly with P extracted from  
195 the sediment in bio-assays with algae (Jordan-Meille, 1998). The Pt concentrations were  
196 measured after a hot sodium hydroxide digestion. The total inorganic and organic P in the  
197 samples were determined according to Saunders and Williams (1955) as described by Fares et

198 al. (1974). The spectrophotometric determination of P-PO<sub>4</sub> in all of the extracts/digestates was  
199 carried out using the molybdenum blue method (Murphy and Riley, 1962) at 882.0 nm on a  
200 Cary 50 UV-Vis spectrometer (Agilent).

201

## 202 **2.3 Isotopic analyses and data treatment**

### 203 *2.3.1 Extraction and purification protocol*

204 We used the extraction and purification protocol initially developed by Tamburini et al. (2010)  
205 for soils (hereafter referred as to Tb10), which we adapted for sediments. The Tb10 protocol  
206 consists of the following six steps:

207 i) extraction of phosphate from the soil/sediment with 1 M HCl, which in sequential extraction  
208 mainly targets the P-PO<sub>4</sub> pool (HCl-P) associated with Ca, but in our case (singular extraction)  
209 also P sorbed onto Fe and Al oxides;

210 ii) dissolved organic matter (DOM) removal from the solution using an Amberlite resin,

211 iii) ammonium phospho-molybdate (APM) precipitation and subsequent dissolution,

212 iv) magnesium ammonium phosphate (MAP) precipitation and dissolution,

213 v) cation removal by a cation exchange resin (BioRad, AG50 x8),

214 vi) silver phosphate (Ag<sub>3</sub>PO<sub>4</sub>) precipitation through the addition of an Ag-ammine solution to  
215 the purified phosphate solution.

216 The goal of these steps, after HCl extraction, is to avoid sources of oxygen (i.e. DOM, nitrate,  
217 etc.) other than P-PO<sub>4</sub> and other contaminants, e.g. chloride, in the final silver phosphate  
218 precipitate.

219 We modified the Tb10 protocol in several ways as follows (Fig. 2 in the supplementary  
220 material). First, the samples were sieved at 200 µm instead of 2 mm, since this finer fraction  
221 contains the bulk of the bio-geochemically active P in river sediments (Poulenard et al., 2008).  
222 The liquid-to-solid ratio in the extraction step was modified to 100:1 instead of 50:1, since the

223 pre-tests showed that, with our highly carbonated sediments, the solution did not reach the  
 224 targeted pH ( $1 \pm 0.5$ ). The quantity of sediment was adjusted to have at least 10  $\mu$ moles of P for  
 225 the purification, corresponding to 10 to 20 g of sieved sediments. The DOM removal step using  
 226 an Amberlite resin was not performed as the subsequent steps successfully eliminated the  
 227 organic matter (see below). Finally, for samples with a high calcium carbonate content, an  
 228 additional step had to be introduced to account for the formation of a calcium molybdate  
 229 precipitate during the APM precipitation. To eliminate the precipitate, we repeated the  
 230 subsequent MAP precipitation step twice.

231 The 1 M HCl extraction may hydrolyze some of the organic or condensed P (Turner et al.,  
 232 2005) and the released Pi may bias the isotopic composition of the inorganic P pool targeted  
 233 here. To assess the existence of such a possible artifact, extractions were run in duplicate using  
 234  $^{18}\text{O}$ -labeled and unlabeled reagents. An  $^{18}\text{O}$ -labelled 1M HCl solution was prepared by adding  
 235 approximately 0.05 ml of  $^{18}\text{O}$ -enriched water (97‰, Aldrich) to 1 liter of 1 M HCl solution.  
 236 Labeled and unlabeled solutions were analyzed for  $\delta^{18}\text{O}_w$ . If hydrolysis occurred, it should  
 237 result in different isotopic compositions depending on whether labeled or unlabeled reagents  
 238 were used, i.e. a higher  $\delta^{18}\text{O}_p$  for the labeled sample compared to the unlabeled one. In this case,  
 239 we calculated the “real” isotopic value by a revised version of the mass balance equation used  
 240 by McLaughlin et al. (2006b):

$$241 \quad \delta^{18}\text{O}_p = \frac{(\delta^{18}\text{O}_{pl} \cdot \delta^{18}\text{O}_{wnl} - \delta^{18}\text{O}_{pnl} \cdot \delta^{18}\text{O}_{wl})}{(\delta^{18}\text{O}_{pl} - \delta^{18}\text{O}_{pnl} - \delta^{18}\text{O}_{wl} + \delta^{18}\text{O}_{wnl})} \quad (1)$$

242 where  $\delta^{18}\text{O}_{pl}$  and  $\delta^{18}\text{O}_{pnl}$  are the isotopic composition of P- $\text{PO}_4$  for the labeled and unlabeled  
 243 samples, respectively, and  $\delta^{18}\text{O}_{wl}$  and  $\delta^{18}\text{O}_{wnl}$  are the isotopic composition of water for the  
 244 labeled and unlabeled reagents, respectively.

245 The efficiency and reliability of the modified Tb10 protocol used here were evaluated by  
 246 considering the repeatability of the extraction/purification process, the final  $\text{Ag}_3\text{PO}_4$  yield and  
 247 its purity. The purity was assessed by i) dissolving the obtained  $\text{Ag}_3\text{PO}_4$  and comparing the

248 actual concentration of phosphate in the solution with the theoretical concentration, i.e. the  
249 amount expected if all of the solid mass was  $\text{Ag}_3\text{PO}_4$ , and ii) determining the C and N  
250 concentration of the obtained  $\text{Ag}_3\text{PO}_4$  on the elemental analyzer (Flash 2000, Termo  
251 Scientific); these two concentrations are used as indicators of possible contamination by  
252 organic matter and/or nitrate. The purity of the obtained  $\text{Ag}_3\text{PO}_4$  crystals was also assessed  
253 through quantification of the oxygen yield during mass spectrometry analyses. This was done  
254 by graphically comparing the weight of the  $\text{Ag}_3\text{PO}_4$  standards and samples introduced into the  
255 machine against the peak area of the produced CO (“oxygen yield”), which is proportional to  
256 the oxygen content in the sample. If other sources of oxygen or contaminants were present in  
257 the sample, the oxygen yield should be different from the standard  $\text{Ag}_3\text{PO}_4$ . P- $\text{PO}_4$  losses at  
258 each step of the purification were also quantified (see Pistocchi et al. 2014 for more details).

### 259 *2.3.2 Isotopic analyses and calculation of the theoretical equilibrium values*

260 The oxygen isotope composition of the purified  $\text{Ag}_3\text{PO}_4$  was measured using a thermal  
261 conversion elemental analyzer (Variopyro Cube Elementar), coupled to an IsoPrime100  
262 isotopic ratio mass spectrometer (IRMS) at the Plant Nutrition Group laboratory (ETH Zurich).  
263 Two to three replicates of each sample were weighed (300 to 600  $\mu\text{g}$ ) in silver capsules with a  
264 small added amount of glassy carbon powder. The temperature of the TC/EA furnace was kept  
265 at 1450°C for pyrolysis.  
266 During each run, repeated measurements of an internal standard (Acros Organics  $\text{Ag}_3\text{PO}_4$  >  
267 97.5%,  $\delta^{18}\text{O}_p = +14.2\text{‰}$ ) and two international benzoic acid standards (IAEA 601 and IAEA  
268 602,  $\delta^{18}\text{O} = +23.3\text{‰}$  and  $+71.4\text{‰}$ , respectively, IAEA, Vienna, Austria) were included and  
269 used to calibrate and correct the instrumental drift. The  $\delta^{18}\text{O}_p$  values presented in this paper are  
270 reported in the standard  $\delta$  notation with respect to VSMOW (Vienna Standard Mean Oceanic  
271 Water), i.e. as ‰ deviation values. Analytical uncertainties, as determined from the replicate  
272 analysis of the standards, were estimated to be below 0.3‰.

273 In order to assess the extent to which the sedimentary P-PO<sub>4</sub> pools investigated in this study  
 274 could be secondary recycled P-PO<sub>4</sub> (i.e. P-PO<sub>4</sub> that has been cycled by the river biota), a  
 275 theoretical equilibrium  $\delta^{18}\text{O}_p$  value – i.e. the oxygen isotopic composition the phosphate should  
 276 have if it is in equilibrium with the ambient water – was calculated for each sample using the  
 277 following equation (Chang and Blake, 2015):

$$278 \quad \delta^{18}\text{O}_p = (\delta^{18}\text{O}_w + 10^3) \cdot e^{\frac{\left[\left(\frac{14.43 \cdot 10^3}{T}\right) - 26.54\right]}{10^3}} - 10^3 \quad (2)$$

279  
 280 where  $\delta^{18}\text{O}_p$  is the calculated oxygen isotopic composition of the P-PO<sub>4</sub> sample at equilibrium  
 281 in ‰,  $\delta^{18}\text{O}_w$  is the measured oxygen isotopic composition of the water in ‰, and T is the  
 282 temperature in degrees Kelvin.

283 Considering the stability of the  $\delta^{18}\text{O}_w$  values during low-flow periods in the Redon River due to  
 284 the dominance of the ground water contribution to the discharge during these periods (Al-  
 285 Charideh, 1988), we used the  $\delta^{18}\text{O}_w$  of the river water measured at each sampling date as the  
 286 reference isotopic value for water in equation 2. We considered both the minimum and  
 287 maximum temperatures measured over the period running between the sampling date and the  
 288 closest big storm flow event (around 10-20 days; Table 1 in the Supplementary Material), since  
 289 the sediments are (partially) renewed at each storm flow. The temperatures measured at the  
 290 time of sampling fell within these ranges. For samples 9 to 12 (sources), we used the  $\delta^{18}\text{O}_w$   
 291 values measured in the local water sources, including rain, groundwater and soil water data,  
 292 and the extremes for the soil temperature measured within the previous 10 days at an agro-  
 293 meteorological station close to the studied sites. This allowed us to have a conservative and  
 294 time-integrated estimate of the equilibrium interval. For these samples, we also calculated the  
 295 deviation from equilibrium using the average stream water temperature and isotopic  
 296 composition, to verify whether or not their  $\delta^{18}\text{O}_p$  values would fall within the equilibrium range  
 297 once transferred into the river. For each sample, the deviation from equilibrium was calculated

298 as the interval (in ‰) between the measured  $\delta^{18}\text{O}_p$  values and the closest values for the  
299 equilibrium range calculated from equation 2

300

### 301 **3. Results**

302

#### 303 **3.1 Method validations**

304 Repeated extractions and purifications ( $n = 3$  or  $4$ ) on the same sediment sample yielded  $\delta^{18}\text{O}_p$   
305 values with a standard deviation  $< 0.4$  ‰. This repeatability can be considered as acceptable,  
306 since it is within the range of the precision obtained on the repeated standards measurements on  
307 the TC/EA-IRMS.

308 The purity of  $\text{Ag}_3\text{PO}_4$  was 97%, as determined by analyzing the P- $\text{PO}_4$  in the redissolved  
309  $\text{Ag}_3\text{PO}_4$  (see section 2.3.1, Table 2 in the Supplementary Material). The carbon and nitrogen  
310 concentrations were generally lower than 1% and 0.1%, respectively (Table 2 in the  
311 Supplementary Material). The C content was comparable to that measured in the internal  
312 standard  $\text{Ag}_3\text{PO}_4$  (0.5%) and much lower than the 23-29% or 0.2% reported by Weiner et al.  
313 (2011) for C and N, respectively. The efficiency of the purification procedure was confirmed  
314 by the oxygen yield given by the mass spectrometer (Fig. 1 in the Supplementary Material). All  
315 of the samples were close to the regression line defined by the  $\text{Ag}_3\text{PO}_4$  standards, indicating  
316 that the amount of oxygen in the samples was close to the stoichiometry of the pure compound.  
317 Most of the labeled/unlabeled pairs did not show significantly different  $\delta^{18}\text{O}_p$  values. Only four  
318 samples (2, 5, 8 and 9) showed different labeled and unlabeled values, meaning that the organic  
319 P or condensed phosphate was hydrolyzed during the HCl extraction. These values have been  
320 corrected with equation 1, as all of the other values are the average of the labeled and unlabeled  
321 results.

322



### 323 **3.2 P speciation**

324 Table 3. P speciation and isotopic data

325

326 The P content and speciation varied strongly from one sample to another, with the Pt value  
327 ranging from 459 to 1256 mgP/kg, Pi from 179 to 631 mgP/kg and Po from 36 to 450 mgP/kg.  
328  $P_{\text{Olsen}}$  accounted for 0.7 to 10.6 % of Pt depending on the samples. The highest  $P_{\text{Olsen}}$   
329 concentrations (65-67 mgP/kg, representing 8.4 to 10.6% of Pt) were found in the river  
330 sediments sampled immediately downstream from the WWTP discharge. These variations are  
331 the consequence of differences in the source, erosion and deposition conditions of the  
332 sediments as well as differences in the P inputs that accumulate along the river (Table 1). The  
333 downstream sediments (samples 5 to 8) and streamlet sediments (samples 1 and 2) were  
334 enriched in Pt, Pi and  $P_{\text{Olsen}}$  compared to the sediments coming from the head of the catchment  
335 (samples 3) or to the riverbank material (sample 12). The same relative enrichment is observed  
336 for the cultivated topsoil (sample 10) compared to its lower horizon (sample 11).  
337 Pt and  $P_{\text{Olsen}}$  were weakly and positively correlated ( $r^2 = 0.49$ ,  $p < 0.01$ ) as well as the  $P_{\text{Olsen}}/P_i$   
338 ratio with Pt concentrations of  $r^2 = 0.76$  ( $p < 0.01$ ), providing evidence that the P enrichment  
339 process recorded by the downstream sediments is accompanied by an increase in the most  
340 reactive fraction of the total P- $\text{PO}_4$  pool. The highest Po concentrations were found in the  
341 topsoil samples (9 and 10).

342

### 343 **3.3. Measured isotopic signatures and comparison with the equilibrium values**

344 The measured  $\delta^{18}\text{O}_p$  values for the HCl extracts varied between +11.8‰ (deep soil horizon,  
345 sample 11) and +18.3‰ (wetland soil, sample 9; Fig. 2a). No significant statistical  
346 relationships were found between the  $\delta^{18}\text{O}_p$  values for the samples and their Pt,  $P_{\text{Olsen}}$  or Pi  
347 content or ratios, nor with the water characteristics, notably the SRP concentrations. The

348 riverbank sample collected in the head of the catchment (sample 12) corresponds to a weakly  
349 weathered moraine, as confirmed by field observations, i.e. no visible weathering and no  
350 vegetation or biofilm on the banks, since they are eroded at each storm flow, and by the P  
351 speciation data (very low  $P_{O_{isen}}$  and  $P_o$  fractions; see Table 3). This sample is representative of  
352 both the principal source of the upstream, unpolluted sediments and the parent material of most  
353 of the soils in this catchment (CIPEL, 1988). Its  $\delta^{18}O_p$  value (+15.9‰) indicates that it has a  
354 mixed igneous/sedimentary origin, as this value is intermediate between the  $\delta^{18}O_p$  values  
355 reported for igneous or metamorphic apatite (approximately +7‰; Tamburini et al., 2012) and  
356 for sedimentary apatite (between +17 and +23‰; Tamburini et al., 2014).  
357 Interestingly, the variability in the  $\delta^{18}O_p$  values for the allochthonous sources that may possibly  
358 contribute toward the river particulate-P load was higher than the variability in the riverbed  
359 sediments. Whereas while the soil, riverbank and riverbed sediments collected in the head of  
360 the catchment (samples 1 to 3 and 9 to 12) are representatives of these sources (Dorioz and  
361 Ferhi, 1994) and exhibit  $\delta^{18}O_p$  values varying by  $> 6$  ‰ (from +11.8 to +18.3‰), this variation  
362 is reduced to approximately 2‰ for the sediments immediately upstream/downstream the  
363 WWTP and to 1‰ for the outlet sediments (+12.8 to +13.8‰; Fig. 2a), the  $\delta^{18}O_p$  signatures of  
364 which were comparable to those recorded by the lake sediments (samples 13 and 14; Table 3).

365

366 [Figure 2]

367

368 We compared the measured  $\delta^{18}O_p$  values with the theoretical equilibrium calculated with  
369 Equation 2 (see Table 2). The deviation between the calculated and measured  $\delta^{18}O_p$  values is  
370 presented in Fig. 2b. The  $\delta^{18}O_p$  value for the riverbank moraine sample (sample 12) was above  
371 the calculated equilibrium by ca. 2‰, as was the wetland soil sample (9). This was not the case  
372 for the cultivated soil samples that showed  $\delta^{18}O_p$  values that were either very close to (-0.1‰,

373 topsoil, sample 10) or slightly lower than (-0.7‰, deep horizon, sample 11) the calculated  
374 equilibrium values.

375 The riverbed sediment samples collected in the streamlets of the agricultural sub-catchment  
376 (samples 1, 2) exhibited  $\delta^{18}\text{O}_p$  values that were lower than the calculated equilibrium values by  
377 ca. 2‰. For the sediments located within the main hydrogeological network, the differences  
378 between the measured and calculated equilibrium values generally tended to be lower (see  
379 Table 3 and Fig. 2b): ca.  $\pm 1.5\%$  in the middle part, upstream and downstream from the  
380 WWTP discharge point (samples 4 to 6) and  $< 0.5\%$  for the sediments collected at the outlet  
381 (samples 7 and 8) and the lacustrine sediments (samples 13 and 14).

382

#### 383 **4. Discussion**

384

##### 385 **4.1 Significance and variability of the measured and equilibrium $\delta^{18}\text{O}_p$ values**

386 The present study shows that the method originally established and optimized by Tamburini et  
387 al. (2010) for soils is suitable for  $\text{CaCO}_3$ -rich riverbed sediment samples, following some  
388 modifications to the methodology. However, as for soils, it should be kept in mind that the  
389 extraction is not entirely specific with regards to the nature of the extracted  $\text{P-PO}_4$ . First, 1 M  
390 HCl can be used to extract the fraction that is primarily composed of Ca-bound P, which can  
391 contain a broad spectrum of P compounds (more or less crystallized apatite forms,  $\text{CaCO}_3$ -  
392 bound P, etc.). This reagent can also extract some  $\text{P-PO}_4$  sorbed onto Fe and Al oxides, if used  
393 as a single extractant. Thus, the  $\delta^{18}\text{O}_p$  signatures obtained with this method correspond to a  
394 pooled  $\text{P-PO}_4$  isotope signature of a mixture of different P fractions with a different degree of  
395 stability, exchangeability and bioavailability (Jarvie et al., 2002) rather than to the signature of  
396 a geochemically specific  $\text{P-PO}_4$  compartment. Despite this limitation, the oxygen isotope  
397 composition of  $\text{P-PO}_4$  extracted from sediment using HCl can provide information that is not

398 available with other methods, including the extent to which a P pool has been biologically  
399 processed or derived from primary inputs or secondary anthropogenic sources (Tamburini et al.,  
400 2014; Davis et al., 2014).

401 The calculation of the equilibrium  $\delta^{18}\text{O}_p$  values is another critical point for the interpretation of  
402 the  $\delta^{18}\text{O}_p$  isotope data. Information regarding the kinetics of the equilibrium is only derived  
403 from cell-free or soil incubation experiments (Blake et al., 2005; Melby et al., 2013), but not  
404 from conditions that are close to the natural state. Our strategy was to take samples during low-  
405 flow periods because of the stability of the  $\delta^{18}\text{O}_w$  value and the temperature, but there is still  
406 the question of how to choose appropriate time points when calculating the theoretical  
407 equilibrium values with equation 2. This is also relevant for soils where sharp variations in the  
408  $\delta^{18}\text{O}_w$  value in the soil surface layers due to the progressive evaporation of the soil water  
409 (Hsieh et al., 1998) might be a major source of uncertainty. Amelung et al. (2015) suggest  
410 calculating the equilibrium  $\delta^{18}\text{O}_p$  value with the annual average  $\delta^{18}\text{O}_w$  for rainwater, arguing  
411 that the biological recycling of Ca-bound P in soil is a long-term process. Using an annual  
412 rainwater  $\delta^{18}\text{O}_w$  value and temperature values, the calculated equilibrium value for the  
413 agricultural soil samples (10 and 11) would increase by approximately +2‰, resulting in  
414 measured signatures below the equilibrium.

415 Except for a small number of studies (Elsbury et al., 2009; Gooddy et al., 2016; Markel et al.,  
416 1994; McLaughlin et al., 2006a), freshwater ecosystems have been poorly explored in terms of  
417  $\delta^{18}\text{O}_p$ . Our results expand the global  $\delta^{18}\text{O}_p$  library for these systems, comparing, for the first  
418 time, the oxygen isotope signatures of HCl-P extracted from river sediment samples from  
419 upstream to downstream with those of the main potential allochthonous sources in the  
420 catchment. The isotope signatures of the analyzed river sediments, ranging from +12.2 to  
421 +15.8‰, lie in the interval reported in previous studies, i.e. +8.6 to 22.5 (Markel et al., 1994),

422 +12.4 to 23.5‰ (Davies et al., 2014) and +9.7 to +20.0‰ (Tamburini et al., 2014). The same is  
423 true for our soil samples (Amelung et al., 2015; Tamburini et al., 2014; Zohar et al., 2010).

424

## 425 **4.2 Biological recycling versus tracing the sources using $\delta^{18}\text{O}_p$ values**

### 426 *4.2.1 Soils*

427 Tamburini et al. (2012, 2014) have shown that the HCl-P fraction in the upper and most  
428 biologically active part of the oldest soil of a chronosequence was formed by secondary  
429 minerals precipitated from  $\text{P}_i$  that were previously cycled by biota. The close-to-equilibrium  
430  $\delta^{18}\text{O}_p$  values measured in the agricultural soil samples (samples 10 and 11) can be viewed as  
431 evidence that biological cycling had a large impact on the HCl-P fraction (which represents  
432 roughly half of the  $\text{P}_t$ ) in these samples. The soil P is supplied by the parent material and  
433 fertilizations and is redistributed throughout the soil profile via biological activities. As the  
434 parent material is low in  $\text{P}_t$ ,  $\text{P}_{\text{Olsen}}$  and  $\text{P}_o$ , the high concentrations observed in the topsoil  
435 sample (see Table 3), as in other cultivated soils, is the long-term result of the accumulation  
436 and transformation of P favoring a high P bioavailability, which supports the assumption of an  
437 intense biological activity.

438 The most common fertilizers used in the area have  $\delta^{18}\text{O}_p$  values that are higher (16.2-22.5‰) or  
439 lower (9.5‰; Guyard 2010) than the values for the studied agricultural soils. Even if we cannot  
440 exclude that the observed equilibrium values were only the result of a mixing between the  
441 parent material (close to +15.9‰) and a lighter fertilizer input, we assume that the fertilizer has  
442 little influence because the fertilizer inputs are almost totally cycled over the long-term (> 1  
443 year) and the amount applied is too small (2-3% of the HCl-P soil stock) over the short-term  
444 (one season).

445 The high and above-equilibrium  $\delta^{18}\text{O}_p$  value in the wetland surface soil (sample 9) can also  
446 reflect the impact of biota, e.g. plants. The  $\delta^{18}\text{O}_p$  value for this sample (18.3‰) cannot be

447 explained by the mixing of any of the potential input materials present in the area (i.e. bank  
448 material or cultivated soil), as +18.3‰ is too far outside the range of the values (11.8 and  
449 15.8‰) recorded by these input materials. The only source with a sufficiently high  $\delta^{18}\text{O}_p$  value  
450 would be plant-derived phosphate. Phosphate in plants is enriched in  $^{18}\text{O}$ , with the  $\delta^{18}\text{O}_p$  values  
451 ranging from 28 to 31‰ (Pfahler et al., 2013; Tamburini et al., 2014). This suggests that the  
452 extracted fraction might partially originate from the release of P from plant litter, either directly  
453 or via decomposition. A significant fraction of this enriched P pool that is not taken up by  
454 microbes (Liang and Blake, 2006; Oberson and Joner, 2005) might have precipitated or been  
455 sorbed into the soil mineral pool. Interestingly, this sample had a particularly high Po  
456 concentration. Preferential sorption/desorption reactions with soil minerals could also have  
457 helped increase the  $\delta^{18}\text{O}_p$  value for this sample. However, previous studies have reported both  
458 negative and positive fractionation associated with sorption (Jaisi et al., 2010; Melby et al.,  
459 2013b). In any case, this effect is probably too small (<1 ‰) to justify the observed value by  
460 itself.

461 When compared to the equilibrium values calculated using the average river conditions (the  
462 values in brackets in Table 3, Fig. 2b), the subsoil/embankment sample and the wetland topsoil  
463 are no longer found within the equilibrium range. The situation is different for the agricultural  
464 topsoil sample, the actual  $\delta^{18}\text{O}_p$  value of which falls within the equilibrium range, meaning that  
465 under these conditions the oxygen isotopic composition alone would not help to distinguish  
466 between a sedimentary P- $\text{PO}_4$  pool inherited from this soil and in-stream recycled P- $\text{PO}_4$ .

467  
468 *4.2.2 River sediments upstream and downstream from the wastewater treatment plant*  
469 *discharge*

470 The September sampling showed that the input of P from wastewater was marked by a  
471 threefold increase in the SRP concentration in the river water sampled upstream and

472 downstream from the discharge point (Table 1), accompanied by a drastic change in the Pt, Pi  
473 and  $P_{\text{Olsen}}$  concentrations in the corresponding sediments (Table 3). No changes were detected  
474 in the Po content, meaning that the impact of the point discharge on this pool in the bed  
475 sediments was negligible (Dorioz et al., 1998a). Interestingly, despite the small distance (< 100  
476 m) separating the upstream and downstream sites, the  $\delta^{18}\text{O}_p$  values were also distinct, with a  
477 difference of 3‰. Upstream from the WWTP discharge point, the P pools in the sediment are  
478 mainly impacted by sources comprising a mixture of riverbanks and agricultural soil erosion  
479 (materials similar to samples 12 and 1, 2). The  $\delta^{18}\text{O}_p$  value (12.2‰) and P speciation for  
480 sample 4 were intermediate between those of these source materials.

481 The two September samples (4 and 5, immediately upstream and downstream from the WWTP)  
482 deviate from the isotopic equilibrium values (by -1.5 and +1.4‰, respectively; Fig. 2b),  
483 suggesting the existence of conditions that prevent the complete biological turnover of P at both  
484 sites (also see Goody et al., 2016). Under the assumption that biological equilibration is the  
485 dominant process during sewage treatment (Gruau et al., 2005), the  $\delta^{18}\text{O}_p$  value for dissolved  
486 Pi from the WWTP would vary between 13.0 and 14.8‰ using equation 2 (confirmed by the  
487 value of 14.0‰ measured by Guyard, 2010), meaning that the higher  $\delta^{18}\text{O}_p$  value downstream  
488 (15.8‰) cannot be interpreted as the result of a mixing between wastewater derived P and P  
489 from the upstream sediments. One possible explanation for this could be that part of the Pi  
490 injected into the river by the WWTP is rapidly (within a few meters from the injection point)  
491 and continuously sorbed onto the sediment particles, as also hypothesized by Dorioz et al.  
492 (1998a), and that the heavier isotopologues are preferentially sorbed. However, there is no  
493 consensus regarding the associated fractionation (Jaisi et al., 2010; Melby et al., 2013b). An  
494 alternative explanation would be that the upstream sample 4 represents a local anomaly, and the  
495 signature of the downstream sediments, sample 5, derives from local riverbank erosion. Sample  
496 12 can be considered as representative of riverbank material and its  $\delta^{18}\text{O}_p$  value is comparable

497 to that of sample 5. However, this hypothesis is unlikely as it does not explain the drastic  
498 change in P speciation observed in the sediments downstream from the WWTP (see Table 3).  
499 Only the locality situated immediately downstream from the WWTP discharge point could be  
500 sampled in March 2012 (sample 6). The SRP, Pt, Pi and P<sub>Olsen</sub> concentrations were as high as  
501 those found in sample 5, which was collected in September, and much higher than the  
502 concentrations measured further upstream, at location 3, once again providing evidence for the  
503 strong impact of the WWTP discharge on the river and sediment P pool. The  $\delta^{18}\text{O}_p$  value for  
504 sample 6 was similar to the September sample (sample 5), although it was close to the  
505 calculated isotopic equilibrium. The lack of a reference value equivalent to sample 4 in March  
506 means that it is not possible to conclude whether the observed value resulted from the complete  
507 equilibration of the sedimentary P-PO<sub>4</sub> pool or if it was instead produced by selective sorption  
508 processes as we suggest for sample 5. A repeated seasonal sampling of the sediments  
509 immediately upstream and downstream from the WWTP could possibly clarify this point.

510

#### 511 *4.2.3 Head and outlet sediments*

512 The possible mixing of isotopically different sediment source materials coupled with  
513 differences in the sediment retention time complicates the interpretation of the  $\delta^{18}\text{O}_p$  values for  
514 the riverbed sediments. Under conditions of low biological activity and a short residence time,  
515 the isotopic signature of the sediments is expected to represent primary source signatures. In  
516 this context, an isotopic disequilibrium would indicate the existence of factors that limit the in-  
517 stream P cycling, intended as uptake by the biota, intracellular equilibration and subsequent  
518 release. As judged from the present data, a limiting situation for in-stream P recycling could  
519 have been prevalent in the head of the Redon River catchment, where strong out-of-equilibrium  
520  $\delta^{18}\text{O}_p$  signatures have been recorded (see Table 3 and Fig. 2b). This could be first related to the  
521 physical conditions in this part of the catchment - high-energy stream (total renewal of the



522 sediments with each storm flow) and a short distance from the sources, i.e. riverbanks - which  
523 both determined the short residence times. The oligotrophic state of the running water (SRP <  
524 10 µg P/l, NO<sub>3</sub> < 0.5 mg N/l) and the low P bioavailability of the riverbed sediments (as  
525 indicated by low P<sub>Olsen</sub> concentrations) could contribute to limit the development of benthic  
526 biota and thus P uptake and subsequent release. For the same reasons the contribution of  
527 extracellular mineralization of organic P is expected to be negligible. The lack of an isotopic  
528 difference and the small differences in the Pt, Pi and Po content between the riverbank sample  
529 (12) and the sediment sample (3) collected immediately downstream further confirms the direct  
530 filiation between the source and the sediment.

531 The hypothesis that a rapid transfer does not significantly modify the δ<sup>18</sup>O<sub>p</sub> signature is further  
532 supported by the disequilibrium signature recorded in the streamlet samples 1 and 2 and their Pi,  
533 Po and P<sub>Olsen</sub> concentrations, as all of these parameters are intermediate between the values  
534 found in the analyzed deep and topsoil samples. This suggests i) a mixed origin for these two  
535 sediment samples derived from both superficial soils and ditch banks or field embankments;  
536 and ii) that the biological activity was not intense or not long enough to equilibrate the original  
537 P oxygen isotope signatures, consistently with the expected very short residence time of the  
538 sediments in the streamlets (stream order of 1).

539 The situation was different for the sediments that were collected closer to the river outlet or in  
540 the lake (stream order higher than 3). Close-to-equilibrium values were found in these samples.  
541 A corresponding increase in the SRP concentration in running water (a factor of 8 between  
542 upstream and the outlet; see Table 1), as the result of the cumulative anthropogenic inputs in  
543 the river, was also observed.

544 The equilibrium values observed in the lowest part of the Redon River suggest the occurrence  
545 of processes that are able to efficiently recycle the sedimentary P-PO<sub>4</sub>. This hypothesis would  
546 be consistent with the decrease in the δ<sup>18</sup>O<sub>p</sub> values observed during the September campaign

547 downstream from the WWTP discharge point, i.e. between samples 5 and 8. There was a 3‰  
548 decline in the  $\delta^{18}\text{O}_\text{P}$  value between sample 5, which was collected immediately downstream  
549 from the WWTP discharge point, and sample 8, which was collected approximately 2 km  
550 further downstream. This decrease was accompanied by a strong narrowing of the gap between  
551 the measured and calculated equilibrium  $\delta^{18}\text{O}_\text{P}$  values. Whereas this gap was +1.4‰ for  
552 sample 5, it was reduced to < 0.4‰ for sample 8 (see Table 3). The isotopic equilibration  
553 inferred by these data would likely occur in the sediments, given that in this kind of torrential  
554 rivers most of the biological activity essentially takes place in the benthic system. Equilibration  
555 could be further promoted by the longer residence time of the sediments in the downstream part  
556 of the river (Doriz et al., 1989). It may have been also driven by the enhanced bio-availability  
557 of P in the lower part of the river, notably downstream from the WWTP, as evidenced by the  
558 sharp increase in the  $\text{P}_{\text{Olsen}}$  concentration starting from this point (see Table 3, Figs. 2a and 2b).  
559 Most likely, the overall process should be viewed more as the integration of a succession of  
560 partial equilibrium occurring over different storage stages under similar equilibrium conditions,  
561 as it is unlikely that the bulk of the P- $\text{PO}_4$  could be completely cycled by the benthic biota  
562 during a single low-flow period.

563 However, we lack information about the real residence time of the sediments and the P  
564 recycling rates in the Redon River. Considering that the measured  $\delta^{18}\text{O}_\text{P}$  values for the  
565 sediments collected near the river outlet (samples 7 and 8) are identical within error to those  
566 measured in the upstream streamlet sediments (samples 1 and 2) and are within the range of the  
567 values found in the possible sediment sources (see Fig. 2a), we cannot entirely exclude that the  
568  $\delta^{18}\text{O}_\text{P}$  values in these downstream sediments are simply source-inherited isotopic values. More  
569 direct evidence for the real residence time of the sediments and the ability of aquatic biota to  
570 efficiently recycle P- $\text{PO}_4$  in the downstream part of the river is needed to decide between these  
571 two hypotheses.

572

### 573 **4.3 Potential and limits of the oxygen isotopes tool**

574 We hypothesize that is possible to trace the potential sources of sediment particulate-P-PO<sub>4</sub>  
575 using oxygen isotopes, assuming a limited number of potential sources and under conditions of  
576 low biological activity and a short residence time in the river network. In the studied torrential  
577 system, it appeared possible to trace these sources in the head of the catchment (only two  
578 sources, rapid and high-energy transfer, limiting conditions for the biological activity). It would  
579 be also possible at the catchment scale, during winter high-flow periods, when the sediments  
580 that originate from banks and agricultural soil erosion are rapidly transferred as suspended  
581 matter and without significant interaction with point source discharge and biological activity. In  
582 this case, the  $\delta^{18}\text{O}_\text{p}$  value would primarily be the result of a two end-members mixing between  
583 the cultivated soil (values close to 11-14‰) and moraine material (values close to 15-16‰).  
584 However, the isotopic tracing is expected to be less straightforward during the summer and  
585 early fall periods, as the storm flows that occur at that time primarily mobilize riverbed  
586 sediments. Therefore, it could become more difficult to perform a quantitative assessment of  
587 the original particulate-P sources using  $\delta^{18}\text{O}_\text{p}$  due to an overprinting of the sediment isotopic  
588 signature induced by the high biological activity. During summer low flows, an even stronger  
589 overprinting of the original isotopic signature is expected to occur in the downstream part of  
590 the river, due to the input of anthropogenic, bioavailable P, a longer sediment residence time  
591 and increased biological activity. This being the case,  $\delta^{18}\text{O}_\text{p}$  could then instead be used as a  
592 tool to assess the in-stream biological turnover of the P.

593

594

### 595 **5. Conclusion**

- 596 • In this study, we successfully adapted Tamburini et al.'s method (2010) for soil for use  
597 with river sediments to provide the first assessment of the variability in  $\delta^{18}\text{O}_\text{p}$  from HCl-  
598 extracted P in river sediments along a gradient of increasing anthropogenic impact.
- 599 • We observed a contrast between the  $\delta^{18}\text{O}_\text{p}$  signature and the speciation of the sediments  
600 collected in the head and at the outlet of the catchment. The sediments that were collected  
601 upstream have isotopic signatures that are far from isotopic equilibrium and within the range of  
602 the signatures found in the possible sediment sources (i.e. riverbank material), while those  
603 collected at the outlet showed  $\delta^{18}\text{O}_\text{p}$  values close to isotopic equilibrium. We related this  
604 difference to the contrast between the highly dynamic character (short residence time) of the  
605 transfer system in the head of the catchment (preservation of the isotopic signature of the  
606 source material in the sediments) and the “quieter” sediment transport system in the lower  
607 catchment, which, along with the input of the bioavailable anthropogenic P, likely fostered the  
608 recycling of P by benthic biota, leading to an equilibration in the  $\delta^{18}\text{O}$  signatures. However,  
609 with the available data, we cannot exclude that the signatures at the outlet were the result of the  
610 mixing of sediments with higher (upstream/wetland sediments) and lower signatures  
611 (agricultural soil erosion).
- 612 • Uncertainties remain about the calculation of the equilibrium window. For aquatic  
613 environments, the duration of the in-stream equilibration processes should be taken into  
614 account, together with the perturbation caused by changes in the hydrological regime. This  
615 would require an experimental approach for a proper estimation.
- 616 • In terms of the quantitative tracing of potential particulate-P sources, future  
617 investigations should focus on winter high flows, when rapid sediment transfer maximally  
618 reduces the possibility of biological interactions. On the other hand, the sensitivity of  $\delta^{18}\text{O}_\text{p}$  to  
619 biological processes opens up new promising perspectives for studying in-stream processes. In

620 situ labelling experiments could help develop these studies by providing unique information  
621 about the biological uptake, turnover and/or organic P mineralization.

622 • More insights about the way and extent the biological activity controls P speciation in  
623 the soil and sediments could be possibly provided by the development of refined extraction  
624 methods that are able to selectively and quantitatively extract other P pools like the one  
625 associated with Al and Fe hydroxides,  $P_{\text{Olsen}}$  and  $P_o$  or finer fractions (e.g.  $< 50 \mu\text{m}$ ,  $< 2 \mu\text{m}$ ).

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### Table and Figure captions

Table 1. Characteristics of the sediment sampling sites.

Table 2. Characteristics of the soil sampling sites.

Table 3. P speciation and oxygen isotope composition.

Figure 1. Redon River catchment and sampling sites; the capital letters refer to the sampling locations, the numbers are the sample code.

Figure 2. Oxygen isotope composition ( $\delta^{18}\text{O}_p$ ) and deviation from isotopic equilibrium. The gray circles represent potential source samples, white circles represent riverbed sediment samples, and  $\text{Pols} = \text{P}_{\text{Olsen}}/\text{P}_t$  in % (note that the deviation of the potential sources from equilibrium in this image have been calculated according to the average within-river conditions).

Figure 3. Conceptual model describing the evolution of  $\delta^{18}\text{O}_p$  for the riverbed sediments in the studied torrential system.

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Table 1 Characteristics of sediment sampling sites

| Site | SO | Sample code | Sampling date     | Q   | EC    | SRP    | Altitude | Drainage area | Land use of the drainage basin | Main sources of stream P                     |
|------|----|-------------|-------------------|-----|-------|--------|----------|---------------|--------------------------------|--|
|      |    |             |                   | L/s | mS/cm | mg P/L | m a.s.l  | ha            |                                |  |
| A    | 1  | 1           | September 3, 2012 | <1  | 543   | 0.0012 | 576      | 4             | Cultivated land                | NPS (erosion of cultivated soils and tiches) |
| B    | 1  | 2           | September 3, 2012 | 1   | 557   | 0.009  | 577      | 17            | Cultivated land                | NPS (erosion of cultivated soils and tiches) |
| C    | 2  | 3           | March 26, 2012    | 35  | 598   | 0.008  | 696      | 510           | Steep forest                   | NPS (natural: mainly river bank erosion)     |
| D    | 3  | 4           | September 3, 2012 | 55  | 7203  | 0.027  | 510      | 2310          | Forest and cultivated land     | NPS (natural, agricultural)                  |
| E    | 3  | 5           | March 26, 2012    | 105 | 654   | 0.056  | 508      | 2311          | Forest and cultivated land     | NPS (natural, agricultural) + PS (WWTP)      |
|      |    | 6           | September 3, 2012 | 55  | 753   | 0.092  |          |               |                                |  |
| F    | 4  | 7           | March 26, 2012    | 340 | 597   | 0.062  | 405      | 3300          | Forest and cultivated land     | NPS (natural, agricultural) + PS (WWTP)      |
|      |    | 8           | September 3, 2012 | 85  | 975   | 0.067  |          |               |                                |  |

SO = Strahler stream order (Strahler, 1957)

Q = water discharge

EC = electric conductivity

SRP = soluble reactive phosphorus concentration in water, measured on filtered samples (0.45  $\mu$ m) by the molybdate-blue method (Murphy and Riley, 1962)

NPS = Non-point source, PS = Point source

WWTP = waste-water treatment plant

Table 2 Characteristics of soil sampling sites

| Sample code | Date of sampling  | Location                                  | Soil depth | Altitude | Sampling conditions                    |
|-------------|-------------------|---|------------|----------|--|
|             |                   |   | cm         | m a.s.l  |  |
| 9           | September 3, 2012 | Wetland topsoil                           | -1 to -5   | 552      | Organic soil, saturated                |
| 10          | September 3, 2012 | Cultivated topsoil <sup>a</sup>           | -10 to -30 | 558      | Cambisol on moraine deposit, dry       |
| 11          | September 3, 2012 | Cultivated soil (embankment) <sup>a</sup> | -60 to -70 | 558      | Cambisol on moraine deposit, saturated |
| 12          | September 3, 2012 | Riverbank in a forested upland area       | -          | 980      | Würm moraine deposits, saturated       |

<sup>a</sup> samples collected within an agricultural sub-catchment of 33 ha (Figure 1)

Table 3. P speciation and oxygen isotopes composition

| Sample code | Sample type                      | Pt     | P <sub>Olsen</sub> | Pi     | Po     | P <sub>Olsen</sub> /Pt <sup>a</sup> | $\delta^{18}\text{O}_p$ | St. Dev. | n | Deviation from equilibrium (‰) <sup>b</sup> |
|-------------|----------------------------------|--------|--------------------|--------|--------|-------------------------------------|-------------------------|----------|---|---|
|             |                                  | mgP/kg | mgP/kg             | mgP/kg | mgP/kg | %                                   | ‰                       | ‰        | - | ‰   |
| 1           | River bed sediments <sup>d</sup> | 791    | 14                 | 401    | 180    | 2.4                                 | 12.5                    | 0.3      | 3 | - 2.4                                       |
| 2           |                                  | 751    | 20                 | 323    | 208    | 3.8                                 | 13.3 <sup>s</sup>       | 0.3      | 2 | - 1.6                                       |
| 3           |                                  | 528    | 8                  | 411    | 78     | 1.7                                 | 15.5                    | 0.6      | 4 | + 1.3                                       |
| 4           |                                  | 609    | 18                 | 398    | 202    | 2.9                                 | 12.2 <sup>s</sup>       | 0.4      | 2 | - 1.5                                       |
| 5           |                                  | 1018   | 65                 | 586    | 194    | 8.4                                 | 15.8 <sup>s</sup>       | 0.7      | 2 | + 1.4                                       |
| 6           |                                  | 962    | 67                 | 470    | 166    | 10.6                                | 15.2                    | 0.3      | 3 | < 0.4                                       |
| 7           |                                  | 734    | 26                 | 439    | 138    | 4.5                                 | 13.8                    | 0.4      | 2 | < 0.4                                       |
| 8           |                                  | 736    | 21                 | 324    | 90     | 5.2                                 | 12.8                    | 0.1      | 3 | - 0.6                                       |
| 9           | Wetland soil                     | 1163   | 32                 | 179    | 383    | 5.7                                 | 18.3 <sup>s</sup>       | 0.2      | 2 | + 2.1 (+ 3.6) <sup>c</sup>                  |
| 10          | Agric. topsoil                   | 1256   | 43                 | 631    | 450    | 6.2                                 | 14.0                    | 0.4      | 2 | < 0.4 (< 0.4) <sup>c</sup>                  |
| 11          | Embankment/<br>deep soil         | 755    | 5                  | 363    | 76     | 1.1                                 | 11.8                    | 0.4      | 2 | - 0.7 (- 2.0) <sup>c</sup>                  |
| 12          | Riverbank<br>(moraine)           | 511    | 3                  | 305    | 36     | 0.7                                 | 15.9                    | 0.2      | 2 | + 1.9 (+ 1.2) <sup>c</sup>                  |
| 13          | Lake                             | 459    | 15                 | 365    | 121    | 3.5                                 | 12.9                    | 0.3      | 2 | < 0.4                                       |
| 14          | sediments                        | 539    | 17                 | 420    | 70     | 3.1                                 | 13.9                    | 0.5      | 2 | + 0.8                                       |

<sup>a</sup> As a sum of Pi and Po fractions

<sup>b</sup> See section 2.3.2 and equation 2

<sup>c</sup> Values not in brackets are calculated according to the temperature and water isotopes conditions at the sampling site, the values in brackets are calculated according to the “within-river” conditions

<sup>d</sup> Sediment samples are ranked according to the corresponding drained area

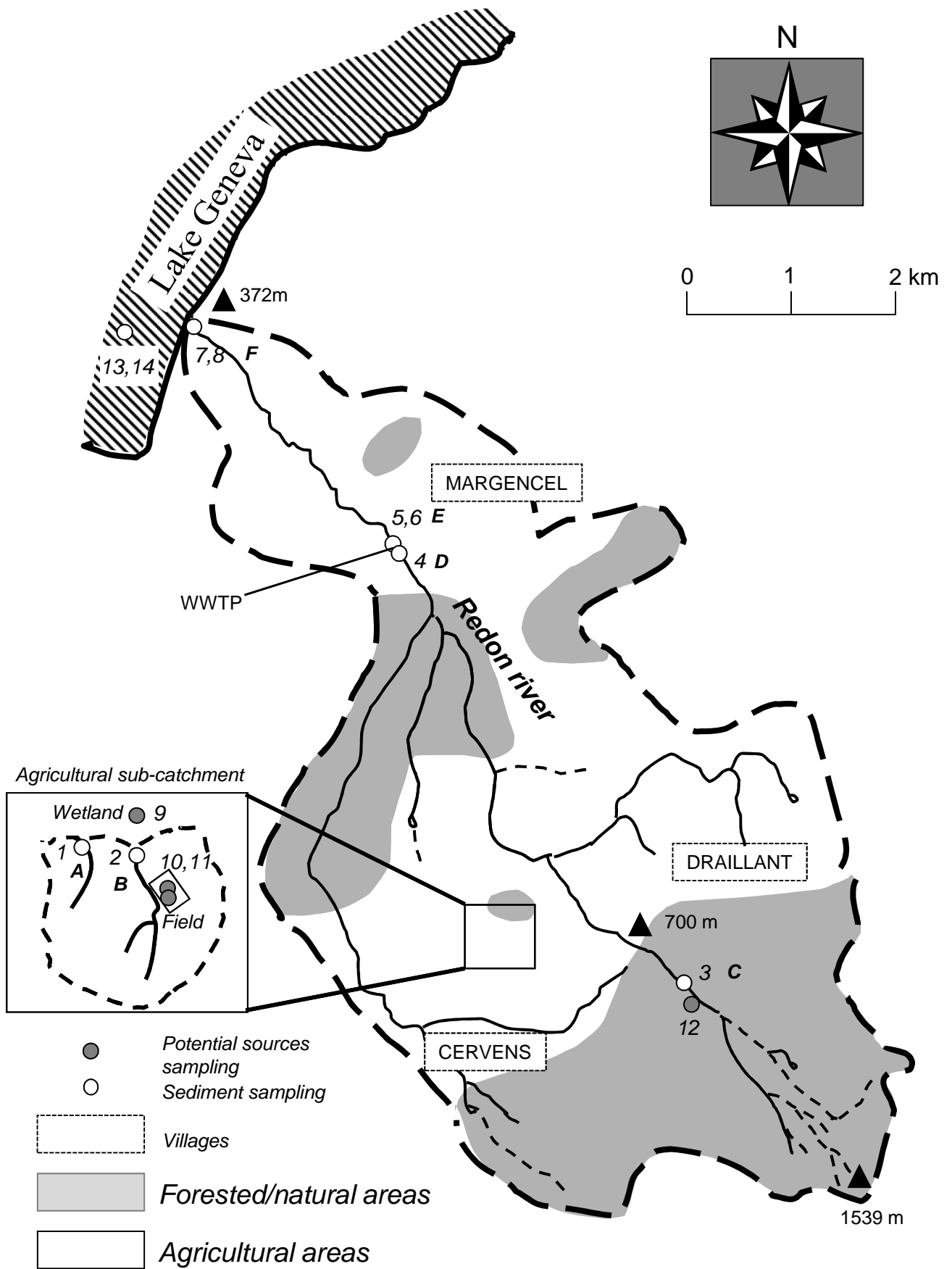


Figure 1. Redon River catchment and sampling sites, capital letters refer to sampling locations, numbers to sample code

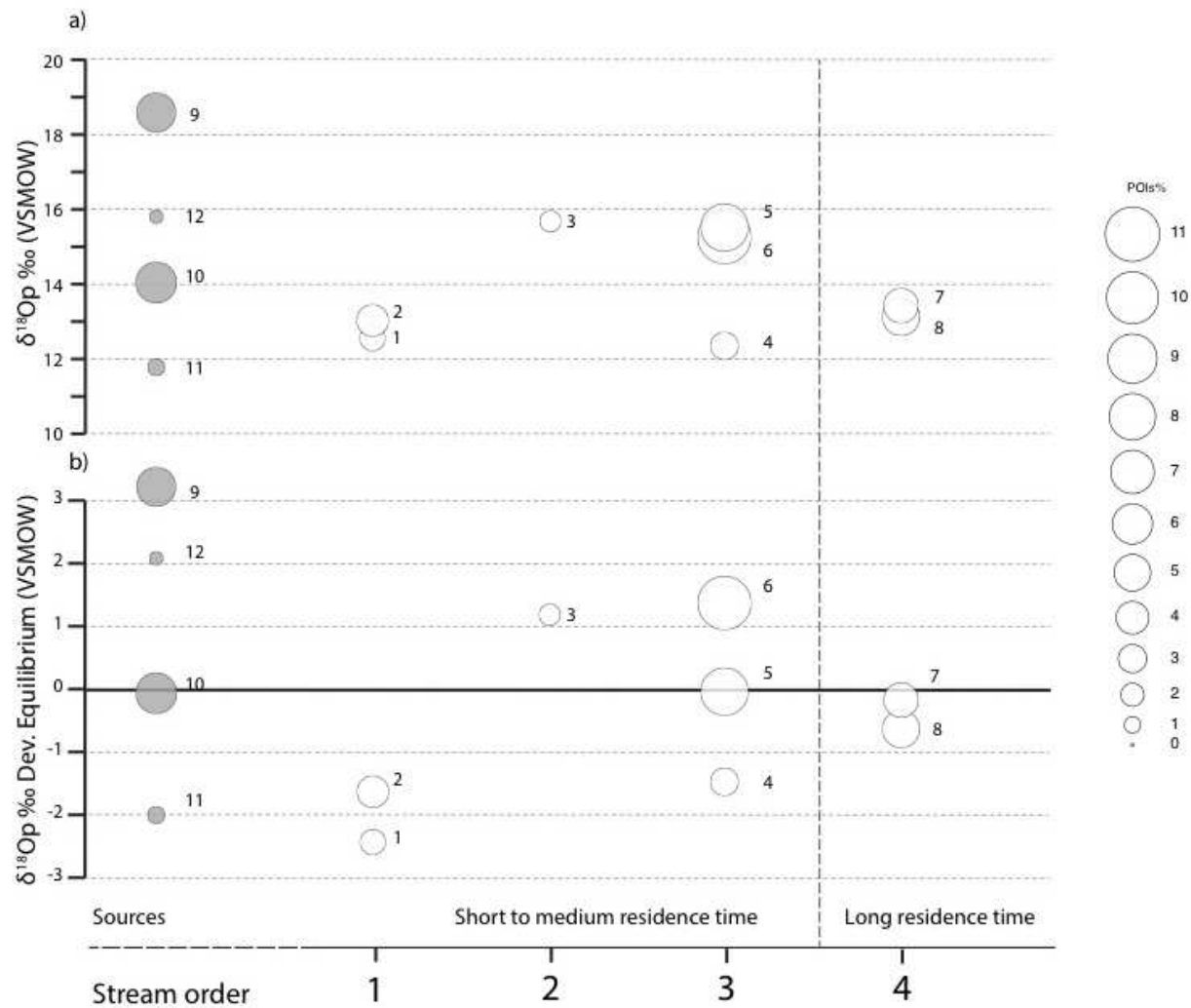


Figure 2. Oxygen isotopes composition  $\delta^{18}\text{Op}$  and deviation from isotopic equilibrium. Grey circles represent potential sources samples, white circles represent riverbed sediments samples,  $P_{\text{ols}} = P_{\text{Olsen}}/P_t$  in % (note that deviation from equilibrium of potential sources in this picture have been calculated according to average within-river conditions)

**Highlights**

- We provided the first assessment of the oxygen isotopic composition of HCl-extracted phosphate in river sediments
- We observe a contrast in the oxygen isotope composition between upstream and downstream sediments
- This contrast can be related to anthropogenic impacts on the river system and to the sediment residence time
- We have shown that in some situations it is possible to trace particulate-P sources of sediments with this isotopic tool