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**Tracing sources and fate of nitrate in multilayered karstic hydrogeological catchments using natural stable isotopic composition ( $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$ ). Application to the Toulon karst system (Dordogne, France)**

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► **To cite this version:**

Guillaume Lorette, Mathieu Sebilo, Damien Buquet, Roland Lastennet, Alain Denis, et al.. Tracing sources and fate of nitrate in multilayered karstic hydrogeological catchments using natural stable isotopic composition ( $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$ ). Application to the Toulon karst system (Dordogne, France). *Journal of Hydrology*, 2022, 610, pp.127972. 10.1016/j.jhydrol.2022.127972 . hal-03974793

**HAL Id: hal-03974793**

**<https://hal.science/hal-03974793>**

Submitted on 22 Jul 2024

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1           **Tracing sources and fate of nitrate in multilayered karstic**  
2           **hydrogeological catchments using natural stable isotopic**  
3           **composition ( $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$ ). Application to the Toulon**  
4           **karst system (Dordogne, France)**

5  
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20  
21 **Abstract**

22  
23       In groundwater management, the determination of the origins of nitrate ( $\text{NO}_3^-$ )  
24 endmember sources is the first step in improving the management over a hydrogeological  
25 catchment. The generally multiple source contamination by nitrate over the karst systems  
26 makes it difficult to identify the exact nitrate origins in surface water and groundwater.

27       This work aims to assess nitrate vulnerability of a karst system fed by several karst  
28 aquifers. The Toulon karst system offers the opportunity to study the relationships between  
29 two multilayered karst aquifers. It shows an increase in mean  $\text{NO}_3^-$  concentrations over  
30 decades. However, the origins of the  $\text{NO}_3^-$  remain unclear. Natural stable isotopic  
31 composition of  $\text{NO}_3^-$  ( $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$ ) measurements combined with concentrations  
32 were used to identify the origins of nitrogen causing this increase. This information was also  
33 integrated into the local hydrogeological situation to improve knowledge of the  
34 contamination of both confined and unconfined karst aquifers.

35  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  in potential nitrate sources over the hydrogeological catchment  
36 were analysed in order to identify the range of values that can be measured in both rivers and  
37 groundwater. Over the Toulon Springs catchment, rivers are contaminated with nitrate  
38 coming from N synthetic fertilizer mineralized in agricultural soils. Most of the springs are  
39 also contaminated by this nitrate source, but some record an isotope composition of nitrate  
40 coming from sewage effluents. Toulon Springs are only contaminated with nitrate from N  
41 synthetic fertilizer mineralized in agricultural soils. Finally, from a hydrogeological point of  
42 view, the analysis of  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values improves knowledge on the functioning  
43 of the study area and shows that the upper Jurassic karst confined aquifer has nitrate of the  
44 same origins (sources) as the upper Cretaceous unconfined aquifer. The results here highlight  
45 that the lower aquifer can be fed by the Upper Cretaceous unconfined aquifer, contrary to  
46 previous belief that the lower aquifer was confined.

47 From a more fundamental standpoint, this work provides a set of parameters adapted  
48 for karstic hydrogeological catchment. It lays the foundation for the use of isotope  
49 biogeochemistry as a powerful tool for understanding the recharge of drinking water  
50 catchments and beyond the efficient management of water quality at the scale of karstic  
51 hydrogeological catchment.

52

53 **Keywords:** nitrate, karst, multilayered aquifer, nitrate isotopes, hydrochemistry.

54

55 **Highlights:**

56

- 57 • Nitrate contents and isotopes are measured in a large multilayered karst aquifer
- 58 • Primary and secondary sources of nitrate are determined, at the local scale, using  
59 nitrate isotopes

- 60 • Origins of nitrate, over a karstic hydrogeological catchment, are assessed by  
61 nitrate isotopes
- 62 • The fate of nitrate in a karst aquifer is specified/ elucidated/ quantified/ combining  
63 isotopes and hydrogeological approaches

## 64 **1. Introduction**

65

66 Karst aquifers are one of the most important water supply sources worldwide.  
67 Approximately 20-25% of the world's population consumes water obtained from karst  
68 aquifers (Ford and Williams, 2007; Goldscheider et al. 2020). These aquifers are  
69 distinguished from porous aquifers by the high degree of heterogeneity that can be identified  
70 in both surface and underground environments (White, 1988; Bakalowicz, 2005). This  
71 heterogeneous characteristic implies a high flow rate into karst systems which could reach  
72 several hundred m h<sup>-1</sup> (Mudarra et al. 2014; Marin et al. 2015). These unique hydrogeological  
73 characteristics make karst aquifers particularly vulnerable to surface contamination and  
74 difficult to manage (Stevanović, 2015, 2019).

75 Nitrate (NO<sub>3</sub><sup>-</sup>) is the most persistent and mobile form of nitrogen in the subsurface  
76 environment. Continuous exposure to nitrate contamination can be dangerous for humans,  
77 leading to several types of cancer (De Roos et al. 2003; Ward et al. 2005) and an increase of  
78 methemoglobin in the blood (Johnson et al. 1987). For several decades, the increase of nitrate  
79 concentrations in groundwaters has caused degradation of water quality all over the world,  
80 and especially in karst environments (Nolan and Stone, 2000; Li et al. 2010; El Gaouzi et al.  
81 2013; Schwientek et al. 2013; Alexia et al. 2015; Musgrove et al. 2016; Opsahl et al. 2017;  
82 Bu et al. 2017; Briand et al. 2017; Yue et al. 2019; Yang et al. 2019; Wang et al. 2020).  
83 Nitrate load export at the output of karst system can be high (Eller et al. 2017) and can imply  
84 a degradation of water quality in the receiving environment.

85           The origin of nitrate nitrogen needs to be identified in order to identify water resource  
86 hazards due to nitrate contents, either through direct inputs (atmospheric deposition,  
87 fertilizer) or through the transformation of nitrogen applied and transformed from the  
88 unsaturated zone to the groundwaters. Hence, knowledge about origins of the nitrate  
89 contamination is the first step for implementation of remediation measures to protect the  
90 water resources and avoid further water quality deterioration. In a complex hydrogeological  
91 context, the measurement of nitrate concentrations alone does not allow to determine their  
92 origin. On the other hand, the combined approach of hydrochemistry and isotope  
93 biogeochemistry could be a very relevant tool to better determine the origin of nitrogen in a  
94 particularly difficult context, with complex hydrogeological relationships between several  
95 karst aquifers both confined and unconfined. For several years, natural abundance stable  
96 isotope measurements of nitrate ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) have been used to identify its origins,  
97 including karst environment (Panno et al. 2001; Liu et al. 2006; Briand et al. 2017; Valiente  
98 et al. 2020). Typically, as a function of the pedo-climatic context and linked with land-use,  
99 nitrate can arise from: (i) primary sources such as atmospheric nitrate (Panno et al., 2001) or  
100 synthetic nitrate fertilizer; (ii) secondary sources such as synthetic ammonium fertilizer  
101 (Panno et al. 2001; Liu et al. 2006; El Gaouzi et al. 2013), sewage (Liu et al. 2006; Li et al.  
102 2010; El Gaouzi et al. 2013; Briand et al. 2017; Yang et al. 2019), or manure (Li et al. 2010;  
103 Heaton et al. 2012) with potential volatilization and subsequent nitrification. Due to the  
104 extent of these processes and the associated isotopic fractionation, the range of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$   
105 values of nitrate can vary significantly especially for  $^{15}\text{N}$  (Kendall et al. 2007).

106           These nitrate sources have distinct isotopic signatures, which can be used to semi-  
107 quantify the relative contribution of a single nitrogen (N) source (Aravena et al. 1993;  
108 Yakolev et al. 2015; Grimmeisen et al. 2017). In the case of a secondary source of N, where  
109 nitrate is produced after transformation in the soil that modify its concentration, fractionate

110 its isotopes and which can blur the initial signature of the N sources (Böttcher et al. 1990;  
111 Kendall et al. 2007; Briand et al. 2017). Besides their widespread use for determining the  
112 origins of nitrate,  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  can also be extended as a tool to characterize the  
113 way of functioning of multilayered karst aquifers and can be used as tool for better water  
114 management.

115 The proposed approach is applied to the Toulon multilayered karst system, located in  
116 the Southwest of France. It is a good example of a multilayered karst aquifer, with an outlet  
117 fed by two multilayered karst aquifers (Lastennet et al. 2004; Lorette et al. 2017, 2018,  
118 2020). For more than 60 years, increasing nitrate concentrations at the outlet of Toulon karst  
119 system have justified the implementation of the isotopic approach all over the  
120 hydrogeological catchment. This is a foremost concern as it serves as a major source of  
121 drinking water for approximately 55 000 people.

122 The objective of this paper is to use an isotopic approach to evaluate nitrate  
123 vulnerability in karst aquifers in order to sustainably manage the water resource.

124 Specifically, this research would like to: (i) assess nitrate sources in the Toulon Springs  
125 hydrogeological catchment; (ii) identify relationships between confined and unconfined karst  
126 aquifers using nitrate isotopes proposing a conceptual framework of the hydrogeochemical  
127 functioning of the Toulon multilayered karst system associated with nitrogen transport ; and  
128 (iii) from a more fundamental standpoint, provide a set of parameters adapted for karstic  
129 hydrogeological catchment and lays the foundation for the use of isotope biogeochemistry as  
130 a powerful tool for understanding the recharge of drinking water catchments and beyond the  
131 efficient management of water quality at the scale of karstic hydrogeological catchment.

132

133

## 134 2. Site description

135

### 136 2.1 Geology and hydrogeology

137

138 The Toulon Springs have supplied drinking water to the city of Périgueux (France)  
139 since 1832. It is a Vaclousian-type spring, located on a major faulted anticlinal structure,  
140 oriented N145° (Lastennet et al. 2004). The Toulon Springs are the main perennial outlet of  
141 the Toulon karst system and can reach almost 1 000 L.s<sup>-1</sup> during rainfall events, with a mean  
142 annual daily discharge of 450 L s<sup>-1</sup> (Lorette et al. 2018; Lorette, 2019).

143 The geology of the area is composed of carbonated rocks from upper Cretaceous and  
144 middle-upper Jurassic carbonate rocks (Fig. 1), the Cretaceous rocks are 200-250 meters in  
145 thickness (Turonian, Coniacian, Santonian, Campanian) and the Jurassic rocks 300-350  
146 meters in thickness (Bajocian, Bathonian, Oxfordian, Kimmeridgian) (Von Stempel, 1972;  
147 Lorette, 2019). The Cretaceous rocks at the top are limestones, while the Jurassic rocks  
148 below are dolomitic limestone and dolomites. There is an epikarst above the carbonate rocks  
149 topped by a soil layer of few meters. The Toulon karst system also presents a fold and faults  
150 mainly oriented NW-SE (Fig. 1).

151 The hydrogeological situation comprises three main multilayered aquifers: (i) an  
152 unconfined karstic upper Cretaceous aquifer: Turonian, Coniacian and Santonian aquifers,  
153 (ii) a confined karstic upper Jurassic aquifer: Oxfordian and Kimmeridgian aquifer and (iii) a  
154 confined karstic middle Jurassic aquifer: Bajocian and Bathonian aquifer. Cenomanian marls,  
155 considered impermeable, separate the upper Cretaceous aquifer and the upper Jurassic  
156 aquifer. The upper Cretaceous aquifer and the upper Jurassic aquifer are separated by  
157 Cenomanian marls, considered impermeable, Spatial thickness (4-20 meters) and facies

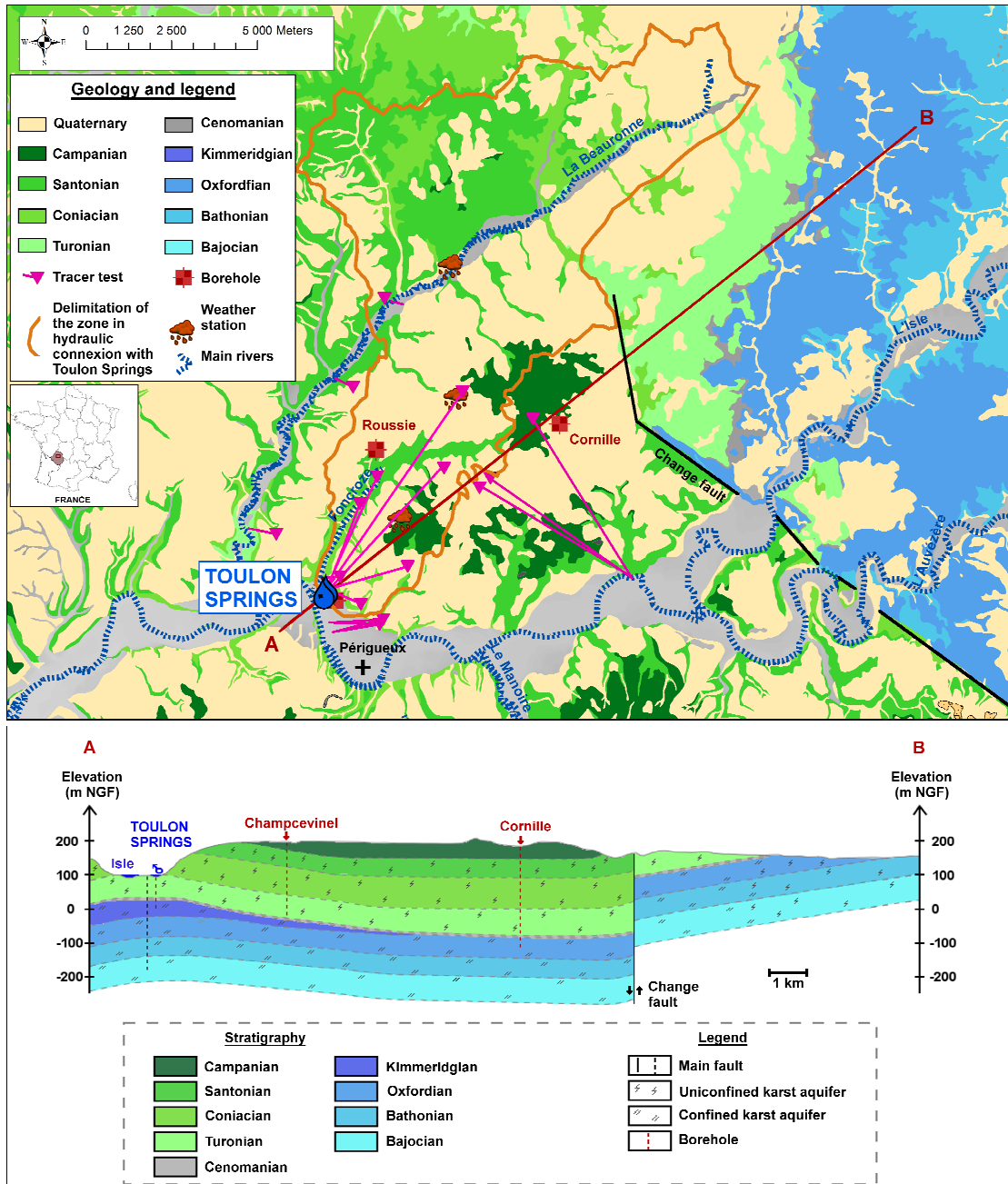
158 variations (marls-sand) over the study area entail additional difficulties in understanding the  
159 relationship of the two main aquifers.

160 The Toulon Springs hydrogeological catchment is composed of three main rivers: (i)  
161 the Isle River, consider as the regional base level; (ii) the Beauronne River in the northern  
162 part of the study area; (iii) the Foncroze River, in the southern part of the aquifer.

163 The hydrogeological dynamic of the Toulon Springs has been explained by [Lastennet](#)  
164 [et al. \(2004\)](#) and [Lorette et al. \(2017, 2018, 2020\)](#). The system is an example of springs  
165 originating from a confined Jurassic aquifer and an unconfined Cretaceous aquifer according  
166 to the hydrogeological conditions. During the low-flow period, there is a large water input  
167 from the confined Jurassic aquifer and the saturated part of the unconfined Cretaceous  
168 aquifer (Turonian). This implies that the Jurassic aquifer has a reservoir function. In a high-  
169 water period, infiltration water activates the drainage network in the formations above the  
170 Turonian (*i.e.* Coniacian and Santonian), thus suggesting that the Cretaceous aquifer has a  
171 transmission function. The Cretaceous aquifer is responsible for the hydrodynamic and  
172 hydrochemical variations. This enables the rapid transmission of the contaminants from the  
173 surface and subsurface. In terms of surface infiltration (*e.g.* NO<sub>3</sub><sup>-</sup>, DOC, bacteria, and  
174 turbidity), the Cretaceous aquifer contributes to the water quality hazards of the Toulon  
175 springs ([Lorette et al. 2020](#)).

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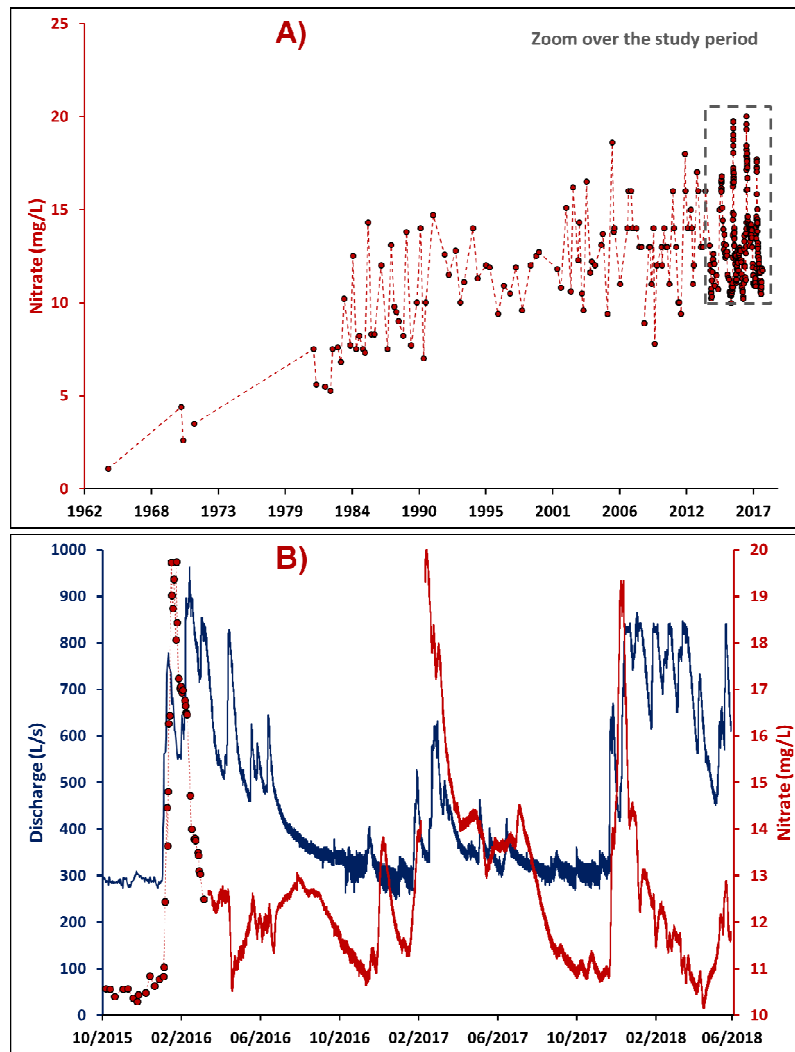
Fig. 1 : Hydrogeological map of the Toulon multilayered karst aquifer. AB: simplified SW-NE geological cross-section. The Cenomanian is considered as non aquifer (adapted from Lorette et al. 2018).

## 184           2.2   Land use and nitrate loads

185

186           Typical karstic landforms can be found on the area's land surface. There are several  
187 exokarstic forms such as sinkholes and surface water loss (*e.g.* stream river loss) on the  
188 plateau above the Toulon springs. Most of the losses are temporary and activated only after  
189 heavy rains. Sinkholes are mostly located on the plateau, in the thin soil layer area. In karst  
190 environments, a tracer test is an effective tool to understand the relationships between karst  
191 landforms and the outlet karst systems (Atkinson et al. 1973; Geyer et al. 2007; Labat and  
192 Mangin, 2015; Mudarra et al. 2014; Marin et al. 2015; Sivelles and Labat, 2019). Over the  
193 study area, tracer tests (Fig. 1) show flow between 6.4 m h<sup>-1</sup> and 130 m h<sup>-1</sup>, testifying to the  
194 karst vulnerability of the hydrosystem.

195           The dominant land uses of the rural area in the northern part of the city of Périgueux  
196 are forestry (47 %) and agricultural (43 %). Urban areas represent only 10 % of the  
197 catchment surface (Lorette, 2019). Agricultural areas are dominated by crops of wheat, corn,  
198 sunflower, and colza. Nitrogen is applied as ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub> - N synthetic  
199 fertilizer) and sewage sludge. Although accounting for only 10 % of land use, the influence  
200 of dwellings on nitrogen support is also noteworthy. On the whole study area, ~1 500 houses  
201 have septic tank, causing concentrated nitrogen inputs. Since the 1960's, the increase of  
202 intensive agriculture with the increase of nitrogen inputs has been responsible for the  
203 significant increase of nitrate concentrations in the Toulon springs (Fig. 2A).



204

205 **Fig. 2 : A) Temporal evolution of nitrate concentration at the Toulon Springs from 1962 to 2018. B) Temporal**  
 206 **evolution of discharge and nitrate concentration at the Toulon Springs from 01/10/2015 to 20/06/2018 (adapted from**  
 207 **Lorette et al. 2018 and Lorette, 2019).**  
 208

209 The local hydrochemical background was  $\sim 1 \text{ mg L}^{-1}$  in 1962 at low-flow conditions  
 210 and is now close to  $\sim 12 \text{ mg L}^{-1}$  on average with a maximum peak concentration of  
 211  $\sim 20 \text{ mg L}^{-1}$  during floods (Fig. 2B). In addition to data provided by administration of  
 212 Dordogne from 1960 to 2016, higher frequency measurements have been taken since 2016  
 213 (Lorette, 2019). Previous studies conducted in the Toulon Springs have demonstrated a  
 214 yearly export of 40 TN (Lorette, 2019). Nitrate stored in the unsaturated zone of the aquifer  
 215 may be transported to the saturated zone after some rainfall events (Lorette et al. 2020). At  
 216 the outlet of the karst system, nitrogen discharge as nitrate from the Toulon springs, come

217 from continuous inputs over the hydrogeological catchment. The resulting dynamic of  
218 changing nitrate concentration over time depends on its storage, transformation, and transfer  
219 from the soil-unsaturated zone to the outlet.

220

## 221 **3. Material and methods**

222

### 223 **3.1 Sampling strategy**

224

225 The main sampling strategy aimed to evaluate the water quality in both surface waters  
226 and groundwaters, with a focus on nitrate contamination. Sampling stations were selected to  
227 be representative of each karst aquifer in both confined and unconfined parts, as a function of  
228 knowledge of the study area and previous field investigations. To do so, hydrogeochemical  
229 and isotope measurements were done in order to characterize nitrate end-members and fate in  
230 the Toulon multilayered karst system.

231 From October 2015 to November 2017, hydrogeochemical data (physico-chemical  
232 parameters, major ion concentrations, nutrient concentrations) and isotopic data ( $\delta^{15}\text{N}_{\text{-NO}_3^-}$ ,  
233  $\delta^{18}\text{O}_{\text{-NO}_3^-}$ ) were measured in springs, boreholes, and rivers over the Toulon multilayered karst  
234 system. In addition,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of nitrate were measured on an ad hoc basis on potential  
235 nitrate sources over the study area.

236

#### 237 **3.1.1 Collection of groundwaters and surface waters**

238

239 At the Toulon Springs, water samples were collected twice a month from October 2015  
240 to January 2017, with daily sampling during groundwater flood events. A total of 51 samples

241 were collected. High resolution analyses of nitrate concentration have also been conducted  
242 since March 2016 using an UV-visible scanning spectrophotometer (Spectro::lyser, S::CAN),  
243 with a precision of 0.1 mg L<sup>-1</sup>, and a 0 to 46 mg L<sup>-1</sup> range of measurement. To avoid  
244 measurement errors due to matrix interference when turbidity increases, a manual calibration  
245 of the Spectro::lyser was done with laboratory measurements (Lorette, 2019).

246 Three sampling campaigns were conducted under different hydrological conditions:  
247 November 2015 (T1), representative of the water quality during low flow conditions; January  
248 2016 (T2), representative of water quality during the first flood of the hydrological cycle;  
249 May 2016 (T3), representative of the water quality during a spring flood. Each campaign was  
250 conducted at least 3 days after rainfall event, to avoid direct contribution of atmospheric  
251 nitrate from rainfall to both surface water and groundwater.

252 17 springs, 4 boreholes, and 7 samples in rivers were sampled during the T1, T2, and  
253 T3 campaigns. A total of 65 samples were collected over the whole study area during sampling  
254 campaigns, 13 from rivers, 45 from springs, 7 from boreholes.

255 Water samples were filtered in the field using a 0.45 µm PVDF membrane and divided  
256 between three bottles: (i) 50 mL polyethylene bottle for anion concentration measurement,  
257 (ii) 50 mL polyethylene bottle and acidified in the field to pH = 2 with HNO<sub>3</sub> for cation  
258 concentration measurement, and (iii) 150 mL was also filtered in the field on a 0.20 µm  
259 PVDF membrane, poisoned with HgCl<sub>2</sub> (6%), and dispatched into polyethylene bottle for the  
260 measurement of δ<sup>15</sup>N and δ<sup>18</sup>O values of nitrate.

261 The groundwater samples were mainly collected in the upper Cretaceous karst aquifer  
262 (96 samples - GW1 to GW15; B2). The other samples were collected in Jurassic karst aquifer  
263 (7 samples - GW16 and GW17; B4). B1 and B3 capture both Turonian aquifer and upper  
264 Jurassic aquifer (Oxfordian, Kimmeridgian).

265

266 **3.1.2 Sewage, fertilizer, soils, and rainwater.**

267

268 In order to better constrain the isotopic range of nitrogen sources, sampling was carried  
269 out before the water reaches the soil, the unsaturated zone and the aquifer.

270 Rainwater samples (NS1) were collected in the rain gauge ([Fig. 3](#)), 3 km north of the  
271 Toulon Springs. Sewage samples (NS2) were collected before discharge into the Beauverne  
272 River ([Fig. 3](#)). Solid samples of ammonium nitrate (NS3), representative of synthetic N  
273 fertilizer used over the hydrogeological catchment, were provided by local farmers, assuming  
274 that all the farmers use the same type of fertilizer and buy from the same supplier.

275 In addition to the characterization of primary sources of N, soil samples were collected  
276 in order to integrate variations in primary sources of N in the soil according to different types  
277 of inputs. An agricultural soil (NS4), representative of soil where N synthetic fertilizers are  
278 amended, was sampled ([Fig. 3](#)). Finally, agricultural soils (NS5), which have received  
279 amendments in the form of sewage sludge applications were sampled ([Fig. 3](#)).

280 The same collection bottles were used for liquid samples (NS1, NS2) as for  
281 groundwater and river water samples.

282 For soils samples (NS4, NS5), extraction of nitrate was performed with 0.5 M KCl to  
283 determine the isotopic composition of nitrate in the soil. Details are given in [Briand et al.](#)  
284 ([2017](#)).

285

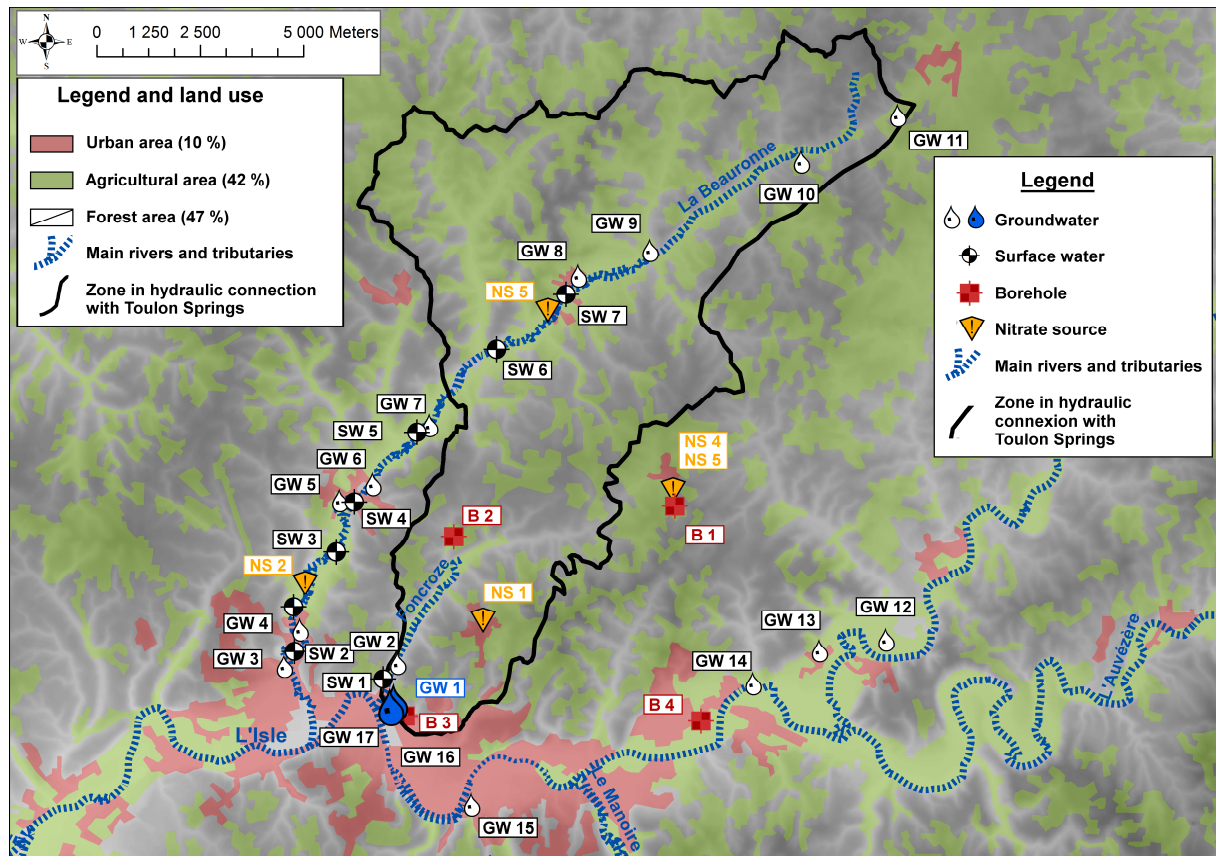


Fig. 3 : Land use and sampling position over the Toulon hydrogeological catchment.

286  
287

288

289

### 3.2 Analytical methods

290

291 In the field, a multiparameter WTW 3430 was used for temperature (T), pH (pH),  
292 electrical conductivity (EC) and dissolved oxygen concentration measurements ( $O_2$ ), precise  
293 to  $\pm 0.1^\circ C$ ,  $\pm 0.05$ ,  $\pm 1 \mu S \text{ cm}^{-1}$ , and  $\pm 0.1 \text{ mg L}^{-1}$  respectively. Alkalinity was measured within  
294 12 hours, by acid titration at the laboratory I2M-GCE in Bordeaux.

295 Ion concentrations ( $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $NO_3^-$  and  $SO_4^{2-}$ ) were determined by  
296 chromatography using a Dionex ICS 1500 for the cations and using a Dionex ICS 1100  
297 equipped (measurement accuracy 2%).

298  $\delta^{15}N$  and  $\delta^{18}O$  values of  $NO_3^-$  were determined using the azide method modified from  
299 McIlvin and Altabet (2005) with a purge and trap IRMS system (DeltaVplus, Thermo

300 Scientific, Bremen, Germany) in continuous-flow with a purge and trap system coupled with  
301 a Finnigan GasBench II system (Thermo Scientific). Details are given in [Semaoune et al.](#)  
302 [2012](#), [Briand et al. 2017](#), and [Sebilo et al. 2019](#). Results are reported in the internationally  
303 accepted delta notation in ‰ with respect to the standards air for  $\delta^{15}\text{N}$  and Vienna Standard  
304 Mean Ocean Water (V-SMOW) for  $\delta^{18}\text{O}$ , respectively. Nitrate and nitrite reference materials  
305 subject to the same analytical procedures were used to calibrate the isotopic composition of  
306  $\text{N}_2\text{O}$  (USGS34,  $\delta^{15}\text{N} = -1.8 \text{‰}$ ,  $\delta^{18}\text{O} = -27.9 \text{‰}$ , USGS35,  $\delta^{15}\text{N} = +2.7 \text{‰}$ ,  $\delta^{18}\text{O} = +57.5 \text{‰}$   
307 and USGS32,  $\delta^{15}\text{N} = +180 \text{‰}$ ,  $\delta^{18}\text{O} = +25.7 \text{‰}$  for nitrate standards; lab nitrite standards  
308 Lb1,  $\delta^{15}\text{N} = -63\text{‰}$  and Lb2,  $\delta^{15}\text{N} = +2.7 \text{‰}$  for nitrite standards). The precision was  $\pm 0.3 \text{‰}$   
309 for  $\delta^{15}\text{N}$  values and  $\pm 0.5 \text{‰}$  for  $\delta^{18}\text{O}$  values of nitrite.

310 For solid samples of fertilizer (NS3), samples were air-dried, ground, and sieved  
311 through a 1 mm mesh. Total nitrogen contents were determined using an elemental analyzer  
312 (Elementar). Isotope abundance ratios of total nitrogen for soils were determined by  
313 continuous-flow isotope ratio mass spectrometry coupled with an elemental analyzer (EA-  
314 CF-IRMS). Cup samples were then analysed N elemental and isotopic compositions using an  
315 elemental analyser (EA; NC2500, CarloErba®) coupled with an isotope ratio mass  
316 spectrometer (IRMS; Isoprime, GV Instruments®). IRMS daily drift was monitored using  
317 homemade standards (caseine, glycine) and, if necessary, data were corrected consequently.  
318 Elemental composition was calibrated against acetanilide and isotopic composition against  
319 homemade standards and reference material (IAEA-N<sub>2</sub>).

320

### 321 **3.3 Hydrological approach**

322

323 The origin of the nitrate present in the Toulon Springs could be inorganic and/or  
324 organic and direct or indirect. Inorganic nitrate is the nitrate itself contained in rainfall and



325 inorganic fertilizers. It can also be nitrate produced from the nitrification of ammonite in  
326 the soil. Organic nitrate arises from the production of nitrate from the ammonification and  
327 nitrification of organic matter and then its nitrification (Sebilo et al. 2013).

328 The isotopic composition of nitrate in the Toulon Springs, as the main outlet of the  
329 karst system, could be an integration of the isotopic signatures of nitrate for different nitrogen  
330 pools within the hydrogeological catchment. According to the large  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of  
331  $\text{NO}_3^-$  range of potential sources measured in literature (Kendall et al. 2007), it was necessary  
332 to: (i) confirm that the data obtained were in agreement with those presented in the Kendall  
333 plot; and (ii) better define these range of values for the studied area to precisely identify the  
334 isotopic signatures of nitrate sources. These range of potential sources are usually represented  
335 by rectangles in  $\delta^{15}\text{N}_v\delta^{18}\text{O}$  diagram (Panno et al. 2001; Kendall et al. 2007; Heaton et al.  
336 2012; Briand et al. 2017), theoretically integrating the set of possible isotopic signatures. In  
337 this work, the redefinition of range of values aimed to reduce the rectangle size in the  
338  $\delta^{15}\text{N}_v\delta^{18}\text{O}$  diagram.

339 The data of potential sources were plotted with data from surface waters, groundwaters  
340 and the Toulon Springs in the  $\delta^{15}\text{N}_v\delta^{18}\text{O}$  diagram. At each corner of the rectangles, arrows of  
341 0.5 slope are drawn, representing the denitrification process. It is considered that any point  
342 which lies between the two arrows from the same potential source (or rectangle) will have the  
343 potential source as an initial isotopic signature and will undergo a denitrification process  
344 causing a deviation from the initial signature.

345

346 Once relationships are established between the parameters, the data is integrated in the  
347 hydrogeological context for a better explanation of the way karst system functions and to  
348 help the water management over the hydrogeological catchment.

349

### 350           **3.4    $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ of potential sources.**

351

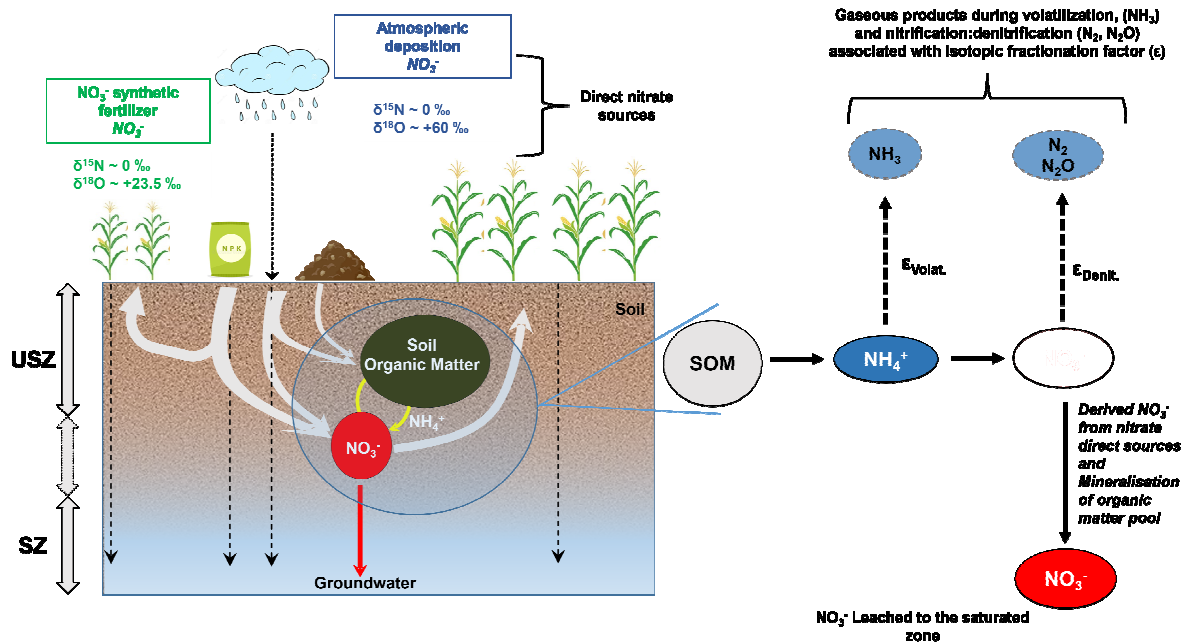
352           In a karstic context of the study, it is thus even more important to take into account the  
353 direct sources of nitrogen, likely to be able to infiltrate directly into the groundwater, and the  
354 indirect sources, likely to confer an isotopic signature of the nitrate that does not reflect the  
355 mixture of the direct sources (Fig. 4). The direct inputs of nitrate nitrogen on the catchment  
356 should be a mixture of atmospheric deposition and inorganic fertilizers (Fig. 4).

357           The basic idea behind the use of isotope biogeochemistry is that the measurement of  
358 the isotopic composition of nitrate at a definite location and time within a catchment reflects:  
359 (i) the endmembers, direct sources from which it originates, and (ii) the processes responsible  
360 for variations in its concentration.

361            $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of nitrate in the unsaturated zone (USZ) integrate on the one hand  
362 the isotopic composition of the potential direct sources of nitrogen (atmospheric deposition,  
363 inorganic fertilizers), and on the other hand the isotopic composition of nitrate resulting from  
364 biogeochemical processes in the soil which represent indirect source of nitrate nitrogen.

365           The ammonification and nitrification of nitrogen contained in organic matter (soils,  
366 manure, slurry) leads to the production of nitrate whose isotopic composition does not reflect  
367 the exogenous nitrogen inputs (atmospheric deposition and inorganic fertilizers), but their  
368 assimilation, production and re-production of nitrate from the dynamic of soil organic matter  
369 (El Gaouzi et al. 2013; Sebilo et al. 2013; Briand et al. 2017). Moreover, volatilization of  
370  $\text{NH}_4^+$ , non-complete nitrification and denitrification in the USZ could induce a significant  
371 isotopic fractioning which could impact the isotopic composition of the nitrate pool leaving  
372 the unsaturated zone and reaching the saturated zone. Finally, nitrate could be a product of  
373 the biological transformation of sewage, sludge spreading and more generally the  
374 ammonification-nitrification of organic matter within the soils (Fig. 4).

375 It has been established that nitrate diffusion and advection do not generate a significant  
 376 isotopic fractionation (Semaoune et al. 2012). Thus, provided that no biogeochemical processes  
 377 take place during the infiltration of nitrate into groundwater, the isotopic composition of  
 378 nitrate reaching the groundwater table should reflect that of nitrate leaving the  
 379 biogeochemically reactive unsaturated zone.



380

381 Fig. 4 : Schematic N transformation from direct nitrate sources in the N cycle associated with isotopic transformation  
 382 in the unsaturated zone (USZ) and transfer to the saturated zone (SZ).  
 383

#### 384 4. Results and discussion

385

386 Tables presenting the data in the following sections are proposed in the supplementary data.

387

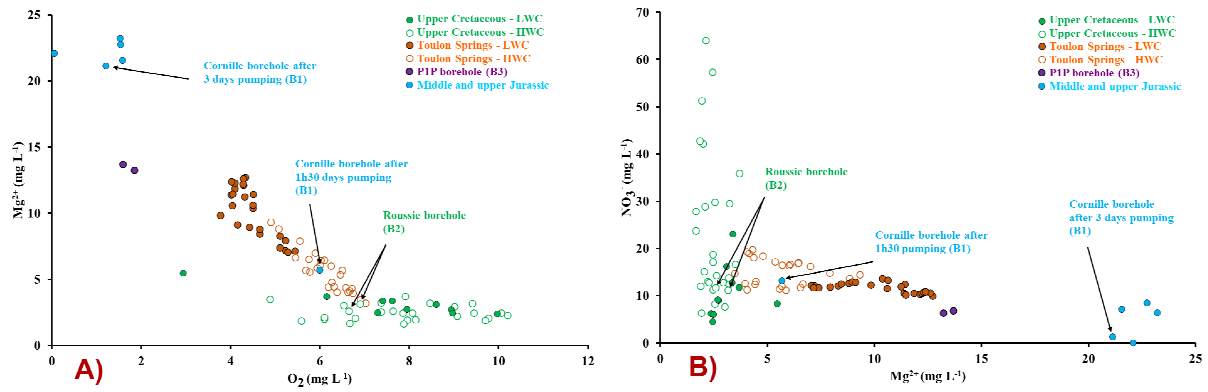
##### 388 4.1 Hydrochemical background and nitrate concentration in groundwater

389

390 Upper Jurassic karst aquifers have a typical deep water signature, with warm water  
 391 (between 17.3 °C and 20.6 °C), and low dissolved oxygen concentrations (between 3.3 mg L<sup>-1</sup>

392 <sup>1</sup> and 0.1 mg L<sup>-1</sup>). Dolomitic limestone and long residence time imply high magnesium  
 393 concentrations in upper Jurassic karst aquifers (between 21.1 mg L<sup>-1</sup> and 23.2 mg L<sup>-1</sup>) (Fig.  
 394 5A).

395



396

397 **Fig. 5 : A) O<sub>2</sub>vs.Mg<sup>2+</sup> binary plot performed with groundwater over the Toulon Springs hydrogeological catchment. B)**  
 398 **Mg<sup>2+</sup> vs.NO<sub>3</sub><sup>-</sup> binary plot performed with groundwater over the Toulon Springs hydrogeological catchment.**  
 399

400 The anoxic condition of these aquifers suggests a confined character, which is not  
 401 typical in karstic environments (Mahler and Bourgeais, 2013; Lorette et al. 2018). Water  
 402 from the Cornille borehole (B1) is a mix between Turonian aquifer and upper Jurassic aquifer  
 403 (Oxfordian, Kimmeridgian). The two samples at the the Cornille borehole were not collected  
 404 under the same conditions. 05/11/2015 sample was performed after 1h30 pumping and  
 405 18/09/2017 after 3 days of pumping, with a same pumping discharge of 100 m<sup>3</sup> h<sup>-1</sup>. Water  
 406 geochemical facies is characteristic of a mix between the two aquifers, with dissolved oxygen  
 407 concentration, magnesium concentration, and nitrate concentration respectively of 2.3 mg L<sup>-1</sup>,  
 408 5.7 mg L<sup>-1</sup> and 13.1 mg L<sup>-1</sup> (Fig. 5B). 18/09/2017 sample was performed after three days  
 409 pumping. Water geochemical facies is characteristic of upper Jurassic aquifer, with dissolved  
 410 oxygen concentration, magnesium concentration, and nitrate concentration respectively of  
 411 1.2 mg L<sup>-1</sup>, 21.1 mg L<sup>-1</sup> and 1.3 mg L<sup>-1</sup>. The Trelassac borehole (B4) captures middle Jurassic  
 412 aquifer (Bajocian, Bathonian). Water hydrogeochemical facies is characteristic of this deep

413 aquifer, with very low dissolved oxygen concentration ( $0.1 \text{ mg L}^{-1}$ ), and high magnesium  
414 concentration ( $22.1 \text{ mg L}^{-1}$ ) (Fig. 5A). No nitrate concentration is recorded in the middle  
415 Jurassic aquifer.  $^{14}\text{C}$  dating have shown a relative age of 12 500 years from middle Jurassic  
416 aquifer (unpublished data).

417 Upper Cretaceous karst aquifers (Turonian, Coniacian, Santonian) are characterized  
418 with a calcium bicarbonate facies. Magnesium concentrations are lower in Cretaceous  
419 aquifers than in Jurassic aquifers, as a result of the absence of dolomite (between  $1.7 \text{ mg L}^{-1}$   
420 and  $5.5 \text{ mg L}^{-1}$ ). As usual in karst environments, water could be considered oxic with  
421  $\text{O}_2 > 6 \text{ mg L}^{-1}$ . (Perrin et al. 2007; Mahler et al. 2011; Musgrove et al. 2011). The Roussie  
422 borehole (B2) is a typical borehole capturing upper Cretaceous aquifer (Turonian,  
423 Coniacian), with a low magnesium concentration compared to Jurassic aquifers ( $3.1 \text{ mg L}^{-1}$   
424 and  $2.6 \text{ mg L}^{-1}$ ). Nitrate concentrations are similar both for the two samples ( $11.2 \text{ mg L}^{-1}$  and  
425  $11.7 \text{ mg L}^{-1}$ ) (Fig. 5B). Dissolved oxygen concentrations highlight oxic water, with similar  
426 concentrations both for the two samples ( $6.9 \text{ mg L}^{-1}$  and  $6.7 \text{ mg L}^{-1}$ ). The P1P borehole (B3)  
427 is representative of the Turonian saturated zone near Toulon Springs. Next to the outlet of the  
428 karst system, upper Jurassic aquifer mixes with Turonian aquifer (Lorette et al. 2018). Mean  
429 dissolved oxygen concentration, mean magnesium concentration, and mean nitrate  
430 concentration are respectively  $1.7 \text{ mg L}^{-1}$ ,  $13.5 \text{ mg L}^{-1}$ , and  $6.6 \text{ mg L}^{-1}$  (Fig. 5B). The Toulon  
431 Springs water hydrogeochemical facies is close to the P1P borehole according to its  
432 hydrogeological characteristics. Dissolved oxygen concentration (minimum value of  $3.8 \text{ mg}$   
433  $\text{L}^{-1}$ ) is consistent with the higher participation of the upper part of the upper Cretaceous  
434 aquifer (Lorette et al. 2018).

435

436 During low water conditions, the surface water (Beauronne River, Foncroze River) has  
437 minimum and maximum nitrate concentrations of  $5.8$  and  $6.2 \text{ mg L}^{-1}$ .

438 During flood events, mean nitrate concentrations have been found to be higher than  
439 during low water conditions, with mean nitrate concentrations of 25.4 mg L<sup>-1</sup> and 18.1 mg L<sup>-1</sup>  
440 respectively, for both January 2016 and May 2016 floods.

441 The mean nitrate concentration for groundwater is higher than in surface water. During  
442 2015 low flow conditions, the mean groundwater nitrate concentration was 17.5 mg L<sup>-1</sup>, with  
443 minimum and maximum values of 8.5 mg L<sup>-1</sup> and 47.4 mg L<sup>-1</sup> respectively (Fig. 5B). During  
444 January 2016 flood, nitrate concentrations increased in most springs, with a mean  
445 concentration of 25.9 mg L<sup>-1</sup>, with a minimum and maximum values of 6.4 mg L<sup>-1</sup> and  
446 64.1 mg L<sup>-1</sup> respectively (Fig. 5B).

447 The Toulon Springs samples which were taken over more than one hydrological cycle  
448 (06/10/2015 to 04/01/2017) allowed us to analyse nitrate concentrations during several flow  
449 conditions (flood, recession, depletion). The mean nitrate concentration value was 13.4 mg L<sup>-1</sup>  
450 <sup>1</sup>, with minimum and maximum concentrations of 10.0 mg L<sup>-1</sup> on 25/11/2015 and 19.8 mg L<sup>-1</sup>  
451 on 18/01/2016 respectively. High resolution monitoring highlights the evolution of nitrate  
452 concentrations at the outlet of the karst system. During flood events, the increase or decrease  
453 in nitrate concentrations recorded at the Toulon Springs, imply no linear relationships  
454 between nitrate and discharge. Increase or decrease in nitrate concentrations have already  
455 been recorded in previous studies and are associated with leaching of the soil and unsaturated  
456 zone of the aquifer (Rowden et al. 2001; Stueber and Criss 2005; Mahler et al. 2008; Pronk et  
457 al. 2009; Pu et al. 2011; Huebsch et al. 2014). During low water conditions, nitrate  
458 concentrations decreased, this is linked with the increased participation of the deep upper  
459 Jurassic aquifer. The earliest analyses of the Toulon Springs suggest nitrate levels were very  
460 low in the 1960's, but rose substantially by the 1970s (Fig. 2). Today the mean concentration  
461 is about 12 mg L<sup>-1</sup> but can reach more than 20 mg L<sup>-1</sup> during some flood events.

462

## 463            **4.2    Isotope data**

464

465             $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of nitrate in surface waters, in groundwaters and in potential  
466 sources are summarized in tables of the supplementary data.

467             $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values for rainwater is -5.4 ‰ and +61.3 ‰ respectively.

468             $\delta^{15}\text{N}$  values for synthetic inorganic fertilizers used over the hydrogeological catchment  
469 of the Toulon Springs are +0.9 ‰ and +1.1 ‰, and are within the typical ranges reported for  
470 fertilizers in other countries (Vitoria et al. 2004; Bateman and Kelly, 2007; Heaton et al.  
471 2012; Briand et al. 2017).

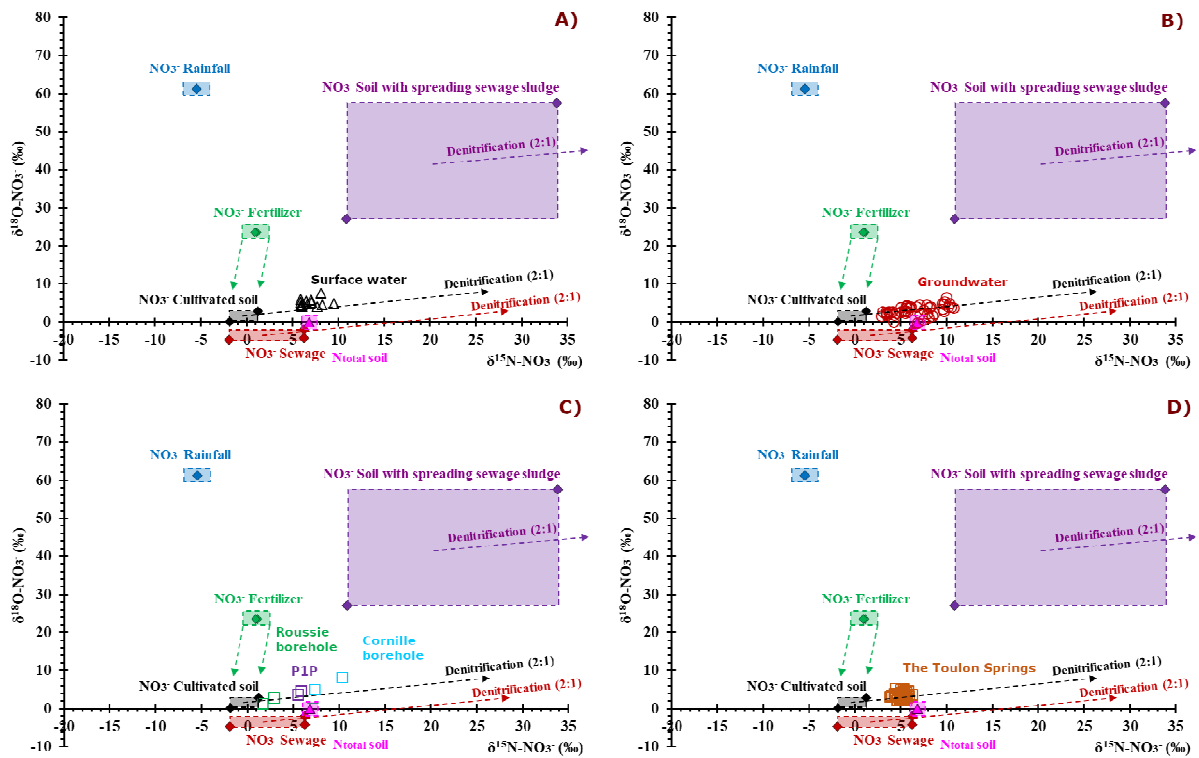
472             $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values for cultivated soil are respectively between -1.9 ‰ and  
473 +1.3 ‰ for  $\delta^{15}\text{N}_{\text{-NO}_3^-}$ , and between +0.2 ‰ and +2.2 ‰ for  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  (Fig. 6).

474             $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values for soil where sewage sludge were applied, are  
475 respectively between +10.9 ‰ and +33.8 ‰ for  $\delta^{15}\text{N}_{\text{-NO}_3^-}$ , and between +27.0 ‰ and  
476 +57.5 ‰ for  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  (Fig. 6).

477             $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values for sewage are respectively between -1.9 ‰ and +6.3 ‰  
478 for  $\delta^{15}\text{N}_{\text{-NO}_3^-}$ , and between -4.2 ‰ and -2.9 ‰ for  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  (Fig. 6).

479            Isotopic signatures from potential sources measured over the hydrogeological  
480 catchment are consistent with the data found in literature but ranges of values (or rectangles)  
481 in Fig. 6 are reduced, illustrating the need for precision in ranges of values. In other words,  
482 the data obtained are in good agreement with those presented in the “Kendall plot” and better  
483 define these ranges of values for the hydrogeological catchment, and are used to accurately  
484 identify the isotopic signatures of nitrate sources. An illustration of our potential sources in a  
485 Kendall plot is given in supplementary information.

486



487

488

489

490

**Fig. 6 :  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  measured in nitrate potential sources, surface water (A), groundwater (B), boreholes (C), and the Toulon Springs (D).**

491

$\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of nitrate in surface water (SW1 to SW7) and in groundwaters

492

(GW1 to GW17) are from +3.4 ‰ to +10.8 ‰ for  $\delta^{15}\text{N-NO}_3^-$ , and from -0.1 ‰ to +7.6 ‰ for

493

$\delta^{18}\text{O-NO}_3^-$  (Fig. 6).

494

For boreholes,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of nitrate vary between +1.7 ‰ and +10.3 ‰ for

495

$\delta^{15}\text{N}$  and between +1.3 ‰ and +9.3 ‰ for  $\delta^{18}\text{O}$  (Fig. 6). The most depleted and enriched

496

were measured respectively for the Roussie borehole (upper Cretaceous aquifer) and in the

497

Cornille borehole (mix between upper Jurassic and upper Cretaceous aquifers).

498

Concerning the Toulon Springs, the isotope compositions of nitrate are between +3.8

499

‰ and +6.2 ‰ for  $\delta^{15}\text{N-NO}_3^-$  and between +2.2 ‰ and +5.4 ‰ for  $\delta^{18}\text{O-NO}_3^-$  (Fig. 6). No linear

500

relationship between  $\delta^{15}\text{N-NO}_3^-$  and discharge (or water condition) is highlighted.

501



502 Isotopic signatures measured in the area environment (surface water, groundwater,  
503 boreholes, and the Toulon Springs) are not located in rectangles. Data are well distributed  
504 along the 2:1 (Fig. 6) expected residual nitrate derived from denitrification (Böttcher et al.  
505 1990; Kendall et al. 2007; Singleton et al. 2007; Liu et al. 2013).

506

#### 507 **4.2.1 Input of nitrate direct sources**

508

509 Rainfall is the main source for aquifer recharge.  $\text{NO}_3^-$  concentrations from rainfall were  
510 measured at  $1.4 \text{ mg L}^{-1}$  on 23/05/2016 with an average concentration of  $1.4 \text{ mg L}^{-1}$  between  
511 October 2015 and October 2018 (Lorette, 2019).

512 Over the study area, the isotopic compositions of rainfall were measured at  $-5.4 \text{ ‰}$  for  
513  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $+61.3 \text{ ‰}$  for  $\delta^{18}\text{O}_{\text{-NO}_3^-}$ , which are close to values measured in other regions  
514 across the world (Panno et al. 2001; Yue et al. 2017; Li et al. 2019). Despite the low nitrate  
515 concentrations, rainfall contributes about 49 tones per year of nitrogen ( $\text{TN year}^{-1}$ ) over the  
516 Toulon Springs hydrogeological catchment (Lorette, 2019). However, this nitrogen source is  
517 very attenuated by biogeochemical processes occurring in the soil. Previous studies (Katz et  
518 al. 2009; Lombardo Associates, 2011; El Gaouzi et al. 2013; Briand et al. 2017) have shown  
519 the isotopic signature of rainfall is totally lost when nitrate reaches the soil because it is  
520 assimilated into soil organic matter. Hence, only occasional infiltration events from sinkholes  
521 can highlight this isotopic composition at the outlet of a karst system (Panno et al. 2001).

522

523 N synthetic fertilizers (ammonium nitrate) have been applied for years over the  
524 catchment. Ammonitrate were produced following the Haber-Bosch process using nitrogen  
525 and oxygen of the air with  $\delta^{15}\text{N}_{\text{-N}_2} = 0 \text{ ‰}$  and  $\delta^{18}\text{O}_{\text{-O}_2} = +23.5 \text{ ‰}$ . N synthetic fertilizer have

526 a  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  close to the initial values record from the fertilizer amended on the study area,  
527 between +0.9 ‰ et +1.1 ‰ (Fig. 6).

528

#### 529 4.2.2 Input of nitrate undirect sources

530

531 The  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  values of crops (Fig. 6) vary between -1.9 ‰ and +1.3 ‰, and the  $\delta^{18}\text{O}_{\text{-NO}_3^-}$   
532  $\text{NO}_3^-$  values vary between +0.2 ‰ and +2.2 ‰, which is similar to values found in several  
533 case studies (Panno et al. 2001; Mayer et al. 2002; Sebilo, 2003; Liu et al. 2006; Xue et al.  
534 2009; Puig et al. 2013; Briand et al. 2017). Such low isotope ratios are typical for nitrate from  
535 cultivated soils that have been intensely amended with synthetic fertilizers having  $\delta^{15}\text{N}_{\text{-N}}$   
536 values near 0 ‰ (Mariotti, 1982). The low  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values indicate that cultivated soil nitrate  
537 is derived from the mineralization of soil organic matter and subsequent nitrification of  
538 nitrogen based mineral fertilizers.

539

540 Nitrate concentrations of sewage effluents can vary, depending on the progress of the  
541 treatment process. Indeed, in 22/03/2017 and 18/11/2017 samples, nitrate concentrations  
542 were high, namely between 169.9 mg L<sup>-1</sup> and 179.7 mg L<sup>-1</sup>, respectively.  $\text{NO}_2^-$  and  $\text{NH}_4^+$   
543 concentrations also varied, illustrating the progress of the treatment process.

544 The isotopic values of derived effluents ranged between -1.9 ‰ and +6.3 ‰ for  $\delta^{15}\text{N}$ ,  
545 and between -4.8 ‰ and -2.1 ‰ for  $\delta^{18}\text{O}$ . Depending on the degree of volatilization and  
546 nitrification during the treatment process, the output signal can vary.

547

548 The  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values are significantly higher in soil amended with sewage  
549 sludge, between +10.9 ‰ and +33.8 ‰ respectively for  $\delta^{15}\text{N}_{\text{-NO}_3^-}$ , and between +27.0 ‰ and  
550 +57.5 ‰ respectively for  $\delta^{18}\text{O}_{\text{-NO}_3^-}$ . The spreading treatment process is based on nitrification-

551 denitrification cycles. These enriched isotopic compositions are typical for nitrate that has  
552 undergone nitrification and partial denitrification during the treatment process (Kendall and  
553 McDonnell, 1998; Derse et al. 2007).

554 After sewage sludge application to soils, another cycle of nitrification-denitrification  
555 process occurs, leading to high  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values. Hence, depending both on  
556 treatment process and on soil biogeochemical, a sewage sludge source can have a very broad  
557 range of values, as illustrated by Fig. 6.

558

### 559 **4.3 Nitrate isotopes for characterization of nitrate sources in both surface** 560 **water and groundwater in karst watershed**

561

562 The isotope compositions of nitrate in both surface waters and groundwaters are plotted  
563 in Fig. 7A, Fig. 8A and Fig. 9 and compared with the isotope compositions of potential  
564 source of nitrate. 0.5 slope arrows are plotted at the corners of potential sources to extend the  
565 zone corresponding to the initial nitrate source.

566

#### 567 **4.3.1 Endmembers of surface nitrate**

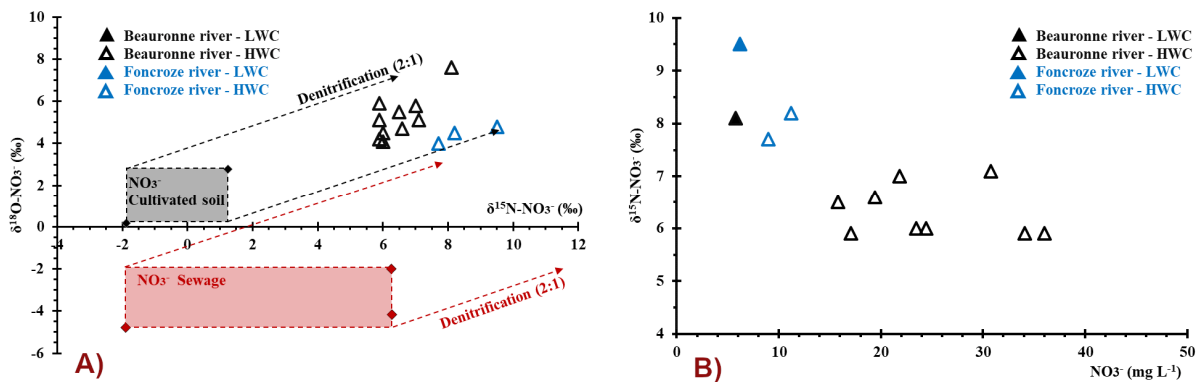
568

569 It is well known in karstic systems that surface water could directly contribute to spring  
570 discharge by infiltration of water from temporary/occasional sinkholes in rivers (Rey et al.  
571 2006; Huneau et al. 2013; Cholet et al. 2018; Lorette et al. 2021). The identification of nitrate  
572 sources in surface waters is therefore a crucial prerequisite for a more general understanding  
573 of the nitrogen dynamic at the catchment scale.

574 Fig. 7A is a magnification of the usual  $\delta^{15}\text{N}_{\text{vs}}\delta^{18}\text{O}$  diagram focusing on two main  
 575 nitrate potential sources: (i)  $\text{NO}_3^-$  from cultivated soil; (ii)  $\text{NO}_3^-$  from sewage. According to  
 576 this figure, it is now considered that nitrate does not come from direct inputs such as  
 577 atmospheric deposition or fertilizers, but rather from indirect input after biogeochemical  
 578 transformation.

579 The isotopic compositions of nitrate ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) for the Beauronne River and the  
 580 Foncroze River seem to reflect the origin of cultivated soil that would have undergone partial  
 581 denitrification, leading the nitrate in these waters having higher isotopic signatures both in  
 582  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ . The highest  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values, associated with water which has  
 583 lower concentrations of nitrate, are measured in low-water conditions (LWC) (Fig. 7B),  
 584 while the less enriched values of  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  are measured in high-water  
 585 conditions (HWC), and are associated with water which has higher nitrate concentrations.  
 586 Nitrate from soils, more depleted in  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  is exported into surface water  
 587 during rainfall events.

588



589

590 Fig. 7 : (A)  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  vs  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  measured in the Beauronne river and the Foncroze river. (B)  $\text{NO}_3^-$  vs  $\delta^{15}\text{N}_{\text{-NO}_3^-}$   
 591 measured in the Beauronne river and the Foncroze river. LWC : low-water condition. HWC : high-water condition.  
 592

### 593 4.3.2 Origins of groundwater nitrate

594

595 Groundwater data are plotted in Fig. 8A. Data are separated into: (i) springs located in  
596 rural areas, (ii) springs located in urban areas.

597 For springs located in rural areas, nitrate records are from the mineralization of  
598 synthetic N fertilizer mineralized in cultivated soil, according to  $\text{NO}_3^-$  isotopic compositions  
599 (Fig. 8A). A denitrification process is identified according to the alignment of points on a  
600 straight 0.5 slope line. Most enriched values have been recorded during low water conditions  
601 (Fig. 8A), and the most depleted have been recorded during floods (Fig. 8A). Soil nitrate of  
602 lower isotope composition enters groundwater during rainfall events. January 2016 flood  
603 water had  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values which were more depleted than the expected theoretical minimum  
604  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values (+4.0 ‰), illustrating a possible quickflow component from the recharge to  
605 the outlet from several springs.

606 Concerning springs located in urban areas, other isotope compositions of groundwater  
607 nitrate are identified (Fig. 8A), implying another nitrate source. The nitrate isotope  
608 concentrations of sewage effluent recorded in springs located in urban areas. As previously, a  
609 denitrification process occurs.

610 Most springs have the same nitrate sources depending on hydrological situation. Two  
611 springs show contamination from several sources: GW9 (Roche Pontissac springs) and  
612 GW10 (Fontamiel spring). During low water conditions,  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values of  
613 GW10 are influenced by “Cultivated soil” type values (Fig. 8A). On the contrary, during high  
614 water conditions, nitrate isotopes values change, and the origins suggest “Sewage” type  
615 values. Similar observations were made for GW9 during the January 2016 flood event,  
616 isotopic compositions of  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  show the influence of “Sewage” type values,  
617 while during May 2016 flood event, these isotopic compositions are closer to those  
618 associated with “Cultivated soil” type values (Fig. 8A). These observations confirm the

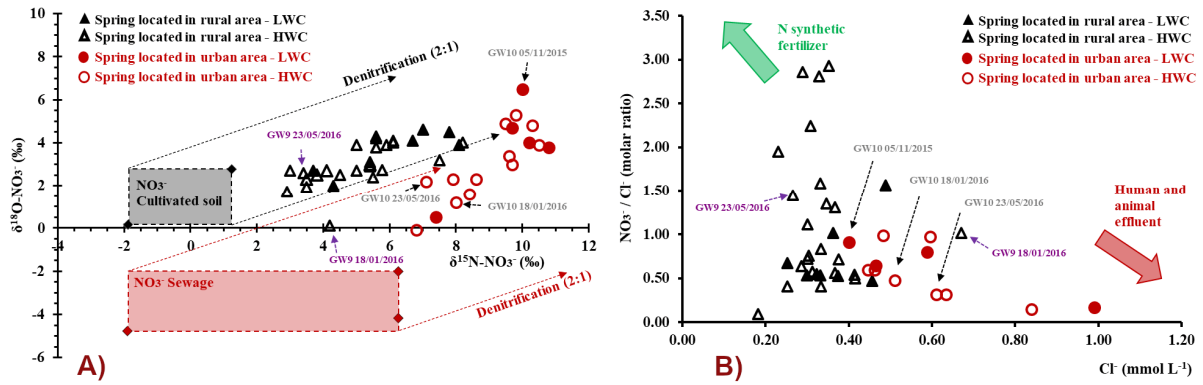
619 multiple sources that can be measured in the aquifer, and the potential mixtures that can  
620 occur in a higher aquifer.

621

622 The chemical behavior of chloride ( $\text{Cl}^-$ ) in natural waters is relatively conservative  
623 because it is not subject to physical, chemical, or biological processes. The  $\text{NO}_3^- / \text{Cl}^-$  ratio  
624 has been used for decades to highlight phenomena of mixtures or biological processes  
625 responsible for the distribution of nitrate concentrations (Mengis et al. 1999; Vitoria et al.  
626 2004; Li et al. 2013). Chemical synthetic N fertilizers are usually characterized by high  
627 nitrogen and low chloride content. Domestic and animal effluents, on the contrary, have  
628 higher  $\text{Cl}^-$  concentrations and lower  $\text{NO}_3^- / \text{Cl}^-$  ratios (Liu et al. 2006; Li et al. 2013). The  
629  $\text{NO}_3^- / \text{Cl}^-$  ratio decreases in the event of denitrification or consumption of the nitrates  
630 without modifying the chloride concentrations. Fig. 8B confirms the results demonstrated by  
631 comparison of nitrate isotopes compositions (Fig. 8A). Indeed, samples located in the bottom  
632 right-hand part of Fig. 8B will characterize contamination by nitrate from domestic waste  
633 (“Sewage” type values), while samples located in the left part of the graph suggest nitrate  
634 origins from synthetic mineral fertilizers (“Cultivated soil” type values).

635 The observations made on Pontissac spring (GW 9) and Fontamiel spring (GW10) are  
636 also made in Fig. 8B. For GW 9, the sample taken during the January 2016 flood event was  
637 close to “Sewage” type values as is highlighted in Fig. 8A. On the contrary, the sample from  
638 May 2016 is closer to the “Cultivated soil” type values. The findings are similar for GW10,  
639 with water quality that changes over the hydrological cycle. During low water conditions, the  
640 main signature seems to be synthetic N fertilizers, while it evolved towards signatures of  
641 domestic wastes in January 2016 and May 2016.

642 The observation of data in  $\text{NO}_3^-/\text{Cl}^-$  vs  $\text{Cl}^-$  diagram consolidates the validity of the use of  
 643 nitrate isotopes to identify the origins of nitrogen contamination in both surface and  
 644 groundwater environments.  
 645



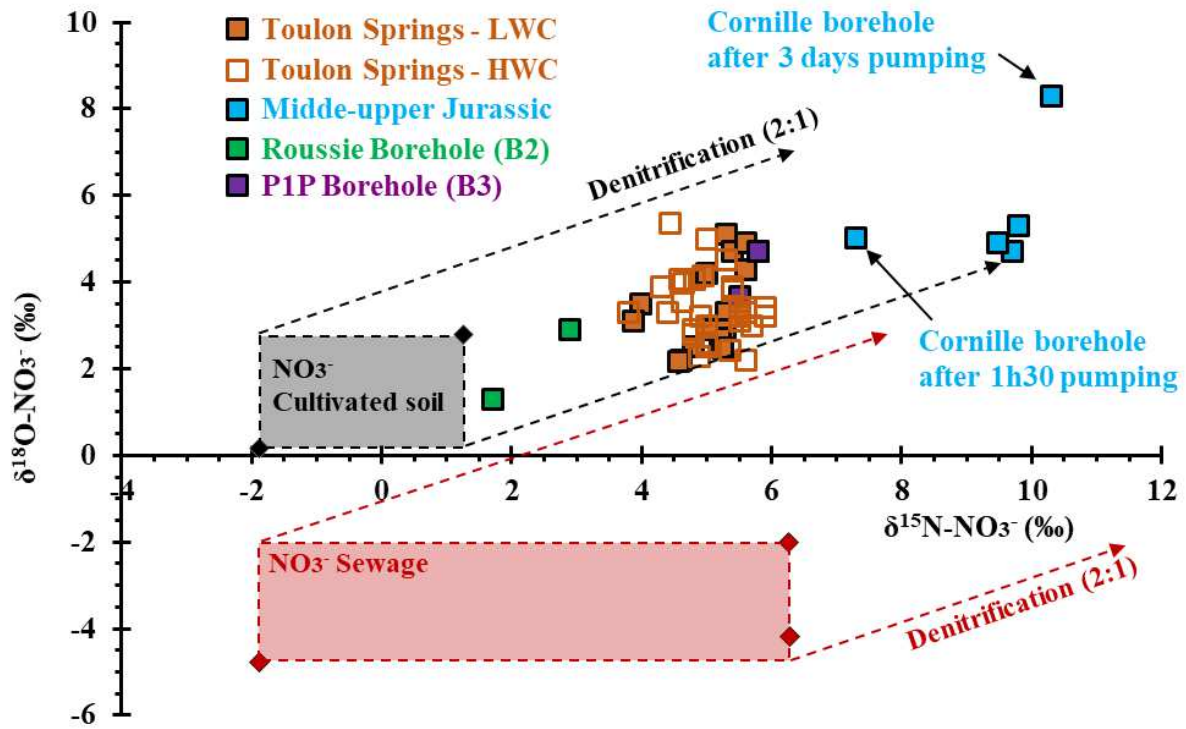
646 **A)**  
 647 **B)**  
 648 **Fig. 8 :** (A)  $\delta^{15}\text{N}-\text{NO}_3^-$  vs  $\delta^{18}\text{O}-\text{NO}_3^-$  measured in springs located over Toulon Springs hydrogeological catchment. (B)  $\text{Cl}^-$   
 649 vs  $\text{NO}_3^-/\text{Cl}^-$  ratio measured in springs located over Toulon Springs hydrogeological catchment. LWC : low-water  
 650 condition. HWC : high-water condition.

#### 651 4.4 Nitrate sources and fate in the Toulon multilayered karst aquifer

652  
 653 Contrary to the springs described above, the Toulon Springs (GW1), which are the  
 654 most important outlet of the study area, probably include all the nitrate isotopic signatures  
 655 measured within the hydrogeological catchment. However, Fig. 9 reveals that the nitrate  
 656 isotopes measured at the outlet are predominantly influenced by synthetic N fertilizers  
 657 mineralized in cultivated soils. No linear relationship between  $\delta^{15}\text{N}-\text{NO}_3^-$  and discharge (or  
 658 water condition) is highlighted. The difficulty to interpret the isotope compositions of the  
 659 Toulon Springs nitrate is probably associated with the complex infiltration process over the  
 660 hydrogeological catchment, the various isotope compositions of the nitrate sources, and the  
 661 various contribution rate of both Jurassic and upper Cretaceous aquifers (Lorette et al. 2018).  
 662 The shift of points on the right-hand side of the graph, which follow a 0.5 slope line of,

663 highlights the high or low extent of the denitrification process. The other sources, such as  
 664 septic effluents or sewage sludge, do not seem to influence the global signal at the outlet of  
 665 the karst system, but are probably present in lower proportions.

666



667

668 Fig. 9 :  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  measured in the Toulon Springs and boreholes over the study area. LWC : low-water  
 669 condition. HWC : high-water condition.  
 670

671 Waters from the Cretaceous aquifer at Roussie borehole (B2) have nitrate isotope  
 672 compositions from the same source as the Toulon Springs (Fig. 9). Proximity to the nitrate  
 673 source in Fig. 9 indicates the low denitrification process of nitrate in water.

674 Water from the Jurassic confined aquifer at Cornille borehole (B1) is very important for  
 675 the study area. As a reminder, B1 is a mix of the upper Cretaceous aquifer (Turonian) and  
 676 upper Jurassic aquifer (Oxfordian, Kimmeridgian). The two samples taken in B1 were not  
 677 obtained under the same conditions. 05/11/2015 sample was obtained after 1h30 of pumping.  
 678 The geochemical facies of the water was characteristic of a mix between the two aquifers,



679 with dissolved oxygen concentration, magnesium concentration, and nitrate concentration of  
680 2.29 mg L<sup>-1</sup>, 5.69 mg L<sup>-1</sup> and 13.14 mg L<sup>-1</sup> respectively. The 18/09/2017 sample was obtained  
681 after three days of pumping. The water geochemical facies was characteristic of the upper  
682 Jurassic aquifer, with dissolved oxygen concentration, magnesium concentration, and nitrate  
683 concentrations of 1.21 mg L<sup>-1</sup>, 21.12 mg L<sup>-1</sup> and 1.32 mg L<sup>-1</sup> respectively. Nitrate isotopes  
684 compositions for these two samples are from the same source (“Cultivated soil” type values)  
685 but have different signatures. The  $\delta^{15}\text{N}_{\text{-NO}_3^-}$  and  $\delta^{18}\text{O}_{\text{-NO}_3^-}$  values of the samples containing  
686 more upper Cretaceous water (05/11/2015) are more depleted than those samples with more  
687 upper Jurassic water (18/09/2017) (Fig. 9).

688 The nitrate isotope composition of water from the mix between the two aquifers is  
689 similar to that found for the Toulon Springs, as illustrated by the P1P borehole (B3) located  
690 on the Toulon pumping plant site. This confirms previous studies demonstrating that the  
691 Toulon Springs are fed by a multilayered karst aquifer from both an unconfined upper  
692 Cretaceous karst aquifer and a confined Jurassic karst aquifer (Lastennet et al. 2004; Lorette  
693 et al. 2017, 2018).

694 Results from the Cornille borehole (B1) are very important to understand the  
695 hydrogeological functioning of the Toulon Springs hydrogeological catchment. Even though  
696 the water is dated at about 2 800 years old in the upper Jurassic aquifer (unpublished data),  
697 the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of  $\text{NO}_3^-$  highlighted the impact of synthetic N fertilizers which have  
698 been applied for several decades. The common origin of the nitrate found in the upper  
699 Cretaceous and in the upper Jurassic still puts a question mark on the impermeable capacity  
700 of the Cenomanian marls and hence on the protective captivity in the upper Jurassic. It is  
701 highly probable that nitrate found in the upper Jurassic comes directly from the upper  
702 Cretaceous.

703

704 According to the previous results of the main Toulon project, from [Lorette et al. \(2017,](#)  
705 [2018, 2020\)](#), and [Lorette \(2019\)](#), a conceptual diagram of the hydrogeochemical functioning  
706 of the Toulon multilayered karst system associated with nitrogen transport is developed  
707 ([Fig. 10](#)).

708  
709 It integrates direct sources of nitrogen (amount and isotopic values), and processes  
710 inducing changes in nitrate concentration within the soil before reaching the groundwater  
711 (detailed in the [Fig. 4](#)): (i) the different potential sources and a quantitative estimation of  
712 nitrogen input and output, (ii) the denitrification process occurring in both the Cretaceous  
713 karst aquifer (low denitrification), and in the upper Jurassic karst aquifer (high  
714 denitrification), (iii) the mix between the aquifers at the Toulon Springs.

715 [Fig. 10A](#) is a schematic N transformation occurring in the soil according to the land use  
716 over the Toulon Springs hydrogeological catchment, where different N input are suggested. It  
717 distinguishes “N applied” and “NO<sub>3</sub><sup>-</sup> infiltrated”. N applied describe the identified and  
718 quantified N potential sources over the hydrogeological catchment. The quantitative annual  
719 estimation is that 349 T year<sup>-1</sup> of nitrogen input come from N synthetic fertilizer, whereas 9 T  
720 year<sup>-1</sup> and 8 T year<sup>-1</sup> come from spreading sludge and sewage respectively. Finally, 49 T year<sup>-1</sup>  
721 of nitrogen input comes from rainfall, but almost the entire quantity is attenuated in the soil  
722 ([Katz et al. 2009](#); [Lombardo Associates, 2011](#)). NO<sub>3</sub><sup>-</sup> infiltrated describe the derived nitrate  
723 which can leak to the aquifer after a number of factors which appear into the soil, such as: (i)  
724 the use of nitrogen by plants for their growth; and (ii) the partial denitrification process which  
725 occurs in the soil and in the saturated zone of the aquifer. Hence, these several factors act to  
726 decrease the quantity of NO<sub>3</sub><sup>-</sup> infiltrated compared to N applied over the surface.

727 Concerning isotopic composition, [Fig. 10A](#) show than  $\delta^{15}\text{N}_{\text{NO}_3^-}$  from potential sources  
728 (or N applied) are different than  $\delta^{15}\text{N}_{\text{NO}_3^-}$  from NO<sub>3</sub><sup>-</sup> infiltrated (or undirect sources as

729 explain previously in Fig.4 and also in sections 3.4, 4.2.1, and 4.2.2). Hence, the mix of every  
730 undirect sources implies the  $\delta^{15}\text{N}_{\text{NO}_3^-}$  signature of the upper part of the Toulon karst aquifer  
731 (upper Cretaceous with low denitrification during short time period).

732 At the outlet of the Toulon karst system, annual nitrogen outputs are similar despite  
733 differences in the total volume recorded. About 40 TN year<sup>-1</sup> have been recorded at the outlet  
734 (Fig. 10B). The  $\delta^{15}\text{N}_{\text{NO}_3^-}$  recorded (+4.5 ‰) is hence a mix between  $\delta^{15}\text{N}_{\text{NO}_3^-}$  from  
735 Cretaceous unconfined karst aquifer (+2.5 ‰) and  $\delta^{15}\text{N}_{\text{NO}_3^-}$  from Jurassic confined karst  
736 aquifer (+10.0 ‰), depending hydrogeological conditions and quick flow component.

737

#### 738 **4.5 Implication for water management and vulnerability of the Toulon** 739 **multilayer karst system**

740

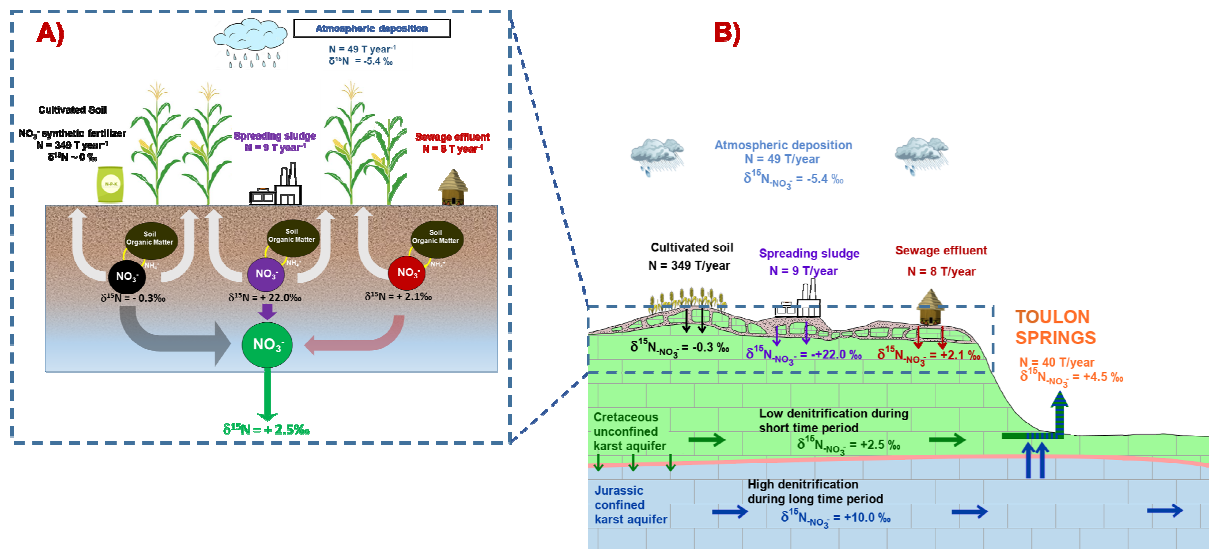
741 Over the Toulon Springs hydrogeological catchment, land use associated with the karst  
742 landscape entails specific water management. Geomorphological features such as sinkholes  
743 or swallowholes imply a potential direct and rapid transfer of any contaminant from the  
744 surface to the outlet.

745 The Toulon Springs are a strategic resource for drinking water in Dordogne. The  
746 increasing trend of nitrate concentration in the Toulon Springs (Fig. 2), means that a specific  
747 methodology to characterize the vulnerability to nitrate contamination in the multilayer karst  
748 system has to be developed. These results have implications for water management in that  
749 they provide a better quantification of nitrate input in surface. For cropland, the survey must  
750 focus on: (i) the use of karst landscapes for cropland, (ii) decreasing anthropogenic nitrogen  
751 input such as synthetic N fertilizer to minimize the leaching of nitrate to the underlying  
752 aquifer. For urban areas, the construction of new residential neighborhoods must be sensitive  
753 to the constraints of the karst environment.

754 In terms of vulnerability, the Cretaceous unconfined multilayer karst aquifer has a low  
755 denitrification capacity as highlighted by the isotope composition of nitrate. This means that  
756 this resource is directly vulnerable to surface pollution. Over the Toulon multilayered karst  
757 system, complex hydrogeological relationships between aquifers ([Lorette et al. 2018](#)) allow  
758 nitrate transfer from the upper Cretaceous unconfined aquifer to the upper Jurassic confined  
759 aquifer. Intense denitrification processes associated with long residence times and anoxic  
760 water make the upper Jurassic karst aquifer less vulnerable to surface pollution. However, a  
761 water quality survey is necessary in order to avoid the increase of nitrate concentrations in  
762 the future. This study on nitrate isotopes has shown that although the upper Jurassic aquifer  
763 seems less vulnerable than the upper Cretaceous aquifer, water from the upper Cretaceous  
764 can nonetheless infiltrate into the upper Jurassic aquifer through the Cenomanian marls.  
765 Thus, the idea that the upper Jurassic aquifer has a protective role would be wrong, implying  
766 that deeper drilling is needed to capture waters from the middle Jurassic.

767 No nitrate concentrations were recorded for the middle Jurassic aquifer, testifying to  
768 the good natural protection of the aquifer. However, particular attention must be paid to this  
769 aquifer. For the past 25 years an increasing number of boreholes have been drilled through  
770 this aquifer for drinking water or industries ([Lorette, 2019](#)), and its possible over-exploitation  
771 could be dangerous for its still protected quality and could lead to a downward infiltration.  
772 Consequently, local public health organizations should also extensively monitor the water by  
773 to ensure that the water quality in the middle Jurassic is maintained.

774



775

776 **Fig. 10 :** (A) Schematic N transformation occurring in the soil over the Toulon Springs hydrogeological catchment (B)  
 777 **Conceptual model of the functioning of Toulon karst system associated with nitrogen transfer and quantitative**  
 778 **estimation of nitrogen fluxes.**

779

## 780 5. Conclusion

781

782 In water management, it is important to know nitrate sources. In this research, an  
 783 approach for tracing nitrate sources in multilayered karst aquifers has been proposed. First,  
 784 the approach involves the identification of water type and nitrate concentrations over the  
 785 hydrogeological catchment, in both surface water and groundwater. The second step consists  
 786 of analyzing every potential source of nitrate over the study area using nitrate isotopes ( $\delta^{15}\text{N}$   
 787 and  $\delta^{18}\text{O}$ ). The third step consists of linking nitrate isotopic compositions of surface water  
 788 and groundwater, to those of potential sources of nitrate. Biogeochemical processes in the N  
 789 cycle, such as nitrification or denitrification, are integrated into the planning process to  
 790 identify nitrate sources in the study area. The final step consists of integrating isotopic values  
 791 in the hydrogeological context to evaluate the fate of nitrate in multilayered karst aquifer, in  
 792 order to propose better water management.

793           The approach was applied on a major karst system in western France: the Toulon  
794 system. Results showed two main nitrate sources: (i) N synthetic fertilizers (ammonium  
795 nitrate) mineralized in cultivated soils and (ii) sewage effluents. Both of these nitrate sources  
796 underwent more or less denitrification before reaching the sampling points. Within the  
797 Toulon Springs hydrogeological catchment, surface waters are contaminated with nitrate  
798 from N synthetic fertilizer (primary source) mineralized in cultivated soils (secondary  
799 source). Most of the springs in the catchment are also contaminated with this nitrate source,  
800 but some indicate nitrate isotope composition of sewage effluent origin. Finally, the Toulon  
801 Springs are only contaminated with nitrate from N synthetic fertilizer mineralized in  
802 cultivated soils.

803           This work highlights soil properties in changing N direct isotopic compositions. Hence,  
804 to have a better idea of nitrate sources affecting surface water or groundwater, a knowledge  
805 of nitrate isotopic compositions of is fundamental. This research suggests discussion about  
806 the fate of nitrate in a karst system with complex hydrogeological relationships. This work  
807 has showed the potential use of nitrate isotopes as a tool for characterization of the  
808 functioning in a multilayered karst aquifer. Over the Toulon Springs hydrogeological  
809 catchment, this work on nitrate isotopes has showed that water from the upper Cretaceous  
810 layers can infiltrate the upper Jurassic aquifer through the Cenomanian marls. This new result  
811 on the study area highlights the value of this work in water management and the vulnerability  
812 of water quality in karst environments. The study on the purifying properties which aquifers  
813 have on nitrate contamination, offers good perspectives for better water management in karst  
814 environments.

815           From a more fundamental standpoint, this work provides a set of parameters adapted  
816 for a karstic hydrogeological catchment. Future steps of this research will provide a common

817 approach for karst environments, focusing on N transformation of primary sources in soil and  
818 consequences on nitrate isotopic compositions, within contrasted karst catchments.

819 This methodology, based on the combination of both nitrate isotope and  
820 hydrogeological tools, provides new information about nitrate sources and fate in karst  
821 environments, and gives tools for implementation of remediation measures to protect the  
822 water resources. The innovative way of implementing the method should encourage its use in  
823 other karst aquifers with similar issues, and hence, lead to an easy method of comparison  
824 between karst systems.

825

## 826 **Acknowledgements**

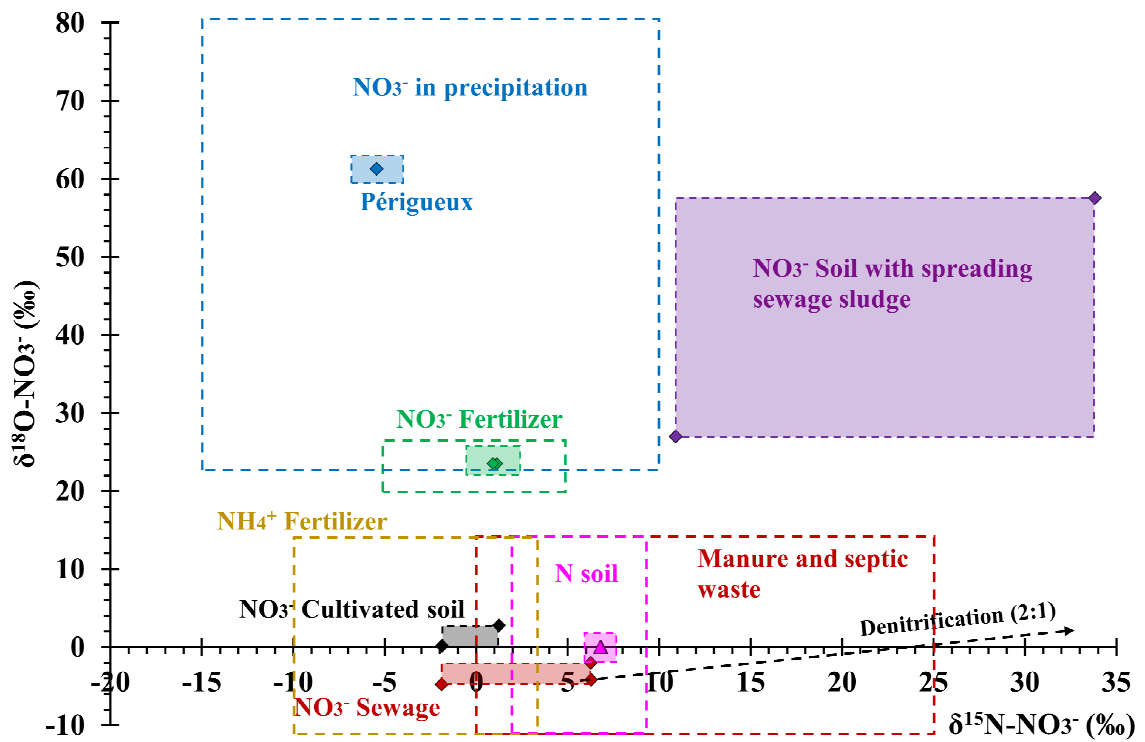
827 This work was supported by the Région Aquitaine, the city of Périgueux, Suez, the  
828 Conseil Général de la Dordogne, and the Adour-Garonne Water Agency. The authors thank  
829 Suez's agents for the technical assistance and the field knowledge they contributed. They  
830 finally want to thank Dr. David VIENNET for his help in proof reading the article.

831 This work benefited from fruitful discussion within the French network of karst  
832 observatories (SNO Karst), initiative from the INSU/CNRS.

833

## 834 **Supplementary information**

835



836

837 Kendall plot vs data obtained from the hydrogeological catchment. Empty rectangles are from Kendall et

838

al. 2007. Full rectangles with points are from this study.

839

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**Sample description, chemical composition (mg L<sup>-1</sup>) and isotopic composition (‰) of surface water samples.**

Sample designation	Sample name	Date	T (°C)	pH (pH unit)	EC (µS cm <sup>-1</sup> )	O <sub>2</sub> (mg L <sup>-1</sup> )	Ca <sup>2+</sup> (mg L <sup>-1</sup> )	Mg <sup>2+</sup> (mg L <sup>-1</sup> )	Na <sup>+</sup> (mg L <sup>-1</sup> )	K <sup>+</sup> (mg L <sup>-1</sup> )	HCO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	Cl <sup>-</sup> (mg L <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (mg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	SiO <sub>2</sub> (mg L <sup>-1</sup> )	δ <sup>18</sup> O <sub>H2O</sub> (‰)	δ <sup>15</sup> N <sub>NO3</sub> <sup>-</sup> (‰)	δ <sup>18</sup> O <sub>NO3</sub> <sup>-</sup> (‰)	Balance (%)
Beauronne - Reymondens	SW 1	03/11/2015	11.9	7.7	566	4.3	109.4	3.3	11.2	3.7	334.3	16.9	5.6	5.8	14.9	-	8.1	7.6	1.3
Beauronne - le Cros	SW 2	25/01/2016	9.4	8.0	625	11.0	118.5	2.4	7.1	1.2	347.7	12.2	8.8	17.1	13.3	-	5.9	5.9	-0.3
Beauronne - Château l'Evêque	SW 3	25/01/2016	9.7	8.0	633	11.3	127.6	2.6	7.0	1.1	345.3	12.5	10.7	23.5	14.9	-	6.0	4.1	2.3
Beauronne - Château l'Evêque	SW 3	26/05/2016	14.7	7.9	594	10.2	114.6	2.3	5.8	1.0	318.4	10.1	8.3	15.8	14.0	-	6.5	5.5	2.2
Beauronne - Preyssac	SW 4	22/01/2016	7.4	8.0	639	11.0	127.4	2.3	6.8	1.2	341.6	12.1	11.2	30.8	15.3	-	7.1	5.1	1.6
Beauronne - Preyssac	SW 4	26/05/2016	14.1	7.8	605	9.8	116.9	2.2	5.8	1.0	334.3	10.2	8.8	19.4	13.7	-	6.6	4.7	0.3
Beauronne - Borie des Cailloux	SW 5	22/01/2016	8.1	8.0	646	10.5	129.1	2.2	6.9	1.2	342.8	12.4	11.7	34.1	14.9	-	5.9	5.1	1.5
Beauronne - Borie des Cailloux	SW 5	26/05/2016	14.1	7.8	613	9.6	115.8	2.3	5.9	1.1	330.6	10.7	9.0	21.8	13.3	-	7.0	5.8	0.0
Beauronne - Agonac	SW 6	22/01/2016	8.5	7.7	645	10.1	132.4	2.1	6.3	1.2	335.5	11.9	12.6	36.0	15.3	-	5.9	4.2	3.1
Beauronne - Agonac	SW 6	26/05/2016	14.1	7.6	617	9.9	115.8	2.3	5.9	1.1	328.2	10.9	9.6	24.4	13.1	-	6.0	4.5	-0.1
Foncroze - Monzie	SW 7	03/11/2015	14.4	7.8	616	7.1	126.3	2.8	10.2	1.7	364.8	15.3	12.6	6.2	14.1	-	9.5	4.8	1.9
Foncroze - Monzie	SW 7	22/01/2016	8.0	7.9	634	10.9	128.0	2.5	9.6	1.0	374.5	15.4	20.7	11.2	12.7	-	8.2	4.5	-0.9
Foncroze - Monzie	SW 7	26/05/2016	14.5	7.7	622	8.9	124.2	2.7	9.4	1.0	352.6	13.7	18.6	9.0	11.8	-	7.7	4.0	1.2

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**Sample description, chemical composition (mg L<sup>-1</sup>) and isotopic composition (‰) of borehole samples.**

Sample designation	Sample name	Date	Aquifer	T (°C)	pH (pH unit)	EC (µS cm <sup>-1</sup> )	O <sub>2</sub> (mg L <sup>-1</sup> )	Ca <sup>2+</sup> (mg L <sup>-1</sup> )	Mg <sup>2+</sup> (mg L <sup>-1</sup> )	Na <sup>+</sup> (mg L <sup>-1</sup> )	K <sup>+</sup> (mg L <sup>-1</sup> )	HCO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	Cl <sup>-</sup> (mg L <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (mg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	SiO <sub>2</sub> (mg L <sup>-1</sup> )	δ <sup>18</sup> O-H <sub>2</sub> O (‰)	δ <sup>15</sup> N-NO <sub>3</sub> <sup>-</sup> (‰)	δ <sup>18</sup> O-NO <sub>3</sub> <sup>-</sup> (‰)	Balance (%)
Comille Borehole	B1	05/11/2015	upper Jurassic - Turonian	15.3	6.9	676	2.3	132.8	5.7	7.0	1.8	414.8	13.1	9.2	13.1	15.1	-	7.3	5.0	-0.7
Comille Borehole	B1	18/09/2017	upper Jurassic - Turonian	17.3	7.1	609	1.2	97.8	21.1	5.2	0.6	395.3	8.9	7.0	1.3	10.6	-	10.3	8.3	0.0
Roussie Borehole	B2	06/11/2015	Turonian - Coniacian	15.0	7.1	590	6.9	118.9	3.2	6.7	1.5	341.6	10.6	7.2	11.2	25.0	-	1.7	1.3	2.4
Roussie Borehole	B2	18/09/2017	Turonian - Coniacian	14.3	7.2	577	6.7	121.2	2.6	6.2	0.4	359.7	9.4	6.3	11.7	24.9	-	2.9	2.9	0.5
PIP Borehole	B3	23/05/2016	Turonian	14.7	7.1	572	1.6	94.5	13.7	6.5	0.7	350.3	10.5	9.6	6.8	15.7	-	5.8	4.7	-1.4
PIP Borehole	B3	20/07/2016	Turonian	15.0	7.1	568	1.9	91.3	13.2	6.2	0.5	345.6	10.6	8.1	6.3	14.8	-	5.5	3.7	-2.3
Trelissac Borehole	B4	18/09/2017	Middle Jurassic	20.8	7.1	562	0.1	85.5	22.1	6.3	0.4	361.4	9.2	7.8	0.0	9.0	-	-	-	0.4

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**Sample description, nitrogen composition (mg L<sup>-1</sup>) and isotopic composition (‰) of nitrate potential sources samples.**

Sample designation	Sample name	Date	NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	δ <sup>18</sup> O-H <sub>2</sub> O (‰)	δ <sup>15</sup> N-NO <sub>3</sub> <sup>-</sup> (‰)	δ <sup>18</sup> O-NO <sub>3</sub> <sup>-</sup> (‰)
Périgueux Rainfall	NS 1	23/05/2016	0.0	1.4	0.6	-4.79	-5.4	61.3
Sewage	NS 2	22/03/2017	0.1	171.8	0.0	-	6.3	-2.9
Sewage	NS 2	19/09/2017	0.1	169.9	0.1	-	6.3	-4.2
Sewage	NS 2	18/11/2017	1.1	179.7	2.3	-	-1.9	-4.8
Ammonium nitrate	NS 3	15/11/2017	-	-	-	-	1.1	23.5
Ammonium nitrate	NS 3	15/05/2016	-	-	-	-	0.9	23.5
Agricultural soil	NS 4	19/09/2017	1.6	0.7	1.3	-	-1.9	0.2
Agricultural soil	NS 4	18/11/2017	1.1	2.2	0.1	-	1.3	2.8
Sewage sludge soil	NS 5	19/09/2017	0.9	0.3	0.5	-	10.9	27.0
Sewage sludge soil	NS 5	18/11/2017	0.5	0.1	0.4	-	33.8	57.5
N <sub>Tot</sub> soil	NS6	19/09/2017	-	-	-	-	6.8	-
N <sub>Tot</sub> soil	NS6	18/11/2017	-	-	-	-	6.8	-

854

855

856 **References**

857

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