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# Evidence of long term biogeochemical interactions in carbonate weathering: the role of planktonic microorganisms and riverine bivalves in a large fluviokarst system

Stéphane Binet<sup>1</sup>, Jean-Baptiste Charlier<sup>2,3</sup>, Nevila Jozja<sup>4</sup>, Christian Défarge<sup>1,4</sup>, Jean-Sébastien Moquet<sup>1</sup>

<sup>1</sup> University of Orléans – INSU/CNRS – BRGM, UMR 7327, Institut des Sciences de la Terre d'Orléans (ISTO), 1A rue de la Férollerie, F-45071 – Orléans Cedex 2, France

<sup>2</sup> BRGM, Univ. Montpellier, Montpellier, France

<sup>3</sup> G-eau, INRAE, CIRAD, IRD, AgroParisTech, Supagro, BRGM, Montpellier, France

<sup>4</sup> University of Orléans, CETRAHE, 8 rue Léonard de Vinci, F-45072 – Orléans cedex 2, France

## ABSTRACT:

The infiltration of organic-rich surface waters towards groundwaters, is known to play a significant role in carbonate weathering and in contributing to the atmospheric continental carbon sink. This paper investigated biogeochemical interactions in karst critical zones, with strong surface water /groundwater interactions, and in particular the role of planktonic microorganisms and riverine bivalves through the analysis of particulate organic matter (OM) oxidation on carbonate weathering. In the large Val d'Orléans fluviokarst aquifer (France), a 20-year monthly dataset of Nitrates, Dissolved Oxygen (DO), dissolved inorganic and organic Carbon (DIC and DOC) fluxes was gathered. The surface water-groundwater comparison of geochemical trends showed that planktonic microorganisms had drastically decreased in surface waters, related to the proliferation of *Corbicula* bivalves spreading and a decrease in nutrients. This decrease in planktonic microorganisms was followed by a DO increase and an DIC decrease at the karst resurgence. The degradation of planktonic microorganisms consumes DO and produces NO<sub>3</sub>, dissolved inorganic carbon (DIC) and a proton that in turn, dissolves calcite and produces DIC. Without the input from planktonic microorganisms, the fluviokarst has lost 29% of this nitrification and 12% of the carbonate dissolution

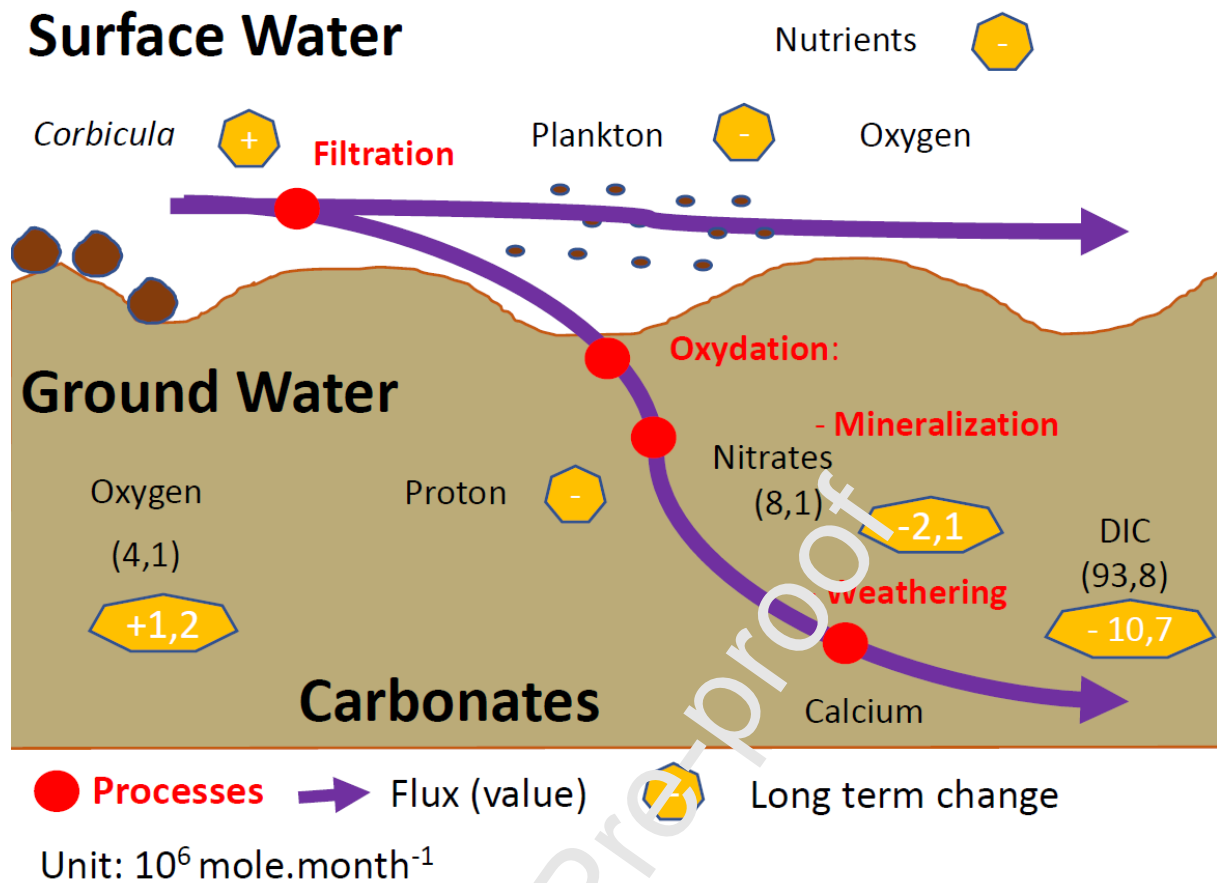
capacities. Thus, the oxidation of particulate organic matter of planktonic microorganisms, which is part of heterotrophic respiration, appears to be a significant source of the inorganic carbon flux in riverine ecosystems. This shows how weathering can remain active under waters saturated versus calcite and suggests that the oxidation of organic matter can be a more appropriate mechanism than autotrophic respiration to explain the relationship between global warming and DIC flux change in rivers. Through the consumption of plankton, the animal life in rivers thus influences the inorganic carbon in groundwaters, creating a negative feedback in the carbon cycle.

**Key words:** inorganic carbon fluxes, particulate organic matter, *Corbicula*, chlorophyll-a, surface water / groundwater interactions, Karst, Critical Zone.

**Highlights :**

- Dissolved carbon fluxes in a fluviokarst are reported in regards to surface water plankton and bivalves dynamics.
- A 12% loss of the DIC export is observed in groundwaters following a plankton decrease in the river
- Planckton decomposition (part of heterotrophic respiration) appears to be a significant source of the inorganic carbon flux

**Graphical abstract:**



## 1 Introduction

Chemical weathering of carbonates plays an important role in the regulation of atmospheric  $\text{CO}_2$  but also in the ocean composition, in the formation of soils, and hence in the feedbacks between continental carbon and atmospheric temperature that drive Earth's climate dynamics (Beaulieu et al., 2012; Gaillardet et al., 2019; Zeng et al., 2019). Carbonate weathering contributes 45-60% of the rock weathering dissolved solute discharged to the ocean at global scale (Gaillardet et al., 1999; Meybeck, 1987). As the main controlling factors of carbonate weathering are climate and vegetation (Calmels et al., 2014; Ulloa-Cedamanos et al., 2020) with a fast response of this weathering process to environmental changes, there is a need for a better understanding of carbonate weathering processes due to the hydro-bio-chemical evolution of waters.

In the soil-water-groundwater continuum of the Critical Zone, carbonate dissolution depends dominantly on pH, air temperature and the saturation state of the solution with respect to abundant minerals. The pH of the solution is, in general, controlled by partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>) produced by ecosystem respiration (Romero-Mujalli et al., 2019; White, 1999), which is the CO<sub>2</sub> produced by the biological activity of soil organisms, through autotrophic (roots) and heterotrophic (organic matter degradation) respiration. To observe the impact of ecosystem changes on pCO<sub>2</sub> and the associated weathering processes requires studying bio-geochemical processes at the catchment scale, where complex feedbacks in the carbon cycle of the Critical Zone can be quantified. Karst aquifers – characterized by carbonate rocks with a high infiltration capacity – promote surface water/groundwater (SW/GW) interactions during low water levels as well as during floods (Bailly-Comte et al., 2012; Binet et al., 2017; Charlier et al., 2013; Mesnil et al. 2022) and are thus ideal study sites to assess the biogeochemical processes that control carbonate weathering in the Critical Zone.

Looking at karst Critical Zone at the catchment scale, several studies have shown pluri-decadal trends in water geochemistry (Binet et al., 2020; Frossard et al., 2020; Jeannin et al., 2016). Analysis of the hydrochemical evolution of spring water has linked water pCO<sub>2</sub> changes and carbonate weathering with surface ecosystem dynamics or with hydro-climatic descriptors such as air temperature (Binet et al., 2020; Ulloa-Cedamuno et al., 2020). These approaches link the pCO<sub>2</sub> change with global descriptors without discrimination of the sources. The associated processes can be related to (1) an increase of organic matter amount, driven by a vegetation change and (2) to an increase of organic matter degradation kinetic driven by microbial activity (heterotrophic respiration) as well as (3) to the adaptation of the vegetation following an increasing autotrophic respiration. However, this discrimination is a key point to correctly identify feedbacks and access the processes that drive weathering.

In river ecosystems, planktonic microorganisms are known to modify nutrients and carbon fluxes, mainly using carbon for photosynthesis (Sun et al., 2021). In the last 50 years, many rivers have been affected by invasive species, partly due to human impacts through intercontinental and river navigation, water pollution or global warming (McDowell et al., 2014). In these rivers, a drastic decrease in planktonic microorganisms in surface waters has been observed following for example the proliferation of *Corbicula fluminea* (*Corbiculidae*, hereafter *Corbicula*) bivalves (Hesse et al., 2015a; Minaudo et al., 2021). Simulations in Loire River, performed by Descy *et al.* 2012, showed a significant impact of invasive *Corbicula* clam species, on phytoplankton biomass. As particulate organic matter (including planktonic microorganisms) in rivers is a key factor to understand C fluxes and reactions in rivers (Hope et al., 1994), and as particulate organic matter can be oxidized (Albéric and Lepiller, 1998), these drastic changes may have an influence on dissolved inorganic carbon (DIC) fluxes. In the case of karst systems characterized by strong (SW/GW) interactions, organic matter (OM) oxidation will react with carbonates and will favor Karst development (Bakalowicz, 2005; Ford et al., 2007; White, 1999). Studying the flux before and after this drastic decrease in organic matter opens up the possibility of pinpointing the contribution of heterotrophic respiration (or OM oxidation) to the weathering rate.

To address this issue, the present paper investigated biogeochemical interactions in a karst Critical Zone, and in particular the significance of plankton biomass oxidation and riverine bivalves proliferation on carbonate weathering. To do so, a monthly 20-yr geochemical dataset of Nitrates ( $\text{NO}_3$ ), Dissolved oxygen (DO), and Carbon (C) fluxes was computed in the large Val d'Orléans fluviokarst system in France, which is influenced by strong SW/GW interactions. Groundwaters are fed by the large river Loire, which is significantly affected by *Corbicula* invasion. Chlorophyll-a, DO, DIC and  $\text{NO}_3$  in surface and groundwaters were compared (before and after plankton biomass decrease) in order to discuss the role of  $\text{CO}_2$  from organic carbon degradation in weathering carbonate rocks and to illustrate the existence of an interaction between animal life and inorganic fluxes in the Critical Zone.

## 2 Study site

### 2.1 Presentation of the fluviokarst system of Val d'Orléans

The unconfined carbonated aquifer of the Beauce Limestone extends over 9000 km<sup>2</sup> between the Seine and the Loire River. The aquifer is hosted within an Oligocene carbonate lacustrine deposit. Around Orléans, this carbonated aquifer is karstified and is considered as a depression of the major bed of the Loire River (Figure 1), overlapped by the quaternary alluvia of the Loire River (Gutierrez and Binet, 2010) and covered by agricultural crops (mainly market gardening) and urban areas. The Val d'Orléans karst system is referred to as a fluviokarst system due to strong surface water-groundwater (SW-GW) interactions with the Loire River. It is one of the largest karst aquifers in France (mean annual discharge of 10 m<sup>3</sup>/s), providing the main water resource of Orléans city (Albéric and Lepiller, 1998).

The Loire River had a mean annual discharge of 334 m<sup>3</sup>.s<sup>-1</sup> between 1964 and 2020 at the Orléans gauging station, for a 37000 km<sup>2</sup> watershed (Figure 1). The Loire feeds between 80% (Joodi et al., 2010) to 96% (Binet et al., 2017) of the groundwater hosted in the karst aquifer, through sinkholes in the river bed located mainly close to Jargeau city (Figure 1). The loss discharge estimation of the Loire River at Jargeau is about 0.3 to 20 m<sup>3</sup>/s (Martin et al., 2003) depending to discharge rate. In the left bank of the Loire River, the groundwater is transferred in 3.5 days through the conduit networks from the Loire losses towards numerous resurgences of the Loiret river (Chery, 1983; Lepiller, 2006; Zunino, 1979), as shown in Figure 1. These resurgences are considered as overflows of the karst aquifer that is mainly drained by the Loire River downstream its confluence with the Loiret river (St Pryvé - St Mesmin in Figure 1). The main resurgence upstream is called the Bouillon spring. The Loiret river is also fed by the Dhuy river, draining shallow clay formations. The difference between local efficient rainfall estimated at 190 mm.yr<sup>-1</sup> and the Dhuy river specific discharge at 110 mm.yr<sup>-1</sup> (Joigneaux et al., 2011) suggests that around 80 mm.yr<sup>-1</sup> can reach the groundwaters. The recharge area of the Val d'Orléans fluviokarst is 284 km<sup>2</sup>.

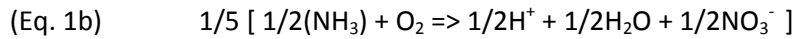
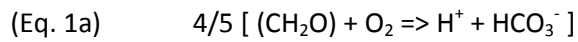
Figure 1: A/ The Val d'Orléans fluviokarst aquifer and B/ The Loire River watershed (modified from Albéric, 2004).

Even if the suspended matter in Loire waters is higher during flood events, the periods of low water levels show high chlorophyll-a concentrations, resulting from high planktonic photosynthetic biomasses (Albéric and Lepiller, 1998). A previous study of the long term evolution evidenced dramatic changes in photosynthetic plankton in the 1990s in response to local and global pressures (Larroudé et al., 2013). Chlorophyll-a was found to have drastically decreased in the 1990s, from  $85 \mu\text{g}\cdot\text{L}^{-1}$  in 1976 to  $50 \mu\text{g}\cdot\text{L}^{-1}$  in 1992-1995, and a second drop was found in 2006 (concentrations  $<1 \mu\text{g}\cdot\text{L}^{-1}$  in the period 2005-2008) (Hesse et al., 2015b). From the 1970s on (first observation in 1976), *Corbicula* has invaded the Loire River but there is no direct correlation between the appearance of *Corbicula* and the decrease in chlorophyll-a in the water. During the first chlorophyll-a decrease (1992-1995), *Corbicula* was observed for the first time in second order tributaries. Then during the second decrease (2005-2008), *Corbicula* was observed for the first time in the head rivers of the Loire watershed (Hesse et al. 2015). The drop in the chlorophyll-a concentration in the Loire River itself is thus an indirect result of the drop in this parameter on all the tributaries of this large watershed (Hesse et al., 2015b). The microbial biomass started to decline before *Corbicula* began to influence it, because of a better control of anthropic Phosphorus inputs, leading to a reduction in the availability of this nutrient for photosynthetic microorganisms. The long term decrease in dissolved Phosphorus concentration is a other main controlling factor of chlorophyll-a decrease (Minaudo et al., 2021).

## 2.2 Origin of the inorganic carbon in groundwaters

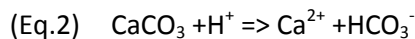
Specific carbonate export rates in the middle Loire watershed were evaluated at close to  $47.10^6 \text{ g}\cdot\text{year}^{-1} \text{ km}^{-2}$  (Grosbois et al., 2000). The conduit network between Loire losses and the Bouillon resurgence is the place of significant oxidation of OM (infiltrated from the Loire River) that controls the carbonate dissolution (around  $7\text{-}29 \text{ mg CaCO}_3/\text{L}$ ), associated with nitrification processes (Albéric and Lepiller, 1998). The magnitude of the exchange between losses from the river and groundwaters

makes this place a hot spot of SW-GW interactions. OM oxidation is observed and can be described as follows



Reactions can be described in this way: (1) the oxidized OM originates from particulate rather than dissolved fractions (Albéric and Lepiller, 1998); (2) the analysis of the C/N ratio of the OM was found in this fluviokarst to be between 7 and 10 (Albéric and Lepiller, 1998). The 4/5 and 1/5 stoichiometric coefficients are related to a C/N =8. One mole of  $(\text{CH}_2\text{O})_8(\text{NH}_3)$  needs 10 moles of  $\text{O}_2$  to be fully oxidized. Eight moles of  $\text{O}_2$  are reacting in eq. 1a ( $8/10=4/5$ ) and 2 moles of  $\text{O}_2$  in eq. 1b ( $2/10=1/5$ ); (3) the carbohydrates of the OM react with  $\text{O}_2$ , given a proton and an  $\text{HCO}_3^-$  ion; (4) at the same time,  $\text{NH}_3$  of the OM is also oxidized to produce  $\text{NO}_3^-$  and  $\text{H}^+$ .

In this Karst medium,  $\text{H}^+$  is buffered by carbonate dissolution following equation 2:



Finally, the protons produced by the oxidation of infiltrated OM (eq. 1) can control carbonate dissolution, and the associated inorganic carbon production (eq.2), in the SW-GW system.

### 3 Data processing

#### 3.1 Hydrological database

Loire River discharge is monitored at an hourly time step at the Orléans [Pont Royal] station (<http://www.hydro.eaufrance.fr/>) (Figure 1). The groundwater level monitoring is carried out at the Bouillon resurgence ( $h_b$ ; Figure 1). Before 2014, only punctual observations are available (frequency: 1 month). From 2014, water level was monitored at an hourly time step using a Campbell® device. The water level measured in the 03982X0006/F borehole Theuriet ( $h_f$ ), located 200 m north of the Bouillon resurgence (Figure 1) enables the missing data to be gapped at the Bouillon resurgence using a linear relationship ( $h_b=h_f$   $R^2=0.76$ .  $N=12$ ). To improve our estimation of the groundwater

discharge in this aquifer ( $Q_{out}$  in equation 3), which is indirectly estimated at present by models (Binet et al., 2017; Joigneaux et al., 2011), 13 dye tracer tests were performed for different stages of the Loire River (Joodi et al., 2010). The 13 dye tracer tests were conducted between 1973 and 2017 for various hydrogeological conditions from 90 to 91.5 m of water table fluctuation. For each experiment, a mass  $M$  (between 0.5 and 13 kg) was injected in the S1 hole and the restitution was observed at the Bouillon resurgence, monitoring the tracer concentration with time ( $c(t)$ ). If we assume that the dye tracer is conserved during the 13 km of the groundwater flow and that the restitution is completed after a duration  $T$ , the discharge estimation with the dilution method (Hongve, 1987) can be expressed as given in equation 3:

$$Q_{out} = \frac{M}{\int_0^T c(t) dt} \quad \text{Equation: 3}$$

The estimation of the underground discharge (Appendix 1) gives results between  $7 \text{ m}^3 \cdot \text{s}^{-1}$  in October 2019 to  $25 \text{ m}^3 \cdot \text{s}^{-1}$  in May 2001. Comparison between the discharge obtained and the groundwater level yielded a rating curve to estimate the groundwater discharge of the karst aquifer. Based on the cross correlation between water level in the karst resurgence and underground discharge, a rating curve is proposed (Appendix 1). The uncertainty between linear regression estimation and discharge calculated by the tracer method was on average about  $1.4 \text{ m}^3 \cdot \text{s}^{-1}$ . Based on this rating curve, the underground discharge can be estimated monthly (Appendix 1).

### 3.2 Hydro-bio-chemical database

Precipitations are recorded in Orléans – Bricy station, (lat. 47.9912/long. 1.7497) (<https://donneespubliques.meteofrance.fr/>). Loire River waters were sampled at least monthly at the JARGEAU, ORLEANS, and ST-PRYVE-ST-MESMIN water quality stations (respectively [04050000], [04050500], [04051010] in <http://www.naiades.eaufrance.fr>). Samples were taken 10 cm below the surface, in the flow close to the border. Groundwaters were sampled in the Theuriet (hf), Gouffre and Bouchet boreholes (respectively [03982X0006/F], [03982X0009/P], and [03982X0006/F] in the <http://www.adcs.eaufrance.fr/> database) and in the Bouillon resurgence.

In-situ measurements of electrical conductivity (EC), pH, water temperature ( $T_w$ ) and dissolved oxygen (DO) were carried out during the sampling periods using WTW® probes. From 2014, hourly measurements of these 4 parameters were monitored using a Campbell® device at the Bouillon resurgence. Total Organic Carbon (TOC) (in the 2002-2004 period) and chlorophyll-a were estimated in the Loire River by fluorescence probes at Jargeau. Particulate organic carbon (POC) was obtained by the difference between TOC and dissolved organic carbon (DOC). Sampled POC values obtained in groundwaters were close to 0 at the resurgence.

Samples of 500 mL were taken using glass bottles immersed in water and filled by the bottom through an inner plastic tube. They were immediately stored at 4° C in the dark until laboratory analyses. Once in the laboratory, within one hour, two aliquots of 60 mL collected by single-use syringes were filtered in pressure at 0.45 µm and analyzed for dissolved anions by ICS-900 and 1100 Thermo Scientific Dionex ion chromatography systems. The chemical analyses of dissolved inorganic carbon (DIC) were conducted on unfiltered waters. The last aliquots of filtered water samples (100 mL) were collected and HgCl was added as inhibitor to avoid the influence of bacteria on determination of DOC. The analyses were performed by laboratories certified by the French government or by the University of Orléans (ISTO/CNRS or CETRAHE).

DIC ( $\text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^{2-}$ ) was calculated from pH, Alkalinity (assuming that Alkalinity in karst waters is dominated by  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ),  $T_w$  and equilibrium constants used in the pHreeqC database (Parkhurst, D.L., Appelo, C.A.J., 1999). The missing DIC concentrations at the Bouillon (53 values in the 150 data presented) and at the Loire River (199 values in the 227 data presented) were gapped when the water electrical conductivity was measured in the sample, using the following relationships:  $\text{Alk} = 0.421 * \text{EC}$ ;  $R^2 = 0.82$ .  $N = 372$  (Appendix 2). Our results are close to the global relationships:  $\text{Alk} = 0.57 * \text{EC} + 17$  found in 1152 samples in 55 French karst springs (Peyraube et al., 2019).

For the surface and groundwaters, we averaged the data to create a monthly database of water quality from 2000 to 2020 (Supplementary material). As the discharge variations at the resurgence are mainly driven by fluctuations in the Loire River level, a monthly frequency enables the seasonal variability in this karst system to be investigated. The standard deviation of monthly concentration was calculated for the period 03/2008 - 03/2010 (high sampling rate period) and averaged. The results gave 0.045, 0.127, 0.026 and 0.017 mmol.L<sup>-1</sup> for NO<sub>3</sub><sup>-</sup>, DIC, DO and DOC respectively, which is at least an order of magnitude lower than the average value of the parameters (see Results section). We assumed that, in a month, the water concentrations between the 4 groundwater sampling points, located in the karst conduit and spaced about 200 m apart, were in the same range, and could therefore be considered as similar regarding the difference with surface waters.

The transformation from gas partial pressure (CO<sub>2</sub> and C<sub>2</sub>) and Tw to concentration was performed using PHREEQC (Parkhurst and Appelo, 1999).

### 3.3 Statistical analysis

Long term change in time series, were investigated following 2 methods. (1) the monotonic trends were assessed with the Mann–Kendall (MK) non-parametric test (Kendall and Gibbons, 1990; Mann, 1945). We calculated Sen trends (Sen's slope estimator; Gilbert, 1987) for each variable in order to compare variations. The Sen trend of a set of two-dimensional points is the median of the slopes determined by all pairs of sample points. The Sen trend is more robust than the least squares estimator, because it is much less sensitive to outliers.

(2) The detection of change points in a time series was carried out using Pettit's test (Pettitt, 1979), a non-parametric test that is used to identify a breaking period in time series. The advantages of Pettit's test over other homogeneity tests are that it is more sensitive to breaks in the middle of a time series and does not assume normality of the data (see table 1). If no break is founded, the mean value of the dataset is given by mean 1 in table 1. If a break is identified, the mean value before and after the break are given by mean 1 and mean 2 respectively.

A Student test was applied to test the differences between average fluxes. The trend, homogeneity and student tests are considered to be significant when the associated p value is  $< 0.01$ .

## 4 Results

### 4.1 Hydrological evolutions

The monthly precipitation on the Val d'Orléans fluviokarst is about  $53.3 \text{ L.m}^2. \text{ month}^{-1}$ . Average values of groundwater discharge  $Q_{out}$  are about  $14.8 \text{ m}^3.\text{s}^{-1}$  with seasonal variations presented in Figure 2. As the average discharge of surface water is  $300 \text{ m}^3.\text{s}^{-1}$  for the Loire River, the groundwater represents 5% of the fluxes flowing at Orléans station. No significant long-term trends were found in the precipitation, groundwater and surface water discharge series over the 2000-2020 period.

Figure 2: Precipitation, surface water discharge and groundwater discharge  $Q_{out}$ . Black circles are monthly average values of  $Q_{out}$ , blue line is hourly automatic probe measurements.

### 4.2 Monthly evolutions of biochemical variables in surface waters

Seasonal variation in surface water quality (DIC, Chl. a,  $\text{NO}_3$ , DO and TOC) was investigated from the monthly interannual average values presented in Figure 3. The lowest DIC values and chlorophyll-a ( $1.74 \text{ mmol.L}^{-1}$  and  $0.3 \mu\text{mol.L}^{-1}$  respectively) and the maximum  $\text{NO}_3$ , DO and DOC concentrations ( $0.22 \text{ mmol.L}^{-1}$ ,  $0.37 \text{ mmol.L}^{-1}$  and  $0.35 \text{ mg.L}^{-1}$  respectively) were recorded in winter (January), when the highest average discharge and the lowest  $T_w$  were recorded (Figure 3). In summer (August) an increase in  $T_w$  (until  $23^\circ\text{C}$ ) was observed,  $\text{NO}_3$  and DO decreased respectively toward  $0.07$  and  $0.34 \text{ mmol.L}^{-1}$  whereas DIC reached its maximum ( $1.96 \text{ mmol.L}^{-1}$ ) and POC concentration  $0.37 \text{ mmol C.L}^{-1}$ .

Figure 3: Average Monthly evolution of carbon in the Loire River/ Discharge, Precipitations, water temperature,  $\text{NO}_3$ , DIC DOC, POC for the period 2000-2010 and Chl-a as a proxy of planktonic biomass for the period 2001- 2006 only, before the drastic decrease. POC is only available from 2001 to 2004.

Trends and breaks in the time series are synthetized for all hydro-bio-chemical variables in Table 1. No breaks were observed in the surface waters in the period 2000-2010, except for Chl-a with a

drastic decrease in 2006 and for DO. Dissolved organic carbon in the Loire River showed a break in 2010 of about  $0.07 \text{ mmol.L}^{-1}$  (from  $0.36$  to  $0.29 \text{ mmol.L}^{-1}$ ), whereas all the other parameters (pH,  $\text{NO}_3$ , DIC and Tw) remained relatively constant throughout the period.

#### 4.3 Relationship between Chl-a in surface water and groundwaters content

Figure 4 shows the evolution of Chl-a in the Loire River and DO in groundwater at the karst outlet.

Chl-a exhibited a seasonal variation with a maximum value in summer and a lower value in winter. In the early 2000s the summer peaks reached  $70$  to  $100 \text{ }\mu\text{g.L}^{-1}$ . During the 2010-2020 period, peaks decreased drastically and remained below  $50 \text{ }\mu\text{g.L}^{-1}$ . The statistical analysis shows a break in 2006 (Table 1) with inter-annual average values falling from  $31.8 \text{ }\mu\text{g.L}^{-1}$  before 2006 to  $8.5 \text{ }\mu\text{g.L}^{-1}$  after this break.

Figure 4: Chl-a in the Loire River (left axis, orange color) and dissolved oxygen (DO) in the aquifer (right axis, black color) (points are monthly average values, blue line is an automatic probe for DO)

In the same period, DO at the groundwater resurgence showed a seasonal pattern (Figure 4), with high values in winter and low values in summer. In the early 2000s anoxic conditions (DO close to 0) were reached each summer. Since 2006-2007, due to an increase in minimal annual values, anoxic conditions have no longer been observed. In groundwaters, the average values evolved from  $0.10 \text{ mmol.L}^{-1}$  to  $0.15 \text{ mol.L}^{-1}$  with a break estimated by statistical analysis in 2009 (Table 1).

Before 2008, the groundwater DOC was 2-fold lower ( $0.19 \text{ mmol.L}^{-1}$ ) than in surface waters ( $0.36 \text{ mmol.L}^{-1}$ ). After 2008, DOC decreased to  $0.29 \text{ mmol.L}^{-1}$  in the surface water whereas the concentration remained stable in groundwater ( $0.16 \text{ mmol.L}^{-1}$ ), given a smaller outlet-inlet difference of  $-0.13/-0.17 \text{ mmol.L}^{-1}$  in this fluviokarst. In groundwater,  $\text{NO}_3$ , DOC and pH changed with a break. The break positions of the long-term analysis ranged between May 2003 and July 2010 (Table 1). DIC concentration was the only parameter that did not record a significant break in the 20-yr period.

Table 1: Trend and breaks in the observed variables. P-values < 0.001 are in bold

Looking at the evolution from input surface water to output groundwaters, both DO and pH means decreased while both  $\text{NO}_3$  and DIC means increased (Table 1). Water was enriched in DIC from 1.81 to 2.27  $\text{mmol.L}^{-1}$  between surface and groundwater (Table 1) given a  $+0.46 \text{ mmol.L}^{-1}$  increase of DIC in the fluviokarst.

#### 4.4 Relationship between DIC, $\text{NO}_3$ , Oxygen and Chl-a

To check if the processes observed from our monthly dataset over a 20 yr period were coherent with the observations on OM oxidation proposed by Albéric and Lepinier (1998) on the basis of a flood event survey, groundwater DIC and  $\text{NO}_3$  were compared to the DO (Figure 5). As expected, surface waters showed the highest values in DO.  $\text{NO}_3$  dispersion is explained by the seasonal evolution of the concentrations linked to farming practices and leaching periods (Frossard et al., 2020). The maximum DIC concentration in surface water was observed in August (see average values defined in Table 1). The lowest observed values are close to a theoretical water composition in equilibrium with atmospheric gas ( $\text{pCO}_2 = -3.5$  and  $\text{pO}_2 = -0.7$ ) that results in a DIC concentration of  $0.95 \text{ mmol.L}^{-1}$  and DO concentration at  $0.25 \text{ mmol.L}^{-1}$ . This water type is a lower bound of the observed low concentrations. Groundwaters showed lower DO values whereas DIC and  $\text{NO}_3$  varied seasonally.

In theory, according to equations 1a and 1b, during OM oxidation, when  $1 \text{ mol.L}^{-1}$  of DO is consumed,  $4/5 * 2 + 1/5 * 1/2 = 1.7$  moles of DIC are produced and  $1/5 * 1/2 = 0.1$  mole of  $\text{NO}_3$ . Based on the January and August surface water concentration, a theoretical decrease in the DO content in groundwaters can be computed (green line in Figure 5). These lines bound the observed values in the groundwaters, showing the consistency of the theoretical reactions with data.

Figure 5: DIC and  $\text{NO}_3$  versus the log of the DO, in groundwater (black) and surface water (orange). The circles represent average surface water for composition in

equilibrium with atmosphere ( $\text{PCO}_2 = -3.5$  and oxygen-saturated); Average value for the months of January and August. Green lines show the stoichiometric evolution of the concentration when the OM of surface water is oxidized in the groundwater (equation 1). The length of the lines is related to the available OM.

#### 4.5 Underground mass fluxes

Combining concentration with discharge data, the mass fluxes can be estimated for groundwaters.

$\text{NO}_3$ , DO and DIC fluxes are presented in Figure 6. In the same way as for the concentrations, seasonal variations dominate the pattern. The periods with a high Chl-a concentration ( $> 5 \text{ mg/L}$ ) in surface water are indicated in blue. We observe that these periods most often correspond to low DIC, low DO, and  $\text{NO}_3$  fluxes, because fluxes are driven by discharge.

Figure 6: Monthly average flux outflowing the fluvio-karst. Blue zones represent the periods when Chl-a in the surface water is higher than  $5 \text{ mg.L}^{-1}$ . Red dashed lines show the breaks identified by statistical trend analysis.

To observe changes induced by Chl-a average values of the fluxes were calculated before and after November 2006, i.e. the date of the Chl-a break (Figure 7). DIC fluxes decreased from  $93.8$  to  $83.1 \cdot 10^6 \text{ mol.month}^{-1}$  showing a  $-11$  (-12%)  $10^6 \text{ mol.month}^{-1}$  decrease compared to the average flux.  $\text{NO}_3$  fluxes decreased from  $8.1$  to  $6.0 \cdot 10^6 \text{ mol.month}^{-1}$  (i.e.  $-2 \cdot 10^6 \text{ mol.month}^{-1}$  or -29%). DO fluxes increased from  $4.1$  to  $5.3 \cdot 10^6 \text{ mol.month}^{-1}$  (i.e.  $1.2 \cdot 10^6 \text{ mol.month}^{-1}$  or +24%) and last, DOC fluxes decreased from  $7.2$  to  $6.3 \cdot 10^6 \text{ mol.month}^{-1}$  (i.e.  $-0.9 \cdot 10^6 \text{ mol.month}^{-1}$  or -14%).

Figure 7: Box plot of groundwater fluxes, for 2000-2006 (grey) and for 2007-2020 (yellow). All differences are statistically significant for a p-value  $< 0.01$ . Values of the average flux are given in  $10^6 \text{ mole.month}^{-1}$  under the box. The year 2006 was chosen as a limit regarding the Chl-a break evidenced by statistical analysis (Table 1)

## 5 Discussion

### 5.1 Groundwater fluxes in the fluviokarst

The question of water fluxes through this fluviokarst aquifer has been discussed since 1880. Using differential discharge measurements in the surface water (Loire River), Sainjon (1880) estimated that the inflow in the Karst aquifer reached  $11 \text{ m}^3/\text{s}$  for low water. Martin et al. (2003) estimated a mean discharge of 12 for the months of August between 1984 and 2001 in a low water period (with monthly average discharge of the surface water  $> 106 \text{ m}^3 \cdot \text{s}^{-1}$ ) and evidenced that this method is not sustainable during high waters. Lalot et al. (2015) proposed a quantification of the regional aquifer (Beauce limestone) contribution to the surface water discharge using satellite infrared imagery. Around the Loire / Loiret confluence where resurgences are known in the surface water, the authors estimated an underground outflowing discharge of about  $15 \text{ m}^3 \cdot \text{s}^{-1}$  for a surface flow measured at  $478 \text{ m}^3 \cdot \text{s}^{-1}$ . The karst water-level time series used in this paper improve the existing punctual estimations, and the obtained average value at  $14.8 \text{ m}^3 \cdot \text{s}^{-1}$  for an average surface water discharge at  $334 \text{ m}^3 \cdot \text{s}^{-1}$ , or 4% of the flux. Globally our results are consistent with Martin et al. (2003) and Lalot et al. (2015) and show the importance of considering SW-GW interactions, as they can represent almost 4% of surface fluxes for large rivers (watershed of  $36970 \text{ km}^2$ ) and represent more than 80% of the flux for this  $282 \text{ km}^2$  karst aquifer. SW-GW interactions are the key hydrological processes to discuss the geochemical processes that take place in the global hydrosystem including the surface water and groundwater continuum (Filipo et al., 2012).

### 5.2 From Chl-a, plankton biomass to *Corbicula* evolutions in surface water.

Long term changes in surface waters showed by Sen slopes (Table 1) for  $\text{NO}_3$  and Tw are in the same range as those observed by Minaudo et al. 2021 but are not significant for this data set. Only the 2006-2007 break for Chl-a and DO is significant. The Chl-a is assumed to characterize the living plankton biomass (Kasprzak et al., 2008; Minaudo et al., 2016). In the surface water the same tipping point as for Chl-a was observed in 2005 by Minaudo et al. 2021 for plankton biomass, confirming the strong link between these two variables. The main factors driving total phytoplankton and cyanobacteria biomasses were found to be the abundance of *Corbicula* clams (from absence before

1998 to 250–1250 individuals·m<sup>-2</sup> after 2010) (Hesse et al., 2015a; Minaudo et al., 2021) and the total phosphorus content. The filter-feeding clams' role in controlling the surface water plankton biomass was evidenced by a modelling approach (Descy et al., 2012), but its relative importance with respect to a decrease in phosphorus remains uncertain (Minaudo et al., 2021).

### 5.3 Organic matter oxidation of plankton biomass in surface waters

The oxidation of organic matter in surface water cannot be evidenced easily, compared to groundwaters where organic carbon decrease can be observed (Binet et al., 2006). At surface, consumed DO is quickly balanced by exchanges with the atmosphere. The fact that DIC does not evolve significantly in surface water suggests that OM oxidation (equation 1) is not fully buffered by carbonates (equation 2). The last indicator can be the NO<sub>3</sub>, but no break correlated with Chl-a break was observed in SW. The low NO<sub>3</sub> content in the river water is due to the biological consumption of aquatic plants and algae (Grosbois et al., 2000), but as groundwater is the major contributor to total nitrate load in the streams, (Bouraoui and Grizzetti, 2008) anthropogenic inputs cannot be ruled out, masking the nitrification processes. The seasonal variability of the NO<sub>3</sub> and DIC in surface water is higher than the amount produced in groundwater by OM oxidation and masks the long-term change. Dilution effect also occurs in DIC concentration change as observed in Qin et al. (2020) and DIC concentrations seems affected by primary production, co-occurred in rivers with large amounts of water and DIC being rapidly turned over in the water column (Zon et al, 2021).

To discuss the role of the plankton biomass oxidation on DIC in comparing low and high plankton biomass concentration periods will lead to a bias because of the coevolution of the discharge with Chl-a. The break for Chl-a observed in 2006 enabled a Before/After comparison to overcome this problem. 2006 will be assumed as a single break for all the dataset (no missing data in this timeline) and dispersion in the position of the breaks is assumed to be owing to uncertainty in the statistical analysis because of missing data. The change in 2006 makes it possible to discuss changes in chemical reaction with the same sampling of discharge conditions, knowing that no significant trend was

detected in surface hydrological variables during the same period. The limitations mentioned above concerning OM oxidation in surface water are not that strong in groundwaters.

#### 5.4 Organic matter oxidation of plankton biomass in groundwaters

The oxidation reaction of the OM was already described in estuary environments, where an increase in total alkalinity was found, due to both anaerobic respiration and a carbonate dissolution coupled to aerobic respiratory  $\text{CO}_2$  generation. (Abril et al., 1999) and in the studied fluviokarst (Albéric and Lepiller, 1998). The OM oxidation (equation 1) gives stoichiometric coefficients that explain groundwater evolution partly controlled by surface water variations. Input surface water concentrations range between two end members (low value for  $\text{CO}_2$  equilibrated with atmosphere and high DIC (August) and high  $\text{NO}_3$  (January) waters represented by circles in Figure 5). OM oxidation (equation 1) explains  $\text{NO}_3$ , DIC and DO variability in groundwaters. According to (Albéric and Lepiller, 1998), our analysis shows that OM oxidation is the main factor driving groundwater concentration for the 20 years considered here.

The outlet-inlet difference of  $-0.13/-0.17 \text{ mmol.L}^{-1}$  for the DOC is not enough to explain the  $+0.46 \text{ mmol.L}^{-1}$  increase of DIC in Karst. This confirms that particulate matter is the substrate of the oxidizing processes. However, seasonal POC evolution (Figure 3) is not consistent with DIC evolution, suggesting that not all the POC participates in the reaction. Changes in the plankton biomass and cyanobacteria blooms (associated with Chl-a) are coherent with DIC changes in groundwaters. Thus groundwater DIC changes are closer to plankton biomass variations in the river than to the POC variations. Overall, Chl-a and POC are never observed at the resurgences, while surface waters can be Chl-a and POC concentrated. Assuming a higher residence time of particles than water in karst, this could explain how organic particles can be stored and how low velocity degradation processes of the OM such as depolymerization or hydrolysis can take place despite the 3.5 days of mean water residence time (Albéric and Lepiller, 1998; Gounot, 1994).

The theoretical C/N ratio of 8 (Albéric and Lepiller, 1998) is appropriate to explain  $\text{NO}_3$ , DO and DIC concentrations in groundwaters. This C/N ratio and time variations of concentrations confirm that

the OM degraded in groundwater originated either from particulate microbial biomass, including plankton rather than from larger particulate organic clasts (characterized by the POC). This is in line with Cleveland and Liptzin, 2007 that observed microbial biomass with low C/N ratio close to 8 when tree foliage or soil organic matter can reach  $C/N < 50$ .

One added value of this paper is the estimation of groundwater mass fluxes. Groundwaters evidence that DO was the limiting factor during the summers before 2006, but anoxic conditions were not observed between 2006 and 2020. The new limiting factor is the amount of OM in water. The different patterns observed for C and N suggest that fluxes are driven by low discharge in summer associated with photosynthetic blooms, rather than by OM oxidation. This synchronicity between low DO values and Chl-a peaks highlights that plankton biomass is a key factor in underground DO consumption.

#### 5.5 Dissolved exports change in 2006: evidence of heterotrophic respiration

Significant changes (Figure 7) were observed for DIC,  $NO_3$  and DO fluxes before and after 2006. The significant trend decreases observed in groundwater discharge seem to compensate the change in DIC concentration, explaining why the DIC concentration does not record a significant break, whereas DIC flux change is significant (Table 1). The ratio between flux changes for DIC and  $NO_3$  in Figure 7 ( $DIC/NO_3$ ) is 5. This is lower than the  $C/N = 8$  ratio.  $NO_3$  can also be attributed to inputs in the local watershed or in the surface waters linked to a long-term trend without a break. However, other reactions or origins of elements, such as denitrification, cannot be ruled out. Denitrification can explain why some groundwater  $NO_3$  concentrations are lower than surface water input, and pollution by local recharge explains why some groundwater concentrations are higher than river ones. Lastly, part of the DIC may be lost by  $CO_2$  degassing. The DO change in 2006 is too low regarding the stoichiometry of the reaction and regarding the DIC and  $NO_3$  change. This suggests that the fluvio karst is not fully closed to surface oxygen or that the long-term trend observed in surface water reduces the impact of this DO change.

This  $11.10^6 \text{ mol.month}^{-1}$  change in 2006, compared with the increasing concentration in groundwater ( $+0.46 \text{ mmol.L}^{-1}$ ) multiplied by the average discharge ( $14.8 \text{ m}^3 \cdot \text{s}^{-1}$ ), leads to the conclusion that 61% of the DIC produced in this fluviokarst originated from plankton biomass oxidation. This is a rough estimate, because input discharge values are unknown and the input of local water also exists (between 1 to 15% according to Binet et al., 2017), but this estimate is in the same range as the ratio given between heterotrophic and autotrophic respiration in soil (48/52) (Kuzyakov and Larionova, 2005). In river, as in soil, heterotrophic respiration is a significant part of DIC production.

When compared to the DIC flux ( $93.8 \cdot 10^6 \text{ mol.month}^{-1}$ ), the fluviokarst lost 12% of the DIC export following the drastic decreases in plankton biomass and associated particulate OM oxidation. This value highlights the importance of river reactivity in the global carbon balance. Indeed, global estimates of the contribution of soil ecosystems to fluvial carbon fluxes are not yet balanced by the carbon transferred to oceans (Rosset et al., n.d.). It is interesting to note that this 12% ratio is also in the same order of magnitude as the 15% ratio calculated in Chinese rivers (Sun et al., 2021) for biological carbon consumption (cycling of OM formed mainly by plankton during photosynthesis, as well as by the cycling of  $\text{CaCO}_3$  during plankton growth), suggesting that the balance between plankton growth vs plankton death and degradation is close to neutral.

#### 5.6 From *Corbicula* to DIC export

*Corbicula* filter the surface water and contribute to reducing the plankton biomass in rivers. Less plankton biomass induces less OM oxidation which in turn reduces the carbonate dissolution known to mineralize organic carbon which is a sink for atmospheric carbon (Amiotte Suchet et al., 2003; Liu and Zhao, 2000). Equation 1 and 2 suggest that 4/5 of the DIC is mineralized during OM oxidation. This leads to a loss of  $8.5 \cdot 10^6 \text{ mol.month}^{-1}$  for atmospheric carbon pumped via photosynthesis and thus the mineralization of the OM by oxidation. Related to the lateral inorganic carbon flux of the surface water in Orléans ( $1.8 \text{ mmol.L}^{-1} \cdot 300 \text{ m}^3 \cdot \text{s}^{-1} = 1424 \cdot 10^6 \text{ mol.month}^{-1}$ ), this value is weak. But on comparing this flux with the estimated  $\text{CO}_2$  evasion rates ( $13 \text{ g.m}^2 \cdot \text{yr}^{-1}$ , (Raymond et al., 2013)) of the middle Loire River surface (Length 200 km Width =  $0.423 \exp Q + 12.9$  (Raymond et al., 2013) = 140 m),

we found an evasion of about  $2.6 \cdot 10^6 \text{ mol}\cdot\text{month}^{-1}$ , suggesting that this mineralization flux is becoming significant at the regional scale. Note that, at the same time, the production of Clam shells is a new way to mineralize organic carbon. Assuming a clam biomass about of  $2.5 \text{ g C m}^{-2}$  in the middle Loire (km 500–700) (Descy et al., 2012) for the same middle Loire River surface, we found that  $0.45 \cdot 10^6 \text{ mol}\cdot\text{C}\cdot\text{month}^{-1}$  could be stored by Clam shells, an order of magnitude under the OM oxidation flux loss caused by the decrease in plankton biomass and the proliferation of *Corbicula*. Even if the significance of the feedback is characterized in this study at the watershed scale, in terms of carbon balance, our results are an illustration of the complexity of the process at the atmosphere/ecosystem/rock interface.

## 6 Conclusion

This paper, from an example where the interactions between surface water and ground water are known, highlights the role of particulate organic matter oxidation on dissolved organic carbon flux. Based on the estimated  $14 \text{ m}^3\cdot\text{s}^{-1}$  exchange rate between surface and ground water, the Val d'Orléans aquifer can be classified as one of the largest fluviokarsts in France in terms of discharge, whereas most of the outflowing water is diffused, making this area a hot spot of surface water / ground water interactions.

In terms of DIC export, carbonate dissolution is mainly associated with  $\text{pCO}_2$  in soil. Our results show that organic matter oxidation, i. e., heterotrophic respiration, can explain at least 12% of the DIC production in waters. This opens a new perspective to explore the role of increasing air temperature in changing  $\text{CO}_2$  flux in rivers. Most of the time an increase in DIC is explained by an increase in biological activity with air warming. As the oxidation of organic matter is driven by bacterial activity, this process may be more appropriate than the respiration process to explain the observed changes.

OM oxidation produces protons that can modify the calco-carbonic balance and occurs even if the saturation of the calcite is reached. In this context, carbonate dissolution is possible even if equilibrium vs. calcite is reached. Numerous weathering processes are observed in zones where the

saturation vs carbonate is reached. In this zone, OM oxidation can be considered as a significant weathering source, and a better characterization of the DOC and POC fluxes and storage may improve the understanding of the dissolution processes in these zones. A water saturated with respect to calcite minerals can retain a weathering potential if it contains Organic Matter. This provides arguments to understand karst conduit development in saturated zones where waters are equilibrated versus calcite.

The observed feedback from atmospheric CO<sub>2</sub> to CO<sub>2</sub> sink by a decrease in carbonate weathering, partly explained by the spread of invasive *Corbicula* which filter OM in the river, is an example of the complexity of the Critical Zone. It links animal life in the river to mineral flux and suggests that more in-depth investigations are needed at the interface between geochemical and ecosystem sciences.

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## 8 Appendix:

- 8.1 Discharge rating curves estimated from (1973;1998;2000;2001; 2007;2008;2009;2010;2011;2013;2016;2017) artificial tracer tests.
- 8.2 Relationship between Alkalinity and electrical conductivity (EC) in groundwaters

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Journal Pre-proof

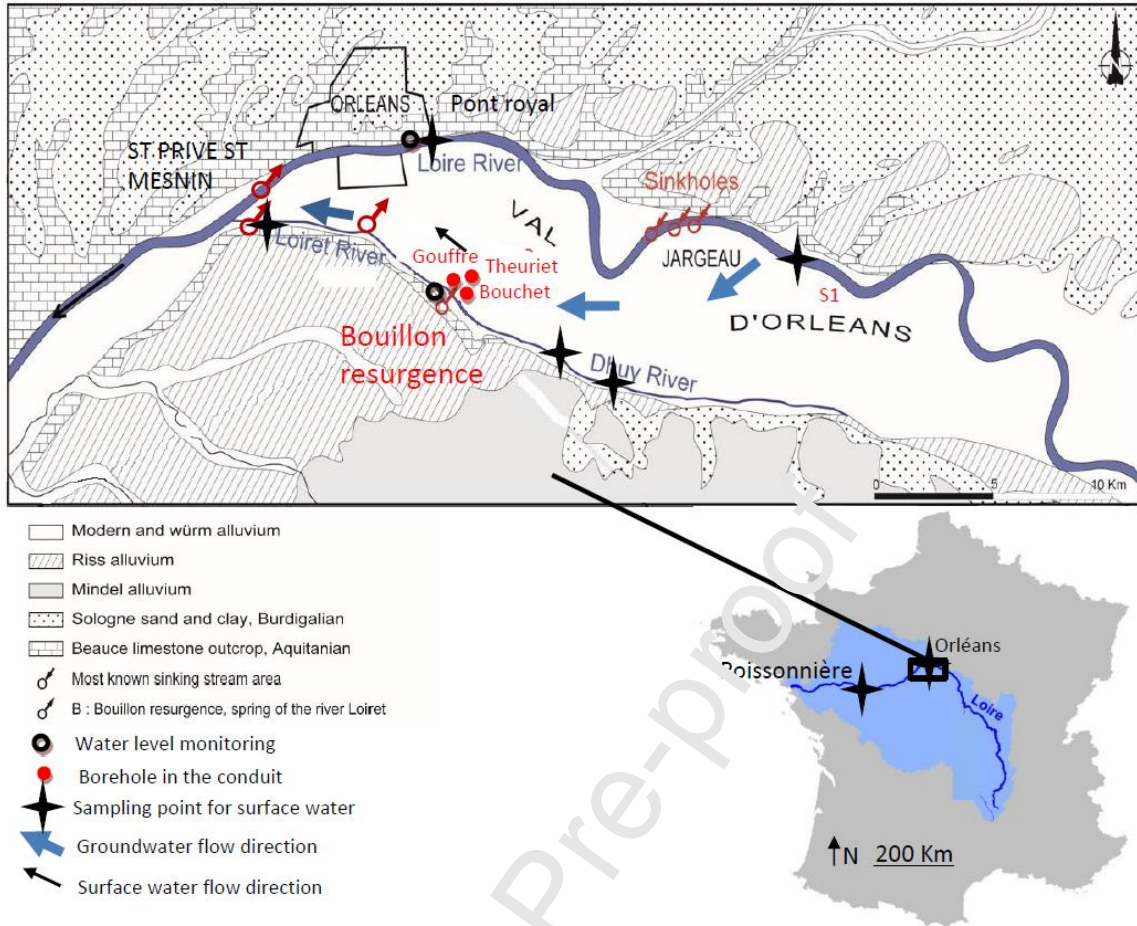


Fig. 1

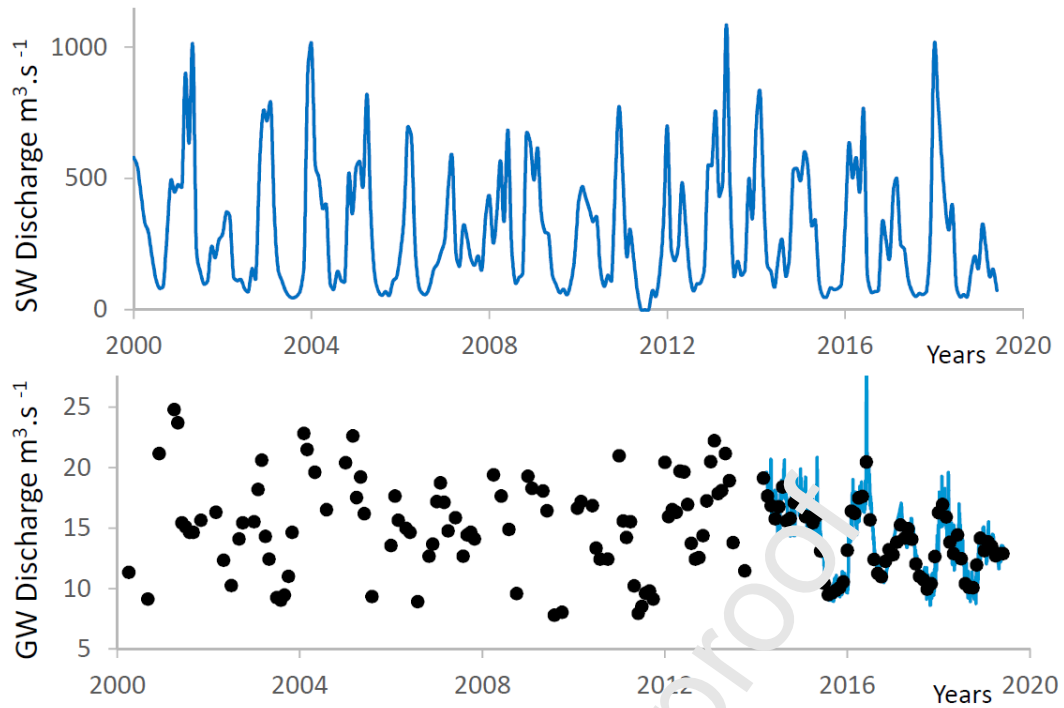


Fig. 2

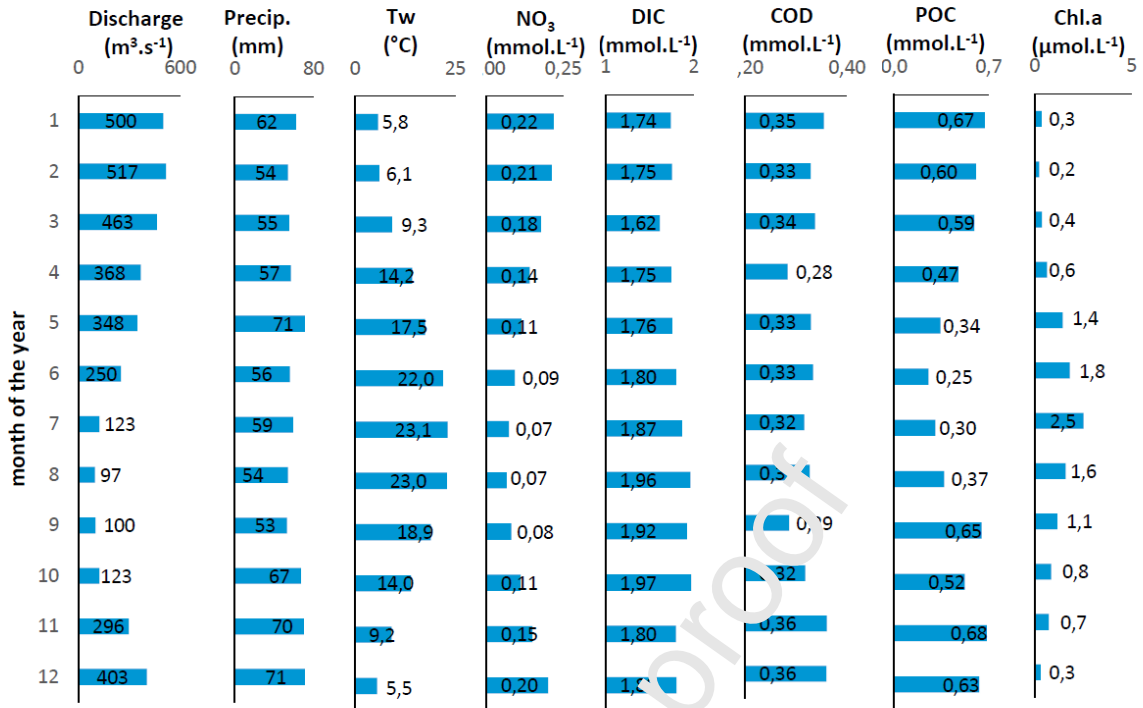


Fig. 3

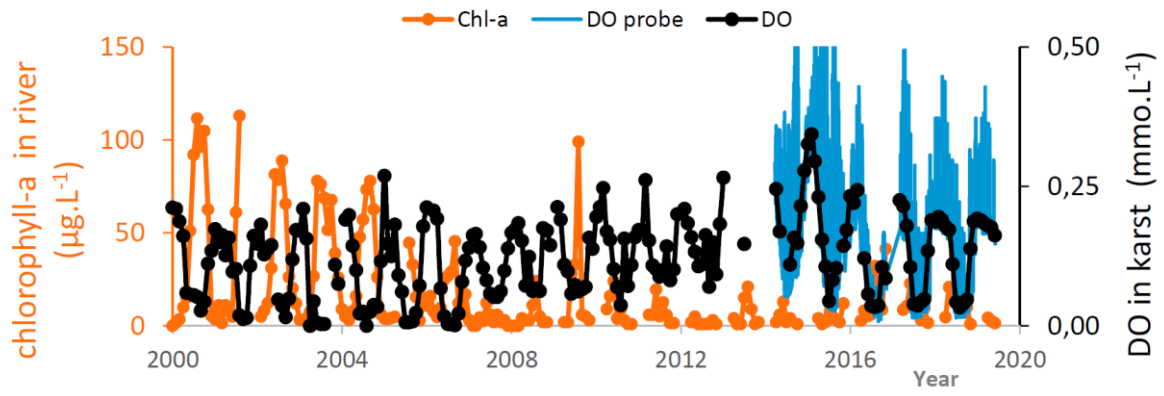


Fig. 4

Journal Pre-proof

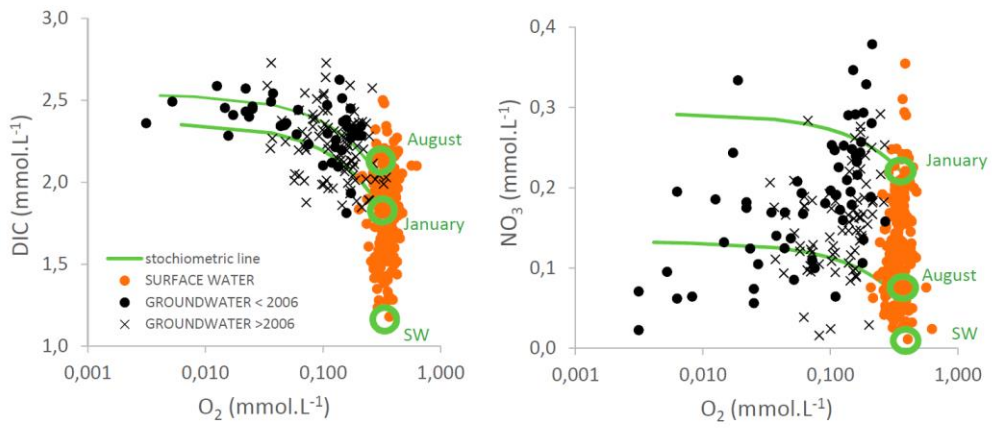


Fig. 5

Journal Pre-proof

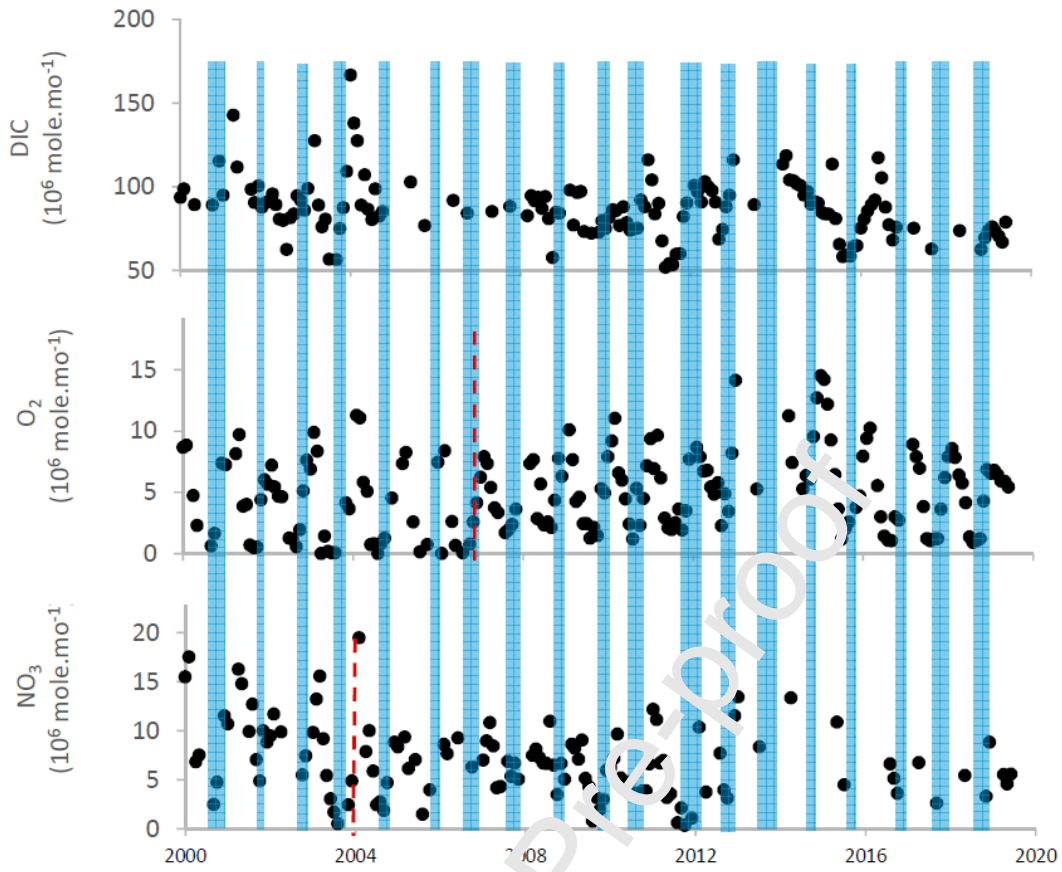


Fig. 6

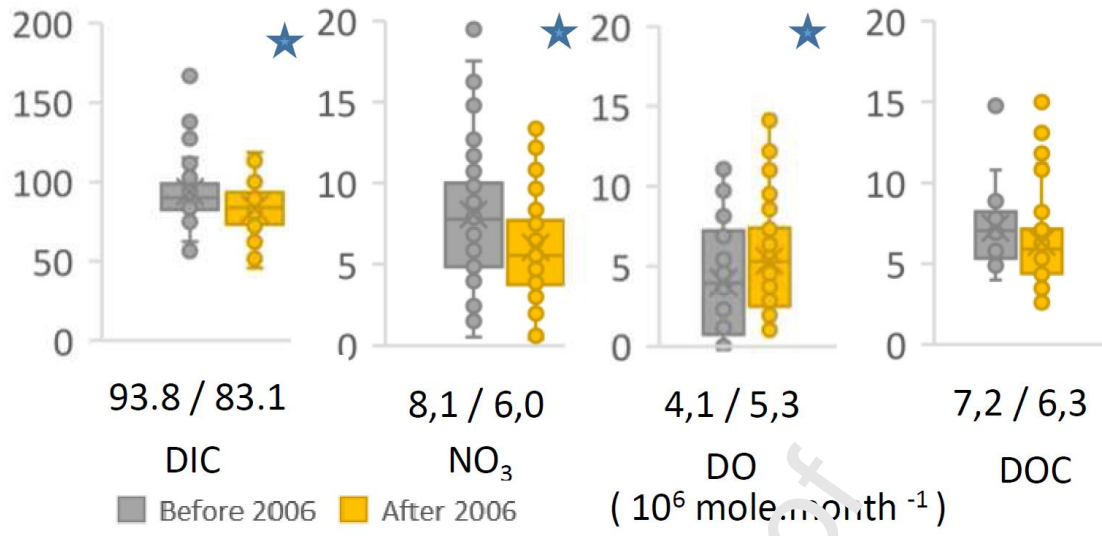
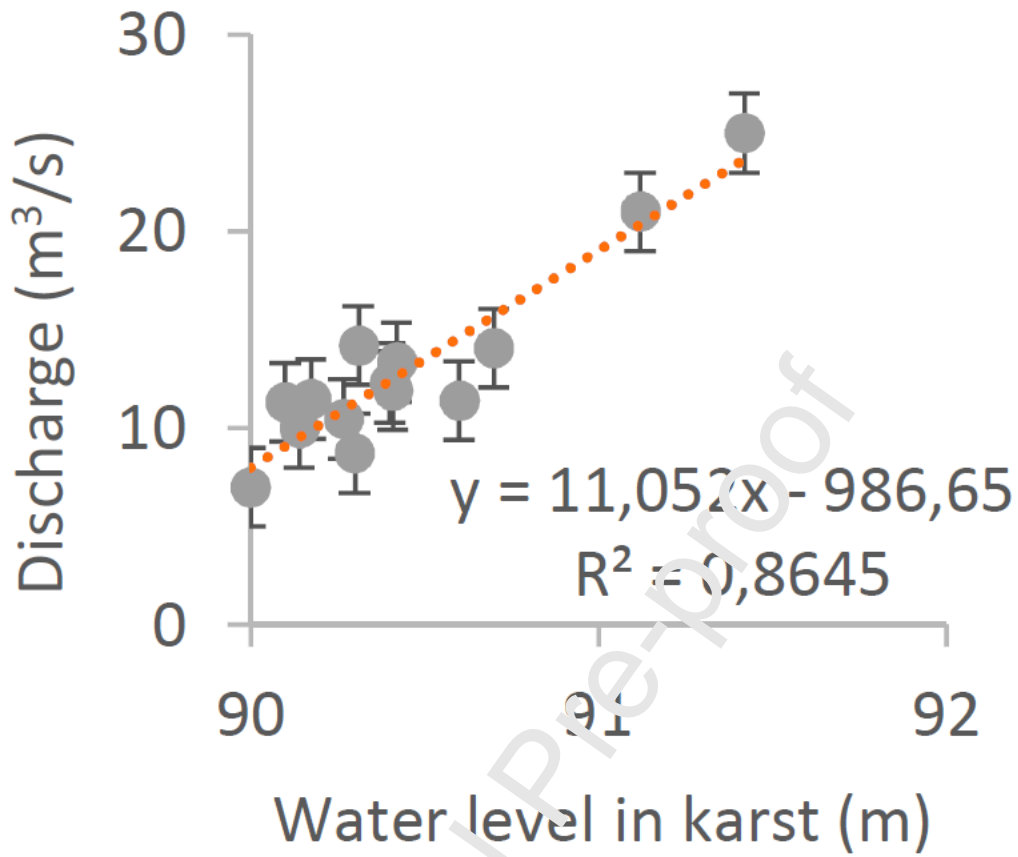
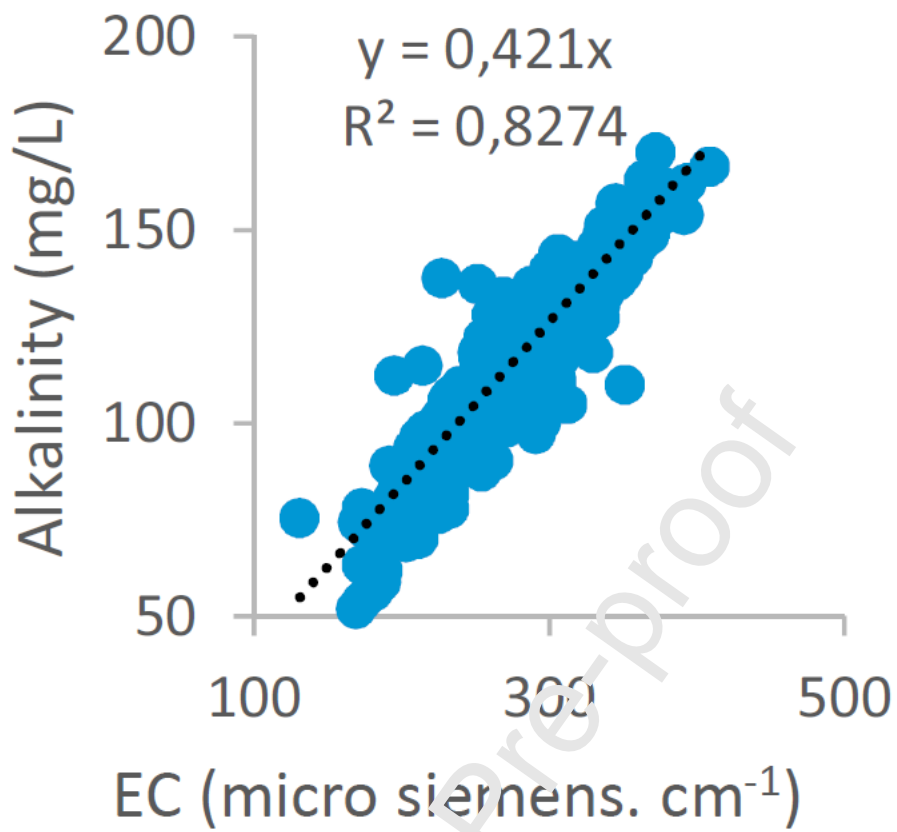


Fig. 7



Appendix 1



Appendix 2

Table 1

Data	Site	Unit	Trend		Homogeneity			Synthesis	
			Mann Kendall test		Pettitt test				
			Sen trend	p-value	Break	Mean 1	Mean 2		p-value
Chl-a	Loire	$\mu\text{g L}^{-1}$	-3,57E-01	<b>1.7.10-6</b>	nov-06	31,8	8,5	<b>&lt;1.10-4</b>	Decreasing trend with a break in 2006
Rainfall		$\text{mm month}^{-1}$	-2,00E-03	0,291	No break	53,3		0,2	No trend, no break
Discharge	Loire	$\text{m}^3 \text{s}^{-1}$	-6,00E-03	0,191	No break	300,1		0,617	No trend, no break
	Groundwater	$\text{m}^3 \text{s}^{-1}$	-3,51E-04	<b>3.5.10-4</b>	No break	14,6		0,001	Decreasing trend without break
DO	Loire	$\text{mmol.L}^{-1}$	4,72E-06	<b>4.2.10-3</b>	juil-10	0,33	0,36	<b>&lt;1.10-4</b>	Increasing trend with a Break in 2007
	Groundwater	$\text{mmol.L}^{-1}$	8,13E-06	<b>0.6.10-3</b>	oct-09	0,1	0,15	<b>&lt;1.10-4</b>	Increasing trend with a Break in 2009
pH	Loire	-	+1.3.10-5	0,6	No break	8,2		0,202	No trend, No break
	Groundwater	-	+1.2.10-5	<b>7.1.10-3</b>	oct-07	7,6	7,7	<b>1.10-3</b>	Increasing trend with Break in 2007
NO <sub>3</sub>	Loire	$\text{mmol.L}^{-1}$	-3,06E-05	0,14	No break	0,13		0,472	No trend, No break
	Groundwater	$\text{mmol.L}^{-1}$	1,10E-05	<b>2.9.10-3</b>	may-03	0,23	0,15	<b>1.10-3</b>	Decreasing trend with Break in 2003
DIC	Loire	$\text{mmol.L}^{-1}$	9,02E-08	0,45	No break	1,81		0,369	No trend, No break
	Groundwater	$\text{mmol.L}^{-1}$	-1,20E-07	0,53	No break	2,27		0,268	No trend, No break
DOC	Loire	$\text{mmol.L}^{-1}$	-1,33E-05	<b>1.2.10-7</b>	dec-10	0,36	0,29	<b>&lt;1.10-4</b>	Decreasing trend with Break in 2010
	Groundwater	$\text{mmol.L}^{-1}$	-8,00E-06	<b>1.3.10-3</b>	juin-08	0,19	0,16	<b>5.10-3</b>	Decreasing trend with Break in 2008
Tw	Loire	$^{\circ}\text{C}$	+1.7.10-4	0,46	No break	14		0,645	No trend, no break
	Groundwater	$^{\circ}\text{C}$	+8.3.10-5	0,5	No break	13,4		0,453	No trend, no break