Eutrophication mitigation in rivers: 30 years of trends in spatial and seasonal patterns of biogeochemistry of the Loire River (1980-2012)

C. Minaudo[1], M. Meybeck[2], F. Moatar[1], N. Gassama[1], F. Curie[1]

[1]{University of Tours, E.A. 6293 GéHCO, Tours, France}

[2]{Paris VI University, UMR METIS, Paris, France}

Correspondence to: C. Minaudo (camille.minaudo@etu.univ-tours.fr)

Abstract

Trends and seasonality analysis since 1980 and longitudinal distribution from headwaters to estuary of chlorophyll *a*, nitrate and phosphate were investigated in the eutrophic Loire River. The continuous decline of phosphate concentrations recorded since 1991 both in the main river and in the tributaries led to the conclusion that it was responsible for the significant reduction in phytoplanktonic biomass across the whole river system, although *Corbicula* spp. clams invaded the river during the same period and probably played a significant role in the phytoplankton decline. While eutrophication remained lower in the main tributaries than in the Loire itself, they were found to contribute up to ≈35% to the total nutrient load of the main river. The seasonality analysis revealed significant seasonal variations for the different eutrophication metrics and called into question the classical monthly survey recommended by national or international authorities. Reducing P-inputs impacted these seasonal variations: the decline of seasonal amplitudes of chlorophyll *a* reduced the seasonal amplitude of orthophosphate and of daily variations of dissolved oxygen and pH but did not significantly affect the seasonal amplitude of nitrate. Thus, the influence of phytoplankton on seasonal variations of nitrate was minor throughout the period of study.

1 Introduction

For several decades, eutrophication has become a major issue affecting most surface waters (Smith et al., 1999; Hilton et al., 2006; Smith and Schindler, 2009; Grizzetti et al., 2012; Romero et al., 2012). The regulation of nutrient inputs in waters by the elimination of N and P during waste-water treatment, better agricultural practices and restriction of the use of phosphorus products (EEC 1991a and b) led to a decrease in phosphate and/or nitrate content which is recorded in several European rivers presenting temperate and continental regimes since the mid-1990s, including the Elbe (Lehmann and Rode, 2001), the Seine (Billen et al., 2007), the Thames (Howden et al., 2010), the Danube (Istvánovics and Honti, 2012), the Rhine (Hartmann et al., 2007) as well as some Mediterranean rivers (Ludwig et al., 2009) and Scandinavian rivers (Grimvall et al., 2014).

Surface water quality is also affected by variations in hydro-climatic conditions (Durance and Ormerod, 2010) and nutrients availability is not the only limiting factor of phytoplanktonic growth in rivers: successful phytoplankton species in rivers are selected on their ability to survive high frequency irradiance fluctuations and the important determinants are turbidity (or its impact upon underwater light) and the water residence time (Istvánovics and Honti, 2012; Krogstad and Lovstad, 1989; Reynolds and Descy, 1996; Reynolds et al., 1994). In Europe, both climatic models and observations show a general rise in air and water temperature since the 1970s (Moatar and Gailhard, 2006; Whitehead et al., 2009; Bustillo et al., 2013) and models predict lower water discharge and rising temperatures during summer, potentially intensifying the risk of eutrophication (Arheimer et al., 2005; Barlocher et al., 2008; Lecerf et al., 2007; Whitehead et al., 2009) as shallow rivers are particularly susceptible to eutrophication (Istvánovics et al., 2014). Besides, phytoplanktonic biomass remains at a high level in many water bodies, evidencing that leaching of long last stored nutrient in soils is still significant: if nutrient mobility should increase with global warming because of the acceleration of organic matter mineralization and of higher soil leaching (Bouraoui et al., 2002; Arheimer et al., 2005), the river system response time to a nitrogen input reduction is limited by the time required for nitrate to be released from soil to receiving waters (Jackson et al., 2008; Bouraoui and Grizzetti, 2011). Therefore we should expect that changes in current agricultural practices may improve water quality only after several decades (Behrendt et al., 2002; Howden et al., 2010).

The first regulatory studies of the largest French river eutrophication, i.e. the Loire River, were made in the 1980s in the Middle and Lower segments (Crouzet, 1983; Meybeck et al., 1988; Lair and Reyes-Marchant, 1997; Etcheber et al., 2007). The Middle reaches (Fig. 1) were recognized as being the most eutrophic sector (Lair and Reyes-Marchant, 1997) resulting from high P levels (Floury et al., 2012), low river velocity and shallow waters, its multiple channels morphology with numerous vegetated islands slowing down flow velocity (Latapie et al., 2014). In recent years, Loire eutrophication indicators and their trends recorded several variations: (i) decline of chlorophyll *a* in the Middle segment since the late 1990s (Floury et al., 2012), (ii) decline of phosphorus as well in the Middle Loire (Gosse et al., 1990; Moatar and Meybeck, 2005; Oudin et al., 2009), (iii) development of *Corbicula fluminea* as an invasive species since the 1990s (Brancotte and Vincent, 2002) and (iv) dominance of small centric diatoms and green algae in phytoplankton population, for most of the year in the Middle and Lower river sectors (Abonyi et al., 2012, 2014; Descy et al., 2011).

Most previous studies focused on the Middle Loire, which represents only 25% of the total drainage basin and excluded the main tributaries and their possible influences on the main river course. Besides, most studies on river eutrophication stayed at the inter-annual variations and did not investigate how long term trends might affect the river biogeochemistry at the seasonal or the daily scale, while seasonal and daily cycles are especially amplified in eutrophic rivers (Moatar et al., 2001). This paper examines longitudinal distributions and long-time trends of chlorophyll *a* and nutrients over three decades (1980-2012) and for the whole Loire basin. Thus, it includes the study of the main tributaries variations and their potential influences on the Loire main stem. It also focuses on how the noticeable long term changes affected the biogeochemical functioning of the river at the seasonal scale, exploring the seasonal variations of chlorophyll *a* and nutrients since 1980 and examining both seasonal and daily fluctuations of dissolved oxygen and pH since 1990.

2 Study area and data compilation

2.1 Geographical and physical characteristics

The Loire River basin (110,000 km^2) covers 20% of the French territory. Its hydrological regime is pluvial with some snow-melt influences because of high headwater elevation (6% of the basin area is over 800 m above sea level). The main stem can be divided into three parts

(Fig.1, Table 1): (i) the Upper Loire (18% of basin area; stations 1 to 9) extending from the headwaters to the confluence with the River Allier; (ii) the Middle Loire (24%; stations 10 to 18) from the Loire-Allier confluence to the Loire-Cher confluence which receives only minor inputs from small tributaries; (iii) the Lower Loire (65%; stations 19 to 21) which receives major tributaries (Cher, Indre, Vienne and Maine Rivers) doubling the river basin area and the average river water discharge.

As summer low flows can reach critically low levels in the Middle reaches where four nuclear power plants are located (Fig. 1), two dams were constructed on the Allier and Upper Loire (Naussac 1981 and Villerest, 1984) to maintain low flows over a minimum of 60 m^3 s^{-1}. Grangent dam was constructed in 1957 for electricity production purposes. The median annual discharge over the last 30 years is 850 m^3 s^{-1} at the basin outlet (station 21) and the median in the driest period from July to September is only 250 m^3 s^{-1}, corresponding to only 2 L s^{-1} km^{-2}. The driest years were 1990, 1991, 2003 and 2011 with a daily discharge average at station 21 reaching sometimes 100 m^3 s^{-1}.

The headwater catchment is a mountainous area and the Loire itself runs through narrow gorges and valleys (Latapie, 2011). After the confluence with the Allier, the geomorphology of the Middle Loire favors phytoplankton development, its multiple channels with numerous vegetated islands slowing down flow velocity and the valleys becoming wider (Latapie et al., 2014). As a consequence, average water depth can be low in the summer (≈ 1 m), contributing to warming and lighting up the water column.

The temperature is always at least 2°C lower in the Upper part than in the lower reaches (annual medians are around 15°C in the Upper Loire during April-October versus 19°C in the Middle and Lower segments) and is affected by global warming. Hence, Moatar and Gailhard (2006) showed that mean water temperature has increased by 2.4 to 3 °C in spring and summer since 1975 due to rising air temperature (Gosse et al., 2008) without a significant impact on phytoplanktonic development (Floury et al., 2012). Approximately 60% of this general rise in water temperature during the warm period was explained by rising air temperature and 40% by a decrease in the May/June river discharge since 1977 (Moatar and Gailhard 2006, Floury et al., 2012). The water returning to the Loire from the nuclear power plants only raises the temperature by a few tenths of a degree thanks to an atmospheric cooling system (Vicaud, 2008) and does not influence the thermal regime of the river studied here.

Urban pressure is significant with 8 million people living in the Loire Basin (2008 population census by the French National Institute of Statistics and Economic Studies, INSEE), mainly concentrated near the main river course. It corresponds to an overall population density of 73 inhabitant km^{-2}. The density is greater in the Upper Loire (144 inhab. km^{-2}, Table 1) due to the city of Saint Etienne (180,000 inhabitants). The Middle and Lower catchments contain some major riparian cities (Fig. 1) with a stable population density around 76 inhab. km^{-2}.

Agricultural pressure is defined here with two indicators: the percentage of the basin occupied by arable land and the Agricultural Pressure Indicator (API) represented as the quotient of (pasture + forest) over (pasture + forest + arable land). According to the Corine Land Cover database (2006), the headwater areas are mostly forested or pastureland (Table 1). Arable land increases from headwaters going downstream to reach 30% of the total basin area at station 21. Land use distribution in the major tributaries differs widely (Table 2): the Allier (catchment at station A) is mostly composed of pasture; the Cher at station B has similar amounts of pasture and arable land, most of the rest being forested; half of the Indre basin at station C is arable land, but this tributary drains only 3% of the total basin; the Vienne and the Maine contribute very significantly to the total area of arable land in the Loire basin. Urban pressure is also significant in the Maine catchment due to the cities of Le Mans and Angers (Fig.1).

2.2 River monitoring datasets

Water quality databases from regulatory surveys (Loire Brittany river basin agency, AELB) used here (chlorophyll a, pheopigments, nitrate (NO_3^-), nitrite (NO_2^-), Kjeldahl nitrogen (NKj), orthophosphate (PO_4^{3-}) and total phosphorus (Ptot)) are available online (http://osur.eau-loire-bretagne.fr/exportosur/Accueil). Sixty-nine monitoring stations were set up along an 895 km stretch. Stations sampled at least monthly between 1980 and 2012 (bi-monthly or weekly for some variables) were selected for analysis in this paper (17 stations, Fig. 1). To take into account the influence of major tributaries, five sampling sites at each of the major tributary outlets were also included (stations A to E).

The water quality of the Loire River has also been assessed during several other surveys, generally with high sampling frequency, but these data have seldom been used and/or compared in previous studies. They included:

i) Water quality surveys upstream and downstream of nuclear power plants carried out since the early 1980s by the French Electricity Company (EDF) (Moatar and Gailhard, 2006; Moatar et al., 2013); see stations 12, 14, 16 and 19 on Fig. 1. These datasets were used to improve the spatial analysis. These surveys included temperature, dissolved oxygen and pH recorded hourly at station 19 enabling us to analyze possible changes in day/night variations (variables hereafter named *delta* O_2 and *delta pH* corresponding to the daily range of O_2 and pH).

ii) The Orléans city experimental survey carried out by the Loire basin authority at station 15 from 1981 to 1985, measuring nutrients and chlorophyll a every three days (Crouzet, 1983; Moatar and Meybeck, 2005).

River flow datasets on a daily basis were taken from the national "Banque Hydro" database (http://www.hydro.eaufrance.fr/). The local population census (INSEE, 2008) and the Corine Land Cover (2006) were also used to estimate the general characteristics at different water quality stations (Tables 1 and 2).

3 Methods

3.1 Data pre-processing

To validate the AELB datasets and eliminate remaining outliers, log-log relationships between concentration and discharge were analyzed and compared with previous research studies carried out during targeted periods (Grosbois et al., 2001; Moatar and Meybeck, 2005). The separation of living phytoplankton biomass (characterized by chlorophyll a) and algal detritus (characterized by pheopigments) depends on the protocol used and since this protocol may have changed over the last 30 years, we worked with the sum of chlorophyll *a* and pheopigments, which increased the robustness of the data and corresponded better to phytoplanktonic biomass as an active biomass and organic detritus (Dessery et al., 1984; Meybeck et al., 1988). Thus, for clarity further in the text, "Chl. *a*" corresponds to the sum chlorophyll *a* + pheopigments.

PO_4^{3-} time series included periods reaching the limit of quantification. When evidenced, such data were not taken into account to avoid mis-interpretation of such constant values. The datasets also included periods with missing values. In all cases, no infilling were realized.

Sampling frequencies were most of the time monthly (only 10% of datasets were sampled on average every two weeks or more often), but in order to homogenize the time series, the following analysis was conducted on monthly medians.

To assess longitudinal distribution of nutrients and phytoplanktonic biomass, each year was divided into two seasons: "*summer*", here considered as the phytoplankton growth period from April to October, when more than 90% of the phytoplankton bloom is observed (Leitão and Lepretre, 1998) and "*winter*", here November to March when Chl. *a* concentrations are usually under 20 µg L^{-1} (average *winter* Chl. *a* in the Middle Loire ≈ 20 µg L^{-1} for the considered period).

Uncertainties on estimates of concentration averages were assessed using Monte Carlo random draws (Moatar and Meybeck, 2005) on experimental high frequency data at Orléans city (station 15). Uncertainties on seasonal means varied between 10% (NO_3^-) and 30% (PO_4^{3-}) in *summer* and between 6% (NO_3^-) and 10% (PO_4^{3-}) in *winter*.

When both river discharge and nutrient concentration datasets were available during the period considered, average annual fluxes were calculated to assess the contribution of each major tributary to the Loire. This calculation was possible during 1980-86 and 1994-2006 for the Allier input, 1985-90 and 1999-2009 for the Cher, 2006-2011 for the Vienne and 1981-2012 for the Maine.

In order to assess potential changes in the nitrogen to phosphorus molar ratio (N:P further in the text) and make the link with possible nutrient limitation of phytoplankton, this ratio was calculated using Ntot (sum of NO_3^-, NO_2^- and NKj) and Ptot.

3.2 Building up spatio-temporal diagrams

Time series were represented with a 2D spatial x-axis and seasonal y-axis. This allowed the observation of both longitudinal and seasonal distribution during a certain period, between the river headwaters to the estuary and from January to December. When needed and possible, missing data were interpolated both spatially and temporally to represent a smoother diagram. Three periods were defined and separated the last three decades in three sub-periods on the basis of Chl. *a* concentrations: 1980-1989, 1990-2001 and 2002-2012.

3.3 Time series decomposition

Long-term trends and seasonal variations analysis were carried out using Dynamic Harmonic Regression (DHR) technique, extensively described in Taylor et al., (2007) (a brief outline of it is also explained in Halliday et al., 2012 and 2013). It decomposes an observed time series into its component parts:

$$f(t) = T(t) + S(t) + Irr(t) \qquad (1)$$

where f is the observed time series, T is the identified trend, S the seasonal component and Irr the "irregular" component defined as white noise, representing the residuals.

The trend was defined using an Integrated Random Walk model. It is a special case of the Generalized Random Walk model (GRW) and has been shown to be useful for extracting smoothed trends (Pedregal et al., 2007). This provided the identified trend and the slope of the trend.

The seasonal components were defined as follow:

$$S(t) = \sum_{i}^{N/2} \left[a_{i,t} \cos(\omega_i t) + b_{i,t} \sin(\omega_i t) \right] \qquad \omega_i = \frac{2\pi \cdot i}{N} i = 1, 2, \ldots, \left[\frac{N}{2} \right] \qquad (2)$$

where ω_i are the fundamental and harmonic frequencies associated with the periodicity in the observed time series chosen by reference to the spectral properties. For instance, the period 12 was corresponding to a monthly sampling in an annual cycle.

The phase and amplitude parameters were modeled as GRW processes and estimated recursively using the Kalman Filter and the Fixed Interval Smoother. These parameters were defined as non-stationary stochastic variables to allow variation with time i.e. allow non-stationary seasonality and represent better the dynamic of the observed parameters.

Significance of the seasonality was based on the squared correlation coefficient between calculated seasonal component and detrended data. Similarly, significance of the trend was determined based on the squared correlation coefficient between calculated trend and deseasonalized data.

Stations 4 (Upper Loire), 18 (Middle) and 21(Lower) presented a large amount of data and were selected here to present and discuss the DHR analysis. Similarly, water discharge data at station 15 was daily and continuous since 1980 and was selected for the DHR analysis presented in the Results section.

4 Results

4.1 Long term trends and longitudinal distributions of Chl. *a* and nutrients

Chl. *a summer* medians (used as the prime indicator of eutrophication) showed a very clear longitudinal increase from headwaters to river mouth (Fig. 2a). At the headwaters, Chl. *a* concentrations remained below 30 µg L^{-1} between 1981 and 2012. It has been shown in other studies that in the Upper Loire reservoirs which have always been eutrophic since the 1980s (Aleya et al., 1994; Jugnia et al., 2004), the phytoplankton assemblage is lake-like and these species do not survive very long in the turbulent and quite turbid river downstream (Abonyi et al., 2011, 2014), explaining why Chl. *a* remains at low levels. In the lowest reaches of the Upper Loire (station 9), Chl. *a* was higher but showed a descending trend for the whole period. In the Middle segments, Chl. *a* levels increased between 1981 and 1990 by a factor of two (Table 3). The maximum ever measured occurred at station 18 in early October 1990 (365 µg L^{-1}). The next decade, the situation already started to decrease in the Middle Loire (-5 µg L^{-1} $year^{-1}$) and even more in the Lower (-9 µg L^{-1} $year^{-1}$). Finally, since 2002, the decline generalized to the whole river and trends slopes were ≈ -5 µg L^{-1} $year^{-1}$ in the Middle Loire and -4 µg L^{-1} $year^{-1}$ in the Lower reach.

Winter medians of phosphate concentrations increased downstream of station 2 (Fig. 2b) and the maximum for the Upper segment was reached at station 4, where population density is 143 inhab. km^{-2}, a maximum for the whole basin. Population density decreased to 75 inhab. km^{-2} between stations 4 and 9, with a corresponding reduction in the phosphate levels. PO_4^{3-} levels were stabilized in the Middle Loire (stations 10 to 18).

The general phosphorus decline during the last decade can be observed along the whole longitudinal profile. Phosphate was at its maximum in the 1980s (above 100 µg P L^{-1}) for almost the whole main stem. It then decreased gradually to reach lower levels <70 µg P L^{-1}. In the urbanized Upper part (stations 3 and 4), from a *winter* median of 190 µg P L^{-1} during 1980-1989, phosphate decreased to its current level (60 µg P L^{-1}). Average phosphate in the Middle and Lower reaches has reduced at least two-fold since 1980. At the Lower Loire outlet (station 21), phosphate contents increased during 1980-1989 and then decreased at the rate of ≈ -4 µg P L^{-1} y^{-1}. Downstream the main reservoirs (Upper Loire), a noticeable decrease in phosphorus concentration was observed. This was probably partly due to P retention between stations 4 and 5 (Fig. 1) as a large part of the particulate matter is stored in the reservoir.

The *winter* nitrate longitudinal profile showed a regular increase from 1 mg N L^{-1} in the headwaters to 3.5 mg N L^{-1} at the river mouth (Fig. 2c). This longitudinal rise could be observed throughout the period of study. The upstream reservoirs did not seem to impact the nitrogen concentration as nitrate represented most of the total nitrogen and the phytoplanktonic uptake within these reservoirs is not questioned here: Fig. 2c present *winter* nitrate concentration. Annual median nitrate concentration remained stable in the Upper Loire, with no significant trends since 1980. In the Middle segment, it only presented an increasing trend during the 1990s (+0.1 mg N L^{-1} y^{-1}) but the more significant variations were observed in the Lower reaches at station 21 where nitrate increased on average at +0.3 mg N L^{-1} y^{-1} during the 1980s, a bit less the next decade (+0.1 mg N L^{-1} y^{-1}) and finally slightly decreased since 2002.

These trends provided by the DHR model were always significant and explained at least 50% of the variations in the deseasonalized time series (Table 3). The most significant trends were observed in Chl. *a* and PO_4^{3-}. The long term variations in NO_3^- were less pronounced justifying a lower corresponding strength.

4.2 Seasonal shifts across the longitudinal distribution of Chl. *a* and nutrients

Throughout the period of study, Chl. *a* concentrations reached their maximum in July or August for the whole Loire River. During the 1980s and 90s, phytoplankton production usually started in early April, reached a peak in early May with a second peak in late August (Fig. 3a) suggesting different phytoplankton communities growth (Abonyi et al., 2012, 2014). After mid-November, Chl. *a* concentrations were very low. A slight change is nevertheless evidenced: between 1980 and 2000 in the Middle and Lower Loire, Chl. *a* concentrations reached occasionally their maximum in October (it is the case of the years 1985, 1988, 1989, 1990, 1995); since 1996, it never happened again.

Phosphate spatio-temporal variations showed inverted seasonal patterns between the Upper and Middle-Lower Loire (Fig. 3b). Maximum phosphorus levels were observed in the middle part of the Upper section (stations 3 to 5) as a result of urban pressure, previously mentioned in the longitudinal profile description. In this upstream reach where phytoplankton development is limited, the seasonal maximum level was observed in *summer* when low flows cannot dilute urban phosphorus inputs; during the period 2002-2012, PO_4^{3-} medians reached 140 µg P L^{-1} at station 4 in June. In the lower reaches of the Upper Loire, the Middle and the

Lower reaches (stations 8 to 21), the seasonality of phosphate was inverted compared to the Upper Loire and clearly controlled by eutrophication with a minimum (<30 µg P L^{-1}) occurring during *summer* due to phytoplankton uptake.

Nitrate concentrations had a very clear seasonality (Fig. 3c) with maximum levels during *winter* (leaching) along the whole Loire River. In *summer*, nitrate was very low with concentrations around 1 to 2.5 mg N L^{-1} along the whole river profile and the lowest concentrations were recorded in August in the Middle Loire. The *summer* nitrate minima have increased since 1980: around 0.4 mg N L^{-1} in the Middle Loire between 1980 and 1999, the average *summer* 10% percentile increased to 1 mg N L^{-1} this last decade. A seasonal Kendall test analysis (station 15, 1980-2012) revealed that water discharge explained 26% of the nitrate variance.

The Dynamic Harmonic Regression model represented well the time series, depending on the river reach and the type of variable (Table 4). Seasonal components were stronger in Middle and Lower Loire than in Upper, with better correlations between detrended time series and calculated seasonal component (45 to 85% variance explained by the seasonal component in the Middle and Lower against 15-45% in the Upper). Chl. *a* series were well represented by the seasonal component, whereas PO_4^{3-} was sometime poorly explained, illustrating the high variability of this parameter. Nitrate time series presented the best fits, with around 80% of the variance explained by the seasonal component in the Middle and Lower reaches.

4.3 Analysis of the main tributaries variations and their impacts on the Loire long-term trends

Trends in the main tributaries of the Loire River (stations A to E) mimicked the Loire River variations with high signs of eutrophication during the 1980s and 1990s followed by a general decline (Table 5).

Chl. *a* in the tributaries remained under the Loire main stem levels in each of the major tributaries except for the Cher River (station B): its highest Chl. *a* concentrations during the 1990s were very close to the extreme values reached at the same time in the Middle Loire (average seasonal variation ≈ 190 µg L^{-1} during the 1990s). Nonetheless, trends in Chl. a concentrations were everywhere following the same pattern, with high seasonal variations and high annual medians between 1980 and 2001, and then clearly declined the last decade.

Phosphate concentrations decreased everywhere continuously from high values in the 1980s (≈ 200 µg P L^{-1}) down to ≈ 50 µg P L^{-1} except at station E (Maine River) where PO_4^{3-} first increased during the 1980s from 200 µg P L^{-1} to peak in 1992 at 300 µg P L^{-1} and finally declined towards 50 µg P L^{-1}.

Like in the Loire River, nitrate concentrations in the main tributaries increased slightly since 1980, but levels and seasonal amplitudes progressed differently: quite low in the Upper tributary (station A, annual medians ≈ 1.5 mg N L^{-1}), NO_3^- reached higher concentrations in the other tributaries and extreme values in the Maine River with *winter* maximums over 10 mg N L^{-1} during the 1990s. At each station but station A, NO_3^- seasonal amplitudes slightly started to decrease since 2002 i.e. the *summer* minimum slightly increased.

At each major tributary confluence, the tributaries inputs could contribute on average to 35% of the main river nutrient fluxes. The more significant inputs were coming from the Allier River (station A) discharging almost the same amount of NO_3^- and PO_4^{3-} as the Upper Loire River. Because of the lack of data allowing nutrient fluxes calculations on a fine temporal scale, these results are to be considered with caution. But they are certainly giving good approximations of how much these tributaries can influence the Loire main stem eutrophication trajectory.

4.4 Seasonal amplitudes of Chl. *a*, nutrients, O_2 and pH in the Middle Loire

As described above, Chl. *a*, nitrate and phosphate concentrations presented different patterns of seasonality depending on the location. This paragraph focuses on seasonality of nutrients and Chl. *a* at station 18 and on dissolved oxygen, pH and temperature at station 19. Both of these stations are representative of the Middle Loire reach where the highest signs of eutrophication occurred in the early 1990s.

Chl. *a* seasonal variation at station 18 increased during the 1980s (Fig. 4a) from 150 to 240 µg L^{-1} (1990) and then presented a spectacular decline in two steps: first, it went down to 150 µg L^{-1} in 1992 and remained at the same level the next 8 years; then, it kept on decreasing since 2000 to finally reach levels of amplitude around 50 µg L^{-1}. Phosphate seasonal variations decreased continuously from 150 µg P L^{-1} in 1980 to 30 µg L^{-1} in 2012 (Fig. 4b), at the rate of -6 µg P L^{-1} $year^{-1}$ in the 1980s, -4 µg P L^{-1} $year^{-1}$ in the 1990s and finally reached a stable variation since 2008 (Table 4). The seasonal variations of NO_3^- presented another pattern through the last 30 years (Fig. 4c): it increased from 2.2 mg N L^{-1} in 1980 to 2.8 mg N L^{-1} in

1991, then remained stable around 2.9 mg N L^{-1} the next 7 years to finally decrease slightly down to 2 mg N L^{-1}.

Interannual dissolved oxygen concentration and pH at station 19 did not present any significant trend (Fig. 4d and 4e): since 1990, annual average O_2 = 10.8 mg L^{-1} and pH = 8.3. At the daily scale, the variations of O_2 were synchronous with water temperature: the typical O_2 daily cycle corresponded to a minimum at sunrise, followed by a rapid increase and a maximum observed two hours after solar mid-day; the daily range could reach 10 mg L^{-1}, with oxygen saturation ranging from 60% to 200%. These daily variations greatly challenge the validity of O_2 measurements as a water quality indicator within the regulatory monthly survey of such eutrophic river. Alongside daily oxygen cycles, significant daily pH cycles were observed (see also Moatar et al., 2001). Dissolved CO_2 and/or bicarbonate uptake by primary producers during the solar day led to increasing pH. By contrast, night-time respiration was reducing pH. In the Loire, daily pH cycles were pronounced with the same phase as the O_2 cycle. The common daily pH range in *summer* was 0.8 unit and could reach 1 pH unit. Because these variations are linked to the in-stream biological activities, daily O_2 and daily pH amplitudes presented a well-defined seasonality, with maximum reached in *summer*.

DHR model applied on water temperature (T°C) successfully represented the observations with squared correlation coefficients R^2 of 0.96. Performances were lower for discharge (Q) with $R^2 = 0.57$. Both T°C and Q trends were weak (only 20% of the variances); however, T°C was increasing, and Q slightly decreasing.

5 Discussion

5.1 Role of agricultural and urban pressures on the Loire long-term variations

The population density profile (Fig. 2) illustrates well the fact that phosphate concentrations are linked with urban P-inputs. Thus, most changes in phosphate levels are connected to more efficient sewage treatment plants (de-phosphatation steps were set up) and the use of phosphate-free detergents. De-phosphatation technologies were not implemented at the same time across the basin, explaining different trends for different catchments. These observations support previous studies highlighting the need for phosphorus control (Gosse et al., 1990; Oudin, 1990). This control has considerably reduced phosphate concentration in the surface waters of the Loire basin (Bouraoui and Grizzetti, 2011). Nevertheless, Descy et al. (2011)

assessed the biogeochemical processes using numerical models of the Middle reaches during the year 2005 and the phosphorus reduction could not totally explain the phytoplankton diminution: it was necessary to introduce the effect of grazing by a benthic lamellibranch, *Corbicula fluminea*. The role played by this invasive clam definitely needs to be assessed, as it has propagated dramatically in the Loire Basin since 1990 (Brancotte and Vincent, 2002) like it did in some other European rivers with significant impacts on the phytoplankton biomass (Hardenbicker et al., 2014; Pigneur et al., 2014). Trends in orthophosphate concentrations were sometimes poorly explained by the DHR model. In summertime, very small increases in water discharge could resupply the system with more available phosphorus, allowing more phytoplankton development, but this would only be observed at a fine temporal scale. Our study uses monthly datasets, which is not sufficiently detailed to discuss variations expected at the daily scale: PO_4^{3-} concentrations are strongly sensitive to TSS concentration and consequently to water discharge variations.

The relationship between the *winter* nitrate levels and the percentage of the catchment under arable land is strong (Fig. 2), illustrating the fact that nitrate levels originate mainly from diffuse agricultural sources. The slightly increasing trend in nitrate could partly be explained by the delayed response of the environment to external changes (Behrendt et al., 2002; Howden et al., 2010), or, according to Bouraoui and Grizzetti (2008), this could be showing a lack of appropriate agro-environmental methods, or a delay in implementing the 1991 European Nitrates Directive. It has been shown that mitigation measures in agriculture did decrease nitrogen loads in several Swedish rivers (Grimvall et al., 2014) and in the Rhine and Danube Rivers (Hartmann et al., 2007) making a great contrast with many other temperate lowland rivers where nitrate increasing trends are still recorded: the Mississippi (Sprague et al., 2011), Ebro, Po and Rhone Rivers (Ludwig et al., 2009) and also the Thames (Howden et al., 2010). Another potential reason for this increase could be climate change: higher mineralization of organic matter in the arable soils is expected and caused by an increased temperature over time (Arheimer et al., 2005) together with higher soil mineralization (Bouraoui et al., 2002). This hypothesis would seem reasonably concomitant with the rising water temperature which was recorded in the Loire River (Fig 4f), but it seems too early to fully determine the link between climate change and nitrate trends.

Such diffuse N sources are seasonal and this depends on leaching of bare soils by rainfall in winter and retention by vegetation in the growing season. Thus, it is possible that the decrease of nitrate seasonal amplitude recorded since 2005 was linked to lower discharge variations, however the increasing nitrate trend contradicts with a slightly descending discharge trend. Figure 3 clearly indicates the antivariation of phytoplankton and nitrate in their seasonal cycle: nitrate minima were reached when Chl. *a* concentrations were maximum, i.e. in *summer* in the Middle and Lower sectors. In addition, increasing of nitrate concentration have been seen in *summer* in the Middle and Lower sectors (see section 4.2), which is concomitant with a reduced phytoplankton biomass. However, seasonal amplitudes of nitrate did not decrease significantly in the Middle Loire while the decline of phytoplanktonic biomass started since the 1990s and was generalized to the whole basin since 2002 (section 4.3). Hence, it is likely that N uptake by phytoplankton had only a minor influence on nitrate seasonal variations. Denitrification could play a significant role on nitrate seasonal variations, like in the neighboring Seine basin (Curie et al., 2011), but further investigations would be needed to fully assess the processes involved. A complete N budget in the watershed plus the development of a N-surplus model would better explain why nitrate levels remain this high in the Loire Basin.

5.2 Nutrient limitation variation since 1980

The N:P molar ratio allows to determine whether the system studied is potentially under nitrate or phosphate limitation (Koerselman and Meuleman, 1996; Ludwig et al., 2009) and may constitute the basis of some indicators to assess the risk of eutrophication in freshwaters (Dupas et al., 2015). Given other controlling factors as non-limiting factors of phytoplankton growth, if N:P is under 14, the system is limited by N; over 16, it is considered P-limited. In-between, N and P availabilities might be sufficient or the ecosystem might be co-limited by N and P (Koerselman and Meuleman, 1996).

In the Loire River, a slight increase in annual concentrations of nitrogen during the last 30 years while phosphorus inputs decreased greatly resulted in the modification of the N:P molar ratio (Fig. 5). In the Middle Loire, the annual average ratio kept on increasing since 1980. In *summer* during the 1980s, the lowest values observed were occasionally under the Redfield limitation but most of the time over the limitation. Since 1992, the system never reached again the Redfield limit and remained in the P-limitation domain as a result of reducing significantly phosphorus direct inputs. Similar variations were observed in other river systems (e.g. the Ebro, Rhone, Po, Danube, Ludwig et al., 2009; the Seine, Billen and Garnier, 2007; the Mississippi, Turner et al., 2003) where similar trends in N and P were recorded. The N:P ratio was subjected to a significant seasonality. Its pattern and strength has changed from low seasonal variations during the 1980s and a minimum reached in *summer* to a well-defined seasonality since 2002 in the Middle and Lower reaches with a maximum reached in *summer*, reinforcing the P-limitation characteristic of the Loire River during the phytoplanktonic growth period. These results indicate that P-limitation of phytoplankton growth has become a significant factor. When the river hydrology remains stable in the summer, phytoplankton is potentially under P-limititation. This is suggesting a potential explanation for the apparent shift in seasonal phases of Chl. a concentrations (late summer blooms no longer occur, described in section 3.2): in those cases, the P-limitation is reached before any other limitation. This shift could also be related to a significant impact of grazing by invasive *Corbicula* spp. clams, which would substantially decrease the phytoplankton biomass (Pigneur et al., 2014).

5.3 Daily O_2 and pH amplitudes as indicators of eutrophication mitigation

The *delta* O_2 and *delta pH* seasonal amplitudes decreased greatly since 1990: around 3.5 mg L^{-1} in 1990-95, *delta* O_2 amplitude declined down to 1.25 mg L^{-1}. Similarly, from a seasonal amplitude at 0.25 pH unit, *delta pH* seasonal amplitude was maximum in 1998 (0.35) and went down to 0.3 since 2007. These descending trends are linked to the apparent decrease of phytoplanktonic biomass: the seasonal amplitude of Chl. *a* concentrations explained 80% of the seasonal variations of *delta* O_2 and only 59% for *delta pH* amplitudes. Continuous records of O_2 and pH take into account the whole in-stream primary activity, that is to say not only the phytoplankton respiration but also macrophytes and periphyton activities. While Chl. *a* concentrations kept on declining since 1991, *delta* O_2 and *delta pH* stopped decreasing suggesting that a non-phytoplanktonic activity was rising. Besides, one would expect that since phytoplankton biomass declined, water column irradiance increased and macrophyte abundance would have risen. We unfortunately lack data about macrophyte and periphyton developments in the Loire River, but the biological reserve Saint-Mesmin located near Orléans City (station 15) studied the development of macrophytes species since 1998 on 24 river sections (60 m long by 5m width) and showed the increasing abundance and biodiversity of such aquatic plants since 2002. Two species were dominant, *Myriophyllum spicatum* and *Ranunculus fluitans*. The role played by fixed aquatic vegetation on the river biogeochemistry is probably very significant as macrophytes are known to get nutrients contained in the water compartment as well as in the sediments (Carignan and Kalff, 1980; Hood, 2012). Hence, during low PO_4^{3-} concentration in *summer*, macrophyte growth is not limited by the in-stream nutrient limitation.

A major change occurred in the seasonal patterns of daily maximum of dissolved O_2. From a maximum reached in June or July at least between 1990 and 2001, the seasonal pattern of daily maximum shifted dramatically to a maximum reached in *winter*. On the contrary, daily O_2 and pH minimum always reached their maximum in *winter* and their minimum in *summer* (due to biomass respiration). Such a spectacular change in daily O_2 maximum because of a declining eutrophication has never been shown in other major European rivers.

When unusual late floods occurred, higher flow velocity, increased turbidity and a reduced water column irradiance probably disrupted the well-established dominance of production/respiration cycles. Therefore both dissolved oxygen and pH levels dropped for a few days. Such episodes happened in 1992 (event described in Moatar et al., 2001), 1998 and 2008. In those cases, phytoplankton growth is under hydrologic limitation.

6 Conclusions

The Loire River is a relevant case of a river recovering from severe eutrophication by controlling phosphorus direct inputs. However, other recent changes should also be considered. For example, it would be interesting to investigate the impact of the development of *Corbicula* clams (Brancotte and Vincent, 2002) on the biogeochemistry of the Loire basin surface waters. A potential numerical model of the Loire basin eutrophication should not only take into account climate and land-use changes, but also recent ecological changes (Descy et al., 2011; Pigneur et al., 2014) and this model would probably be able to answer many questions about the occurrence of invasive grazers in the Loire River.

This study highlighted how contrasted can be the different long term trajectories of Chl. *a* and nutrient concentrations in the different reaches of a eutrophic river and contributed to better understand the current biogeochemical functioning. Although the Upper Loire received the highest concentrations of phosphorus, the signs of eutrophication were expressed only in the lowest part of the Upper River because of its morphology. The Middle Loire is very favorable to eutrophication and the Lower reach functioning and trends remained close to the Middle Loire trajectory although it receives most of the tributaries inputs. Signs of eutrophication remained lower in the major tributaries than the main river stem, but it has been shown that their influence on the Loire River nutrient fluxes (and consequently on the phytoplanktonic biomass) at the confluences can reach up to 35%.

This study also support the previous works on the Loire eutrophication, but the analysis of the long term changes in seasonality in this paper could bring more elements:

i) Controlling P-inputs also impacted the river biogeochemistry at the seasonal scale: seasonal amplitudes of Chl. *a* and orthophosphate greatly decreased; and this impacted O_2 and pH both daily and seasonally. However, nitrate amplitudes remained quite stable, evidencing the fact that phytoplankton growth had a minor influence on nitrate seasonal variations questioning the exact role played by fixed aquatic vegetation and denitrification on the nitrogen cycle.

ii) When hydrologic conditions remain favorable for phytoplankton growth in *summer*, orthophosphate concentration becomes the limiting factor.

iii) Combined to Chl. *a* concentration time series, *delta* O_2 and *delta pH* are relevant metrics for studying eutrophication variations. High frequency records of Chl. *a*, O_2 and pH could potentially enable the separation between phytoplankton and macrophytes impacts on the river biogeochemistry.

In addition, this study highlights the temporal variability of the different eutrophication metrics: in *summer*, the river biogeochemistry is essentially controlled by production/respiration processes. Thus, daily and seasonal variations are very significant and call into question the classical monthly survey recommended by national or international authorities.

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Table 1. Loire main stem stations characteristics. Kilometric point (KP): distance from headwaters; Drained area; Q: average annual discharge; population density in 2008; arable land as percentage of the drained catchment; API: agricultural pressure indicator = (pasture + forest) / (pasture + forest + arable land) expressed in percentage. See paragraph 1.2. for source information.

	Upper Loire									Middle Loire						Lower	
Station	1	2	3	4	5	6	7	8	9	10	11	13	15	17	18	20	21
KP (km)	44	92	200	224	273	292	344	417	451	465	500	564	633	712	772	822	895
Drained area (10^3 km^2)	0.5	1	4	5	7	8	13	15	18	33	34	36	37	41	43	82	109
Q (m^3 s^{-1})	6	10	-	47	67	-	89	-	180	300	320	327	-	360	366	680	850
Population density (inhab.km^{-2})	13	50	144	143	122	128	101	91	80	75	74	74	73	80	83	-	73
Arable land (%)	0.6	3	1	3	4	4	3	4	6	9	11	13	13	15	17	24	30
API (%)	99	97	99	96	96	96	96	96	93	90	89	87	86	84	82	75	69

Table 2. Major tributaries station characteristics.

Station	A	B	C	D	E
Drained area (10^3 km^2)	14	13	33	21	22
Average discharge (m^3 s^{-1})	143	81	37	-	135
Population density (inhab. km^{-2})	67	52	76	55	82
Arable land (%)	13	36	52	25	49
API (%)	87	63	46	74	50

Table 3. Long term trends at three stations representative of the Upper, Middle and Lower Loire.

		Annual median			Trend			Significance of trend 1980-2012 (%)		
	years	Chl. *a* µg L^{-1}	PO_4^{3-} µg P L^{-1}	NO_3^- mg N L^{-1}	Chl. *a* µg L^{-1} y^{-1}	PO_4^{3-} µg P L^{-1} y^{-1}	NO_3^- mg N L^{-1} y^{-1}	Chl. *a*	PO_4^{3-}	NO_3^-
Upper Loire Station 4	80-89	9	183	1.4	+2	+16	0.0	74	87	77
	90-01	12	169	1.8	0	-16	0.0			
	02-12	11	88	1.4	-1	-3	0.0			
Middle Loire Station 18	80-89	47	121	1.8	+3	-6	0.0	82	91	53
	90-01	83	58	1.9	-5	-3	+0.1			
	02-12	17	26	2.2	-5	-2	0.0			
Lower Loire Station 21	80-89	50	79	2.5	+5	+12	+0.3	83	76	71
	90-01	58	89	3.3	-9	-3	+0.1			
	02-12	14	37	2.6	-4	-5	-0.1			

Table 4. Seasonality analysis and changes since 1980 at three stations representative of the Upper, Middle and Lower Loire.

		Seasonal amplitude			Significance (%)			Amplitude trend		
	years	Chl. *a* µg L^{-1}	PO$_4^{3-}$ µg P L^{-1}	NO$_3^{-}$ mg N L^{-1}	Chl. *a*	PO$_4^{3-}$	NO$_3^{-}$	Chl. *a* µg L^{-1} y^{-1}	PO$_4^{3-}$ µg P L^{-1} y^{-1}	NO$_3^{-}$ mg N L^{-1} y^{-1}
Upper Loire Station 4	80-89	61	101	0.7	41	16	25	0.0	-0.2	+0.1
	90-01	114	107	0.9	31	33	38	-0.2	+0.7	0.0
	02-12	17	26	2.2	24	41	42	-1.2	+0.5	+0.1
Middle Loire Station 18	80-89	182	123	2.2	61	44	80	+7.8	-5.6	+0.1
	90-01	152	71	2.8	64	43	85	-9.8	-3.7	0.0
	02-12	57	38	2.1	55	47	84	-8.1	-2.1	0.0
Lower Loire Station 21	80-89	184	125	3.2	68	46	78	-2.7	+2.0	+0.4
	90-01	82	120	5.5	52	62	81	-9.6	-1.6	-0.2
	02-12	53	65	3.2	62	51	85	-1.1	-10.4	+0.1

Table 5. Annual medians, DHR-model seasonal amplitudes and nutrients flux contributions of the main tributaries.

		Annual median			Seasonal amplitude			Nutrient flux contribution	
		Chl. *a* µg L^{-1}	PO_4^{3-} µg P L^{-1}	NO_3^- mg N L^{-1}	Chl. *a* µg L^{-1}	PO_4^{3-} µg P L^{-1}	NO_3^- mg N L^{-1}	PO_4^{3-}	NO_3^-
	1980-89	20	124	1.4	85	180	1.7	54%	47%
A	1990-01	23	83	1.5	134	112	2.1	44%	43%
	2002-12	17	51	1.7	83	74	2.3	42%	36%
	1980-89	44	108	3.6	147	190	4.3	17%	32%
B	1990-01	61	79	4.1	197	181	5.9	31%	37%
	2002-12	13	45	4.7	57	57	3.9	33%	33%
	1980-89	28	166	4.0	104	234	3.7	-	-
C	1990-01	44	90	4.2	109	144	5.0	-	-
	2002-12	16	59	4.6	37	79	4.2	-	-
	1980-89	43	126	3.0	102	137	2.2	-	-
D	1990-01	50	68	2.7	107	87	2.8	-	-
	2002-12	6	30	2.8	18	36	2.5	27%	35%
	1980-89	50	191	4.0	142	326	4.2	38%	24%
E	1990-01	62	181	4.4	132	236	8.1	33%	23%
	2002-12	21	73	4.1	51	102	5.6	35%	27%

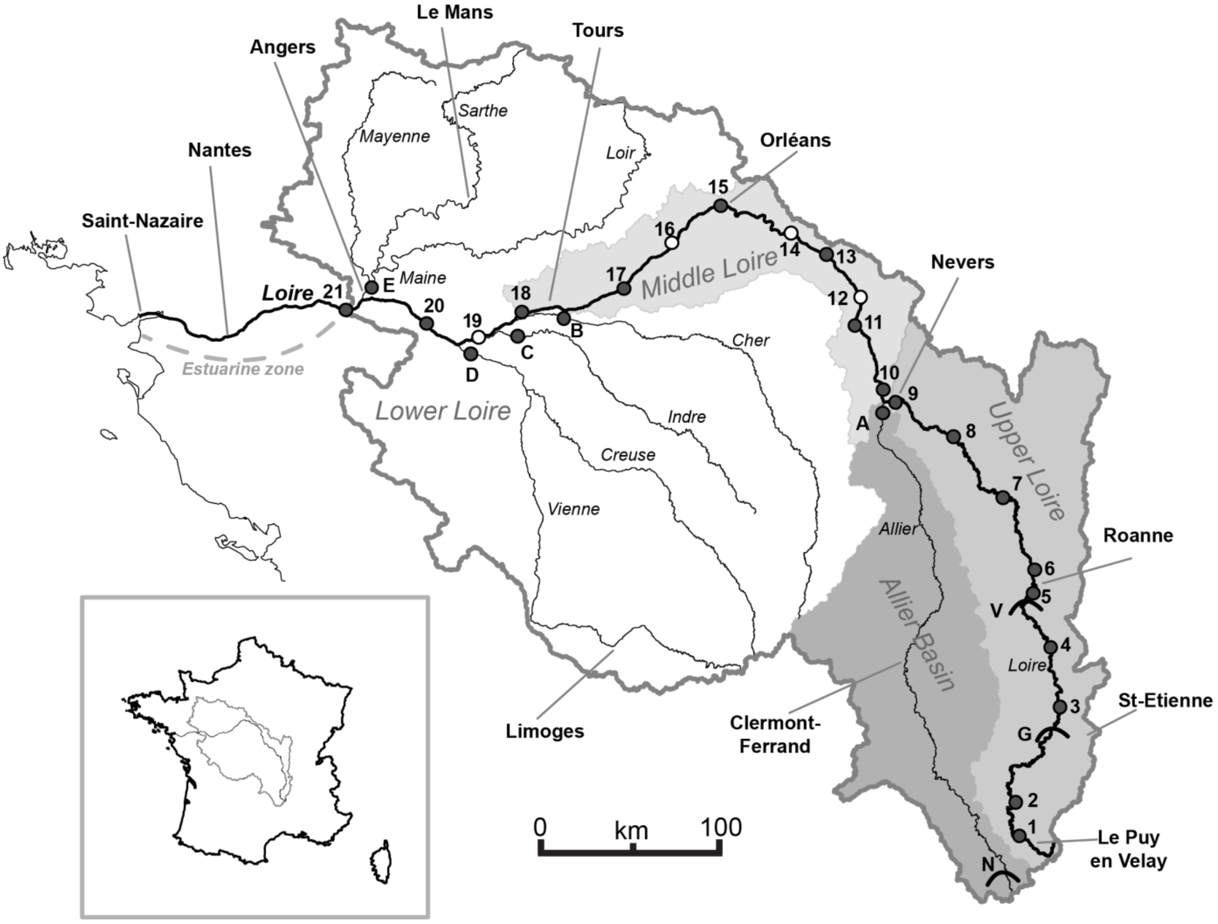

Figure 1. Loire River Basin. Dark circles: sites of regulatory surveys. White circles: Nuclear Power Plants sampling sites. A to E: regulatory survey stations at tributaries outlets. G, V, N: three major dams, respectively Grangent, Villerest and Naussac. The estuarine influence begins downstream of station 21.

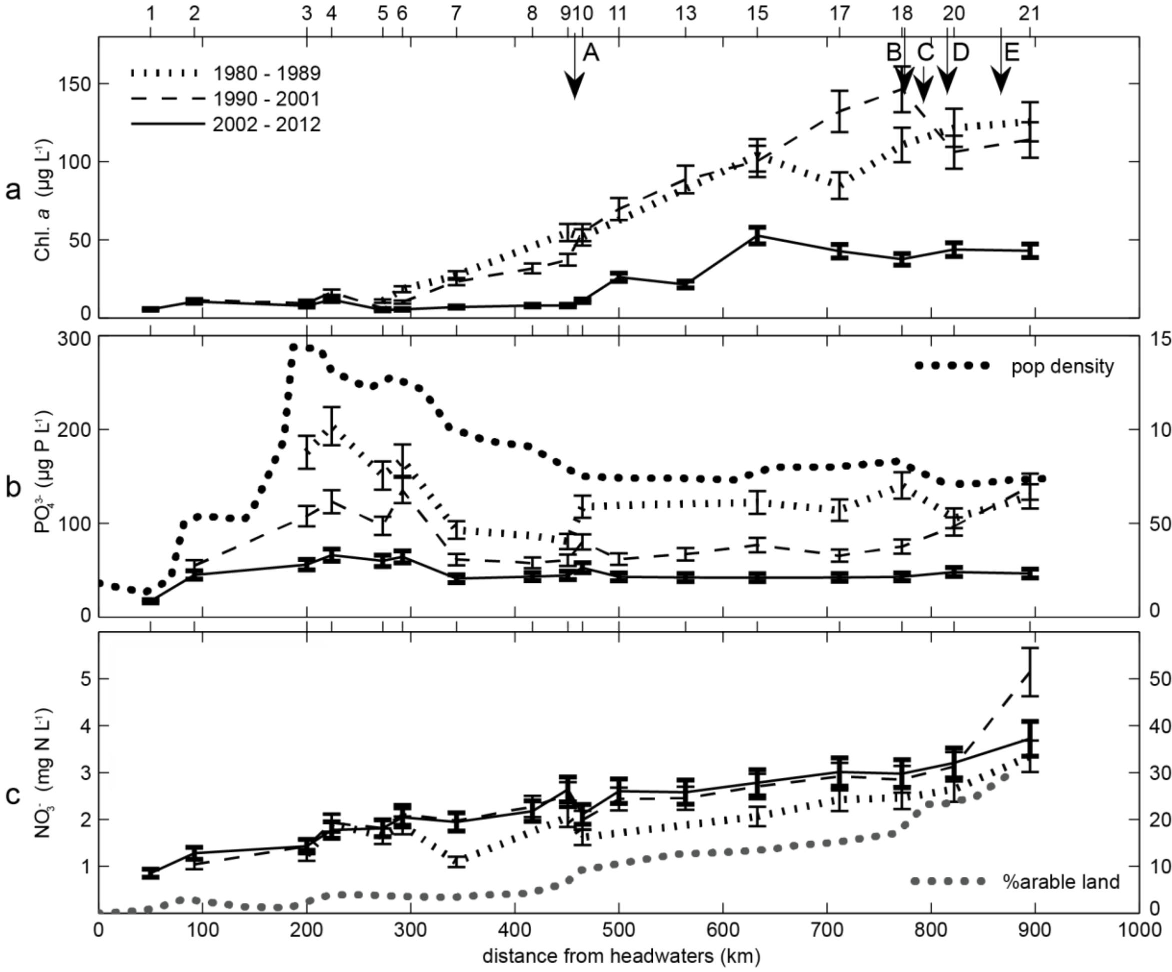

Figure 2. Longitudinal profiles of *summer* median Chl. *a* (a), *winter* median PO_4^{3-} (b) and NO_3^- (c). Averages for three periods, in relation to % arable land (2006) and population density (2008) tested as eutrophication control variables. Uncertainty bars are due to sampling frequency. Arrows and capital letters (A to E) represent confluences with major tributaries (Fig. 1).

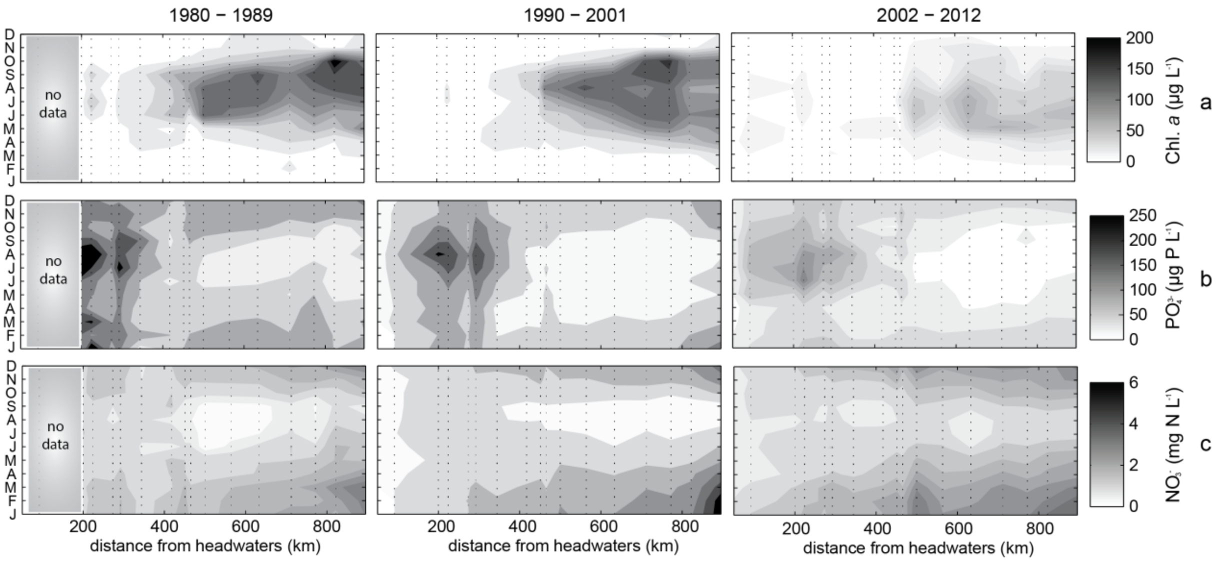

Figure 3. Spatio-temporal diagrams of monthly median levels of Chl. *a* (a), PO_4^{3-} (b) and NO_3^- (c) during three periods along a longitudinal profile. Dotted vertical lines correspond to the monitoring stations (Fig.1).

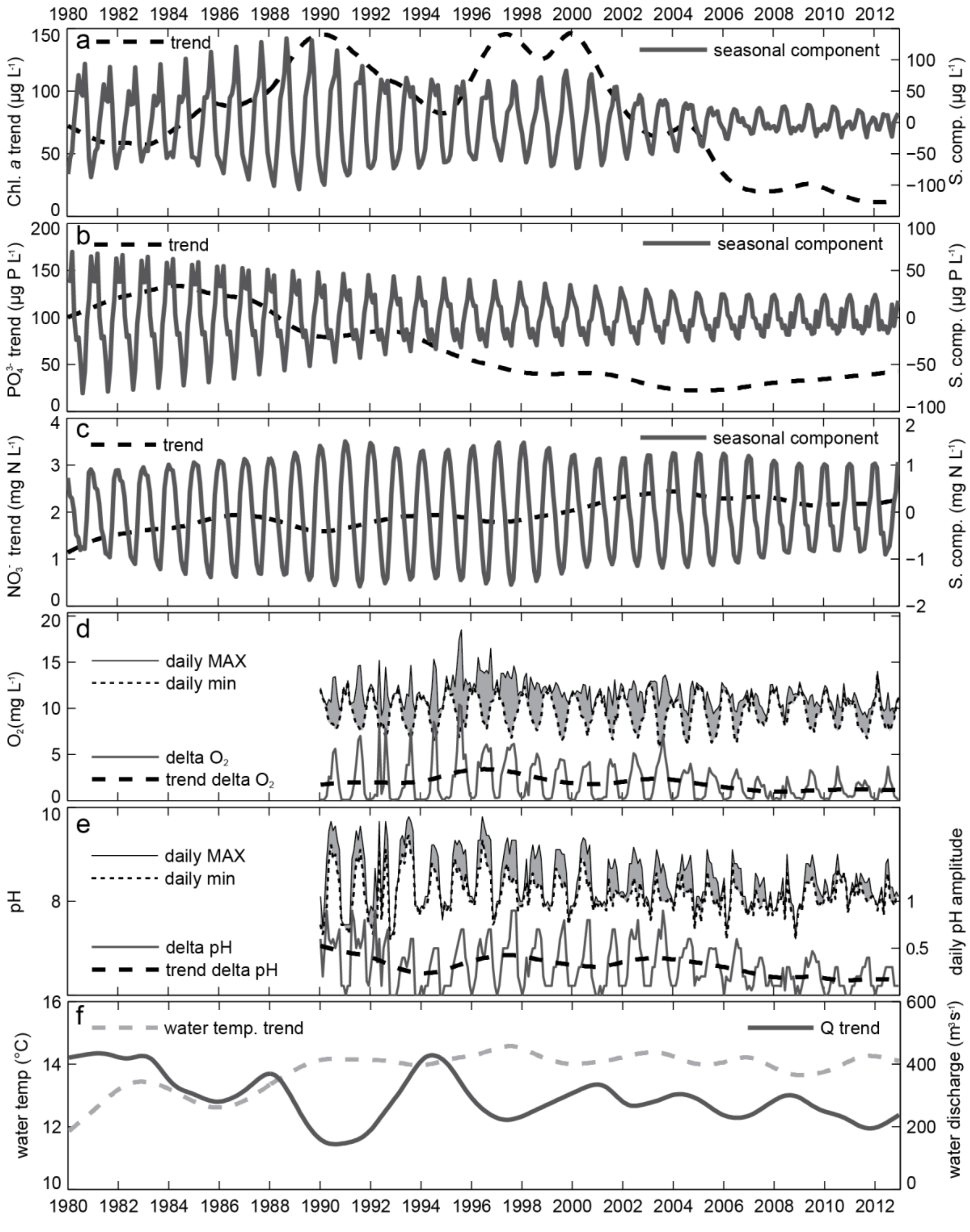

Figure 4. Trends and seasonal components at station 18 of Chl. *a* (a), phosphate (b) and nitrate (c). Corresponding time series of monthly medians of both daily min and max of O_2 (d) and pH (e) and their amplitude dynamics at station 19 (i.e. *delta* O_2 and *delta pH*). Water temperature trend at station 19 (f) and discharge trend at station 15 since 1980.

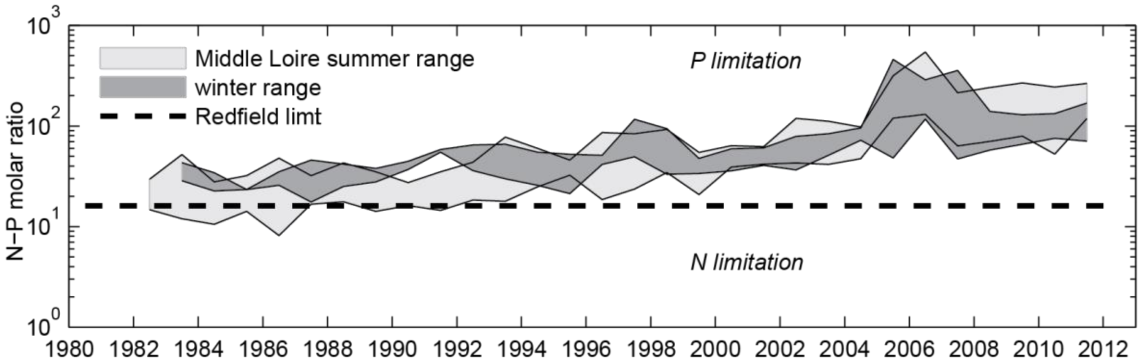

Figure 5. Variations of total nitrogen over total phosphorus molar ratios ranges during *summer* and *winter* in the Middle Loire (station 18) since 1980 and compared to the Redfield limit (dotted line). Each patch is composed at the bottom by the percentile 10% of the recorded data and percentile 90% at the top, and y-axis is logarithmic.