Space and time variations of watershed N and P budgets and their relationships with reactive N and P loadings in a heavily impacted river basin (Po river, Northern Italy)

Pierluigi Viaroli[1,2]*, Elisa Soana[3], Silvano Pecora[4], Alex Laini[1], Mariachiara Naldi[1,2], Elisa Anna Fano[2,3], Daniele Nizzoli[1]

[1]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy

[2] CoNISMa, piazzale Flaminio 9, 00196 Roma, Italy

[3]Department of Life Sciences and Biotechnology, University of Ferrara, via L. Borsari, 46 44121 Ferrara Italy

[4]ARPAE SIMC – Hydrology Unit, Via Garibaldi, 75, 43121 Parma

*corresponding author: pierluigi.viaroli@unipr.it

ABSTRACT

The aim of the present study is to analyze relationships between land uses and anthropogenic pressures, and nutrient loadings in the Po river basin, the largest hydrographic system in Italy, together with the changes they have undergone in the last half century. Four main points are addressed: 1) spatial distribution and time evolution of land uses and associated N and P budgets; 2) long-term trajectories of the reactive N and P loadings exported from the Po river; 3) relationships between budgets and loadings; 4) brief review of relationships between N and P loadings and eutrophication in the Northern Adriatic Sea.

Net Anthropogenic N (NANI) and P (NAPI) inputs, and N and P surpluses in the cropland between 1960 and 2010 were calculated. The annual loadings of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) exported by the river were calculated for the whole 1968-2016 period.

N and P loadings increased from the 1960s to the 1980s, as NAPI and NANI and N and P surpluses increased. Thereafter SRP declined, while DIN remained steadily high, resulting in a notable increase of the N:P molar ratio from 47 to 100. In the same period, the Po river watershed underwent a trajectory from net autotrophy to net heterotrophy, which reflected its specialization toward livestock farming.

This study also demonstrates that in a relatively short time, i.e. almost one decade, N and P sources were relocated within the watershed, due to discordant environmental policies and mismanagement on the local scale, with frequent episodes of heavy pollution. This poses key questions about the spatial scale on which problems have to be dealt with in order to harmonize policies, set sustainable management goals, restore river basins and, ultimately, protect the adjacent coastal seas from eutrophication.

Key words: NANI, NAPI, N surplus, P surplus, dissolved inorganic nitrogen, soluble reactive phosphorus

1. INTRODUCTION

In the last century, the global biogeochemical cycles of nitrogen (N) and phosphorus (P) have been manipulated with beneficial effects for the human society, especially due to the supply of food and other provisional goods (Galloway et al., 2003; van Dijk et al., 2016). However, the exploitation of N and P for various uses has led to a considerable increase in the soluble reactive forms of N and P in surface and ground waters, with a cascade effect throughout the river basin – coastal seas continuum (Hong et al., 2017; Meybeck and Vörösmarty, 2005; Romero et al., 2013).

Water fluxes and nutrient loadings in the most developed countries have changed greatly in the last century, due to the increasing impacts of human activities (Meybeck et al. 2016; Vörösmarty et al., 2015). From the 1950s onwards, the increasing exploitation of reactive nitrogen and phosphates has led to high nutrient pollution with a surge in diffuse eutrophication phenomena and groundwater contamination. In the most recent decades, the implementation of environmental policies, wastewater treatment plants and both preventive and restoration measures have mitigated pollution and improved water quality, although in many cases they have not yielded the expected goals (Jarvie et al., 2013; Glibert 2017).

Both N and P play a major role in the surge and evolution of eutrophication. Processes occurring in rivers can evolve in time, with long lag periods often followed by sudden and exponential phases, with cascade effects on the receiving inland and coastal marine waters (Meybeck and Vörösmarty 2005). Moreover, conceptual models of eutrophication have been built on a single nutrient, generally P in inland waters and N in coastal marine waters. Thus, ecological stoichiometry, interactions and biogeochemical feedbacks among nutrients have often been neglected, thereby ignoring the complexity of eutrophication (Duarte et al., 2009; Howarth et al. 2011; Glibert, 2017).

Studies mainly based on mass balances in several European watersheds have demonstrated that ~60% of reactive nitrogen derives from synthetic fertilizers employed in agriculture, and ~20% from feed and food imports into the watershed, which are linked to agriculture itself (Billen et al., 2011). Formerly, the P pollution was the result of point sources, especially large urban areas, which decreased due to the implementation of wastewater treatment plants and the reduction in polyphosphates in detergents (van Dijk et al., 2016). Currently, the major sources of reactive phosphorus are from agriculture and livestock, i.e. from synthetic fertilizers and manure (Hong et al., 2012; Kronvang et al., 2007).

Nutrient pollution and stoichiometry and related eutrophication processes differ greatly among regional watersheds, e.g. due to climate conditions and land use, and their evolution in time (Romero et al., 2013). Recent studies have identified major hot spots of N and P pollution in Europe, among which the main cropland and livestock districts (Billen et al., 2011; van Dijk et al., 2016). In addition to land uses, the alteration of hydrological regime, river morphology and lateral connectivity, and the increased longitudinal fragmentation have further amplified the instability of the biogeochemical processes and the contamination extent, especially from the nitrogen sources (Pinay et al., 2002). In particular, hydro-morphological alterations have hampered river metabolism, amplifying the nutrient transport and delivery to coastal seas, but also triggering eutrophication in rivers themselves (Dodds, 2006).

In this context, key questions are to what extent changes in land use and related anthropogenic pressures and governance influence N and P availability in watersheds and their capacity to process, transform and retain the loadings, and what effects N and P excess has on water quality and aquatic ecosystem functioning (Billen et al 2013; Romero et al., 2013; Withers and Jarvie, 2008).

Among others, one of the most impacted areas in Europe is the Po river basin, in Northern Italy (Cozzi and Giani, 2011; Ludwig et al., 2009; Romero et al., 2013; Viaroli et al., 2013; 2015).

In-depth studies on water quality in the Po river were carried out between the late 1960s and the 1990s, when along the Northern Adriatic coast of Italy a dramatic surge in phytoplankton and mucilage blooms occurred, often followed by benthic anoxia, and mass kill of benthic and fish fauna (Vollenweider et al., 1992). Relationships between water quality deterioration and the main anthropogenic activities in the watershed were identified and addressed (Marchetti, 1992;

Marchetti, 1993; Marchetti et al., 1989; Provini and Binelli, 2006; Provini et al., 1992). These studies led to important legislative acts, such as the ones aimed at reducing phosphates in detergents and improving the urban wastewater treatment plants, which were followed by an appreciable reduction in phosphorus loadings (Palmeri et al., 2005). The measures for controlling and reducing the contribution of the widespread agricultural and livestock sources were much less effective, especially for nitrogen (de Wit and Bendoricchio, 2001; Palmeri et al., 2005; Pirrone et al., 2005). The scenarios analyses by Palmeri et al. (2005) showed how the measures introduced by the nitrate (91/676/EEC) and urban waste water treatment plants (91/271/EEC) directives were not sufficient to obtain the expected reduction in N and P loads in the Po river basin. More recent studies have identified hot spots of pollution in the watershed, highlighting how N and P sources are affected by great patchiness, which is ultimately linked to land uses (Bartoli et al., 2012; Delconte et al., 2014; Soana et al., 2011; Viaroli et al., 2013, 2015). Cozzi and Giani (2011) stressed the impact of the Po river on the Northern Adriatic Sea, with the river accounting for almost 65% of freshwater discharge and nutrient loadings.

This study aims to analyze relationships among land uses and anthropogenic pressures, N and P budgets and reactive N and P loadings in the Po river basin, and how they have changed in the last half century, by specifically addressing the following points:

1- spatial distribution and time evolution of land uses and associated N and P budgets;

2- long-term trajectories of the reactive N and P loadings exported from the Po river;

3- relationships between N and P budgets and loadings.

Finally, the evolution of main impacts of nutrient loadings on the North Adriatic Sea will be briefly reviewed.

2. MATERIALS AND METHODS

2.1. Study area

The Po river, one of the major rivers in the Mediterranean region, is 652 km long (Fig. 1). The watershed is 74,000 km^2, of which 71,000 km^2 (~46,000 km^2 as lowland) are in Italy. Its surface is almost one fourth of the surface of Italy, where ~40% of the Italian GDP is produced (Viaroli et al., 2010). Agriculture interferes heavily with the hydrological cycle, because ~$17x10^9$ m^3 yr^{-1} of water are used for irrigation, which represents approximately 50% of the average annual discharge of the Po river and is almost equivalent to the summer water flux in the watershed (Montanari, 2012). The south side of the river is affected by water scarcity, and streams and rivers have an extremely variable flow regime. The north side of the river has a great number of both high altitude small lakes and reservoirs, and four large deep subalpine lakes fed by Alpine glaciers (from West to East: Maggiore, Como, Iseo and Garda). Overall, the four lakes account for ~70% of the water volume of surface freshwater in Italy and feed the main tributaries of the Po river (from West to East: Ticino, Adda, Oglio and Mincio rivers), which make up about 50% of its total water discharge.

In this study land uses and N and P budgets were estimated for the watershed upstream of Pontelagoscuro (PLS in Fig. 1), where the closing station of the river basin is located. The territory of the provinces of Ferrara and Rovigo was excluded from N and P budget computation, because it geographically belongs to the Po river delta, downstream the closing station of the watershed. A fraction of the Po river basin, located in Switzerland, and to a much lesser extent in France, was also unaccounted, it representing ~4% of the total watershed surface, for which only recent data were available.

2.2.Temporal and spatial evolution of land uses

We analyzed the evolution of land use and anthropogenic pressures in the Po river watershed at 10-year intervals from 1961 to 2010 by collecting data on total agricultural land (AL), AL surface areas occupied by different crop types and respective production (six main categories: cereals, industrial crops, vegetables, temporary meadows, permanent meadow, permanent woody crops,), numbers of farmed animals (seven main categories: cattle, pigs, horses, goats, sheep, poultry, rabbits), synthetic fertilizer application, and human population.

Statistical data on agricultural activities were extracted, at provincial resolution, from the databases of the National Institute of Statistics (ISTAT), the main supplier of official statistical information in Italy, collected for the General Census of Agriculture and the Annals of Agrarian Statistics (ISTAT, 1961, 1970, 1982, 1990, 2000, 2010). Census databases provided data for livestock numbers, while the Annals of Agrarian Statistics provided data for agricultural areas, crop production and fertilizer application. A total of 32 provinces (areal range from 405 to 6,896 km^2), which are either totally or partially included within the Po river watershed boundary, were considered. Census data were collected by searching the ISTAT online databases (years 1982, 1990, 2000 and 2010, http://dati-censimentoagricoltura.istat.it) and consulting the census printed volumes for years 1961 and 1970. On-line access to the Annals of Agrarian Statistics, published yearly by ISTAT at the provincial level for the whole national territory, was possible for year 2010 only (http://agri.istat.it/), while for previous years only printed volumes were available. Older data (1961 and 1970) were less detailed than those of the following decades. Therefore, they were firstly reorganized in order to homogenize the historical series and the N and P budgets obtained from them.

Census data on population were extracted from the ISTAT Census of Population and Housing (years 1961, 1971, 1981, 1991, 2001, 2011, http://dati-censimentopopolazione.istat.it). Province-level data were then aggregated at the catchment scale by weighting each province based on the percentage of area included in the watershed (Han and Allan, 2008) with QGIS 2.18 software (QGIS Development Team, 2017). Shape files of the Po River watershed (Po River Basin Authority, WebGIS application, http://www.adbpo.gov.it/) and of administrative boundaries (ISTAT, http://www.istat.it/it/archivio/104317) represented the cartographical material used in this study. Even though the censuses were performed within the third year of every decade, we will refer to them as the first year of that decade (for example, the year 1982 census is referred to as 1980).

2.3. Nitrogen and phosphorus mass balances at watershed and agricultural land scale

The effect of land use changes on N and P cycles was evaluated by computing nutrients budgets at the watershed and AL scale. N and P budgets of the whole catchment were computed at 10-year time intervals from 1960 to 2010 with the Net Anthropogenic Nitrogen Input (NANI) and Net Anthropogenic Phosphorus Input (NAPI) accounting approach (Hong et al., 2012; Howarth et al., 1996; Russell et al., 2008). These budgets represent the new N and P entering the watershed as a consequence of anthropogenic activities and were calculated as follows:

$$NANI = N_{Dep} + N_{Fert} + N_{Fix} + N_{Trade} \qquad (1)$$

$$NAPI = P_{Dep} + P_{Fert} + P_{Det} + P_{Trade} \qquad (2)$$

where

N_{Dep} and P_{Dep} = atmospheric N and P deposition on total watershed area

N_{Fert} and P_{Fert} = synthetic N and P fertilizer applied to AL

N_{Fix} = agricultural N_2 fixation associated with N-fixing crops

$P_{Det\ =}$ non-food use of P by human (detergents)

N_{Trade} and P_{Trade} = net exchange of N and P as food and feed.

In addition to NANI and NAPI, we also calculated detailed N and P budgets for AL using a method previously applied to some of the sub-basins of the Po River system (Castaldelli et al., 2013; Soana et al., 2011) and to other Italian rivers (De Girolamo et al., 2017). Nutrient budgets were determined by computing the differences between N and P input and output across the productive agricultural land in the catchment. These differences represent the excess of N and P which is not used by crops and remains in the soil (surplus), i.e. the nutrient use efficiency in the agricultural system. They are net of losses to atmosphere. For this reason, they are also an indicator of the potential pollution risk for surface and ground waters.

The AL nutrient budgets were calculated as follows:

$$\text{AL N budget} = N_{Dep(AL)} + N_{Fert} + N_{Fix} + N_{Man} - N_{Harv} - N_{Vol} - N_{Den} \quad (3)$$

$$\text{AL P budget} = P_{Dep(AL)} + P_{Fert} + P_{Man} - P_{Harv} \quad (4)$$

where:

$N_{Dep\ (AL)}$ and $P_{Dep\ (AL)}$= atmospheric N and P deposition on AL

N_{Fert} and P_{Fert} = synthetic N and P fertilizer applied to AL

N_{Fix} = agricultural N_2 fixation associated with N-fixing crops

N_{Man} and P_{Man} = N and P in livestock manure applied to AL

N_{Harv} and P_{Harv} = N and P exported from agricultural soils with crop harvest

N_{Vol} = NH_3 volatilization

$N_{Den\ =}$ denitrification in AL

A detailed description of data sources, computational methods and uncertainty assessment of NANI, NAPI and N and P budgets of AL is reported in the supplementary materials.

2.4. River discharge and reactive N and P loadings

The data on river discharge were obtained from Hydrological Annals-Part 2, published by the Environmental Agency (ARPAE) of the Emilia-Romagna region (https://www.arpae.it/documenti.asp?parolachiave=sim_annali&cerca=si&idlivello=64). The data of reactive N and P loadings were obtained from a monitoring activity for 2015-2016 and from different sources for 1968-2014. Since total nitrogen and total phosphorus concentrations were available only starting from 1977 with frequent gaps, loadings were estimated for dissolved inorganic nitrogen (DIN=N-NO_3^-+N-NO_2^-+N-NH_4^+) and dissolved phosphorus reactive to molybdate (SRP=soluble reactive phosphorus) only. Water sampling was performed at Pontelagoscuro station, at the closing section of the Po river watershed (Fig. 1). Sixty-six water samples were collected in 2015 with frequency ranging from daily to fortnightly, depending on flow rates. In 2016 the sampling was nearly fortnightly, for a total 24 samples.

On each date, triplicate water samples were collected at 0.5-1.5 m depth. An aliquot of each sample was filtered immediately (Whatman GF/F), refrigerated, and brought to the laboratory in less than 2 hours. Water samples were then analyzed for ammonium (Koroleff, 1970), nitrite and nitrate (APHA, 1998), and soluble reactive phosphorus (Valderrama, 1981).

The data for 1992-1998 (fortnightly) and 2008-2014 (monthly) were provided by ARPAE of Emilia-Romagna, whilst for 1999-2008 the daily to fortnightly data from a previous project (Naldi et al., 2010) were reanalyzed and used.

For 1968-1991 loadings were estimated with load-flow relationships (Provini et al., 1992), and compared for consistency with data from Marchetti et al. (1989), Crosa and Marchetti (1993) and Provini and Binelli (2006).

All the sampling techniques and analytical methods used in the different periods were also checked for consistency (see also Provini and Binelli, 2006).

Annual loadings were calculated as the product of the discharge weighted mean concentration by the mean annual discharge (Quilbè et al., 2006) as follows:

$$L = k\frac{\sum_{i=1}^{n} CiQi}{\sum_{i=1}^{n} Qi} Q_m \quad (5)$$

where:

L= annual loading (t yr^{-1})

Ci = concentration at day i (g m^{-3})

Qi = mean daily discharge at day i (m^3 s^{-1})

Qm = mean annual discharge (m^3 s^{-1})

k = factor ($31.53*10^6$) to calculate L

N and P retention (R, %) in the watershed was estimated for each decade as:

$$R = \frac{B-L}{B} * 100 \quad (6)$$

where:

B = average N or P budget in terms of NANI, NAPI, N-surplus and P-surplus (kt yr^{-1}) estimated as the arithmetic mean of the budgets of two subsequent decades (e.g. 1960 and 1970; 1970 and 1980, etc.)

L = average N or P loading at the closing section of the watershed (kt yr^{-1}) estimated as arithmetic mean of the loading data of each decade.

2.5. Statistics

A change-point analysis was performed in order to find the location of change points in the time series of DIN and SRP loadings, and molar DIN:SRP ratio. The binary segmentation algorithm (Edwards and Cavalli-Sforza, 1965) was used to this purpose and the results were visually checked to ascertain their reliability. We also identified periods of change in the time series studied by using the approach proposed by Monteith et al. (2014). Briefly, a generalized additive model (GAM) was first fitted on the target time series, then periods of change were detected on the trend identified by GAM where the rate of change of the trend was significantly different from 0 (*inflection point analysis*).

All statistical analyses were performed using the statistical computing software R (R Core Team, 2017) with the packages *changepoint* (Killick and Eckley, 2014) and *mgcv* (Wood, 2017). In addition, the Pearson correlation analysis was performed on the main land use and livestock data with R.

3. RESULTS

3.1. Human population and relevant changes in land use in the Po river watershed in the last half century

The main changes in land uses in the Po river watershed over the last half century are summarized in Table 1 and Figure 2.

From 1960 to 2010 the total agricultural land (AL) decreased progressively from 62% to 43% of the total watershed surface, with a net loss of ~1.3×10^6 ha. The AL loss was accompanied by relevant changes in crop typologies, especially by a net loss of ~1.1×10^6 ha of both permanent and temporary meadows. The total AL and meadows surface areas were significantly correlated (Table 2), indicating that the AL loss was mainly due to the disappearance of grass coverage. Until the 1980s, alfalfa meadows were widespread over the central plain, where the typical dairy production of Parmesan and Grana cheeses took place (Fig. 2A). In the final two decades, alfalfa crops shrank to the Parmesan cheese district only, where fresh grass and hay are mandatory for feeding dairy cows, and other fodders (silage, maize, etc.) are forbidden.

Cereal crop areas (1.11×10^6 - 1.28×10^6 ha) have remained substantially stable over time, although the breakdown in the various crop typologies has changed markedly from 1982 to date, with a 58% decrease in winter wheat and the concurrent increase in areas planted with maize (+ 47%) and rice (+ 102%). Up to the 1980s, winter wheat was a common widespread crop which was alternated with alfalfa and was associated with traditional cattle breeding (Fig. 2B). Maize was typically cultivated north of the Po river due to the large water availability and was a subsidiary crop in the rest of the basin. Since 2000 it has become the dominant crop in the irrigated lowland occupying up to 40% of agricultural land (Fig. 2C). Here, an intensive monoculture is currently performed for non-food production too, e.g. for bioenergy and bioplastic production. Rice, a highly demanding culture, expanded along with maize mainly between the regions of Piedmont and Lombardy, and along the Po river. The total area occupied by rice and maize was inversely correlated to both wheat and meadows areas (Table 2).

In the Po river basin, cattle were a common livestock, of which 33-45% was devoted to the typical and renowned dairy production of Grana and Parmesan cheeses. However, since the 1980s the total cattle stock has declined progressively with a net loss of ~1.37×10^6 heads, ~31% (Table 1, Fig.

2D). The decline in cattle correlates with the decrease in meadows, for both total cattle stock and dairy cattle only (Table 2). The cattle stock is also inversely related to both maize+rice areas and pig stock, the cattle loss coinciding with an abrupt rapid growth in the pig population, from ~$1.2x10^6$ heads in 1960 to ~$5.2x10^6$ heads in 1980, with a ~300% net increase. The cattle to pigs ratio as Livestock Units (LSU), decreased from 11.1 in 1960 to 1.3 from 2000 onwards, documenting the growing impact of pigs (Table 1). The temporal trajectory of the livestock density, pigs especially, has been exacerbated by its spatial distribution (Fig. 2E). In the last 20 years both cattle and pig densities have risen in a relatively small area South-East of Milan while they have decreased in most of the basin.

The human population in the watershed increased from $16.2x10^6$ (1960) to $17.3x10^6$ (1980), and has been almost steady in the following decades (Table 1, Fig. 2F). More than 50% inhabitants lived in the Lombardy region, which accounts for ~35% of the watershed surface. In 2010, the average density was 243 inhabitants km^{-2}, with great differences between the Alpine and Apennine areas (<30 inhabitants km^{-2}) and the main metropolitan areas (>2,000 inhabitants km^{-2}), i.e. Milan and its hinterland (Fig. 2F).

3.2. NANI and NAPI in the watershed, and N and P budgets in its agricultural part

Between the 1970s and the 1980s, NANI in the Po river basin abruptly increased from 330±57 to 739±72 kt yr^{-1}, mainly due to synthetic fertilizers and feed import (Fig. 3A, Table 5S). Biological fixation, which was the main N source until the 1980s, almost halved in the next three decades. The watershed was net autotrophic until the 1970s, then it turned completely heterotrophic as autotrophic organic N production within the catchment was not sufficient to meet the N needs of the livestock population. N was thus imported to the watershed as animal feed. By contrast, the Po basin maintained a net food export which was indeed <10% of the total feed+food import.

NAPI followed a similar trend, with a steep increment from 43±5 to 111±8 kt yr^{-1} from 1960 to 1980, which was supported by feed import and synthetic fertilizers (Fig. 3B, Table 6S). The contribution of detergents and atmospheric deposition to NAPI was one order of magnitude smaller, but wastewaters with P from detergents were delivered directly into surface waters. Phosphorus was exported steadily from the watershed as food products, but the export was comparatively much lower than feed import.

The N surplus in the cropland was statistically correlated to NANI (Table 2) and increased until the 1980s up to ~300 kt yr^{-1}, more than twice the surplus in 1960 (Fig. 3A, Table 7S). Manure and synthetic fertilizers equally contributed to the increase, N fixation being steadily constant until the 1980s, and decreasing thereafter. The N outputs were mainly due to crop harvesting, and to a lesser extent, to denitrification to N_2.

The P surplus in the agricultural land was correlated to NAPI (Table 2). It increased nearly five-fold from 1960 to 1980 and peaked in 1990, halving thereafter (Fig. 3B, Table 8S). The increment of P surplus was mainly due to manure, while the quantity of synthetic fertilizers was almost constant until 1990 and has decreased in the last two decades. The P output by crop harvesting was steadily constant over time.

The spatial distribution of NANI (Fig. 4A) and NAPI (Fig. 4B) has highlighted both great patchiness and temporal trends in the N and P inputs to the watershed. In the 1960s and 1970s, the mid-western part of the basin, especially the mountain areas showed approximately NANI<10,000 kg km^{-2} yr^{-1} and NAPI<1,500 kg km^{-2} yr^{-1}. Only in the mid-eastern provinces and in the Milan area NANI reached 45,000 kg km^{-2} yr^{-1} from 1980 to 1990, and NAPI up to ~5,000 kg km^{-2} yr^{-1}. This pattern was consistent with the surpluses of N and P in agricultural land. The average N surplus was < 20 kg ha^{-1} yr^{-1} until 1980. Afterwards, diffuse pollution impacted the central and eastern parts of the basin, with N surplus reaching 160-185 kg ha^{-1} yr^{-1} (Fig. 4C). The P surplus showed a similar heterogeneous distribution across the basin (Fig 4D). In the 1960s and 1970s, wide areas were P deficient (<0 kg ha^{-1} yr^{-1}), especially in the alpine arc. From 1980 onwards, P surplus increased in the eastern part of the basin, especially north of the Po river. Thereafter, the maximum P surplus, up to 50 kg ha^{-1} yr^{-1}, was reached in the same zone with the highest N surplus. However, large areas in the basin maintained a condition of P deficit or very low surplus < 5 kg ha^{-1} yr^{-1}.

3.3. Long term trajectories of river discharge and DIN and SRP exported from the Po river

From 1968 to 2016, the mean annual discharge (Q) of the Po river at the closing station of the watershed (Pontelagoscuro, PLS in Fig. 1) was subject to wide inter-annual variability between 821 and 2,630 m^3 s^{-1}, with average 1,530 m^3 s^{-1} and standard deviation 365 m^3 s^{-1}. Wet years with 1,900<Q<2,700 m^3 s^{-1}, e.g. 1977, 1996, 2000 and 2014, alternated with very dry periods with Q<1,000 m^3 s^{-1}, e.g. in 2003-2007 (Fig. 5).

At PLS, the dissolved inorganic nitrogen (DIN) loading, consisting of nitrate for more than 75%, grew suddenly from ~50,000 to ~100,000 t N yr^{-1} between 1970 and 1980, in parallel with NAPI and N surplus increases (Fig. 3A). Afterwards it remained steadily elevated with wide oscillations from low values in dry years and peaks in wet years.

The soluble reactive phosphorus (SRP) experienced a dramatic surge from the late 1960s to the mid 1970s, from less than ~2,000 t P yr^{-1} up to over ~5,000 t P yr^{-1}, as NAPI and P surplus increased dramatically (Figs. 3B and 5B). Since late 1980s, SRP decreased progressively, reaching values in the range 1,500-2,000 t yr^{-1} in the dry 2003 and 2005-2007, which were close to that measured in the dry 1970.

Until 1990, the atomic DIN:SRP ratio was relatively constant, then increased step-by step reaching the highest values in the last decade (Fig. 5C). Over time, the ratio deviated many-fold from the Redfield ratio (N: P = 16: 1), indicating an excess of dissolved inorganic nitrogen relative to soluble reactive phosphorus.

The time changes of DIN and SRP fluxes and DIN:SRP ratio were assessed with a Generalized Additive Model, through an inflection point analysis. Changes in DIN loading were statistically significant from 1968 to 1985 (Fig. 5D). SRP loading underwent a significant increase from 1968 to 1978, while it decreased from 1982-1989 and again in 1998-2008 (Fig. 5E). The molar DIN:SRP ratio increased significantly from 1985-2001 (Fig. 5F). The inflection point analysis outcomes were consistent with the change point analysis, which allowed the calculation of the mean DIN and SRP loadings and their ratio of each phase, documenting their time trajectories (Table 3; Fig. 1S).

Responses of riverine fluxes in DIN to time changes in NANI, and SRP to time changes in NAPI followed opposite trajectories (Fig. 6). Responses of DIN to N surplus and SRP to P surplus were almost identical to NANI and NAPI, respectively. Since NANI and NAPI and related N and P surpluses in cropland were significantly correlated, the latter are not displayed here.

Initially, DIN loadings increased until late 1970s in response to NANI (Fig. 3A). Once NANI started to decrease, DIN fluxes remained persistently high without showing recovery (Fig. 6A). By contrast, SRP loadings increased until late 1980s as a direct response to NAPI and P surplus increment (Fig. 3B). Afterwards, SRP loadings decreased as NAPI was reduced, but with P surplus still increasing. Overall, SRP loadings made a clockwise hysteresis in response to both NAPI (Fig. 6B) and P surplus (data not shown) recovering in 2010 riverine fluxes similar to those measured in the late 1960s. This relevant reduction of SRP loadings was achieved with a 29% decrease in NAPI and 49% P surplus.

4. DISCUSSION

4.1. Anthropogenic inputs and surplus of N and P in the Po river watershed

Anthropogenic N and P inputs to the Po river watershed and the resulting N and P surpluses in the agricultural land were high and underwent temporal and spatial variations related to changes in land use and farming practices. Overall, the comparison of the Po river basin with other watersheds worldwide highlighted how its N and P budgets occupied the upper limit of the range (Table 4). In 2010, the average values were similar to those of European countries, North America, China and

India, while in the most impacted area, average NANI≅26,000 kg km^{-2} yr^{-1} and NAPI≅4,000 kg km^{-2} yr^{-1} were much greater than in the most impacted areas worldwide (Table 4). Moreover, NANI and NAPI were in the upper range even in the 1960s, perhaps a legacy of the long term exploitation of this watershed (Marchetti, 1993; Viaroli et al., 2010). N and P surpluses followed a similar pattern, resulting among the highest values from agricultural areas in Europe and America (Table 4). The breakdown of components of NANI, NAPI and N and P surpluses was similar to other watersheds dominated by agricultural activities (Billen et al., 2013; Kronvang et al., 2007; Lassaletta et al., 2012). The correlation between NANI and N surplus, and NAPI and P surplus can be also assumed as evidence of how N and P fluxes in the watershed were mainly affected by agriculture and livestock.

NANI exhibited clear temporal variations related to changes in land uses and livestock. Two main phases can be evidenced. Until the 1970s, the Po river basin was autotrophic and exported both feed and food. Coherently, NANI was mainly supported by nitrogen fixation from alfalfa and meadows. Since 1980, it has turned heterotrophic due to the increasing feed demand to sustain livestock. NAPI followed a similar pattern, with a greater contribution of P fertilizer in the first three decades, and of feed imports thereafter. As such, the Po river watershed underwent a trajectory from net autotrophy to net heterotrophy which reflected its specialization toward livestock farming, similar to other watersheds, e.g. Scheldt and Ebro (Billen et al., 2013). This outcome was consistent with the N and P surpluses in the agricultural land, where the main N and P sources were fertilizers and manure, evidencing a relevant contribution of the livestock component. These time changes highlighted a main shift which occurred between the 1970s and the 1980s, when the pig population increased dramatically and traditional dairy farming declined. From the 1980s, trends of NANI, NAPI and N and P surpluses reversed, mainly due to the reduction in N-fixing crops and P fertilizer use. In other words, the 1970s represented a transition between the traditional farming practices, in which dairy farms integrated husbandry and agriculture, and large scale industrial livestock farming, in which husbandry and agriculture were decoupled.

Permanent meadows and the rotation of cereals and temporary meadows were the backbone of the traditional farming mode. Fresh fodder and hay were used as feed, and cereal straw, especially wheat straw, was used as litter for maintaining healthy conditions in stables. The resulting high quality manure was used to support soil fertility, while the raw pig slurry was much less suitable for agronomic purposes and more exposed to runoff. The decrease in cattle and the concomitant increase in pigs were also accompanied by a significant growth of farm size over time, changing from a typically family-run management to an industrial mode. This led to changes in the management of manure and slurry, which from resources turned into wastes. Furthermore, when local authorities in a given area imposed restrictions on manure and sewage emission and usage, e.g. spreading on cropland, livestock and farming were relocated to another zone with less restrictive rules. For this reason, livestock moved from Emilia-Romagna region, where restrictive regional standards were enforced to contrast coastal eutrophication, to south eastern Lombardy region (see Fig. 2). Here, the increased animal density also added to impacting crops, such as maize. Accordingly, NANI, NAPI and N and P surpluses decreased in Emilia-Romagna and increased and became concentrated in the farmland along the northern side of the Po river in Lombardy (see Fig. 4). Here, due to the huge livestock load compared to cropland availability, management and controls of manure and wastewaters were often unsustainable due to the imbalance between inputs to cropland and removal capacity by crops and natural processes, such as denitrification (Bartoli et al., 2012; Soana et al., 2011).

Additionally, the loss of approximately 1/3 of the agricultural land was accompanied by the concurrent sprawl of urban and industrial areas, and infrastructure development (Gardi et al., 2013). The largest sprawl was in the metropolitan area of Milan, and in the neighbouring provinces both North and East, thus creating hot spots of urban and industrial wastewaters too. These urban related sources were not considered in the N and P surpluses, which deals with the agricultural land only, and were only indirectly accounted by NANI and NAPI as food.

4.2. Relationships between anthropogenic inputs, cropland surplus of N and P and riverine nutrient fluxes

Riverine fluxes of DIN and SRP responded differently to NANI and NAPI, and N and P surpluses in cropland. While SRP fluxes followed changes of both NAPI and P surplus without time lags between inputs and riverine loads, the decrease of NANI and N surplus did not result in the reduction of DIN fluxes (Fig. 6B). The origin of such N to P asymmetry can be searched in the different N and P sources and biogeochemical cycling of the two elements, which resulted also in a different retention within the watershed (Table 5). Both retention values of N and P were in the range of worldwide assessments (Han et al., 2011; Hong et al., 2012; Lassaletta et al., 2012; Swaney et al., 2012). NANI retention was nearly constant at 80-86%, although a slight decrease can be seen from 1990 onwards, while NAPI retention was 95-97%.

We hypothesize that the SRP increase from the mid 1970s to the late 1980s was mainly due to the direct delivery of P into rivers, e.g. from untreated point sources and/or wastewater treatment plants (see also Jarvie et al., 2006). In fact, during this period, the steep surge of SRP was related to a comparable increase in P input from detergents (Table 6S). Thereafter, the reduction of SRP loading followed mainly the enforcement of environmental policies aiming to contrast emissions from point sources with wastewater treatments and preventive measures, such as the reduction of polyphosphates in detergents. The SRP increase was also related to the steep increase in manure spreading until 1980, combined with persistently high mineral P fertilization (Tables 6S and 8S). The fate of this P is difficult to evaluate, because responses of SRP export to P inputs to cropland are affected by the capacity of soil and sediments to bind and retain P, which can induce even decades-long time lags (Jarvie et al., 2013).

These trends only deal with SRP, which on average was 30% of the total P in the Po river (Viaroli et al., 2013). Indeed, this SRP bulk was the most reactive P source, which can immediately affect primary productivity of both macrophytes and phytoplankton. However, the missing time-lag between P emission and SRP loadings must be studied further taking into account particulate P.

The SRP decrease from 1990 onwards has also been documented for the Danube river under base-flow conditions, while total P fluxes were affected by flood conditions (Zoboli et al., 2015). Further controversial issues are how to disentangle the contribution of the main P sources, i.e. urban wastewaters and agricultural runoff, and to what extent total P, especially particulate P, is really available to primary producers (Jarvie et al., 2006; 2013). Preliminary studies in the Po river have documented that most of the particulate P was released during flood events (Naldi et al., 2010), and only <10% of such particulate P was promptly available to primary producers (Giordani et al., 2010).

Contrarily to SRP, riverine loads of DIN first increased and then remained stable, once NANI and N surplus decreased. Likely, the increase from the 1960s to 1980s was directly affected by ineffective wastewater treatments and the considerable change in agricultural practices and livestock management. We hypothesize the persistently high loadings in the following decades to be accounted for as hydrologic legacy, i.e. N retention in groundwater and unsaturated zone followed by its release to surficial waters (van Meter et al., 2016; van Meter and Basu, 2017). This assumption is supported by water quality data from few hundred wells in the lowland of the Po river basin, which attest an accumulation of nitrate in groundwaters from the mid 1980s to the mid 1990s, when nitrate concentrations increased twofold from 3 up to 6 mg N L^{-1} (Cinnerella et al., 2005). Twenty years later, an extensive study of groundwaters in the Po plain documented further how nitrate contamination had increased and was correlated with agriculture and livestock, especially with pig population (Martinelli et al., 2018).

Replacement of meadows with seasonal crops, e.g. cereals and tomatoes, could have exacerbated nitrogen leaching to groundwaters. These crops have high fertilization requirements, and are potential sources of diffuse pollution. Furthermore, among cereals, the replacement of winter wheat with the more profitable maize and rice was relevant for water management and pollution, because wheat is a non-irrigated winter crop, while maize and rice are summer crops requiring large quantities of water and nitrogen fertilizers. In the northern side of the Po river, nitrate leaching from cropland to groundwater was also accelerated by the extensive irrigation with submersion of heavily fertilized and manure loaded soils (Perego et al. 2012; Provolo et al., 2005). Here, among others, maize crops were found to contribute leaching of up to 300 kg N ha^{-1} yr^{-1} (Perego et al., 2012). We do not have evidences of biogeochemical legacy due to retention and transformations in the root zone of organic N from crop residuals and manure (van Meter, 2016), but we can speculate that it was incorporated in the hydrologic legacy, due to N mineralization and nitrification, with nitrate ultimately leached from the root zone into groundwaters or lost by denitrification (Bartoli et al., 2012; Martinelli et al., 2018; Provolo, 2005). Manure was a further source of diffuse N pollution, especially in zones with a high livestock density, where the traditional dairy cattle farming was substituted by industrial pig breeding (Martinelli et al., 2018; see Fig. 2).

In addition to livestock manure, sludge from urban wastewater treatment plants (WWPT) were also spread on the cropland. Experimental assessment documented relevant N leaching, proportional to the sludge ammonium content (Fumagalli et al., 2013). However, the WWPT sludge contributed to N contamination much less than livestock manure, the ratio of N-sludge to N-manure being nearly 1:50 (Soana et al., 2011).

The groundwater supply of reactive N, mainly nitrates, was explicitly documented in the Oglio river, one of the tributaries of Po river which flows in mid-eastern Lombardy, where the highest NANI and N surplus within the Po river basin were estimated (Bartoli et al., 2012; Soana et al., 2011). In the last decade, in the river reach crossing the spring belt area in the transition zone between high- and lowland, nitrate concentration in river waters underwent a steep ten-fold increase from about 1 to 10 mg N L^{-1} (Bartoli et al., 2012).

Compared to P, the control of N emissions was therefore less effective, due to widely diffuse livestock and agricultural sources as already suggested (de Wit and Bendoricchio, 2001; Pirrone et al., 2005). The persistently high DIN loadings combined with the SRP decrease, accounted for largely unbalanced DIN:SRP molar ratios with possible consequences for coastal ecosystems.

4.3. Linking the Po river watershed to Northern Adriatic Sea

Previous studies analyzed series of loading data from the Po river and responses of the Northern Adriatic Sea, within a limited time period, and neglected processes in the watershed. Among others, Ludwig et al. (2009) and Cozzi and Giani (2011) highlighted how the Po river accounted for ~65% of freshwater, nitrogen and phosphorus loads to the Adriatic Sea. In the present study, relationships between land uses and nutrient loading were also considered for the whole data set available (1968-2016), highlighting how timing and intensity of SRP and DIN loadings in the Po river were related to changes of NANI and N surplus and NAPI and P surplus.

In turn, increased DIN and SRP fluxes from the river impacted the Adriatic Sea, triggering eutrophication processes which extent depended on circulation structure and short-term climatic fluctuations too (Bernardi Aubry et al., 2004; Degobbis, 2005; Fonda Umani et al., 2005; Grilli et al., 2005; Giani et al., 2012). When the western current is active, the Po river plume impacts the western coast of the Adriatic sea, where eutrophication severely impaired water quality from 1970 to 1990 (Marchetti, 1992; Vollenweider et al., 1992). Here, the high primary productivity fuelled benthic microbial processes and caused frequent hypoxia and anoxia in the deep waters, especially in late summer-autumn from the 1970s to the 1980s and, to a much lesser extent, in the 1990s (Degobbis et al., 2000). From the late 1980s through the early 1990s, frequent mucilage blooms occurred (Rinaldi et al., 1995). Disproportionate N to P ratio, with progressive DIN and SRP exhaustion, was assumed to trigger mucilage formation, but in combination with other factors, e.g. silica availability, pulsed freshwater inputs, water circulation and stratification (Sellner and Fonda Umani, 1999; Degobbis et al., 2005).

DIN and SRP loadings also affected primary producer communities in the deltaic lagoons, where a shift from pristine phanerogam meadows to macroalgal blooms occurred from the mid 1980s to the late 1990s (Viaroli et al., 2006). Changes in community composition and dominance of macroalgae were related to DIN loadings, although it was difficult to clearly disentangle pressures from Po river watershed from local factors, e.g. fishery and aquaculture (Viaroli et al., 2008).

After peaking in the mid 1980s, eutrophication has apparently started a reversal trend, especially since 2000, when SRP concentrations decreased, along with low chlorophyll-a concentrations and phytoplankton biomass, which reached very low values in dry years, i.e. from 2003 to 2007 (Mozetičet al., 2010; Giani et al., 2012). Afterwards, in very wet years (e.g. 2008-2009 and 2014) river discharge and loadings increased again, adding uncertainty to the recovery trend expected. This pattern is not unexpected, because alternating floods and drought can affect pathways and fate of N and P, and their ratio (Naldi et al., 2010; Zoboli et al., 2015). Drought can also induce a sort of time-lag between delivery of nutrients from catchments and their availability in the final recipient, which indeed can be portrayed as an apparent recovery of healthier conditions. Floods can further remobilize nutrient stored during the drought phase. However, repeated floods can also flush and reduce the nutrient bulk stored, leading to lower concentrations (Zoboli et al., 2015). Moreover, the restoration trajectories towards low nutrient concentrations have to be further evaluated because the achievement of lower DIN and SRP concentrations and imbalanced N:P ratios could not match with recovery of pristine structure and species composition in the primary producer communities (Duarte et al. 2009, Glibert, 2017).

4.4. Concluding remarks and perspectives

DIN and SRP loadings were clearly related to changes of NANI, NAPI and N and P surpluses. The attempts to control N emissions were of little effect, due to widely diffuse livestock and agricultural sources and discordant environmental policies at the local scale. The latter caused a resource relocation in the watershed leading nutrient sources to concentrate in few hot spots. One of the challenges for environmental policies and management is to reduce N pollution throughout the restoration of biogeochemical processes and functions in river and streams (see Pinay et al., 2002). However, this approach is often unreliable, economically unsustainable, and time consuming due to the size of such waterbodies. Likely, the secondary hydrographic network, composed of small irrigation and drainage canal and ditches, is more reliable and can offer opportunities to restore either the hydrological or biogeochemical functionality of the river margins. Studies of small sub-basins in the Po river basin proved that the vegetation management in the lowland canals and ditches can ensure a relevant N removal, especially through denitrification processes (Castaldelli et al., 2013, 2015). This could contribute to a solution of the persistent nitrate pollution problem, given that current policies and management options failed in controlling the emission of nitrogen excess from diffuse sources (Palmeri et al., 2005). Restoration and management of the canal network can be seen as an opportunity to accelerate recovery.

Compared to N, preventive and remedial policies have proved to be successful for P, achieving a notable reduction in reactive P loading. P was likely retained for the most part by soils and sediments in the watershed. Key questions are how long and to what extent this phosphorus bulk will be retained, given that the increasing frequency of short-term and heavy rainfall can increase runoff and flash floods (Vezzoli et al., 2015), which can impact P more than N.

The efforts to address N and P pollution at different scales and independently of one another have proven unsuccessful for the recovery of good water quality, and are producing imbalances in the N to P ratio of loadings. Currently, the large excess of DIN relative to SRP is assumed to perturb primary producer communities, causing shifts from micro - to macroalgae, phenological mismatch between grazers and phytoplankton in the marine food webs, surge of harmful algal blooms, which are deviations from the recovery of healthier conditions (Glibert, 2017). It is therefore of the utmost importance to consider key questions on the spatial scale at which problems have to be addressed for harmonizing policies, setting sustainable management goals, restoring river basins and, ultimately, protecting the adjacent coastal seas.

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Figure captions

Figure 1. Map of the Po river basin with the main hydrographic network. The province borders are also reported, with indicated some reference – MI: Milan, TO: Turin, MN: Mantua, PR: Parma. PLS: Pontelagoscuro, closing section of Po river basin.

Figure 2. Trends of the areal distribution of the crop typologies representative of the main changes of agricultural land use, cattle and pig stocks in the agricultural land, and human population in the Po river watershed from 1960 to 2010.

Figure 3. Temporal trends of the Net Anthropogenic Inputs of Nitrogen (NANI) and Net Anthropogenic Inputs of Phosphorus (NAPI) in the whole watershed of the Po river, and N and P surpluses in the agricultural land from 1960 to 2010. DIN and SRP loadings (continuous line) are also reported for comparison.

Figure 4. Trends of the areal distribution of the Net Anthropogenic Nitrogen Inputs (NANI), Net Anthropogenic Phosphorus Inputs (NAPI) in the whole watershed, and N and P surpluses in the agricultural land of the Po river basin from 1960 to 2010.

Figure 5. Annual loadings exported from the Po river watershed at Pontelagoscuro (PLS in Fig. 1). A) dissolved inorganic nitrogen (DIN), B) soluble reactive phosphorus (SRP), C) molar DIN:SRP ratio. The mean annual discharge is depicted as grey background. Time changes detected with the results of the Inflection Point Analysis are reported. The bold lines represent the time extent of increase (line up) or decrease (line down). D) DIN increased in 1968-1985, E) SRP increased in

1968-1978, decreased in 1982-1989 and 1998-2008; F) DIN:SRP molar ratio: increased in 1985-2001.

Figure 6. Long term trajectories of DIN loading response to NANI and SRP loading response to NAPI. Data are mean values of each considered decade. Standard deviations are reported in Table 5. Arrows indicate the directionality of changes. When they are parallel to axes, no changes occur.