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Research Article

Climate change impacts on eutrophication in the Po River (Italy): Temperature-mediated reduction in nitrogen export but no effect on phosphorus

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ABSTRACT

Rivers worldwide are under stress from eutrophication and nitrate pollution, but the ecological consequences overlap with climate change, and the resulting interactions may be unexpected and still unexplored. The Po River basin (northern Italy) is one of the most agriculturally productive and densely populated areas in Europe. It remains unclear whether the climate change impacts on the thermal and hydrological regimes are already affecting nutrient dynamics and transport to coastal areas. The present work addresses the long-term trends (1992–2020) of nitrogen and phosphorus export by investigating both the annual magnitude and the seasonal patterns and their relationship with water temperature and discharge trajectories. Despite the constant diffuse and point sources in the basin, a marked decrease (-20%) in nitrogen export, mostly as nitrate, was recorded in the last decade compared to the 1990s, while no significant downward trend was observed for phosphorus. The water temperature of the Po River has warmed, with the most pronounced signals in summer (+0.13°C/year) and autumn (+0.16°C/year), together with the strongest increase in the number of warm days (+70%–80%). An extended seasonal window of warm temperatures and the persistence of low flow periods are likely to create favorable conditions for permanent nitrate removal via denitrification, resulting in a lower delivery of reactive nitrogen to the sea. The present results show that climate change-driven warming may enhance nitrogen processing by increasing respiratory river metabolism, thereby reducing export from spring to early autumn, when the risk of eutrophication in coastal zones is higher.

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Introduction

Over the last century, nitrogen (N) and phosphorus (P) inputs have increased dramatically in human-impacted watersheds with a cascade of multiple negative impacts on aquatic environments in terms of water pollution, eutrophication, greenhouse gas emissions, ecosystem functioning, and biodiversity loss (Battye et al., 2017; Glibert, 2017; Yuan et al., 2018). Watershed nutrient budgets provide insight into the relative importance of anthropogenic sources, i.e., the main determinants of riverine loads (Romero et al., 2021), but the amounts of nutrients processed or transported downstream and ultimately exported are closely linked to hydrological dynamics and internal biogeochemical cycling. Several temperature-dependent (e.g., organic matter mineralisation and biogeochemical N pathways in soils and waters) or precipitation-dependent (e.g., runoff and erosion processes) processes occur across landscapes and shape the timing and the magnitude of nutrient mobilisation (Baron et al., 2013; Wagena et al., 2018; Goyette et al., 2019). Many rivers around the world are under stress due to eutrophication and nitrate (NO_3^-) pollution, but their ecological consequences overlap with the effects of climate change and the resulting interactions may be complex, unexpected not yet fully understood (Rozemeijer et al., 2021; Meerhoff et al., 2022; Costa et al., 2022). Climate change may affect the biogeochemical dynamics and the ecological functioning of rivers by influencing the amount and the timing of nutrient delivery from terrestrial ecosystems, and by altering the dilution capacity and the extent of internal dissipation and recycling processes (Goyette et al., 2019; Abily et al., 2021; Zheng et al., 2023).

River networks are disproportionately important relative to their surface areas for processing anthropogenic N inputs. On a global scale, more than 75% of the N load generated in the catchments and transferred to rivers is removed along the terrestrial-freshwater-marine continuum (Seitzinger et al., 2006; Howarth et al, 2012). In these systems, microbial denitrification is considered the main mechanism responsible for permanent N removal through the reduction of nitrate (NO_3^-) to nitrogen gas (N_2) under hypoxic-anoxic conditions (Birgand et al., 2007; Reisinger et al., 2016; Hill, 2023). Warming can affect denitrification both as a direct effect of enzyme activity and as an indirect effect of temperature upon redox conditions. Higher water temperature decreases oxygen solubility and enhances sediment oxygen respiration, limiting oxygen penetration depth and resulting in a synergistic effect that stimulates denitrification (de Klein et al., 2017; Velthuis and Veraart, 2022). In cases where denitrification is strongly dependent on NO_3^- supply from nitrifying bacteria, decreased oxygen at higher water temperatures may result in reduced nitrification, and consequently lower denitrification (Pina-Ochoa and Álvarez-Cobelas, 2006; Birgand et al., 2007).

Simultaneously, a wide range of abiotic and biotic processes (e.g., sorption, particulate sedimentation, periphyton, and phytoplankton uptake) are responsible for P retention in river sediments and account for the temporary storage of this element (Yuan et al., 2018; Goyette et al., 2019). Overall, rivers actively transform, temporarily store, and permanently remove nutrients in a highly dynamic environment largely controlled by microbial activity, which is temperature-dependent and thus sensitive to global warming (Salmon-Monviola et al., 2013; Velthuis and Veraart, 2022; Hill, 2023).

Nutrient transport and transformation are expected to change in response to changes in the seasonality of precipitation patterns and snow accumulation, intensified climatic variability, and increased frequency and severity of extreme thermal and hydrological (floods and droughts) events (Howarth et al, 2012; Baron et al., 2013; IPCC, 2021). A better understanding of the combined and simultaneous effects of hydrological variability and temperature warming on current and future nutrient export from watersheds is essential for defining and implementing policies to effectively tackle eutrophication in an era of climate change.

Nitrogen pollution is a priority challenge in the Po River basin (northern Italy), one of the most agriculturally exploited and densely populated areas in Europe, and eutrophication is a major concern, especially in the receiving coastal lagoons and the Adriatic Sea. Between the 1960s and the 1990s, the temporal trajectory of the input-output N and P budgets in the Po River watershed was deeply affected by changes in land use, agricultural and livestock practices, and wastewater treatment efficiency (e.g., de Wit and Bendoricchio, 2001; Artioli et al., 2008). In recent decades, the excess agricultural nutrients have remained stable (Viaroli et al., 2018; Gervasio et al., 2022a). Therefore, the basin can be considered as a case study to investigate the response of removal and recycling processes and downstream nutrient delivery to recent climatic anomalies. The effects of climate change in the Po River basin are already evident, as shown by alterations in precipitation intensity and patterns, temperature warming, increased evaporation, and frequency of droughts and water stress periods (Appiotti et al., 2014; Coppola et al., 2014; Formetta et al., 2022; Tarolli et al., 2023). Anyway, it is currently unclear whether climate change impacts on the hydrological cycle and temperature of the Po River are already affecting the river functioning in terms of nutrient removal, recycling, and delivery to the coastal zones and the Adriatic Sea. To fill this research gap, the present work addresses the long-term trends (1992–2020) of N and P export from the Po River basin by investigating both the annual magnitude and the seasonal patterns and their relationship with the trajectories of water temperature and discharge. The main hypothesis is that warming and an increase in the duration of low flow periods may enhance the rates of microbial processes and sustain more favorable conditions for denitrification and NO_3^- removal, resulting in a net decrease in N delivery to the Adriatic Sea. On the other hand, the increase in rainfall intensity and frequency may result in enhanced erosion processes and sediment yields, and downstream P delivery may eventually be favored over N.

1. Materials and methods

1.1. *Study area*

The Po River, with a length of 652 km from the Alps to the Adriatic Sea, is the longest river in Italy (Fig. 1) and the largest one with an average annual discharge of ~1,500 m^3/sec mea-

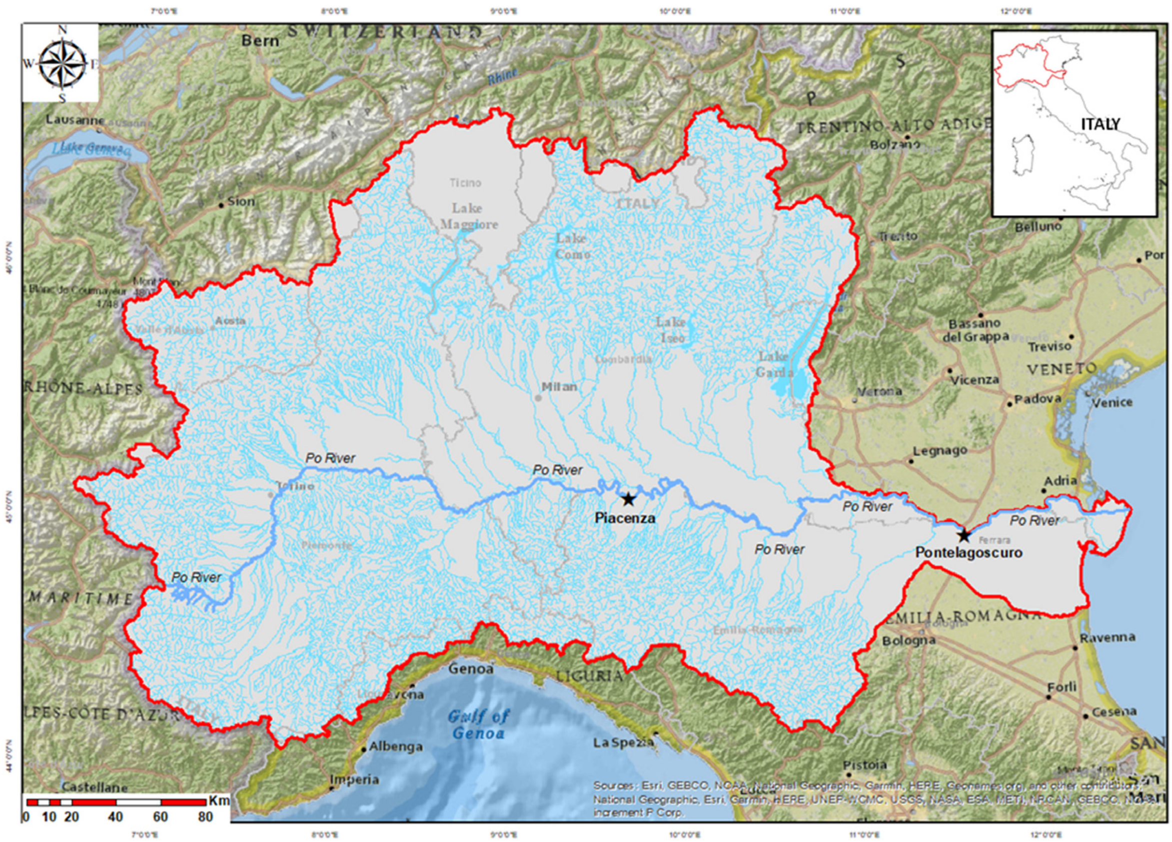

Fig. 1 – Map of the Po River basin (red line), its natural hydrological network (blue lines), and the administrative boundaries of the Northern Italy Regions (grey lines). The two monitoring stations (Piacenza and Pontelagoscuro) are indicated as black stars.

sured at the Pontelagoscuro gauging station, right upstream of the deltaic system (Zanchettin et al., 2008). The Po River basin extends over an area of ~75,000 km^2, from the headwaters on the northern slope of Monviso (3,841 m asl, Cottian Alps) to the delta, which projects into the northern Adriatic Sea. The river is fed from both the Alps and the Apennines by a network of more than 140 tributaries with a total length of over 6,700 km and a secondary network of artificial irrigation and drainage canals that is almost ten times larger (Soana et al., 2019). The hydrological regime is characterized by two periods of low flow (winter and summer) and two flood periods (spring and late autumn) associated with snowmelt and precipitation that feed the tributaries descending from the valleys of the Alps and the Apennines, respectively (Montanari, 2012; Coppola et al., 2014; Ravazzani et al., 2015). The basin crosses the transition zone between the subcontinental climate of Central Europe and the Mediterranean climate (warm-temperate climate). The average annual precipitation is 1,200 mm, varying from a maximum of 2,000 mm in the Alpine sector to less than 700 mm in the deltaic region (Vezzoli et al., 2015; Beck et al., 2018). Water resources in the basin are intensively exploited, particularly for irrigation, hydropower generation, domestic, and industrial purposes (Montanari et al., 2012).

The basin includes the area with the highest economic income in Italy, where approximately 40% of the gross domestic product is produced. Cropland accounts for more than half of the land use in the basin, which encompasses a large part of the Padano–Veneta plain, the widest and most fertile alluvial lowland in Italy (~47,000 km^2). The Po River lowland is the largest cultivated area in Italy and accounts for more than one-third of the national agricultural and livestock production. The Padano–Veneta plain is the most densely urbanized, industrialized, and agriculturally exploited area in Italy, resulting in one of the European regions with the highest N and P inputs to cropland and nutrient losses to surface and groundwater (Viaroli et al., 2018; Romero et al., 2021; Batool et al., 2022). The Po River accounts for approximately two-thirds of the total freshwater discharge and nutrient inputs to the northern Adriatic Sea, making it a well-known eutrophication hotspot (Ludwig et al., 2009; Blaas and Kroeze, 2016; Malone and Newton, 2020).

1.2. *Calculation of nutrient loads exported from the Po River basin*

Monthly, seasonal, and annual N and P loads exported to the Adriatic Sea were calculated using discharge and nutrient concentration datasets collected for the period 1992–2020 at the Pontelagoscuro gauging station (44°53'19.34"N, 11°36'29.60"E; Ferrara, river kilometer 586), which is conventionally considered the basin closing section (Fig. 1). Daily discharge data were obtained from the Hydrological Annals published by the Environmental Protection Agency of the Emilia-Romagna Region (ARPAE), whose electronic versions are available on the ARPAE Open Data Portal (Dexter Portal, https://simc.arpae.it/dext3r/). Observed discharge data were derived from rating curves which convert river water levels, measured every 15 min at the gauged cross section of interest, into flow rates (rating curve uncertainty <10% of discharge value; Domeneghetti et al., 2012). Concentrations of total nitrogen (TN), total phosphorus (TP), NO_3^-, ammonium (NH_4^+), and PO_4^{3-} (reactive phosphorus) were obtained from monthly, and in some cases, fortnightly, samples taken by ARPAE as part of the official surface water monitoring programme (Regional Open Data Portal, https://dati.arpae.it/group/acqua). Dissolved inorganic nutrients were determined on filtered water samples (Whatman GF/F filters) by standard colorimetric methods based on the azo dye reaction for NO_3^- (detection limit >0.2 mg N/L), the indophenol reaction for NH_4^+ detection limit >0.02 mg N/L), and the molybdate reaction for PO_4^{3-} (detection limit >0.01 mg N/L). Precision ranged between ±3% and ±5% for the three nutrient analyses. Total nitrogen and total phosphorus were determined on unfiltered samples by the persulphate oxidation method followed by the colorimetric determinations of NO_3^- and PO_4^{3-}. Sample collection and analytical determinations were performed according to standard methods and analytical protocols outlined by national environmental regulations and adopted by the Regional Environmental Agencies in Italy (APAT—IRSA/CNR, 2003). The analytical methods were comparable to those applied in a wide range of environmental studies (e.g., APHA, 2005). When TN concentrations were not available, TN values were calculated from DIN (NO_3^- + NH_4^+) concentrations using the equation $TN = 0.93 \cdot DIN + 0.75$ ($r^2 = 0.54$; $p < 0.001$), previously obtained by plotting time series with simultaneous measurements of TN and DIN (Gervasio et al., 2022a).

The method used to calculate loads was based on linear interpolation of daily nutrient concentrations between two consecutive sampling events. Previous applications have shown that this method is accurate for large rivers with recurrent seasonal variations of nutrient concentrations (Moatar et al., 2005; Nava et al., 2019). Daily loads were calculated as the product of the measured daily discharge and nutrient concentration (measured or interpolated) and then aggregated monthly according to the following equation:

$$L = k \cdot \sum_{j}^{n} C_i \cdot Q_i \tag{1}$$

where L (tons/month) is the monthly load, C_i (mg/m^3) is the daily concentration on day i measured or linearly interpolated between two consecutive measurements to represent unsampled days, Q_i (m^3/sec) is the average daily discharge on day i, n is the number of days in each month, and k (86.4x10^{-6}, from mg/sec to tons/day) is the unit conversion factor.

Annual loads (tons N/year or tons P/year) were calculated by summing the monthly contributions. Trends in seasonal loads (tons N/season or tons P/season) were evaluated according to the following seasonal breakdowns: winter (January–March), spring (April–June), summer (July–September), and autumn (October–December).

The annual loads calculated by the interpolation concentration method were compared for consistency with those calculated by the flow-adjusted concentration method, previously used to estimate the long-term trajectories of the reactive nutrient loads exported from the Po River basin (Cozzi et al., 2018; Viaroli et al., 2018). The flow-adjusted concentration method is commonly adopted to assess annual loads as recommended by international conventions (e.g., OSPAR-Convention for the Protection of the Marine Environment of the North-East Atlantic; Lenhart et al., 2010) and regional guidelines for the implementation of River Basin Management Plans, as required by EU Directive 2000/60/EC (e.g., Po River District Authority, 2021). In fact, the method allows for the best weighting of the data when the chemical and flow data series have different temporal resolutions. However, this approach is not suitable for the calculation of monthly and seasonal loads because the Regional Environmental Agencies generally conduct only one sampling per month. A very good correlation was found between the annual values calculated using the two methods ($r^2 = 0.99$; $p < 0.001$), with an average discrepancy of less than 5% (Appendix A Fig. S1).

To remove the effect of varying inter-annual hydrological conditions in the trend assessment of annual and seasonal nutrient export, monthly flow-normalized loads (Ln) were calculated as follows (Sileika et al., 2006):

$$Ln = L \cdot Kh \tag{2}$$

where Kh is the hydrological coefficient. The hydrological coefficient was obtained as the ratio of the long-term (period 1992–2020) average outflow for a given month to the monthly outflow for a given year. The annual normalized loads were calculated by summing up the monthly flow-normalized loads.

1.3. *Datasets of Po River water temperature and air temperature*

Water temperature time series representative of the middle-lower reach of the Po River were collected at two monitoring stations located at the cooling water intakes of two thermoelectric power plants near the city of Piacenza (Emilia-Romagna Region, river kilometer 330) (Fig. 1). Daily average water temperature data were recorded at La Casella Power Station operated by the ENEL group (https://www.enel.com/it/media/esplora/ricerca-foto/photo/2020/03/italia-centrale-la-casella) and at Piacenza Power Station operated by the A2A Life Company Group (https://www.a2a.eu/en/group), and integrated to cover the period 1992–2020. Water temperature measurements were carried out using RTD probes with platinum Pt100 resistance thermometers with a nominal resistance of 100 Ω at 0°C, defined according to IEC 751 (EN 60751). Other sensor features: measuring range 0–40°C, accuracy ±0.1°C at 0°C, 4-wire connection, signal conversion electronics with 4-20 mA output in the range 0–40°C.

A validation procedure was applied to reconstruct a continuous three-decade time series from the daily water temperature values recorded at the two sites. The water temperature of the Po River was retrieved from the ARPAE database (https://dati.arpae.it/group/acqua) for the two stations in the official monitoring network. The ARPAE Castel San Giovanni station (45°05′30.0″N, 9°26′44.2″E) is located close to the La Casella Power Station (<2 km), whereas the ARPAE Piacenza statio’ (45”03′37.9′N, 9”42′19.9"E) is located close to the A2A Piacenza Power Station (<1.5 km). Water temperatures were obtained from monthly sampling campaigns conducted on the same day (within < 2 hr) for the two stations as part of the ARPAE environmental monitoring program. The observations made on the same day at the two ARPAE stations were consistent. Because of the very good correlation (Appendix A Fig. S2) and a discrepancy of less than 0.3°C, on average, between the observations, the merging of the two datasets was considered appropriate. Occasional missing data points (up to five consecutive days) owing to temporary maintenance operations of the thermoelectric power plants were linearly interpolated between two subsequent sampling events. From the daily temperature data, annual and seasonal trends in the average, minimum, and maximum values were analysed according to the monthly clustering previously described for nutrient loads. The occurrence of warm days (i.e., the number of days with water temperatures above the long-term average) was assessed on an annual and seasonal scales.

Nutrient loads transported seasonally and annually at the basin closing section (i.e., Pontelagoscuro) were related to the water temperature data recorded at the Piacenza section. Water temperature data that were both sufficiently detailed (daily) and recorded over the entire 1992–2020-time interval were not available for the Pontelagoscuro section. Although in the river reach between Piacenza and Pontelagoscuro the inflow of tributaries and groundwater does not substantially modify the thermal regime (data not shown), it should be noted that Pontelagoscuro is the most suitable section for calculating loads, as it is at the end of the basin before the delta, and Piacenza is the most suitable section for measuring temperature, as it is in the middle of the river, thus providing average values of all the hydrological situations that contribute to the overall nutrient metabolism and denitrification capacity expressed by the river .

Continuous air temperature measurements were obtained from a monitoring station located close to the course of the Po River, near the city of Piacenza (45”02′15.0′N, 9”42′03.5"E). Time series were retrieved from the ARPAE climate database (Antolini et al., 2016; https://dati.arpae.it/dataset/erg5-eraclito) and analysed as previously described for the water temperature datasets.

1.4. *Calculation of nutrient loads from diffuse and point sources in the basin*

Changes in anthropogenic pressures in the Po River basin and in nutrient loads from diffuse (agriculture) and point sources (urban areas) were assessed by collecting census data at 10-years intervals over the last three decades. N and P loads from diffuse sources were quantified by calculating the soil budget (SB), i.e., accounting for the difference between inputs (livestock manure, synthetic fertilizers, atmospheric deposition, and biological fixation in the case of N) and outputs via crop harvest (Romero et al., 2021). Point sources (PS) N and P loads were quantified using resident population data and the per capita nutrient production coefficients corrected for the percentage of sewerage systems connected to wastewater treatment plants and the depuration efficiency. The details of the calculation methods, sources of the census data and coefficients are presented in Appendix A Tables S1, S2 and S3).

1.5. *Statistical analyses*

Parametric (linear regression) and non-parametric (Mann-Kendall, Sens’s slope and Pettitt’s test) tests were applied to the annual and seasonal historical data of temperature, riverine nutrient loads, and discharge (Helsel et al., 2020). The Mann–Kendall test was used to verify the presence of a significant monotonic trend in the time series, whereas Sen’s test quantified the magnitude of this change by calculating the slope of the linear interpolation model. The relationship between the water temperature and nutrient loads of the Po River (monthly values) was tested using Pearson’s correlation analysis. Statistical analyses were performed using the XL-STAT (Addinsoft, 2022). In all cases, the trends and factors tested were considered to be statistically significant at $p < 0.05$.

2. Results and discussion

2.1. *Trajectories of annual and seasonal nutrient export from the Po River basin*

Over the last three decades, the annual TN load exported by the Po River to the Adriatic Sea showed high inter-annual variability, ranging from ~68,000 tons N/year (2017) to ~238,000 tons N/year (1996), while a significant negative trend was observed (Fig. 2a, Appendix A Table S4), with an average decrease of 20% in the last decade compared to the ‘90s. Nitrate was the most abundant form of N, accounting for 62%–86% of the annual TN load (Fig. 2a), a common feature of agriculture-dominated watersheds (Pina-Ochoa and Álvarez-Cobelas, 2006; Hill, 2023; Li et al., 2023). During the period 1992–2020, a significant load reduction was also observed for NO_3^- (23%), although the export varied greatly among years according to hydrological conditions, with the highest values occurring in 1996 and 2014 (+40%−54% of the long-term average annual discharge) and the lowest in 2007 and 2017 (−45% of the long-term average annual discharge). The annual NH_4^+ load was quantitatively much less relevant (1%−5% of TN load), but, similar to NO_3^-, reached a minimum in 2017 and a maximum in 1996. During the study period, annual NH_4^+ export decreased by more than 50%, from ~5,600 tons N/year in the early 1990s to less than 2,700 tons N/year in the last decade (Fig. 2a, Appendix A Table S4). The annual loads of TP and PO_4^{3-}, accounting for on average of 40%, were characterized by a high inter-annual variability, mainly due to the highly variable hydrological conditions; however, in contrast to the loads of TN and its dissolved inorganic forms, without a significant decreasing trend in recent years (Table 1, Fig. 3a).

The decrease in nitrogen export to the Adriatic Sea was not related to changes in anthropogenic pressures in the catchment area or nutrient loads from diffuse (agriculture) and point sources (sewage). The soil N budget in agricultural land showed a steady constant excess in the last three decades

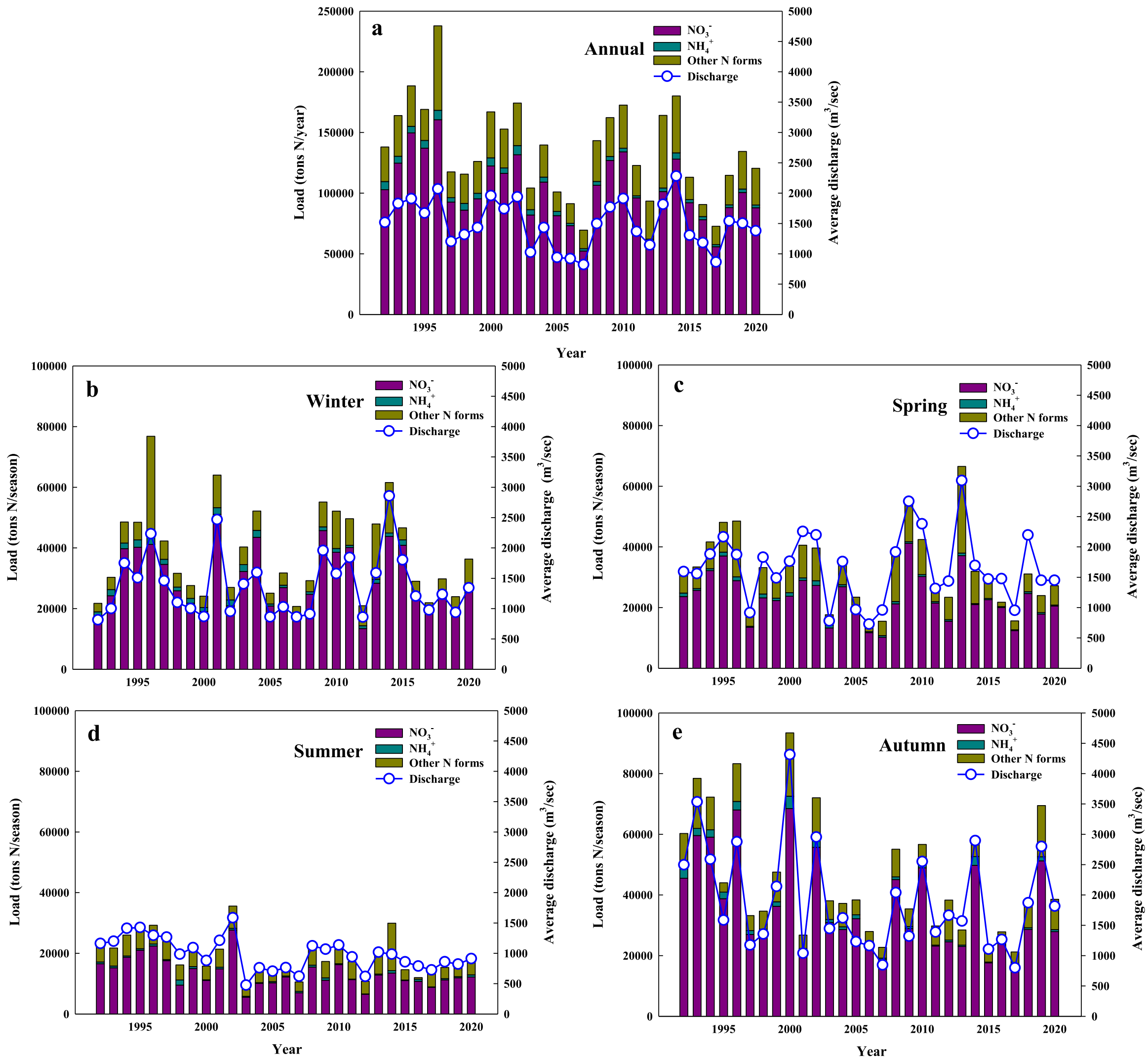

Fig. 2 – Trajectories of annual and seasonal N loads (TN, NO_3^-, and NH_4^+) and average discharge measured at the Po River closing section.

Table 1 – Decadal changes (1990–2020) of the soil N budget (SB-N) and the N load from point sources (PS-N) in the Po River basin. All budget terms are expressed as ktons N/year.

Year	N_{Man}	N_{Fert}	N_{Fix}	N_{Dep}	N_{Harv}	SB-N	PS-N
1990	214.1±30.6	231.0±3.2	194.3±33.3	31.9±4.7	336.8±22.3	**334.5±94.1**	**40.3±1.8**
2000	221.6±31.8	217.8±3.0	174.4±28.0	31.9±4.8	372.6±24.6	**273.1±92.2**	**22.1±2.2**
2010	221.9±29.4	170.9±2.3	149.7±25.5	24.3±3.7	290.8±22.3	**276.0±83.2**	**22.9±2.4**
2020	222.2±31.0	195.4±2.7	126.5±21.2	24.5±3.7	270.1±18.8	**298.4±77.4**	**23.4±2.5**

N_{Man} = N in livestock manure; N_{Fert} = synthetic N fertilizers; N_{Fix} = biological fixation; N_{Dep} = atmospheric N deposition; N_{Harv} = N export with crop harvest

(Table 1). Total N inputs showed a slight decrease in 2010 compared to the previous two decades, but this was also associated with a decrease in crop N harvest, resulting in a net surplus that was not significantly different between the two periods, taking into account the uncertainty associated with the budget terms. In contrast to N, there was a marked decrease in the soil P budget between 1990 and 2000, mainly due to the reduction in the use of synthetic fertilisers, which together with livestock manure are the main inputs to agricultural land (Table 1). While the resident population of the Po River basin has remained almost stable over the last three decades (~17 million), nutrient loads from point sources decreased by about 45% between 1990 and 2000, and then remained almost constant until today. The reduction in nutrient loads from urban areas occurred exclusively in the first decade of the study period and followed a significant abate-

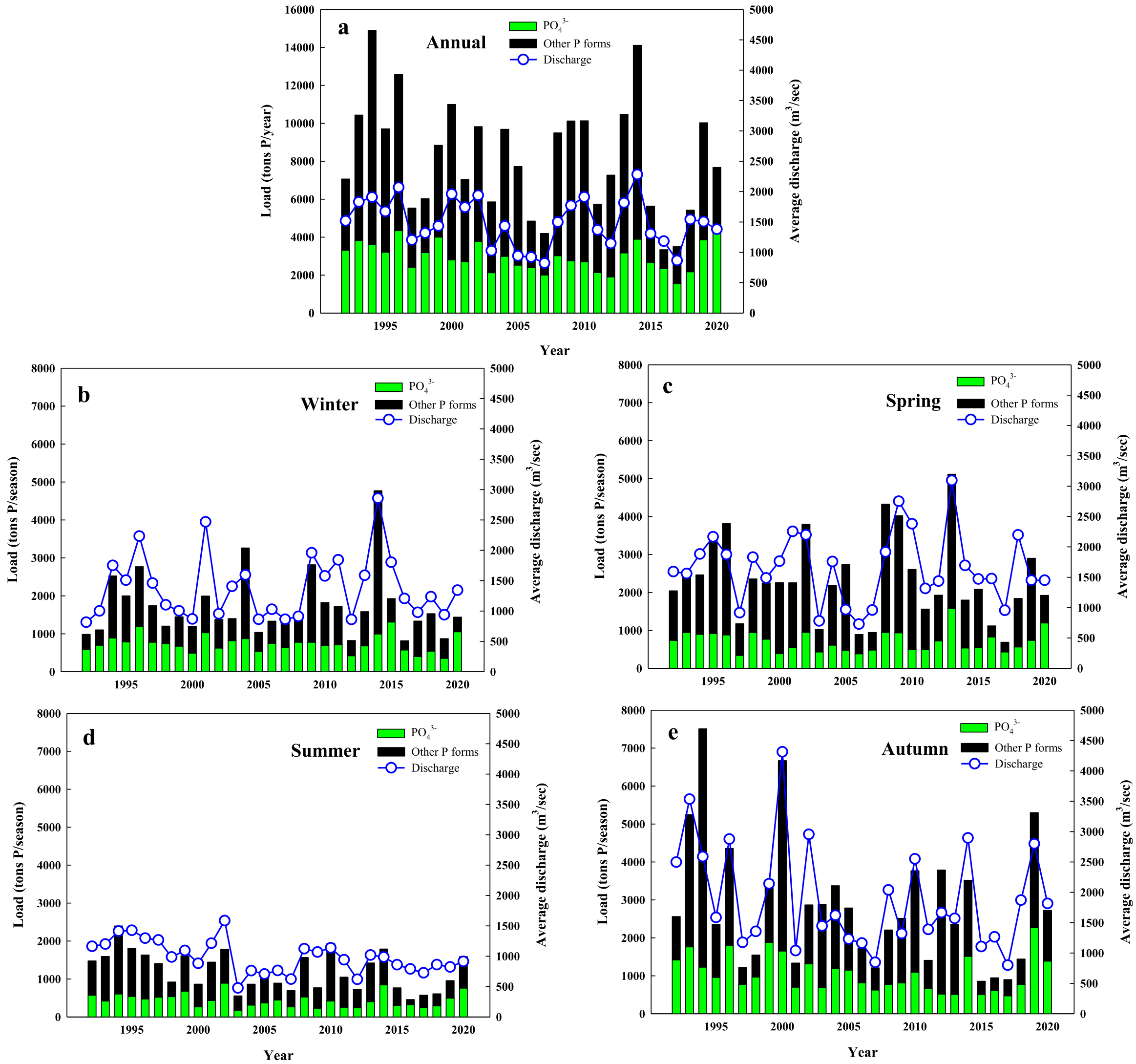

Fig. 3 – Trajectories of annual and seasonal P loads (TP and PO_4^{3-}) and average discharge measured at the Po River closing section.

ment in direct discharges of untreated or poorly treated domestic wastewater into surface waters. Indeed, the enforcement of environmental policies and legislative acts aimed at controlling nutrient emissions from point sources, such as the construction of wastewater treatment plants, the introduction of nitrification/denitrification processes, and the ban on polyphosphates in detergents in the early '90s, led to a marked reduction in N and P loads from domestic sources (de Wit and Bendoricchio, 2001; Palmeri et al., 2005). Nitrogen load decline from urban areas may have been partly responsible for the decrease of riverine NH_4^+ loads, but it was not in the order of magnitude to explain the decrease recorded for the riverine TN loads. Overall, nutrient loads from point sources have accounted for 3%–6% of the total nutrient inputs from diffuse agricultural sources over the last three decades (Tables 1 and 2).

Previous experimental and modelling studies have quantified the historical trajectories of annual nutrient export from the Po River basin (e.g., Cozzi et al., 2018; Viaroli et al., 2018), but none of them have isolated the seasonal contributions and analysed their temporal evolution, although a preliminary investigation of N export during the spring-summer months has been reported by Gervasio and co-authors (2022a). The present

Table 2 – Decadal changes (1990–2020) of the soil P budget (SB-P) and the P load from point sources (PS-P) in the Po River basin. All budget terms are expressed as ktons P/year.

Year	P_{Man}	P_{Fert}	P_{Dep}	P_{Harv}	SB-P	PS-P
1990	62.8±4.2	55.38±0.7	0.67±0.1	53.1±3.1	**65.8±8.1**	**6.5±0.3**
2000	57.4±3.2	37.95±0.5	0.67±0.1	63.36±4.0	**32.7±7.8**	**3.5±0.6**
2010	57.71±3.1	27.92±0.4	0.61±0.1	50.72±4.0	**35.5±7.5**	**3.6±0.6**
2020	49.9±2.7	27.1±0.4	0.50±0.1	51.70±4.0	**25.8±7.1**	**3.7±0.6**

P_{Man} = P in livestock manure; P_{Fert} = synthetic P fertilizers; P_{Dep} = atmospheric P deposition; P_{Harv} = P export with crop harvest results clearly show that considering the overall decline in annual N loads may mask significant differences in the load trends at the seasonal scale. In the Po River, the main contributions to the annual TN loads were autumn (34% on average, range 17%–56%) and winter (29% on average, range 14%–42%), while spring and summer loads represented 15%–41% (23% on average) and 8%–21% (14% on average), of the corresponding annual values, respectively (Fig. 2). The analysis of the seasonal trends showed a significant decline in summer and autumn for TN loads (Fig. 2d, e, Appendix A Table S4), decreasing on average by 26% and 32%, respectively, in the last decade compared to the ‘90s, while this tendency was not detected in winter and spring when the temporal variation was more erratic (Fig. 2b, c, Appendix A Table S4). The seasonal distribution of NO_3^- and NH_4^+ overlapped with that of TN, with an average of two-thirds of the annual loads transported during the autumn-winter months. This is a common finding in watersheds with Mediterranean or temperate climates where most of the riverine N export occurs during the wet season, when hydrological processes overwhelm the in-stream N metabolism (McCrackin et al., 2014; Compton et al., 2020). The time series of NO_3^- loads exhibited a downward trend in summer and autumn (Fig. 2d, e, Appendix A Table S4), decreasing by 32%, whereas no significant decline was observed in spring and winter, although the annual NO_3^- loads were reduced by 12%–22% in the last five years compared to the previous decades. No tendency towards reduced export was observed for P forms, except in summer (Fig. 3d, Appendix A Table S4).

Over the last three decades, the hydrological conditions of the Po River have been characterized by large inter-annual oscillations, but without any significant downward or upward trend. On a seasonal scale, only summer discharge showed a highly significant decrease with a breakpoint in the time series observed in 2002, the same year when the decline in summer TN and NO_3^- loads started (Appendix A Table S4, Table 3). In fact, the Po River experienced temporary phases of low regime during 2003–2007 and 2015–2017 (Zanchettin et al., 2008; Montanari et al., 2012; Marchina et al., 2019), the periods most affected by prolonged droughts with a consequent strong reduction in nutrient loads transported to the coastal zones.

After the flow normalisation procedure, highly significant downward trends were detected for loads of TN and the two dissolved inorganic species, NO_3^- and NH_4^+, at both annual and seasonal scales, with the sole exception of summer (Appendix A Table S4, Fig. 3), highlighting that a considerable contribution to the variability in summer nutrient transport was undoubtedly due to variations in water flow. The most marked decline of TN and NO_3^- loads occurred in spring (15%–19%) and autumn (18%–21%), whereas for NH_4^+ the decrease was generalized and always >30% in the last decade compared to the ‘90s (Fig. 4). In contrast to N, no significant downward trends were detected for the flow-normalized P loads (Fig. 5).

Assessing the intra-annual dynamics of nutrient loads is crucial for identifying those periods when the risk of eutrophication is particularly high. Phytoplankton growth and coastal biogeochemical dynamics are constrained by environmental factors that follow seasonal cycles (Glibert, 2017; Cozzi et al., 2018; Ricci et al., 2022), but seasonal patterns of nutrient loads are rarely analysed (Gervasio et al., 2022a), and there is a lack of systematic research predicting the effects of climate change on river nutrient cycling alongside the expected intra-annual export variations. This study provides the first evidence of long-term changes in N export from the Po River, demonstrating a generalized decline over the years but with different extent at the seasonal scale: lower N loads are transported to the Adriatic Sea throughout the spring-summer-early autumn period, when the risk of eutrophication in the coastal lagoons is higher. Summer N loads declined owing to a reduction in water flow, while downward trends in the other seasons are likely to be related to other drivers that require further investigation.

2.2. *Water temperature trends*

Since 1992, the annual average water temperature of the Po River has increased by almost 3.5°C, corresponding to an overall rate of 0.12°C/year and the warming accelerated in 2004, when a breakpoint in the time series was detected (Table 3). The analysis of the daily river temperatures showed the existence of two phases: a condition of relative stability characterized the first decade of the series with an average value of 13.91±0.24°C and low inter-annual variability, then a marked increase occurred, and the temperature varied widely among the years with a mean value of 15.95±0.97°C (Fig. 6a). Highly significant upward trends were identified in all seasons, with the most pronounced warming signals in summer (0.13°C/year) and autumn (0.16°C/year) starting in 2002 and 2006, respectively (Table 3), while the spring and winter increases averaged at 0.10°C/year (Fig. 6a). The present outcomes were consistent with the analysis of the air temperature trends, which showed a significant warming in annual and seasonal values, with the highest slope in summer and the early 2000s as change point years in the time series (Appendix A, Fig. S3, Table S5). This is in agreement with previous long-term climate studies and model-based climate projections demonstrating that warming has occurred throughout the Po River basin since the ‘80s, with more pronounced changes in summer followed by spring and elevated temperatures maintained until the mid-autumn (Appiotti et al., 2014; Fioravanti et al., 2016; Vezzoli et al., 2015).

The temperature increase measured for the Po River was found to be almost an order of magnitude (or at the very least two times) faster than the increases observed in other large temperate European rivers over similar time periods (Hannah and Garner, 2015; Arora et al., 2016; Hardenbicker et al., 2017; Ptak et al., 2022), suggesting that Mediterranean watercourses may be among the most vulnerable worldwide to the impacts of climate change and require more research attention.

Similar to the trends observed for mean temperature, the minimum and maximum water temperature time series showed marked warming since the 2000s and an increased inter-annual variability for both annual and the seasonal values (Fig. 6b, c, Table 3). The highest annual temperature values (up to 29–31°C) were recorded in 2003–2007 and 2015–2017, two periods characterized by both climatic anomalies (reduced precipitation, high air temperature in summer) and hydrological extremes (phases of low flow regime in

Table 3 – Summary of statistical results from the trend analysis performed on water temperature and discharge datasets. Values in bold are statistically significant at p=0.05. Arrows indicate significant increasing or decreasing trends.

Variable	Period	Linear regression	Mann-Kendall				Pettitt test		
		p-value	Kendall's tau	*p*-value	Sen's slope	Trend	K	*p*-value	Change point year
Average water temperature	Annual	**<0.0001**	**0.695**	**<0.0001**	**0.124**	↑	**200**	**<0.0001**	**2004**
	Winter	**<0.0001**	**0.488**	**0.0002**	**0.103**	↑	**182**	**<0.0001**	**2006**
	Spring	**0.0019**	**0.453**	**0.0006**	**0.093**	↑	**152**	**0.004**	**2002**
	Summer	**<0.0001**	**0.567**	**<0.0001**	**0.134**	↑	**198**	**<0.0001**	**2002**
	Autumn	**<0.0001**	**0.700**	**<0.0001**	**0.160**	↑	**206**	**<0.0001**	**2005**
Minimum water temperature	Annual	**0.0001**	**0.545**	**<0.0001**	**0.120**	↑	**188**	**<0.0001**	**2005**
	Winter	**0.0001**	**0.441**	**0.0008**	**0.101**	↑	**170**	**0.0004**	**2006**
	Spring	**0.0025**	**0.385**	**0.0036**	**0.094**	↑	122	0.052	2003
	Summer	**<0.0001**	**0.647**	**<0.0001**	**0.157**	↑	**196**	**<0.0001**	**2002**
	Autumn	**0.0003**	**0.451**	**0.0006**	**0.120**	↑	**146**	**0.0062**	**2005**
Maximum water temperature	Annual	**<0.0001**	**0.527**	**<0.0001**	**0.162**	↑	**204**	**<0.0001**	**2004**
	Winter	**<0.0001**	**0.438**	**0.0009**	**0.147**	↑	**154**	**0.0024**	**2010**
	Spring	**0.0007**	**0.481**	**0.0003**	**0.160**	↑	**182**	**<0.0001**	**2001**
	Summer	**<0.0001**	**0.527**	**<0.0001**	**0.162**	↑	**204**	**<0.0001**	**2004**
	Autumn	**<0.0001**	**0.621**	**<0.0001**	**0.224**	↑	**210**	**<0.0001**	**2005**
Frequency of occurrence	Annual	**<0.0001**	**0.672**	**<0.0001**	**0.005**	↑	**195**	**<0.0001**	**2005**
	Winter	**0.0005**	**0.388**	**0.0034**	**0.013**	↑	**154**	**0.002**	**2006**
	Spring	**0.0068**	**0.410**	**0.0021**	**0.009**	↑	**143**	**0.0086**	**2002**
	Summer	**<0.0001**	**0.535**	**<0.0001**	**0.018**	↑	**181**	**<0.0001**	**2002**
	Autumn	**<0.0001**	**0.644**	**<0.0001**	**0.014**	↑	**208**	**<0.0001**	**2004**
Average discharge	Annual	0.2225	-0.172	0.196	-11.591		102	0.193	2002
	Winter	0.8338	0.020	0.8955	2.161		56	0.6352	2008
	Spring	0.9554	-0.074	0.5865	-4.158		64	0.9118	2002
	Summer	**0.0025**	**-0.379**	**0.0041**	**-17.511**	↓	**172**	**<0.0001**	**2002**
	Autumn	0.1210	-0.158	0.2373	-23.647		82	0.5262	2000

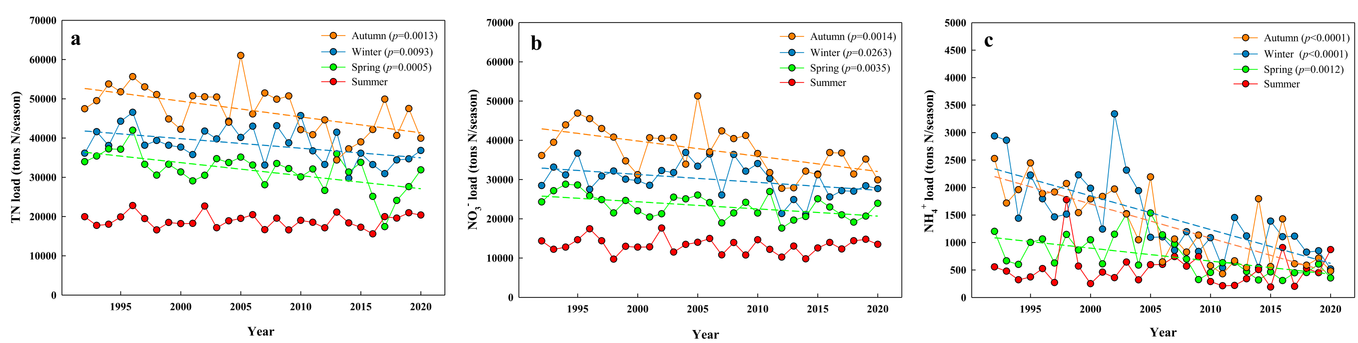

Fig. 4 – Trajectories of flow-normalized seasonal TN (panel a), NO_3^- (panel b), and NH_4^+ loads (panel c) measured at the Po River closing section.

the Po River) (Zanchettin et al., 2008; Appiotti et al., 2014; Marchina et al., 2019). The highest warming rates for the Po River water were observed for maximum temperatures in spring, summer (0.16°C/year) and autumn (0.22°C/year) (Fig. 6), together with the highest increase in the frequency of occurrence, i.e. the number of days in each season with water temperature above the long-term average, that passed from ∼30% in the '90s to ∼80% and ∼70% in summer and autumn nowadays, respectively (Fig. 7). Summer and autumn were also characterised by the most pronounced increase in air temperature and frequency of occurrence of warm days (Appendix A, Fig. S3, Fig. S4, Table S5). In parallel with the positive trends in water temperature, the occurrence of warm days showed a noticeable increase at both the annual and the seasonal scales (Fig. 7; Table 3), suggesting an extension of the vegetative season length, as previously shown for the entire Mediterranean region (Efthymiadis et al., 2011) and other large European rivers (Hardenbicker et al., 2017). Water temperature trends were generally superimposed on air temperature trends, which is the main forcing factor explaining long-term river temperature trajectories and inter-annual variability. The coupled effect of air temperature warming, and reduced flow may explain why water temperature increased faster than air temperature, as found for

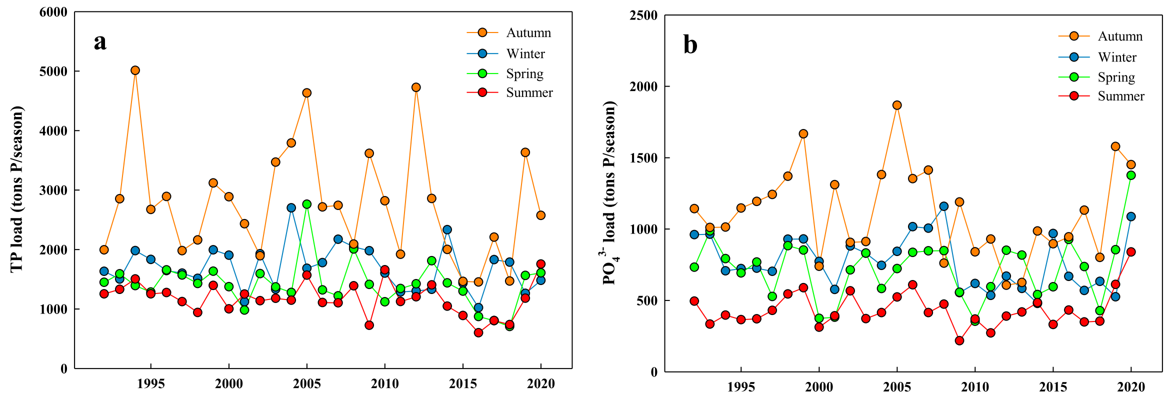

Fig. 5 – Trajectories of flow-normalized seasonal TP (panel a), and PO_4^{3-} loads (panel b) measured at the Po River closing section.

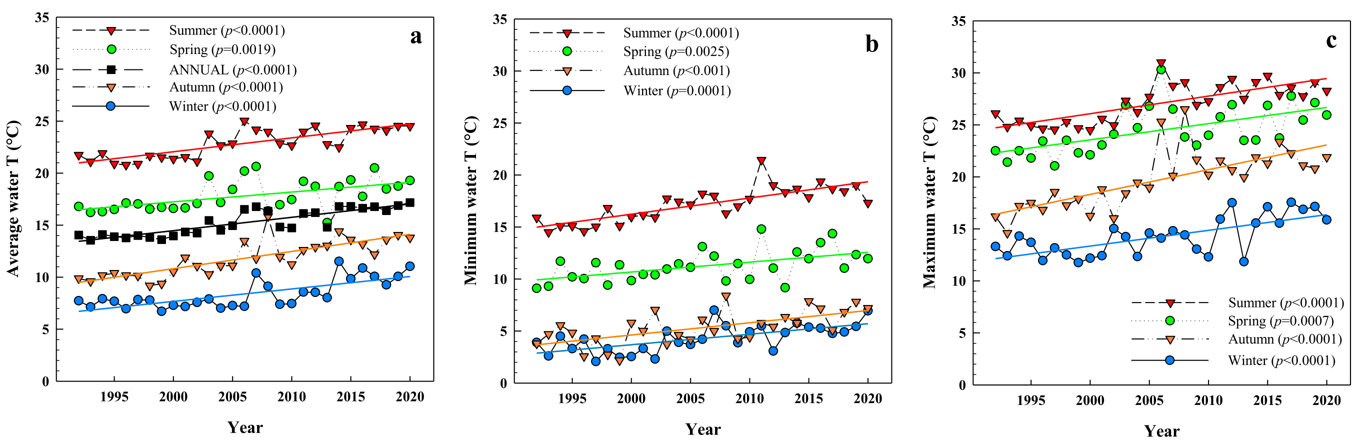

Fig. 6 – Temporal trends of average (panel a), minimum (panel b), and maximum (panel c) temperature of the Po River water.

other large European rivers (Seyedhashemi et al., 2022). This effect is likely to be exacerbated in the future, as the Po River is expected to experience lower flows and more severe summer droughts because of reduced rainfall (Vezzoli et al., 2015).

2.3. *Impact of water temperature warming on in-stream nutrient cycling*

The link between climate change and eutrophication is controversial and currently under discussion. Several previous studies have reported an increased risk of eutrophication due to warming in lentic water bodies (Jenny et al., 2016; Woolway et al., 2022). Although the scientific community has made remarkable progress in addressing the impacts of climate change in lakes and coastal zones (Glibert, 2017; Meerhoff et al., 2022), the potential feedbacks between climatic anomalies, river functioning, and water quality are still understudied and far from being evidenced and proven. Nevertheless, their understanding is crucial for the definition and implementation of effective watershed management strategies to counteract eutrophication in heavily exploited areas, such as the Po basin. The intensification of extreme climatic events causes sharp fluctuations in hydrological conditions, with marked alternation between flash floods and periods of minimum flow, thereby affecting the rates of nutrient export from agricultural soils to aquatic ecosystems, the water residence time and, consequently, the rates of permanent removal or temporary sequestration (Baron et al., 2013; Goyette et al., 2019; Costa et al., 2022). Climate change is generally expected to further delay European water quality goals by altering both the amount and timing of nutrient delivery from terrestrial ecosystems through changes in the magnitude and seasonal patterns of precipitation (Abily et al., 2021; Rozemeijer et al., 2021). Catchment-scale modelling studies developed for northern Europe have predicted boosted and accelerated annual riverine nutrient export in response to projected increase in precipitation and rising leaching effects of increased water flows, and further enhanced by temperature-induced nutrient mineralisation in soils (Øygarden et al., 2014; Plunge et al., 2022). In contrast, for the Mediterranean region, the climate models predicted a shift in the seasonal pattern of precipitation rather than a change in the total amount of precipitation, with more severe droughts in the summer and flash floods in the other seasons (Sperna Weiland et al., 2021). The alternation between large floods and droughts is likely to disrupt biogeochemical dynamics in rivers, resulting in reduced dilution effect during low flow periods and the rapid downstream delivery of large quantities of nutrients, particularly in particulate form, owing to the erosive power of intense

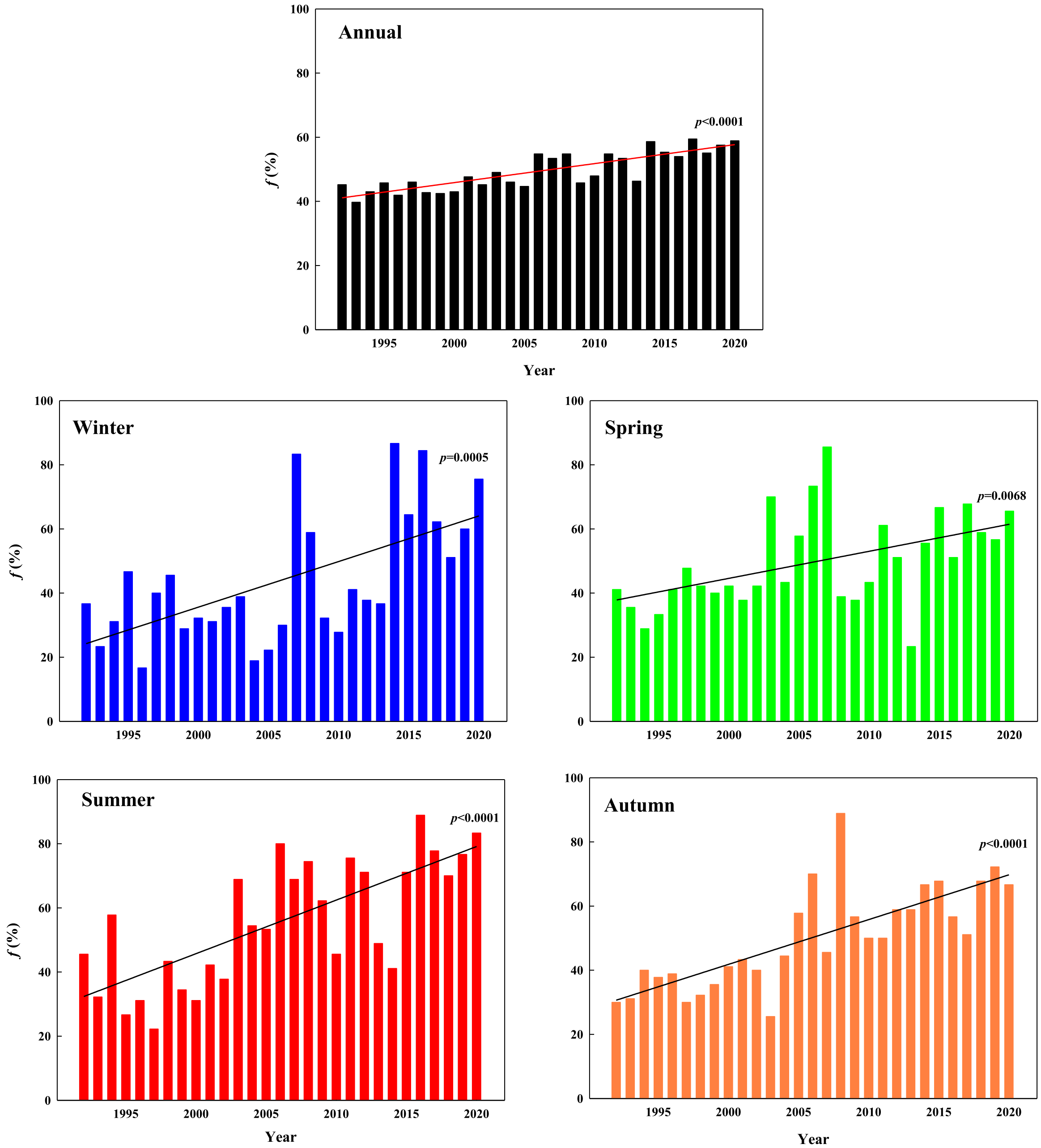

Fig. 7 – Frequency of occurrence of days with Po River water temperature above the long-term average (1992–2020). Long-term average values calculated from daily measurements were 15.2°C, 8.4°C, 17.8°C, 22.9°C, and 11.8°C for the annual, winter, spring, summer, autumn periods, respectively.

rainfall events (Withers and Jarvie, 2008; Goyette et al., 2019; Abily et al., 2021).

Contrary to previous studies in northern European catchments, a generalized negative trend in annual N loads was highlighted for the Po River, associated with a discharge reduction only for the summer season. No significant variations in annual P export were observed and this, together with the hydrological evidence, i.e., no significant long-term trend detected for the annual discharge, strongly supports the hypothesis that the riverine N load decline was not due to variations in load generation and transport but triggered by temperature-dependent effects increasing the river metabolic capacity. Highly significant negative seasonal correlations were found between monthly average water temperature and TN loads and of its main dissolved inorganic species, i.e., NO_3^- and NH_4^+ (Fig. 8). No significant relationship was found between water temperature and P export, highlighting that the main mechanisms responsible for P processing are not as strictly temperature dependent as N, which relies on key microbial processes such as nitrification and denitrification. In large turbid rivers, such as the Po, P cycling is likely less sensitive to temperature warming and the negative relationship found between water temperature and P loads only in summer (Fig. 9) likely reflects increased PO_4^{3-} uptake by

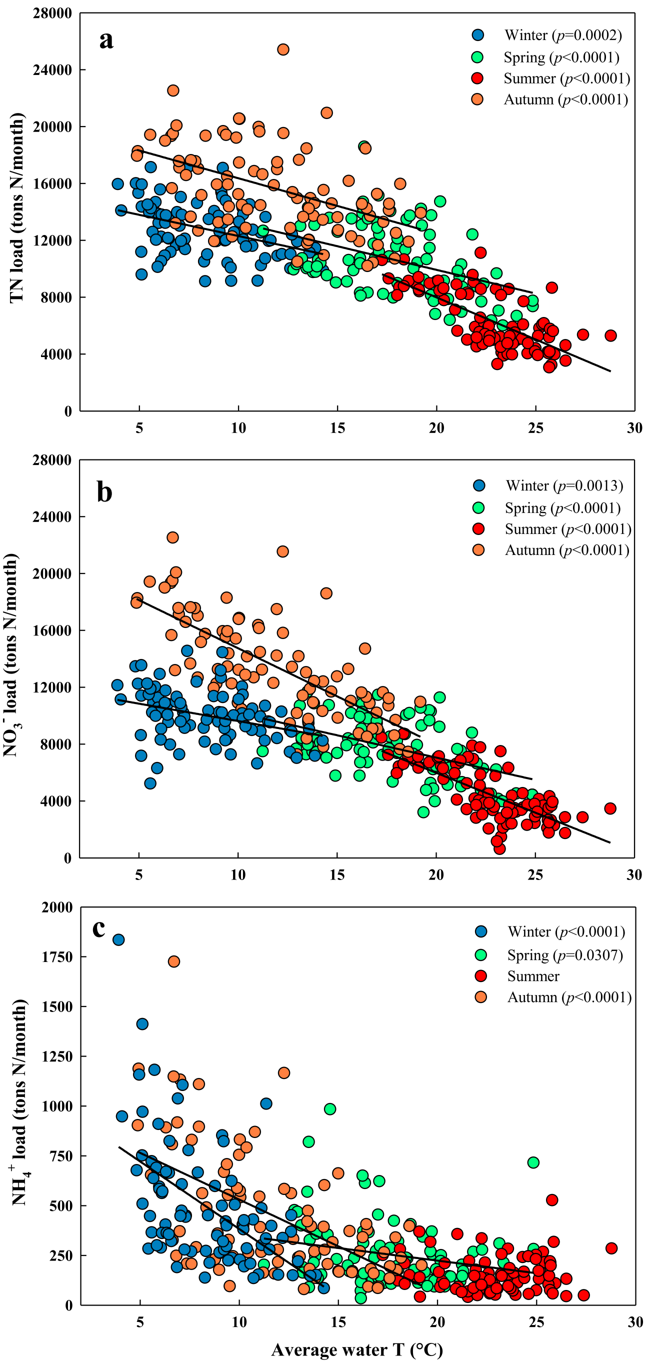

Fig. 8 – Correlations between monthly average water temperature and N loads measured at the Po River closing section.

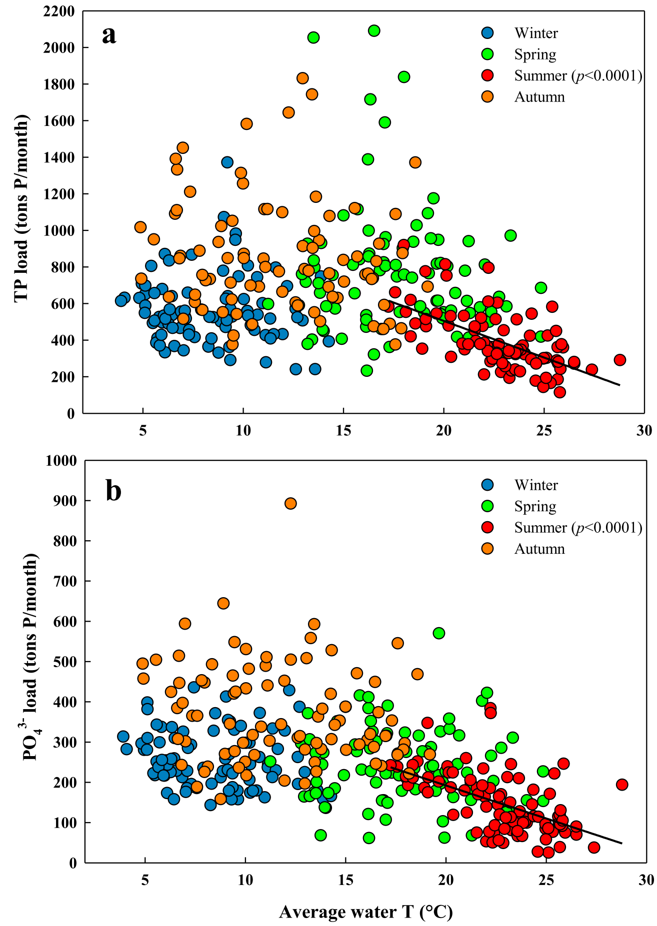

Fig. 9 – Correlations between monthly average water temperature and P loads measured at the Po River closing section.

phytoplankton (Withers and Jarvie, 2008). Differently to P, a 1°C increase in water temperature resulted in a 7% decrease of monthly TN and NO_3^- export in summer, almost 4% in spring and autumn, and about 2% in winter. Underlying biogeochemical processes support the observed inverse relationship between water temperature and N loads (Fig. 8). Water temperature warming is likely to stimulate heterotrophic activity, including denitrification, and create more favourable conditions for permanent NO_3^- removal (de Klein et al., 2017; Velthuis and Veraart, 2022), resulting in lower N delivery to the coastal zones.

The Po River basin is a global hotspot for NO_3^- pollution and eutrophication, and in recent years, denitrification rates have been widely measured in aquatic ecosystems within the basin (e.g., drainage canals, riverine wetlands, pit lakes and lagoons), as recently reviewed by Gervasio and co-authors (2022b). On the contrary, denitrification has been little documented in the riverbed sediments of the main channel. Evidence of riverine denitrification has been observed through the isotopic signature of river water collected from deltaic branches in summer (Marchina et al., 2016). Some preliminary measurements of denitrification were performed recently in the lowland reach in mid-summer by incubation of intact sediment cores. The good agreement between NO_3^- consumption and N_2 production proved that denitrification is the dominant mechanism driving NO_3^- removal from the water column, with rates among the highest found in freshwater and brackish environments (Pina-Ochoa and Álvarez-Cobelas, 2006; Reisinger et al., 2016; Gervasio et al., 2022b).

Data on dissolved organic carbon in the lowland reach of the Po River are scarce (Pettine et al., 1998) and its routine measurement has only recently been included in the institutional monitoring programs. Concentrations average at 2.6 mg/L without any clear seasonal behavior, indicating that organic carbon is in equilibrium with NO_3^- availability (2.1 mg N/L, average value for the period 2018-2020). In fact, denitrification is an anaerobic respiration requiring organic carbon and NO_3^- in an approximate 1:1 ratio (Taylor and Townsend, 2010). When process substrates (i.e., NO_3^- and labile organic carbon) are not limiting, denitrification has a strong positive temperature dependence and is therefore sensitive to warming, as are all biogeochemical reactions controlled by microbial activities (de Klein et al., 2017; Velthuis and Veraart, 2022). This is consistent with the NO_3^- load time-series, which showed the most pronounced decline in summer and autumn, corresponding to the most marked increase in water temperature (~3.5°C and ~4°C in summer and autumn, respectively). Predicting the effects of climate change on denitrification in aquatic ecosystems is challenging because of the intertwined biogeochemical dynamics involved. Furthermore, experimental evidence on the temperature dependence of denitrification is sparse and highly variable between systems. However, a comprehensive synthesis of empirical and modeling results obtained in freshwater sediments has suggested that, in the range 15–25°C, denitrification rates may double upon a three-degree temperature rise (Veraart et al., 2011).

Nitrogen fate in rivers is not only affected by rising temperature, but other hydrological features depending on climate change, are also likely to impact the proximal drivers controlling N processing. Water warming leads to significantly greater N removal efficiency during summer low flow conditions, when denitrification and biological uptake effectively control N loads and prevent delivery to downstream waters due to longer water residence time and higher ratio of bioactive surface area to water volume (Birgand et al., 2007; Reisinger et al., 2016). For the Po River, a significant increase in water temperature in all seasons, together with an extension of the vegetative season length (i.e., an extended seasonal window of warm water temperatures) and prolonged durations of low flow periods, likely resulted in a longer residence time for NO_3^- and more opportunities for its removal in the river sediments along the lowland reaches. This evidence is supported by the inverse relationship between the number of warm days (i.e., days with water temperature above the long-term average) and the export of dissolved inorganic N forms in the spring-summer period (Appendix A, Fig. S5). It cannot be excluded that exceptional conditions of water scarcity and prolonged drought may partially disconnect rivers from floodplains, wetlands, and riparian ecosystems, reducing the extent of flooded areas and thus the possibility of reactive N removal and the denitrification capacity of the whole river system (Bernal et al., 2013; Cerco and Tian, 2021).

The negative relationship between water temperature and NH_4^+ loads in all seasons, except summer, suggests that warming may also have stimulated nitrification (Pina-Ochoa and Álvarez-Cobelas, 2006; Birgand et al., 2007). In the Po River, the water column is indeed generally well mixed, and the dissolved oxygen concentration is generally at or near 100% saturation, making the oxygen status of the surface sediment suitable to support coupled nitrification-denitrification (Gervasio et al., 2022a). This condition differs from that of other Mediterranean rivers which are experiencing declining oxygen levels correlated to upward trends of water temperatures (Diamantini et al., 2018). On the other hand, prolonged summer low flow conditions may promote the establishment of thermal and/or saline gradients and lead to partial or complete stratification, preventing the complete mixing of the water column and the NH_4^+ oxidation in the benthic compartment due to limited oxygen availability at the sediment-water interface (Gervasio et al., 2022b; Tarolli et al., 2023).

Despite the importance of bacterial denitrification in regulating N fluxes along the terrestrial-freshwater-marine continuum, the effect of ongoing hydrological variations and water temperature shifts on this process remains understudied. As data on the response of denitrification to temperature are usually obtained from microcosm experiments, it is challenging to forecast larger scale effects. Inferring how microbial communities will respond to gradual temperature increases, such as those occurring in aquatic ecosystems, is not entirely possible from short-term laboratory studies (Veraart et al., 2011; de Klein et al., 2017), making the outcome of global environmental change on N removal efficiency difficult to predict, despite the need to optimize future N budgets and management strategies. Watershed-scale studies have demonstrated a negative relationship between air temperature and N export to coastal zones (Schaefer and Alber, 2007; Salmon-Monviola et al., 2013; Wagena et al., 2018), suggesting that warming may lead to significantly higher denitrification efficiency across the landscape. Similarly, the results of the present work suggest that increased riverine denitrification associated with rising water temperatures may contribute to mitigate the N loads exported from Mediterranean catchments, with relevant implications to partially prevent coastal eutrophication.

3. Conclusions

The present study showed that for the Po River, downward trends in N export over the last three decades were correlated with upward trends in water temperature and an increasing number of warm days. The reduction of N loads can be seen as an unexpected consequence of climate change, enhancing rates of microbial denitrification in river sediments, with potential negative feedback on coastal eutrophication. In human-impacted watersheds, the study of the generation, transport, and transformation of nutrient loads is a key issue for the implementation of environmental policies to control eutrophication and protect coastal zones. Future scenarios of nutrient export from catchments must consider the simultaneous effects of hydrological changes and temperature increases that are already co-occurring. There is still a lack of scientific understanding of the net effects of climatic and hydrological extremes (e.g., dry summers or springs with unusually high temperatures) on the biogeochemistry of both freshwater and coastal ecosystems. It is crucial to investigate the combined effects of multiple stressors such as temperature, nutrient pulses following flood events, saline intrusion, on the self-depuration capacity and the balance between N removal and recycling processes in lowland stretches and deltas in order to predict how climate change will alter N fate in rivers. Furthermore, to fully understand how climate change will affect eutrophication, it is necessary to incorporate the results of experimental studies on the intrinsic temperature response of key nutrient cycling processes into catchment-scale models.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2023.07.008.

References

Abily, M., Acuña, V., Gernjak, W., Rodríguez-Roda, I., Poch, M., Corominas, L., 2021. Climate change impact on EU rivers' dilution capacity and ecological status. Water Res. 199, 117166. doi:10.1016/j.watres.2021.117166.

Addinsoft, 2022. XLSTAT Statistical and Data Analysis Solution Paris, France. https://www.xlstat.com .

Antolini, G., Auteri, L., Pavan, V., Tomei, F., Tomozeiu, R., Marletto, V., 2016. A daily high-resolution gridded climatic data set for Emilia-Romagna, Italy, during 1961–2010. Int. J. Climatol. 36 (4), 1970–1986. doi:10.1002/joc.4473.

American Public Health Association (A.P.H.A.), American Water Works Association, Water Environment Federation, 2005. Standard Methods for the Examination of Water and Wastewater, 21st Ed. Washington DC.

APAT—IRSA/CNR. 2003. Analytical methods for water. Manuals and guidelines, 29/2003 (in Italian) https://www.isprambiente.gov.it/it/pubblicazioni/manuali-e-linee-guida/metodi-analitici-per-le-acque.

Appiotti, F., Krželj, M., Russo, A., Ferretti, M., Bastianini, M., Marincioni, F., 2014. A multidisciplinary study on the effects of climate change in the northern Adriatic Sea and the Marche region (central Italy). Regional Environ. Change 14 (5), 2007–2024. doi:10.1007/s10113-013-0451-5.

Arora, R., Tockner, K., Venohr, M., 2016. Changing river temperatures in northern Germany: trends and drivers of change. Hydrol. Processes 30 (17), 3084–3096. doi:10.1002/hyp.10849.

Artioli, Y., Friedrich, J., Gilbert, A.J., McQuatters-Gollop, A., Mee, L.D., Vermaat, J.E., et al., 2008. Nutrient budgets for European seas: a measure of the effectiveness of nutrient reduction policies. Mar. Pollut. Bull. 56 (9), 1609–1617. doi:10.1016/j.marpolbul.2008.05.027.

Baron, J.S., Hall, E.K., Nolan, B.T., Finlay, J.C., Bernhardt, E.S., Harrison, J.A., et al., 2013. The interactive effects of excess reactive nitrogen and climate change on aquatic ecosystems and water resources of the United States. Biogeochemistry 114, 71–92. doi:10.1007/s10533-012-9788-y.

Batool, M., Sarrazin, F.J., Attinger, S., Basu, N.B., Van Meter, K., Kumar, R., 2022. Long-term annual soil nitrogen surplus across Europe (1850–2019). Scientific Data 9 (1), 1–22. doi:10.1038/s41597-022-01693-9.

Battye, W., Aneja, V.P., Schlesinger, W.H., 2017. Is nitrogen the next carbon? Earth's Future 5 (9), 894–904. doi:10.1002/2017EF000592.

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. Scientific Data 5 (1), 1–12. doi:10.1038/sdata.2018.214.

Bernal, S., von Schiller, D., Sabater, F., Martí, E., 2013. Hydrological extremes modulate nutrient dynamics in mediterranean climate streams across different spatial scales. Hydrobiologia 719 (1), 31–42. doi:10.1007/s10750-012-1246-2.

Birgand, F., Skaggs, R.W., Chescheir, G.M., Gilliam, J.W., 2007. Nitrogen removal in streams of agricultural catchments—a literature review. Crit. Rev. Environ. Sci. Technol. 37 (5), 381–487. doi:10.1080/10643380600966426.

Blaas, H., Kroeze, C., 2016. Excessive nitrogen and phosphorus in European rivers: 2000–2050. Ecol. Indic. 67, 328–337. doi:10.1016/j.ecolind.2016.03.004.

Cerco, C.F., Tian, R., 2021. Impact of wetlands loss and migration, induced by climate change, on Chesapeake Bay DO standards. JAWRA J. Am. Water Res. Ass. 58 (6), 958–970. doi:10.1111/1752-1688.12919.

Compton, J.E., Goodwin, K.E., Sobota, D.J., Lin, J., 2020. Seasonal disconnect between streamflow and retention shapes riverine nitrogen export in the Willamette River Basin. Oregon. Ecosyst. 23 (1), 1–17. doi:10.1007/s10021-019-00383-9.

Coppola, E., Verdecchia, M., Giorgi, F., Colaiuda, V., Tomassetti, B., Lombardi, A., 2014. Changing hydrological conditions in the Po basin under global warming. Sci. Total Environ. 493, 1183–1196. doi:10.1016/j.scitotenv.2014.03.003.

Costa, D., Sutter, C., Shepherd, A., Jarvie, H., Wilson, H., Elliott, J., et al., 2022. Impact of climate change on catchment nutrient dynamics: insights from around the world. Environ. Rev. 31 (1), 4–25. doi:10.1139/er-2021-0109.

Cozzi, S., Ibáñez, C., Lazar, L., Raimbault, P., Giani, M., 2018. Flow regime and nutrient-loading trends from the largest South European watersheds: Implications for the productivity of Mediterranean and Black Sea's Coastal Areas. Water 11 (1), 1. doi:10.3390/w11010001.

de Klein, J.J., Overbeek, C.C., Juncher Jørgensen, C., Veraart, A.J., 2017. Effect of temperature on oxygen profiles and denitrification rates in freshwater sediments. Wetlands 37, 975–983. doi:10.1007/s13157-017-0933-1.

de Wit, M., Bendoricchio, G., 2001. Nutrient fluxes in the Po basin. Sci. Total Environ. 273 (1-3), 147–161. doi:10.1016/S0048-9697(00)00851-2.

Diamantini, E., Lutz, S.R., Mallucci, S., Majone, B., Merz, R., Bellin, A., 2018. Driver detection of water quality trends in three large European river basins. Sci. Total Environ. 612, 49–62. doi:10.1016/j.scitotenv.2017.08.172.

Domeneghetti, A., Castellarin, A., Brath, A., 2012. Assessing rating-curve uncertainty and its effects on hydraulic model calibration. Hydrol. Earth Syst. Sci. 16 (4), 1191–1202. doi:10.5194/hess-16-1191-2012.

Efthymiadis, D., Goodess, C.M., Jones, P.D., 2011. Trends in Mediterranean gridded temperature extremes and large-scale circulation influences. Natural Hazards Earth Syst. Sci. 11 (8), 2199–2214. doi:10.5194/nhess-11-2199-2011.

Fioravanti, G., Piervitali, E., Desiato, F., 2016. Recent changes of temperature extremes over Italy: an index-based analysis. Theor. Appl. Climatol. 123, 473–486. doi:10.1007/s00704-014-1362-1.

Formetta, G., Tootle, G., Therrell, M., 2022. Regional reconstruction of Po River Basin (Italy) streamflow. Hydrology 9 (10), 163. doi:10.3390/hydrology9100163.

Gervasio, M.P., Soana, E., Granata, T., Colombo, D., Castaldelli, G., 2022a. An unexpected negative feedback between climate change and eutrophication: higher temperatures increase denitrification and buffer nitrogen loads in the Po River (Northern Italy). Environ. Res. Lett. 17 (8). doi:10.1088/1748-9326/ac8497.

Gervasio, M.P., Soana, E., Vincenzi, F., Castaldelli, G., 2022b. An underestimated contribution of deltaic denitrification in reducing nitrate export to the coastal zone (Po River–Adriatic Sea, Northern Italy). Water 14 (3), 501. doi:10.3390/w14030501.

Glibert, P.M., 2017. Eutrophication, harmful algae and biodiversity—challenging paradigms in a world of complex nutrient changes. Mar. Pollut. Bull. 124 (2), 591–606. doi:10.1016/j.marpolbul.2017.04.027.

Goyette, J.O., Bennett, E.M., Maranger, R., 2019. Differential influence of landscape features and climate on nitrogen and phosphorus transport throughout the watershed. Biogeochemistry 142 (1), 155–174. doi:10.1007/s10533-018-0526-y.

Hannah, D.M., Garner, G., 2015. River water temperature in the United Kingdom: changes over the 20th century and possible changes over the 21st century. Prog. Phys. Geog. 39 (1), 68–92. doi:10.1177/0309133314550669.

Hardenbicker, P., Viergutz, C., Becker, A., Kirchesch, V., Nilson, E., Fischer, H., 2017. Water temperature increases in the river Rhine in response to climate change. Regional Environ. Change 17, 299–308. doi:10.1007/s10113-016-1006-3.

Helsel, D.R., Hirsch, R.M., Ryberg, K., Archfield, S., Gilroy, E., 2020. Statistical Methods in Water Resources Techniques and Methods 4 A3. USGS Tech. Methods 458. https://pubs.er.usgs.gov/publication/tm4A3.

Hill, A.R., 2023. Patterns of nitrate retention in agriculturally influenced streams and rivers. Biogeochemistry 163 (2), 155–183. doi:10.1007/s10533-023-01027-w.

Howarth, R., Swaney, D., Billen, G., Garnier, J., Hong, B., Humborg, C., et al., 2012. Nitrogen fluxes from the landscape are controlled by net anthropogenic nitrogen inputs and by climate. Front. Ecol. Environ. 10 (1), 37–43. doi:10.1890/100178.

IPCC, 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Jenny, J.P., Francus, P., Normandeau, A., Lapointe, F., Perga, M.E., Ojala, A., et al., 2016. Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. Global Change Biol. 22 (4), 1481–1489. doi:10.1111/gcb.13193.

Lenhart, H.J., Mills, D.K., Baretta-Bekker, H., Van Leeuwen, S.M., Van Der Molen, J., Baretta, J.W., et al., 2010. Predicting the consequences of nutrient reduction on the eutrophication status of the North Sea. J. Mar. Syst. 81 (1-2), 148–170. doi:10.1016/j.jmarsys.2009.12.014.

Li, X., Xu, Y.J., Ni, M., Wang, C., Li, S., 2023. Riverine nitrate source and transformation as affected by land use and land cover. Environ. Res. 222, 115380. doi:10.1016/j.envres.2023.115380.

Ludwig, W., Dumont, E., Meybeck, M., Heussner, S., 2009. River discharges of water and nutrients to the Mediterranean and Black Sea: major drivers for ecosystem changes during past and future decades? Prog. Oceanogr. 80 (3-4), 199–217. doi:10.1016/j.pocean.2009.02.001.

Malone, T.C., Newton, A., 2020. The globalization of cultural eutrophication in the coastal ocean: causes and consequences. Front. Marine Sci. 7, 670. doi:10.3389/fmars.2020.00670.

Marchina, C., Bianchini, G., Natali, C., Knöller, K., 2016. Geochemical and isotopic analyses on the Po delta water: insights to understand a complex riverine ecosystem. Rendiconti Lincei 27, 83–88. doi:10.1007/s12210-015-0465-7.

Marchina, C., Natali, C., Bianchini, G., 2019. The Po River water isotopes during the drought condition of the year 2017. Water 11 (1), 150. doi:10.3390/w11010150.

McCrackin, M.L., Harrison, J.A., Compton, J.E., 2014. Factors influencing export of dissolved inorganic nitrogen by major rivers: A new, seasonal, spatially explicit, global model. Global Biogeochem. Cycles 28 (3), 269–285. doi:10.1002/2013GB004723.

Meerhoff, M., Audet, J., Davidson, T.A., De Meester, L., Hilt, S., Kosten, S., et al., 2022. Feedback between climate change and eutrophication: revisiting the allied attack concept and how to strike back. Inland Waters, pp. 1–18. doi:10.1080/20442041.2022.2029317.

Moatar, F., Meybeck, M., 2005. Compared performances of different algorithms for estimating annual nutrient loads discharged by the eutrophic River Loire. Hydrol. Processes 19 (2), 429–444. doi:10.1002/hyp.5541.

Montanari, A., 2012. Hydrology of the Po River: looking for changing patterns in river discharge. Hydrol. Earth Syst. Sci. 16 (10), 3739–3747. doi:10.5194/hess-16-3739-2012.

Nava, V., Patelli, M., Rotiroti, M., Leoni, B., 2019. An R package for estimating river compound load using different methods. Environ. Modell. Software 117, 100–108. doi:10.1016/j.envsoft.2019.03.012.

Øygarden, L., Deelstra, J., Lagzdins, A., Bechmann, M., Greipsland, I., Kyllmar, K., et al., 2014. Climate change and the potential effects on runoff and nitrogen losses in the Nordic–Baltic region. Agricult., Ecosyst. Environ. 198, 114–126. doi:10.1016/j.agee.2014.06.025.

Palmeri, L., Bendoricchio, G., Artioli, Y., 2005. Modelling nutrient emissions from river systems and loads to the coastal zone: Po River case study, Italy. Ecol. Modell. 184 (1), 37–53. doi:10.1016/j.ecolmodel.2004.11.007.

Pettine, M., Patrolecco, L., Camusso, M., Crescenzio, S., 1998. Transport of carbon and nitrogen to the northern Adriatic Sea by the Po River. Estuarine Coastal Shelf Sci. 46 (1), 127–142. doi:10.1006/ecss.1997.0303.

Pina-Ochoa, E., Álvarez-Cobelas, M., 2006. Denitrification in aquatic environments: a cross-system analysis. Biogeochemistry 81 (1), 111–130. doi:10.1007/s10533-006-9033-7.

Plunge, S., Gudas, M., Povilaitis, A., 2022. Expected climate change impacts on surface water bodies in Lithuania. Ecohydrol. Hydrobiol. 22 (2), 246–268. doi:10.1016/j.ecohyd.2021.11.004.

Po River District Authority. 2021. Management Plan of the hydrographic district of the River Po. In Italian. https://pianoacque.adbpo.it/piano-di-gestione-2021/

Ptak, M., Sojka, M., Graf, R., Choiński, A., Zhu, S., Nowak, B., 2022. Warming Vistula River–the effects of climate and local conditions on water temperature in one of the largest rivers in Europe. J. Hydrol. Hydromech. 70 (1), 1–11. doi:10.2478/johh-2021-0032.

Ravazzani, G., Barbero, S., Salandin, A., Senatore, A., Mancini, M., 2015. An integrated hydrological model for assessing climate change impacts on water resources of the upper Po river basin. Water Resour. Manage. 29 (4), 1193–1215. doi:10.1007/s11269-014-0868-8.

Reisinger, A.J., Tank, J.L., Hoellein, T.J., Hall Jr, R.O., 2016. Sediment, water column, and open-channel denitrification in rivers measured using membrane-inlet mass spectrometry. J. Geophys. Res. 121 (5), 1258–1274. doi:10.1002/2015JG003261.

Ricci, F., Capellacci, S., Campanelli, A., Grilli, F., Marini, M., Penna, A., 2022. Long-term dynamics of annual and seasonal physical and biogeochemical properties: Role of minor river discharges in the North-western Adriatic coast. Estuarine Coastal Shelf Sci. 107902. doi:10.1016/j.ecss.2022.107902.

Romero, E., Ludwig, W., Sadaoui, M., Lassaletta, L., Bouwman, A.F., Beusen, A.H., et al., 2021. The Mediterranean region as a paradigm of the global decoupling of N and P between soils and freshwaters. Global Biogeochem. Cycles 35 (3). doi:10.1029/2020GB006874, e2020GB006874.

Rozemeijer, J., Noordhuis, R., Ouwerkerk, K., Pires, M.D., Blauw, A., Hooijboer, A., et al., 2021. Climate variability effects on eutrophication of groundwater, lakes, rivers, and coastal waters in the Netherlands. Sci. Total Environ. 771, 145366. doi:10.1016/j.scitotenv.2021.145366.

Salmon-Monviola, J., Moreau, P., Benhamou, C., Durand, P., Merot, P., Oehler, F., et al., 2013. Effect of climate change and increased atmospheric CO_2 on hydrological and nitrogen cycling in an intensive agricultural headwater catchment in western France. Clim. Change 120 (1–2), 433–447. doi:10.1007/s10584-013-0828-y.

Schaefer, S.C., Alber, M., 2007. Temperature controls a latitudinal gradient in the proportion of watershed nitrogen exported to coastal ecosystems. Biogeochemistry 85, 333–346. doi:10.1007/s10533-007-9144-9.

Seitzinger, S., Harrison, J.A., Böhlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B., et al., 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecol. Appl. 16 (6), 2064–2090. https://doi.org/10.1890/1051-0761(2006)016[2064:DALAWA]2.0.CO;2

Seyedhashemi, H., Vidal, J.P., Diamond, J.S., Thiéry, D., Monteil, C., Hendrickx, F., et al., 2022. Regional, multi-decadal analysis on the Loire River basin reveals that stream temperature increases faster than air temperature. Hydrol. Earth Syst. Sci. 26 (9), 2583–2603. doi:10.5194/hess-26-2583-2022.

Sileika, A.S., Stålnacke, P., Kutra, S., Gaigalis, K., Berankiene, L., 2006. Temporal and spatial variation of nutrient levels in the Nemunas River (Lithuania and Belarus). Environ. Monit. Assess. 122 (1), 335–354. doi:10.1007/s10661-006-9186-9.

Soana, E., Bartoli, M., Milardi, M., Fano, E.A., Castaldelli, G., 2019. An ounce of prevention is worth a pound of cure: managing macrophytes for nitrate mitigation in irrigated agricultural watersheds. Sci. Total Environ. 647, 301–312. doi:10.1016/j.scitotenv.2018.07.385.

Sperna Weiland, F.C., Visser, R.D., Greve, P., Bisselink, B., Brunner, L., Weerts, A.H., 2021. Estimating regionalized hydrological impacts of climate change over europe by performance-based weighting of CORDEX projections. Front. Water 143. doi:10.3389/frwa.2021.713537.

Tarolli, P., Luo, J., Straffelini, E., Liou, Y.A., Nguyen, K.A., Laurenti, R., et al., 2023. Saltwater intrusion and climate change impact on coastal agriculture. PLOS Water 2 (4), e0000121. doi:10.1371/journal.pwat.0000121.

Taylor, P.G., Townsend, A.R., 2010. Stoichiometric control of organic carbon–nitrate relationships from soils to the sea. Nature 464 (7292), 1178–1181. doi:10.1038/nature08985.

Velthuis, M., Veraart, A.J., 2022. Temperature sensitivity of freshwater denitrification and N_2O emission–a meta-analysis. Global Biogeochem. Cycles doi:10.1029/2022GB007339, e2022GB007339.

Veraart, A.J., De Klein, J.J., Scheffer, M., 2011. Warming can boost denitrification disproportionately due to altered oxygen dynamics. PLoS One 6 (3), e18508. doi:10.1371/journal.pone.0018508.

Vezzoli, R., Mercogliano, P., Pecora, S., Zollo, A.L., Cacciamani, C., 2015. Hydrological simulation of Po River (North Italy) discharge under climate change scenarios using the RCM COSMO-CLM. Sci. Total Environ. 521, 346–358. doi:10.1016/j.scitotenv.2015.03.096.

Viaroli, P., Soana, E., Pecora, S., Laini, A., Naldi, M., Fano, E.A., et al., 2018. 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). Sci. Total Environ. 639, 1574–1587. doi:10.1016/j.scitotenv.2018.05.233.

Wagena, M.B., Collick, A.S., Ross, A.C., Najjar, R.G., Rau, B., Sommerlot, A.R., et al., 2018. Impact of climate change and climate anomalies on hydrologic and biogeochemical processes in an agricultural catchment of the Chesapeake Bay watershed, USA. Sci. Total Environ. 637, 1443–1454. doi:10.1016/j.scitotenv.2018.05.116.

Withers, P.J.A., Jarvie, H.P., 2008. Delivery and cycling of phosphorus in rivers: a review. Sci. Total Environ. 400 (1-3), 379–395. doi:10.1016/j.scitotenv.2008.08.002.

Woolway, R.I., Sharma, S., Smol, J.P., 2022. Lakes in hot water: the impacts of a changing climate on aquatic ecosystems. Bioscience 72 (11), 1050–1061. doi:10.1093/biosci/biac052.

Yuan, Z., Jiang, S., Sheng, H., Liu, X., Hua, H., Liu, X., et al., 2018. Human perturbation of the global phosphorus cycle: changes and consequences. Environ. Sci. Technol. 52 (5), 2438–2450. doi:10.1021/acs.est.7b03910.

Zanchettin, D., Traverso, P., Tomasino, M., 2008. Po River discharges: a preliminary analysis of a 200-year time series. Clim. Change 89 (3), 411–433. doi:10.1007/s10584-008-9395-z.

Zheng, J., Cao, X., Ma, C., Weng, N., Huo, S., 2023. What drives the change of nitrogen and phosphorus loads in the Yellow River Basin during 2006-2017? J. Environ. Sci. 126, 17–28. doi:10.1016/j.jes.2022.04.039.