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Influence of anthropic pressures and hydrological variability on nitrogen and phosphorus transport throughout catchments in the Po River District

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1 Introduction

Hydrological connectivity (HC) is a fundamental property of aquatic environments linking biotic with abiotic components and involving different ecosystems (Covino et al., 2017). The HC of a given aquatic ecosystem influences the so-called Land Ocean Aquatic Continuum (LOAC), a series of biogeochemically and physically active systems (Xenopoulos et al., 2017). Examples of environments involved within the LOAC include streams, rivers, floodplains, wetlands, lakes and lagoons that transport and transform nutrients and other elements e.g. from the mountains to the oceans (Regnier et al., 2022). Over the last four decades, the concept of river continuum (Vannote et al., 1980; Ward 1989) has evolved towards the incorporation of the linkages and interactions with terrestrial and marine environments. This new paradigm places enhanced emphasis on the relationship between aquatic and terrestrial ecosystems, with factors such as land cover and land use being commonly considered as determinants of the quality of rivers and streams, reservoirs, estuaries and coastal seas. Understanding the transport and transformation of elements along this hydrological continuum, and how hydrological connectivity affects these processes at local, regional, and global scales, has become particularly relevant due to human alterations to the hydrological system and element cycles (Abbott et al., 2019).

Over the last century, human development has changed the pathways and rates of biogeochemical nutrient cycles worldwide (Billen et al., 1991; Covino 2017; Dodds et al. 2020). The increased resources demand caused by the population growth has driven a substantial surge in the use of fertilizers to support agricultural production, as well as the extraction of fresh water and fossil fuels to sustain industry and energy production. These activities have significantly changed the landscape worldwide, resulting in a rise in nutrient inputs with implications on the quality of soil, water, and the atmosphere. The structure and hydrological patterns of the water systems have also been altered. Overall, human action has modified the genesis, transformation, and transport of nutrients, with consequences throughout the LOAC and effects along the entire trophic webs of the ecosystems involved (Abbott et al., 2019; Galloway et al., 2008; Filippelli, 2008; Jickells et al.; Wurtsbaugh et al., 2019).

1.1 Eutrophication: nutrients excess and stoichiometry in aquatic ecosystems

Nutrients availability, together with temperature and light, is one of the main factors regulating the structure and functioning of aquatic ecosystems (Geider et al., 1998). In particular, nitrogen (N) and phosphorus (P) are essential nutrients for primary production, especially when these elements are below the minimum nutritional requirement of a given phytoplankton or vegetal species. Globally, between the 1960s and 1990s anthropic impacts have doubled the nutrient quantity exported by rivers. Over time, anthropogenic impacts have also increased the number of inorganic forms at the expense of organic forms. For example, in just 30 years there has been an increment in dissolved inorganic P from 40% to 51% and a decrease in organic N from 70% to 57%. Additionally, the ratio between N and P has shifted in favour of N, from 19:1 in the 80s to 30:1 in the last decade (Vilmin et al., 2018; Peñuelas & Sardans, 2022).

Eutrophication can occur naturally in aquatic environments, but human activity has significantly contributed to its expansion through the increased input of reactive forms of N and P, as well as modifications in the timing of input to the aquatic compartment, resulting in what is known as "cultural eutrophication" (Serediak et al., 2013; Vilmin et al., 2018; Wurtsbaugh et al., 2019). Overall, this process leads to a rapid transformation of the aquatic system (Le Moal et al., 2019; Serediak et al., 2013), such as the proliferation of opportunistic algae that can result in a decrease in biodiversity, as they outcompete other primary producers (O'Hare et al., 2018; Piazzi et al., 2012). The rapid growth rate of these algae can also result in harmful blooms and the release of toxins (Facca et al., 2014; Glibert, 2017). In addition, the biomass growth alters the absolute rates and balance between primary production and decomposition, which in extreme cases can lead to hypoxia and the formation of dead zones, the accumulation of toxic compounds from anaerobic microbial pathways, and the disappearance of the more sensitive fauna with changes in community structure and loss of biodiversity (Dodds and Cole, 2007; Conley, Björck, et al. 2009; Viaroli et al., 2015; Diaz and Rosenberg 2017; Glibert, 2017; Le Moal et al., 2019).

However, as summarized by Glibert (2017), the eutrophication of aquatic ecosystems is a multifactorial issue, depending not only on the amount of N and P but also on their relative proportions (stoichiometry) and chemical forms. Within the water column, the reference molar ratio (the Redfield-Brzezinski ratio) is C: N:P: Si = 106: 16: 1: 15-10, although it may vary according to the aquatic system under consideration and the nutrient needs of different algal groups. The N:P ratio influences the growth rates and composition of biological communities, as well as the microbial processes in different aquatic environments. N: P ratio in combination with the availability of Silicon (Si), for example, defines the optimal conditions for the growth of diatoms (Billen and Garnier 2007; Brzezinski, 1985; Maranger et al., 2018; Redfield et al. 1963; Keck & Lepori 2012). Thus, when a Si deficit occurs, harmful algal blooms can take place, with detrimental effects on ecosystem suitability for fishing, aquaculture and bathing (Hilton et al., 2006; Le Moal et al., 2019). At the same time, the C: N:P ratio in combination with the availability of key elements such as S, Fe, Mg and Ca along the redox gradient of the sediment, plays an important role in regulating both respiration rates and anaerobic metabolisms (both bacterial-mediated and geochemical). These processes play a key role in shaping the availability and forms of N and P in the water column (Algeo & Li, 2020; Delgado-Baquerizo et al., 2017; Parsons et al., 2017; Robertson et al., 2019).

1.2 Regulation of nutrients transport and retention: local vs catchment scale

The input of nutrients into aquatic ecosystems is closely linked to the characteristics of the catchment area (land use, hydrology and geology) through which the hydrographic network flows. The type and extent of anthropogenic pressures define, for example, the quantity, quality (pool composition and molar nutrient ratio) and temporal distribution of the nutrient stock (Casquin et al., 2021; Goyette et al., 2019; Shousha et al., 2021). One of the most widely used classifications of nutrient sources concerns the subdivision into diffuse and point sources. A diffuse source (e.g., agriculture) differs from a point source (e.g., sewage) not only because of the different origin (fertiliser and/or manure vs. treated sewage) but also because of the different input pathways (erosion or weathering vs. direct input), implicitly deriving the interactions between catchments and the forms of the nutrients introduced into the hydrographic network (Drewry et al., 2006; Vilmin et al., 2018). (Le Moal et al., 2019). Besides direct anthropic inputs, different biogeochemical processes control the cycling and transfer of N and P along the land-sea continuum. P is primarily influenced by sedimentary dynamics and its bioavailability depends on its form. N, on the other hand, is more soluble and can be exchanged with the atmosphere through denitrification or N-fixation, two processes that respectively eliminate or introduce reactive N. The interaction between sources, biogeochemical properties of the element, and hydrology can therefore cause significant changes in nutrient stoichiometry from headwater catchments to the sea (Alexander et al., 2008; Wurtsbaugh et al., 2019; Shousha et al., 2023).

The relationship between nutrient concentration and discharge has long been used at different spatial (catchment and sub-catchment) and/or temporal (interannual and multiannual) scales. This aspect has been investigated to test how catchment characteristics (e.g. land use, hydrology and geology) influence export dynamics, and also to detect long-term changes in hydrological and biogeochemical functioning (Moatar et al., 2017; Mussolf et al., 2015). Depending on the afore mentioned relationship, three main transport patterns can be identified: dilution, enrichment or static. The use of this categorisation allows us to infer the dominant regulatory processes, geochemical or biological, present during the transport phase (Moatar et al., 2017; Mussolf et al., 2015). At the catchment scale, the importance of these processes varies spatially and temporally, responding not only to land use changes but also to short- and long-term hydrological changes (Ebeling et al., 2021; Minaudo et al., 2019).

The interaction between landscape features and weathering is one of the main factors that influence transportation. Precipitation, through erosion and runoff, mediates the transfer of particulate matter and dissolved compounds from the soil to the hydrographic network. Agricultural and uncovered soils are eroded more easily than those covered by shrub and tree vegetation, while urban areas prevent contact with groundwater and amplify the impact of flash floods (Barron et al., 2013; Hrachowitz et al., 2016; Stephens et al., 2021). Most of the transport of solid materials takes place during floods. This aspect seems to be more important for P due to the erosion of particulate P, largely happening in arid catchments or after drought periods (Lisboa et al., 2020; King et al., 2015). N, on the other hand, and especially $N\text{-}NO_3^-$, quickly reach the aquatic compartment after weathering due to its high solubility.

The structure of the hydrographic network also plays a key role in mediating nutrient transport and transformation. The environments through which the hydrological continuum flows can retain and accumulate part of the transported load. Soil is pivotal in retaining P, while groundwater acts as a nitrate reservoir in permeable soils and lake sediments accumulate nutrients with different capacities depending on the element (Dupas et al., 2020; Maavara et al., 2020; Westphal et al., 2019). Natural and artificial environments, such as wetlands, lakes, drainage channels and reservoirs, or irrigation practices, influence residence time and favour biogeochemical transformations (Kleinman et al., 2015; Maranger et al, 2018; Nizzoli et al., 2020; Pinardi et al., 2020; Soana et al., 2018). Water impoundments sustain sedimentation rates, which reduce P and Si by trapping them in the sediment; concurrently, denitrification and mineralisation rates are favoured, with increased N and C elimination to the atmosphere. In addition, assimilation by primary producers converts reactive forms of N and P into organic compounds that act as temporary storage, while microbial mineralisation recycles them into dissolved forms (Covino 2017; Maavara et al., 2020; Romero et al., 2016; Racchetti et al., 2017; Scibona et al., 2022; Von Schiller et al., 2017; Wolf et al., 2013). Apart from the conversion of nutrients to gaseous forms, the other biogeochemical processes only modify the forms and the spatial and temporal dynamics of the loads. Therefore, the release of the accumulated nutrients can occur at different times with estimates that can vary from a few years to a few decades, depending on the characteristics of the catchment (Kusmer et al., 2018; Liu et al., 2021; McCrackin et al., 2017; Van Meter et al., 2017). This phenomenon underlies the observed hysteresis, which specifically describes the delay of a water body in returning to a better ecological status after reducing anthropogenic inputs (Newcomer et al., 2021).

1.3 Temporal trends and research gaps

Although there have been successful initiatives to reduce eutrophication in different countries, many problems related to eutrophication persist in both freshwater rivers and lakes, as well as in coastal oceans (Le Moal et al., 2019; Voulvoulis et al., 2017). Currently, more than half of the water bodies in the European Union do not meet the ecological standards required by the EU Water Framework Directive (2000/60/EC), and nutrients excess is the major cause of this degradation (Poikane et al., 2019). Progress on this aspect has often been hindered by a lack of understanding regarding the factors that control the formation of nutrient loads, as well as scientific controversies surrounding the necessary actions to control nutrient inputs (Le Moal et al., 2019; Wurtsbaugh et al., 2019). Furthermore, managing linked systems across the LOAC can be complicated due to the varying characteristics of the continuum. Aquatic environments can oscillate between limitations of N or P, depending not only on anthropic pressures but also on the system's ability to recycle, store, or eliminate these nutrients (Dodds & Smith, 2016; Mainstone & Parr, 2002; Smith & Shindler 2009; Wurtsbaugh et al., 2019). For example, it has been shown that the control of only one of the two nutrients (N or P) does not lead to a generalized improvement along the continuum. In fact, measures taken to reduce N alone do not take into account the ability to fix or recycle N with high C, while the control of P alone can limit biological processes and decrease the ability to eliminate dissolved N via uptake, sedimentation or denitrification (Pearl et al., 2009, Wurtsbaugh et al., 2019).

A long-term analysis along the Loire-Vilaine-VB continuum (France) has shown that a significant reduction in P inputs without a simultaneous reduction in N was not enough to control eutrophication phenomena (Ratmaya et. al 2019). In the Northern Adriatic Sea, the reduction of exported P decoupled to N was assumed to trigger mucilage formation (Sellner and Fonda Umani, 1999; Degobbis et al., 2005). In coastal lagoons, changes in community composition and dominance of macroalgae were related to excess N loadings (Viaroli et al., 2008). Nonetheless, despite a global deterioration trend, some basins in the United States and Europe have shown improvement in exported load reduction (Bouraoui & Grizzetti, 2011; Oelsner & Stets, 2019). In some cases, this reduction appears to be linked to a decrease or better management of anthropogenic pressures, as seen in the Baltic Sea or the Adriatic Sea (Andersen et al., 2017; Viaroli et al., 2018). Other studies, such as those conducted in the Chesapeake Bay or the Mediterranean basins, have highlighted the potential role of climate and the alternation between dry and wet periods as regulatory factors (Cozzi et al., 2018; Harding et al., 2019).

However, our knowledge of how the frequency and intensity of precipitation impacts nutrients export remains incomplete. In addition, the role of extreme events in regulating nutrient retention or export is not yet fully understood, especially in relation to human pressures, landscape characteristics and antecedent conditions (e.g. number of dry days prior to an extreme event) (McMillan et al., 2018). Sinha et al. (2017) stated that higher rainfalls in certain areas of the US would lead to an increase in the load of N. Indeed, periods of drought can alter the retention capacity of drainage areas but the effect of drought on N transport is complex and likely to be linked to the temporal scale considered (Moatar et al., 2017; Winter et al., 2023). The analysis of the 2018-2019 drought in the Selke catchment (Germany) evidenced an exceptionally low riverine nitrate concentration during dry periods followed by an exceptionally high concentration during subsequent wet periods, with nitrate loads up to 73 % higher compared to the long-term load–discharge relationship (Winter et al., 2023). This pattern could be due to limitations in the transport and decreases in biogeochemical processes ultimately mediating N delivery (e.g., denitrification and assimilation) during drought, followed by mobilization of trapped $N\text{-}NO_3^-$ sources as precipitation augmentation. In certain Canadian catchments, water quality has been affected by both climate change impacts on hydrology and changes in land use (El-Khoury et al., 2015). Likewise, Lheureux et al. (2023) found that changes in nutrient concentrations and ratios in French coastal ecosystems could be attributed to both climatic/continental factors and management policies in France. It has been observed that in some US catchments, stream N, P and sediment concentrations can respond in contrasting ways to changes associated with agricultural management and use. These findings indicate that temporal trends in the region can be moderated, accelerated, or reversed on decadal timescales (Renwick et al. 2018). Furthermore, the fragmentation of rivers has a significant impact on nutrients transport. Although the EU Biodiversity Strategy aims to restore 25,000 km of rivers across the continent, the unpredictability of water resources due to climate change may lead to an increased demand for artificial dams. This, in turn, could further alter the processes of nutrient retention and release (Belletti et al., 2020; Dopico et al., 2022).

Therefore, despite current knowledge of key differences between N and P biogeochemistry, describing and modelling the movement of nutrients along the LOAC is extremely complicated due to the large number of controlling and regulating factors. In fact, the availability of nutrients in dissolved and particulate forms depends on soil characteristics such as land cover, management, geology, and topography, as well as hydrology, amongst others. Additionally, the spatial arrangement of constituent sources within the catchment area and cross-correlations between landscape characteristics and seasonality also influence the nutrient availability (Lintern et al., 2017; Le Moal et al., 2019). While the main drivers are straightforward to pinpoint, their weight and role depend on possible cross-interactions that are highly variable and specifically linked to a characteristic set of conditions. In fact, the hydrological, hydrogeomorphological, and biogeochemical features of a basin are not always generalizable, making it difficult to extrapolate biogeochemical rates. These aspects illustrate how different forcings can result in similar consequences, according to the principle of equifinality. All these factors contribute to the complexity of basin areas and provide challenges for the identification and quantification of nutrient sources, pathways, transformations and fate, and their stoichiometry. All these issues are critical for a better management of heavily exploited catchments (Dupas et al., 2017; Kinkard et al., 2020; Le Moal et al., 2019; Strohmenger et al., 2020).

An improved knowledge of the cause-effect relationship and regulatory factors is therefore timely and would allow us to interpret more precisely the modifications of biogeochemical cycles in the short and long term and along the hydrological continuum. It is also worth considering that the processes driving biogeochemical cycles have a different importance and role depending on the scale with which they are analysed: in time - from seconds in the case of metabolism to decades in the case of the effect linked to legacy, and in space - from local such as the landscape features to global in the case of climate change.

1.4 Research aims and structure

The Overarching aim of this Thesis is to improve the understanding of the processes that regulate the generation, transport, and transformation of nutrients within basins. The three specific objectives developed in order to disentangle such a vast and complex topic are:

- Assess the long-term evolution (1992-2022) of N and P loads exported from the Po River to the Adriatic Sea in relation to precipitation patterns;
- Quantify the spatial variability of anthropogenic nutrients inputs to water basins and riverine loads, and evaluate how different catchment features influence N and P export and their ratios;
- Examine how artificial reservoirs in Mediterranean streams with intermittent fluvial regimes affect the transport of N, P, and Si, as well as their stoichiometry, in relation to hydrology and to the level of reservoir impoundment.

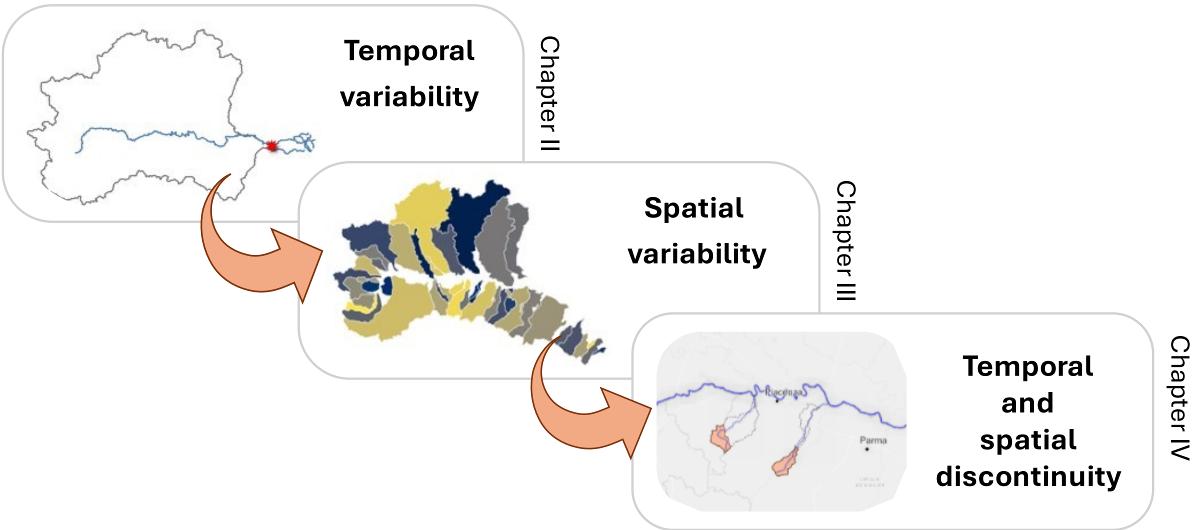

Figure 1.1 *The figure shows the structure of the Thesis evidencing the different spatial and temporal scales and the main topics.*

The relationships between hydrology and pressures, catchment characteristics and seasonality have been analysed at different spatial and temporal scales in the Po River Hydrographic District (PRHD), an impacted region defined under the Water Framework Directive for water management (Legislative Decree 152/2006). The PRHD comprises the Po River basin, one of the largest rivers in southern Europe, which accounts for approximately 85% of the total district surface area (86,800 km^2), and is the major river draining to the Adriatic

Sea. Nutrient loads delivered by the Po River account for ~65% of the N and P to the Adriatic Sea (Viaroli et al., 2018). Between 1970s and 1980s, dissolved inorganic nitrogen (DIN) grew suddenly from ~50000 to ~100000 t N y^{-1} and remained elevated, while the soluble reactive phosphorus (SRP) experienced a dramatic surge from the late 1960s (~2000 t P y^{-1}) to the mid-1970s (~5000 t P y^{-1}) and is now in the range 1500–4000 t y−1 depending on hydrological conditions (Viaroli et al., 2018). Changes in nutrient fluxes impacted the Adriatic Sea triggering eutrophication processes, in particular between the '70 and the '90, which resulted in persistent phytoplanktonic and mucilage blooms along the Northern Adriatic coastline, as well as anoxia and death of benthic and fish fauna (Cozzi and Giani, 2011; Viaroli et al., 2015, 2013). Exported DIN and SRP are undoubtedly related to changes in N and P excess generated by human activities over the last 50 years. Unfortunately, more recent attempts to reduce these loads were effective only for point P sources through the implementation of sewage treatment plants and the reduction of P in detergents (Viaroli et al., 2018). Diffuse N remain high along with total phosphorus (TP) (Viaroli et al., 2018).

Several studies have analysed the spatial and temporal evolution of the N and P loads in the Po River basin either at a single sub-catchment scale (Bartoli et al., 2012; Pinardi et al., 2018) or at the level of the entire water basin (Viaroli et al., 2018). The role of hydrology in N and P load formation has been studied only at the water basin closing station. These data indicated that the amount, forms and stoichiometry of N and P discharged to the Adriatic Sea depends also on water discharge (Cozzi & Giani 2011; Cozzi et al., 2018; Tesi et al., 2013). Different hydrological characteristics between the Alpine side of the water basin – where river hydrology is mainly regulated by subglacial lakes – and the Apennine side – where stream hydrology is mainly regulated by rainfall patterns - exist. In parallel, the role played by lentic aquatic ecosystems in the control of nutrient stock has been analysed mainly in natural lakes (Nizzoli et al., 2018; Scibona et al., 2022), while reservoirs are understudied despite being important variables that could influence the export of nutrient loads.

In this context, the underlying idea of this thesis is that the analysis of the same territory at different scales brings new essential insights into the factors that control the nutrient cycles in a water basin, which would otherwise remain hidden. The aquatic continuum is the connecting element between the different compartments, both in space and time, and it was chosen to analyse the role of seasonality, pressures, and land use in relation to hydrological factors. The analysis was divided into three main parts corresponding to chapters 2, 3 and 4 and briefly described below.

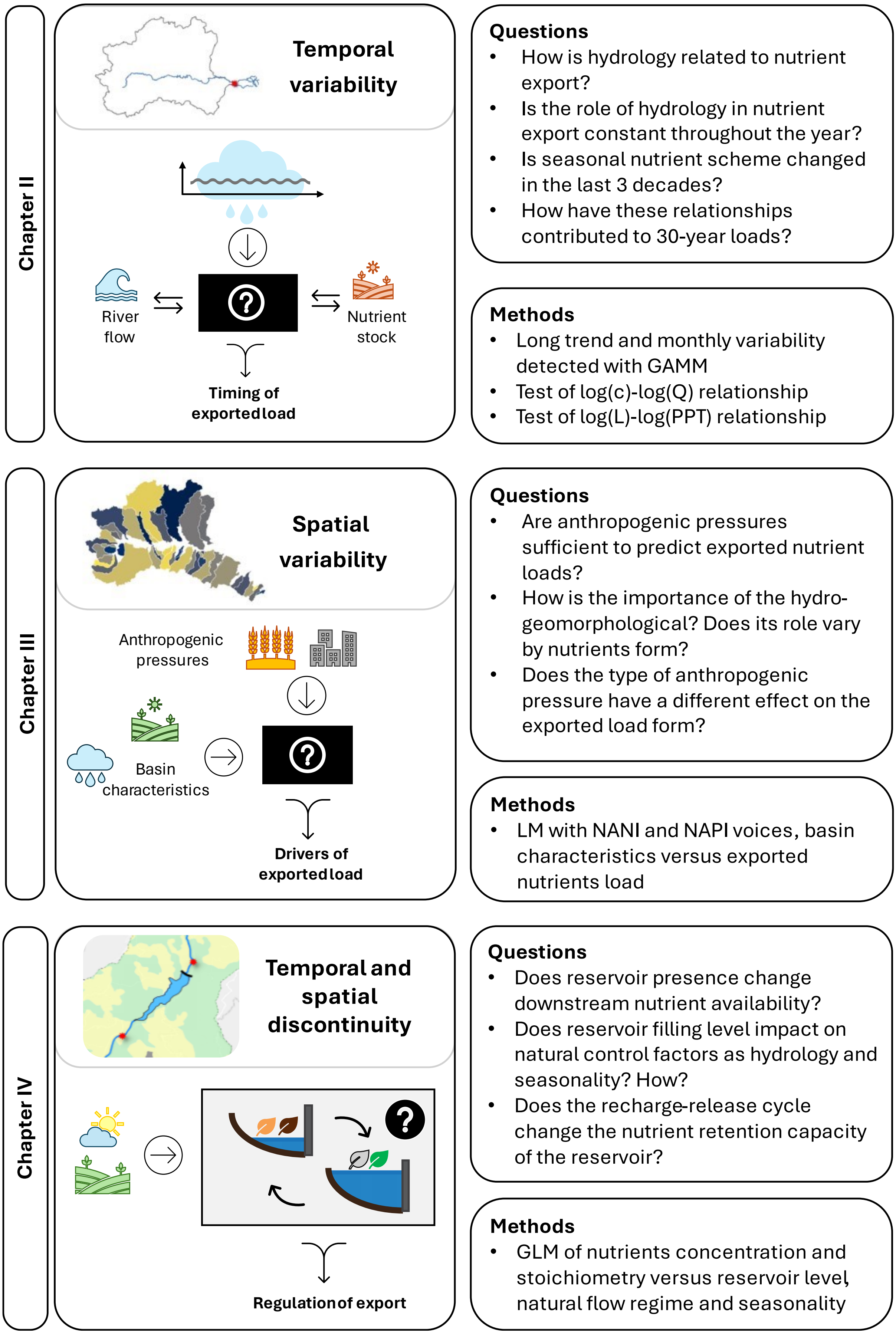
Chapter II
Temporal variability
River flow
Nutrient stock
Timing of exported load
Questions
• How is hydrology related to nutrient export?
• Is the role of hydrology in nutrient export constant throughout the year?
• Is seasonal nutrient scheme changed in the last 3 decades?
• How have these relationships contributed to 30-year loads?
Methods
• Long trend and monthly variability detected with GAMM
• Test of log(c)-log(Q) relationship
• Test of log(L)-log(PPT) relationship
Chapter III
Spatial variability
Anthropogenic pressures
Basin characteristics
Drivers of exported load
Questions
• Are anthropogenic pressures sufficient to predict exported nutrient loads?
• How is the importance of the hydro-geomorphological? Does its role vary by nutrients form?
• Does the type of anthropogenic pressure have a different effect on the exported load form?
Methods
• LM with NANI and NAPI voices, basin characteristics versus exported nutrients load
Chapter IV
Temporal and spatial discontinuity
Regulation of export
Questions
• Does reservoir presence change downstream nutrient availability?
• Does reservoir filling level impact on natural control factors as hydrology and seasonality? How?
• Does the recharge-release cycle change the nutrient retention capacity of the reservoir?
Methods
• GLM of nutrients concentration and stoichiometry versus reservoir level, natural flow regime and seasonality

Figure 1.2 *The figure summarises the organization of the following chapters. For each chapter, the main research topic is identified with the study area. On the left side of the picture the graphical abstract synthetizes the chapter structure indicating the research gap and then the main hypothesis. In the right part of the figure are summed up the single research questions and the applied methods.*

1.4.1 Influence of seasonal and hydrological patterns on the long-term trends of nutrient loads within the Po River basin

This research activity aimed to identify any variation of the seasonal nutrient scheme from ‘90 and to understand how this process is hydrological-driven. To reach this goal, the Po River basin, which represents the main portion of the Po River district, was chosen as the experimental unit. First, the monthly loads of $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, TP and SRP at the water basin closing station (Pontelagoscuro) were calculated from 1992 to 2022. Second, the interaction between long-term and seasonal dynamics was tested. Third, the relationship between nutrients and hydrological variables was also analysed by examining the monthly variation of the c-Q index (concentration-flow rate) and the ratio between the individual coefficients of variation. The results of these analyses identified the export regime of nutrients, which can be classified as chemostatic, dilution or enrichment regime. Finally, the relationship between precipitation and exported load was tested.

The hydrological effect on nutrient export of Po River Basin was only partially described by Cozzi & Giani, (2011), applying a dry/wet years classification and water discharge. This work expands this approach through the direct estimation of the relationships between precipitation and nutrients loads within the seasonal variability. The chapter:

- Defines, for the first time, the effect of precipitation on nutrient export and the export regime scheme at the scale of whole Po River basin.
- Contributes to explain the variability of export regimes of large river basins, which are characterized by more complexity and spatiotemporal interactions.
- Defines an initial framework that investigates the hydrological connectivity of the basin and the dominant processes along the continuum, and identifies dynamics that should be further inspected.

Finally, The variation in export patterns of various nutrients across different seasons suggests that the interaction between landscape and hydrology may play a role in mediating the transport of anthropogenic nutrients.

1.4.2 Net anthropogenic nitrogen/ phosphorus inputs and hydrological factors: effects on genesis, form and stoichiometry of the exported loads

This research activity aimed to examine the anthropogenically-driven alterations to the exported loads of the different pools of total N and P in their inorganic and organic forms. The analysis took into account the hydrological and geographical characteristics of the water basins, and the study focused on 42 sub-basins in the PRHD as experimental units, chosen based on the availability of data to estimate nutrient export. For each sub-basin, the individual components of the net anthropogenic nitrogen (NANI) and phosphorus (NAPI) inputs to water basins (such as N and P fertilization, fixation and N deposition, trade in food for people and animals, and use of detergents) were quantified, as well as the exported load of TN, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, TP and SRP. The main hydrological and spatial variables, such as precipitation, areal hydraulic load, and average altitude, were also derived from the Copernicus datasets.

This work analyses spatial variability for the first time at the scale of the entire Po district, complementing the temporal analysis of Viaroli et al. (2018). By exploiting the robustness of a widely applied methodology, this chapter:

- Shows that without hydrologic variables, anthropogenic inputs are not sufficient to explain the variability of exported nutrients loads, meaning the hydrological components not only regulates but also competes in generating loads.
- Contributes to significantly explain the role of basins structure likewise the difference of autotrophic vs heterotrophic basins;
- Concurs to identify and quantify mains sources and biogeochemical processes that regulate the export of TN, TP and also the compounds that make up the N and P pool at the catchment scale. At a general level, this contributes to increasing the level of detail in the knowledge nutrients pathways at the catchment scale.

1.4.3 Hydraulic management and flood effect regulate sink and source behaviour altering nutrient stoichiometry of irrigation-dammed reservoirs

This research activity examines how artificial reservoirs in Mediterranean streams with intermittent fluvial regimes affect the transport of nitrogen, phosphorus, and silicon, as well as their stoichiometry, in relation to hydrology and to the level of reservoir impoundment. Reservoirs are located on the Arda and Tidone streams in the province of Piacenza and are aged over 90 years. Upstream of the reservoirs, the river has an intermittent regime strongly dependent on rainfall, while downstream the regime is regulated by the seasonal dynamics of recharge and release period, due to irrigation. For 12 months, upstream and downstream sampling was carried out following the hydrological trend and measuring N-NO_3^-, N-NH_4^+, PN, SRP, PP, DRSi, SST and Chl-a.

Reservoirs are among the man-made elements that contribute most to hydrological discontinuity and, given the changes in the hydrological regime in the near future, they are destined to increase for drinking water supply and irrigation (Calderon et al., 2023). This work, which analyses the role of two reservoirs during the 2022 drought in northern Italy, can assume a paradigmatic meaning for the near future. The chapter:

- Expands understanding of reservoirs' impact on nutrients pathways. We observed that reservoirs can function as both nutrients traps and sources, depending on interactions between water management and seasonality, within the same year.
- Contributes to changing the view of reservoirs as only nutrient traps, showing how further studies are needed to investigate possible effects on nutrient pathways in catchments controlled by irrigation reservoirs.

2 Chapter II

Influence of seasonal and hydrological patterns on the long-term trends of nutrient loads within the Po River basin

2.1 Introduction

Many rivers and streams are impacted by altered flow and water temperature, especially in the temperate regions, where the frequency of flood events and prolonged drought periods is dramatically increasing due to climate change (Hirabayashi et al., 2008; Kundzewicz et al., 2018; Kunkel et al., 2003; Satoh et al., 2022). The resulting alternation of such different conditions can deeply modify the mobility and availability of nitrogen (N) and phosphorus (P) forms, which depend on e.g. oxygen availability, sedimentary microbial metabolism, sediment sorption properties, redox-sensitive metals as well as the precipitation effect on soil erosion (Attygalla et al., 2016; Homyak et al., 2017; Merbt et al., 2016; Ramos et al., 2019). In this context, an interesting study case is represented by the rivers and streams of Northern Italy, which are currently facing hydrological intermittence due not only to global change but also to increasing local anthropogenic pressures on aquatic ecosystems (Montanari et al, 2023; Piano et al., 2019).

Widespread alterations of nutrient cycling are also occurring in river basins at regional level due to the increased intensity of land uses, i.e. agriculture, livestock and urbanization, hydro-morphological modifications and climate change (Lheureux et al., 2023; Romero et al., 2013; Smith and Schindler, 2009; van Meter et al., 2020). These pressures result in excessive N and P delivery to both inland and marine coastal waters which triggers degenerative eutrophication processes and unprecedented nitrate pollution, especially in lentic aquatic ecosystems (Grizzetti et al., 2012; Hilton et al., 2006; Le Moal et al. 2019; Smith et al., 1999). The relationships between anthropogenic N/P loadings and eutrophication processes are currently assessed with simple indicators, such as the Net Anthropogenic Nitrogen Inputs (NANI) and Net Anthropogenic Phosphorus Inputs (NAPI) or the diffuse N and P surplus from the farmland. These variables assume the dependence between N and P loadings in running waters and their input in relation to climate and hydrological patterns (Goyette et al., 2019; Grizzetti et al., 2012; Howarth et al., 2012; Lassaletta et al., 2012; Le Moal et al. 2019; Viaroli et al., 2018). However, the environmental pathways and fate of N and P are not simply dependent on their sources, as they are also affected by several other factors including the chemical speciation of N, (e.g. ammonium ($N\text{-}NH_4^+$) to nitrate ($N\text{-}NO_3^-$) ratio), biological availability of P compounds, river metabolism and the interactions between the river and its floodplain, including the interchange with lateral wetlands (Glibert, 2017; Houser and Richardson 2010; Jarvie et al., 2020; Racchetti et al., 2011; Wolf et al., 2013).

The chemical speciation of N, namely the ammonium ($N\text{-}NH_4^+$) to nitrate (N- NO3-) ratio, influences N reactivity and bioavailability, and the inherent risk of $N\text{-}NO_3^-$ pollution of both surface and groundwater. In turn, besides the anthropogenic sources, the prevalence of N-NO_3^- or $N\text{-}NH_4^+$ is often regulated by the so-called "flood pulses" (Tockner et al., 2000). The fluctuation of water level induces the alternation of dry phases, causing exposure to air of soils and riverbeds (oxic-oxidative phase), followed by flooding (anoxic-reducing phase) which supports nitrification and denitrification processes, respectively (Kreiling et al., 2019; Shrestha et al., 2014). Concurrently, inorganic P forms are adsorbed and retained by soil particles in the dry phase, whilst they are released/desorbed and transported by the surface runoff in the wet phase (Jarvie et al., 2013; Withers and Jarvis, 2008). In the river basins of the temperate zone, these processes are expected to undergo nearly regular seasonal patterns depending on a series of factors: precipitation, heat budget/temperature, evapotranspiration, water abstraction for irrigation and river metabolism. Collectively, these components affect the long-term trends of nutrient loadings (Abbott et al. 2018; Ebeling et al. 2021; Viaroli et al. 2018).

Over the last decades, climate changes have deeply modified the seasonality of both river discharge and nutrient loadings, due to the occurrence of severe and prolonged drought, followed by heavy short-term rainfalls or exceptional snowmelt and flash floods (Carrer et al., 2023; Chiarle et al., 2021; Lionello & Scarascia 2018). Under these conditions, river runoff is expected to deliver high amounts of soluble $N\text{-}NO_3^-$ along with particulate P (Heathwaite et al. 2005; Howarth and Marino 2006; Ockenden et al. 2016). Therefore, N and P may differentially impact the downstream ecosystems, $N\text{-}NO_3^-$ being promptly reactive (Galloway, 2003; Matiatos et al., 2021), whereas PO4- is tightly associated with soil/sediment particles which are usually much less reactive and bioavailable than $N\text{-}NO_3^-$ (Withers and Jarvis, 2008).

Under low flow conditions during the warm season, the river itself can develop a relevant metabolism with a substantial impact on nutrient retention, stoichiometry, and speciation (Bernhardt et al., 2022; Cohen et al., 2013; Tzoraki and Nikolaidis, 2007; von Schiller et al., 2011). The river metabolism is greatly amplified by the occurrence of dams. reservoirs and natural lakes which can selectively retain less soluble forms such as particulate P and Si, and release the most soluble N-NO_3^- (Ittekkot et al., 2000; Scibona et al., 2022).

As a result, land use and morphological modifications, along with precipitation and global warming may modify not only the nutrient loading delivery to coastal zones, but also its stoichiometry and timing/seasonality (Kinkaid et al., 2020; Shousha et al., 2021; Strohmenger et al., 2020). Importantly, the onset of the hydrological intermittence is challenging our capacity to discriminate which factors, and at which temporal scale, have the potential to influence the eutrophication of receiving waterbodies (Homyak et al., 2017; Skoulikidis et al., 2017). Variations in the precipitation regime, coupled with a higher frequency of extreme drought and rainfall/storm events, along with snowfall and snowmelt dynamics, may further concur to modify nutrient transport and fate in the catchment.

In this context, an interesting case is represented by Po River basin in northern Italy, which is facing a general trend of increasing drought intensity in the Mediterranean area (Polade et al. 2017, Lionello et al., 2018), as well as the progressive volume loss of alpine glaciers due to climate change (Carrer et al., 2023; Colombo et al., 2022). Furthermore, the global warming-derived temperature increase causes more evapotranspiration, which further depletes water levels in context of constantly increasing increases water demand. Locally, this impact is amplified by the increase of irrigated cropland, e.g. the substitution of the non-irrigated winter wheat by high water-demanding summer crops such as maize and rice (Montanari et al., 2023; Viaroli et al., 2018). The concurrency of these aspects explains why the annual decline of the Po river discharge in summer is more relevant than the decline rate of the annual precipitation (Montanari et al., 2023).

This work aims to analyse long-term trends and relationships of precipitation, river discharge, as well as N and P concentrations and loads exported from the Po river basin. More specifically we aim to: (i) Identify the main temporal patterns, both at decadal and seasonal scale , (ii) define nutrients export regime at the monthly scale and the main transport dynamic of N and P chemical species (hydrogeological vs biogeochemical control), and (iii) estimate precipitation effects on load formation and evaluate whether hydrology regime variations are affecting export of nutrients across the years.

To achieve these goals, we analysed long-term trends of N and P concentrations and loadings exported from the Po river basin and their stoichiometry over 3 decades (1992–2022).

We also analysed interannual changes in precipitation and water discharge and their relationships with nutrient concentrations and loadings. This approach was previously used to capture the integrative influence of climate and hydrology on nutrient transport and it provided useful information on the processes controlling the mobilization and delivery of chemical elements into streams (i.e. export regimes), as well as biogeochemical transformations in river networks (Bieroza et al., 2018; Moatar et al., 2017; Mussolf et al., 2015; Speir et al., 2024).

2.2 Study area

The Po River, one of the major rivers in the Mediterranean region, is 652 km long (Fig. 1). The water basin is 74,000 km^2, of which 71,000 km^2 (~46,000 km^2 as lowland) is in Italy. Its surface is almost one-fourth the surface of Italy, and ~40% of the Italian GDP is generated in the Po region (Viaroli et al., 2010). In this area, agriculture interferes heavily with the hydrological cycle: ~22x109 m^3 y^{-1} of water is 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 water basin (Montanari, 2012). The southern side of the river basin is affected by water scarcity, and streams and rivers have extremely variable flow regimes. Contrarily, the northern side of the river basin has a great number of reservoirs and high-altitude small lakes, as well as four large deep subalpine lakes fed by Alpine glaciers (Maggiore, Como, Iseo, Garda). Overall, these largest four lakes account for ~70% of the water volume of surface freshwater in Italy and feed the main tributaries of the Po River, which make up about 50% of its total water discharge (Turco et al., 2013; Po River basin Authority, 2016).

Nutrient loadings have been studied since the late 1960s (Marchetti et al, 1989) at Pontelagoscuro (PLS in Fig. 1), where the closing station of the river basin is located. Detailed studies were performed in the early 2000s, aiming at relating anthropogenic sources to riverine loadings (Cozzi & Giani, 2011, Viaroli et al. 2018) and studying natural processes of N removal (Soana et al., 2023).

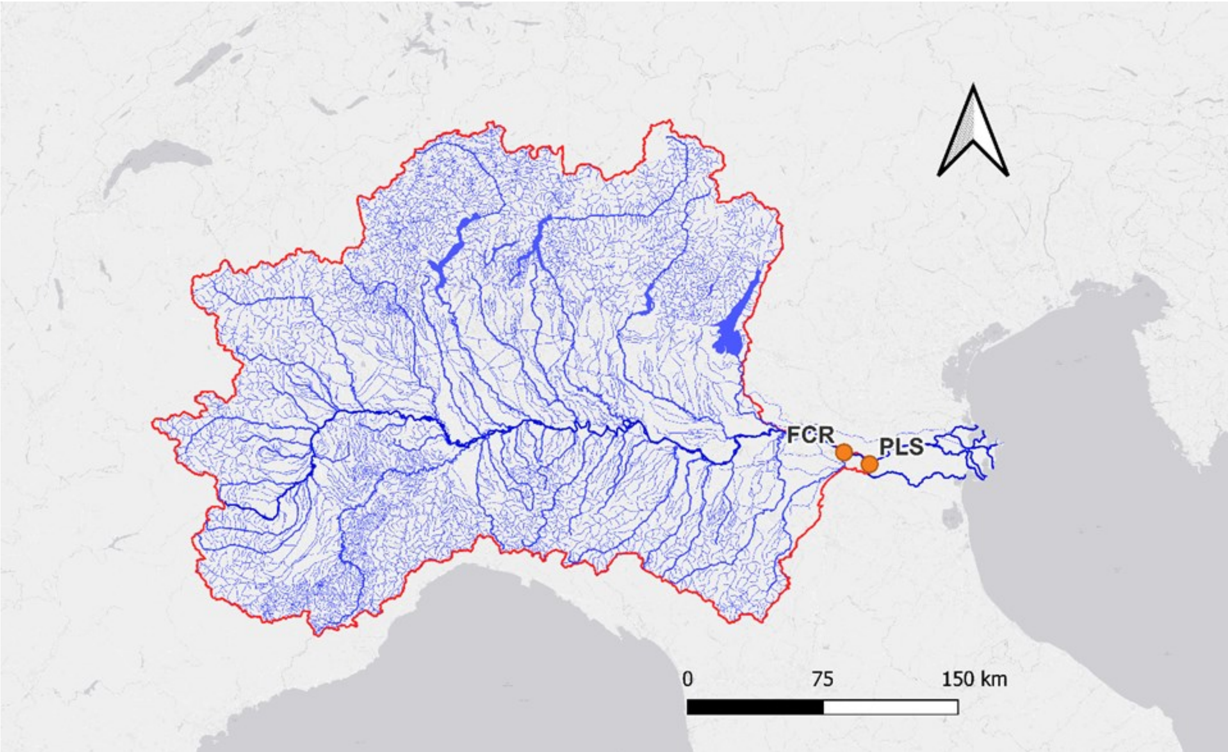

Figure 2.1 *Po River basin with closing stations: Pontelagoscuro (PLS) and Ficarolo (FCR)*

2.3 Materials and Methods

2.3.1 Datasets and temporal resolution

The closing station of the Po River basin is historically positioned at Pontelagoscuro village (PLS) (Figure 2.1). Monitoring of water quality and quantity are routinely conducted by the Emilia-Romagna Regional Agency for Environmental Protection (ARPAE).

Nutrient concentration data from 1992 to 2009 were directly provided by ARPAE, whereas data from 2010 to 2022 are freely available for download from their website (https://dati.arpae.it/dataset/rete-regionale-per-la-qualita-ambientale-acque-superficiali-fluviali-dati). The dataset for this study includes fortnightly sampling from 1992 to 2000, and monthly sampling from 2001 to 2022. Phosphorus (TP, SRP) and inorganic nitrogen ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$) data are available for the entire 30-year period 1992 ¬ 2022.

Daily water discharge data are also freely available for download from the ARPAE online repository DEXT3R (https://simc.arpae.it/dext3r/) and these data were downloaded and analysed over the period 1992 ¬ 2022. Missing data for time intervals shorter than a few days were replaced by linear interpolation, whereas missing data from 2018-07-25 to 2018-10-28

(96 days) were estimated from discharge data of the Ficarolo station, which is positioned ~ 20 km upstream from Pontelagoscuro (Figure 1) and shows highly comparable Q values (slope=0.98, R2=0.99).

Precipitation (PPT) data were extrapolated from E-OBS data files (from the Copernicus Climate Change Service (https://surfobs.climate.copernicus.eu/) (Cornse et al., 2018). E-OBS datafile is composed of daily raster files including the modelized European rainfall at a 0.1° regular grid resolution. The average daily precipitation was calculated at the basin scale and then summed to obtain monthly and annual mean precipitation.

2.3.2 Calculation of export metrics and exported loads

We individuated the nutrients export scheme for each month, applying concentration-discharge metrics (Mussolf et al., 2015; Thomson et al., 2011), by the following power law relationship (Godsey et al. 2009):

$$c = aQ^b$$

$$\log(c) = \log(a) + b\log(Q)$$

where log(*a)* is the intercept and *b* the slope of the log(c) – log(Q) regression.

The slope of the log(c) – log(Q) relationship describes the export pattern: a positive slope (b > 0) indicates an enrichment pattern while a negative slope (b < 0) means a dilution pattern. When the log(c) – log(Q) relationship presents a non significant slope (b ≈ 0) Thomson et al. (2011) proposed to associate the ratio between c and Q coefficient of variation to describe the export regime:

$$\frac{CV_c}{CV_Q} = \frac{\mu_Q}{\mu_c}\frac{\sigma_c}{\sigma_Q}$$

According to previous published works we distinguished a chemostatic behaviour, with a low ratio ($CV_C/CV_Q < 0.5$), from a chemodynamic behaviour with a high ratio ($CV_C/CV_Q > 0.5$), which is considered as an index of high solute reactivity or threshold-driven transport (Mussolf et al. 2015; Ebeling et al. 2021).

Nutrients loads were calculated on both a monthly and annual basis as the product of the discharge-weighted mean concentration by the mean discharge of the period (Quilbé et al., 2006) as follows:

$$L = \frac{\sum_{i=1}^{n} c_i * Q_i}{\sum_{i=1}^{n} Q_i} * \bar{Q} * k$$

where:

L = period loading (kg period^{-1})

C_i = instantaneous concentration measured on day_i (g m^{-3})

Q_i = mean daily discharge on day_i (m^3 s^{-1})

$\bar{Q}$ = mean period discharge (m^3 s^{-1})

k = conversion factor from g m^3 s^{-1} to kg period^{-1}

2.3.3 Statistical analysis

All the statistical analyses were performed using the software R (R Core Team, 2021). To test significance of monthly variability for PPT, Q and nutrients concentration, we applied a one-way ANOVA through the *lm* function. Differences among months were estimated with the *summary* function verifying the contrast against January value. To ensure model assumptions were met and due to the skewness of the distribution of residuals, data were log or root-square transformed on the basis of the graphical evaluation of QQplot.

To assess long-term and periodicity within year of nutrient loads, we applied a Generalized Additive Mixed Model (GAMM) with the packages *mgcv* (Wood, 2017). To verify the significance of monthly trends, months were treated as a factor, and then we tested the general model, with an annual common smoother plus monthly-levels smoothers with different wiggliness, as follows:

$$y_i \sim month + s(year) + s(year, by = month) + \varepsilon_i$$

Reduction of collinearity between the global smoother and monthly trends was achieved by specifying the marginal TPRS basis as described by Pedersen et al. (2019). In order to ensure that model assumptions were met (due to the skewness of the distribution of residuals), data were log or root-square transformed on the basis of the graphical evaluation of QQplot, while to resolve temporal autocorrelation we used the corARMA function. To decide the autoregressive order (p), it was varied from 1 to 10, and then we selected the model with lower AIC (Akaike Information criterion) (Zuur et al. 2009). As a spline function, we adopted cubic regression (bs=”cr”) spline. The accepted model was finally calculated using the Restricted Maximum Likelihood (REML) (Zuur et al. 2009).

2.4 Results

2.4.1 Precipitation and hydrology

In the last three decades (1992-2022), at the closing section of the Po river basin (Pontelagoscuro), the mean annual discharge (Q) ranged from 550 (2022) to 2,282 m^3 s^{-1} (2014), with median = 1,443 m^3 s^{-1} and mean = 1,450 ± 409 m^3 s^{-1} for the whole period (Figure 2.2A). The mean annual discharge was significantly dependent on the mean annual precipitation ($R^2 = 0.71$, $p < 0.001$), which ranged from 673 (2022) to 1,286 (2014) mm y^{-1}, with median = 1,029 mm y^{-1} and mean = 918 ± 169 mm y^{-1} (Figure 2.2A).

The PPT-Q relationship was then analysed with a quartile analysis, which allowed to partition the dataset in dry, wet and normal years (Figure 2.2A). The first quartiles of both PPT and Q were assumed to be the upper thresholds of dry years; the 3rd quartiles were the lower thresholds of wet years and the 25th-75th interquartile comprised the normal years. Based on this classification, six out of seven dry years occurred from 2003 onwards. Both PPT and Q were characterised by a significant intra-annual variability ($p < 0.001$), which can be synthesised by two-phase seasonal patterns (Figure 2.2A). The higher PPT periods occurred from April to June, and from August to November, when they attained significant peaks, i.e. 90 ± 40 mm in May and 118 ± 77 mm in November. By contrast, the lowest values were detected in February (49 ± 35 mm) and July (61 ± 23 mm). Q attained two significant peaks ($p<0.001$) in May (1985 ± 1190 m^3 s^{-1}) and November (2204 ± 1735 m^3 s^{-1}). Significantly lower values ($p<0.01$) were detected in July and August (850 ± 405 m^3 s^{-1}), while Q was relatively constant from January to April (1381 ± 684 m^3 s^{-1}) (Figure 2.2B).

The PPT and Q relationship was also analysed for each month with a linear regression model of log-log transformed data. The slope was significant for all months, except for August. The slope peaked in June-July (b = 0.73-0.77), becomes lower during the rest of the year (b = 0.25-0.47), indicating that in June-July, at low Q values, PPT has greater effects on Q itself, than in the other months. (Figure 2.2C, Figure S. 2-5)

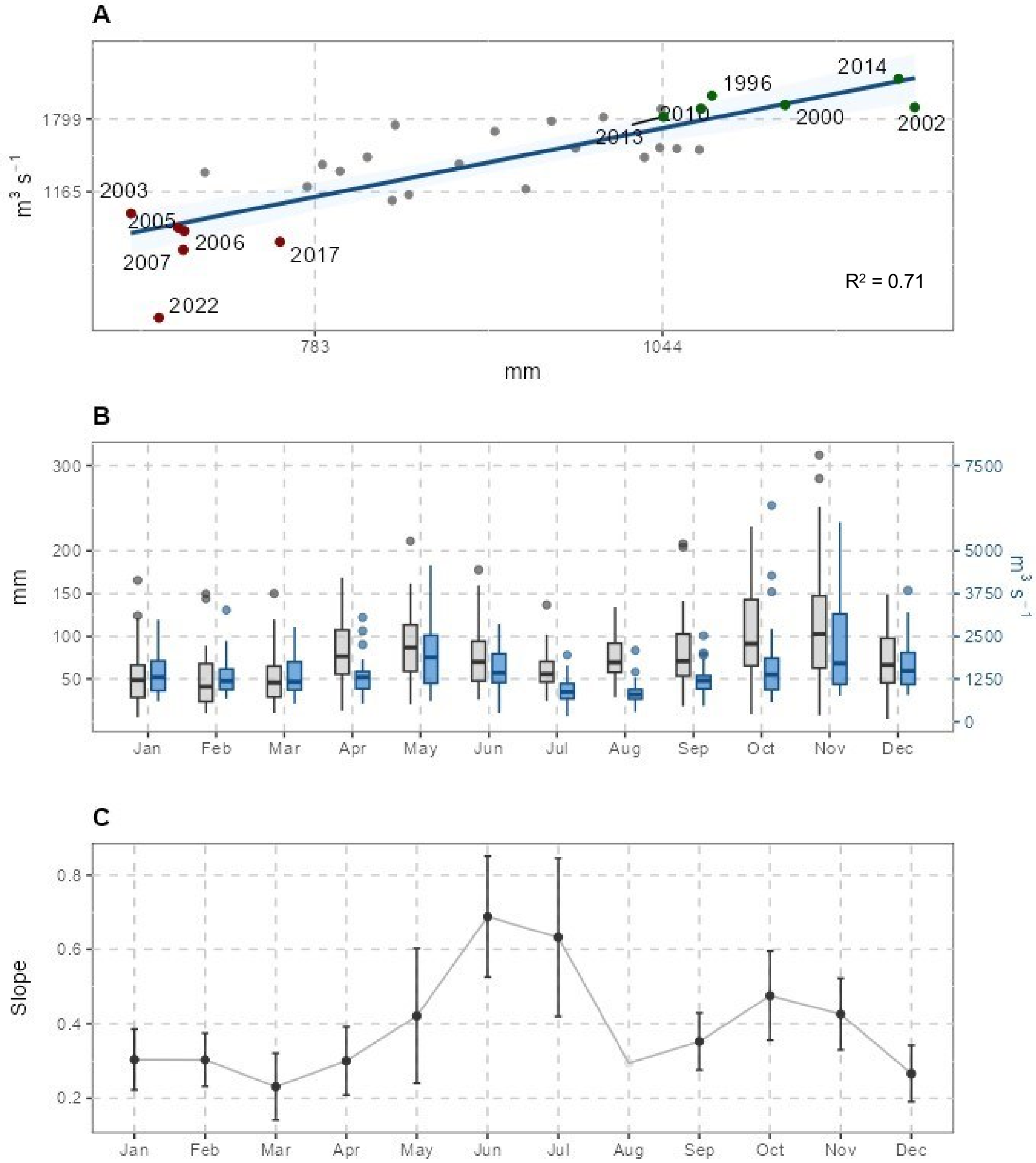

Figure 2.2 *Relationships between precipitation (PPT) and the water discharge rates (Q) in the Po River basin. (**A**) log(Q) - log(PPT) linear regression, with Q = mean annual water discharge and PPT annual cumulated precipitation (p.val < 0.001,* $R^2 = 0.71$*). Dashed lines represent the first and third quartiles of both PPT and Q. Red dots represent dry years, while green dots represent wet years. (**B**) Boxplot of monthly cumulated PPT (grey) and monthly mean Q (blue). (**C**) slope ± st. error of log(Q) – log(PPT) regression, with Q = mean monthly water discharge and PPT monthly cumulated precipitation, black dots represent significant slopes (*$p < 0.05$*). Expanded plots of monthly relationships are available in **Figure S. 2-5**.*

2.4.2 Nutrient concentration

The main N and P compounds underwent a clear periodicity, especially nitrate ($N\text{-}NO_3^-$) and soluble reactive phosphorus - SRP (Figure 2.3). Moreover, $N\text{-}NO_3^-$ accounted for 93-98 % of dissolved inorganic nitrogen (DIN = $N\text{-}NO_3^-$ + $N\text{-}NH_4^+$), while the contribution of ammonium ($N\text{-}NH_4^+$) was almost negligible. On average, $N\text{-}NO_3^-$ concentrations decreased from January (~3 mg N L^{-1}) to July (~1.3 mg N L^{-1}), and returns to growth in the second part of the year. The soluble reactive phosphorus (SRP) followed a similar pattern, with summer minima. By contrast, TP reached very high concentrations in May and from October to December (90th, TP > 0.3 mg P L^{-1}) likely due to flood events, while monthly medians are included between 0.13 and 0.16 mg P L^{-1}. The stoichiometric ratio DIN:SRP was strongly unbalanced, with lower values in June and from August to October (median=71), than the rest of the year (median=87).

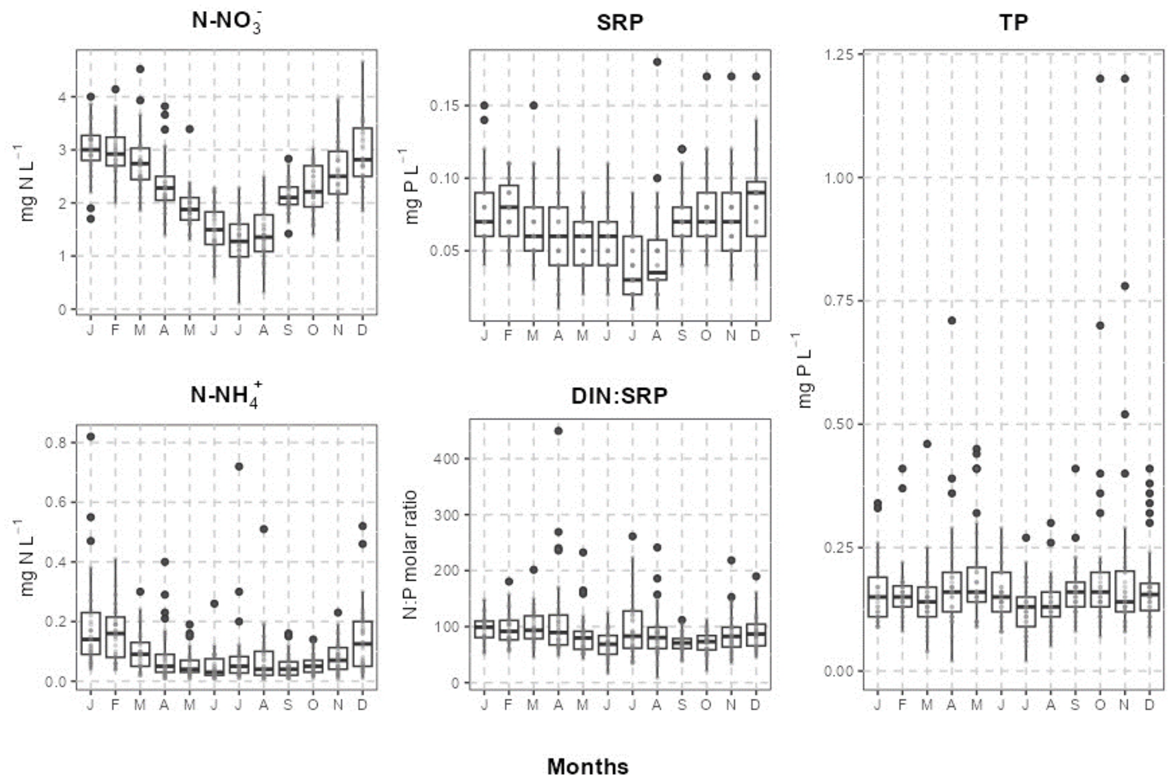

Figure 2.3 *Monthly variations of the concentrations of nitrate ($N\text{-}NO_3^-$), ammonium ($N\text{-}NH_4^+$), soluble reactive phosphorus (SRP), total phosphorus (TP), and DIN: SRP molar ratio. Whiskers represent 5th and 95th percentiles, boxes the 25th and 75th percentiles, horizontal lines the 50th percentile.*

2.4.3 *Nutrient loadings*

Nutrient loadings exported from the Po River at the closing section of the basin were affected by both inter-annual and intra-annual variability (Figure 2.4). At the inter-annual level, N-NO_3^- and SRP were the more conservative compounds (CV = 0.65), while TP was the most variable (CV = 1.6) and ammonium showed an intermediate behaviour (CV = 1.0).

The intra-annual loadings were affected by a clear periodicity which was analysed with month as factor and comparing all the months to January using the GAMM model (Figure 2.4). The monthly N-NO_3^- loadings were significantly lower from June to September ($p < 0.001$) and, to a lesser extent, in April ($p<0.02$). The N-NH_4^+ loadings were persistently low from March to November ($p< 0.01$), and they accounted for less than 10 % of the monthly DIN loadings. The TP loadings attained two peaks in May ($p < 0.01$) and November ($p < 0.01$), while in July and August the loads were lower than in January ($p < 0.001$). Finally, SRP exhibited an intermediate behaviour between N-NO_3^- and TP, with lower loadings in July and August ($p < 0.001$) and higher loads in November ($p = 0.029$) and April ($p = 0.05$). The DIN: SRP molar ratio showed a two-phase trend with low values in June ($p < 0.001$) and September-October ($p < 0.001$). The annual global-smooth term decreased progressively and was statistically significant for N-NO_3^- ($p < 0.01$), N-NH_4^+ ($p < 0.001$) and TP ($p < 0.01$), while SRP changes were not statistically significant (Figure 2.4). The DIN:SRP molar ratio also underwent a significant reduction, especially in the last decade.

Log transformation suggests that the decreasing rate of calculated loads is not homogeneous over the period and has become lower in recent years, indicating that the load decrease occurred mostly in the first decade. Single monthly smooths vary by nutrient and in some cases also by month. The period from November to May and September show the same decreasing trend for N-NO_3^-. June to July period and October present a significantly ($p_{Jun\text{-}Aug} = 0.001$, $p_{Oct} = 0.04$) different trend than the general smooth: June and October are characterized by a greater decrease in the first decade and then became stable in the last two decades, while August substantially shows no temporal trend. For N-NH_4^+ only December and July present a significantly different smooth ($p < 0.05$) from the general one, with a higher decreasing rate for December in the first decade and a stabilization of the load in July. Contrary to N, TP presents no different smooths among months, while for SRP in June ($p_{Jun} = 0.01$, $p_{Jul} = 0.09$, $p_{Oct} = 0.05$) the smooths are similar to those of N-NO_3^- for the same months, suggesting a decreasing trend. This trend is partially confirmed by DIN: SRP ratio which shows a decreasing behaviour in June ($p < 0.05$).

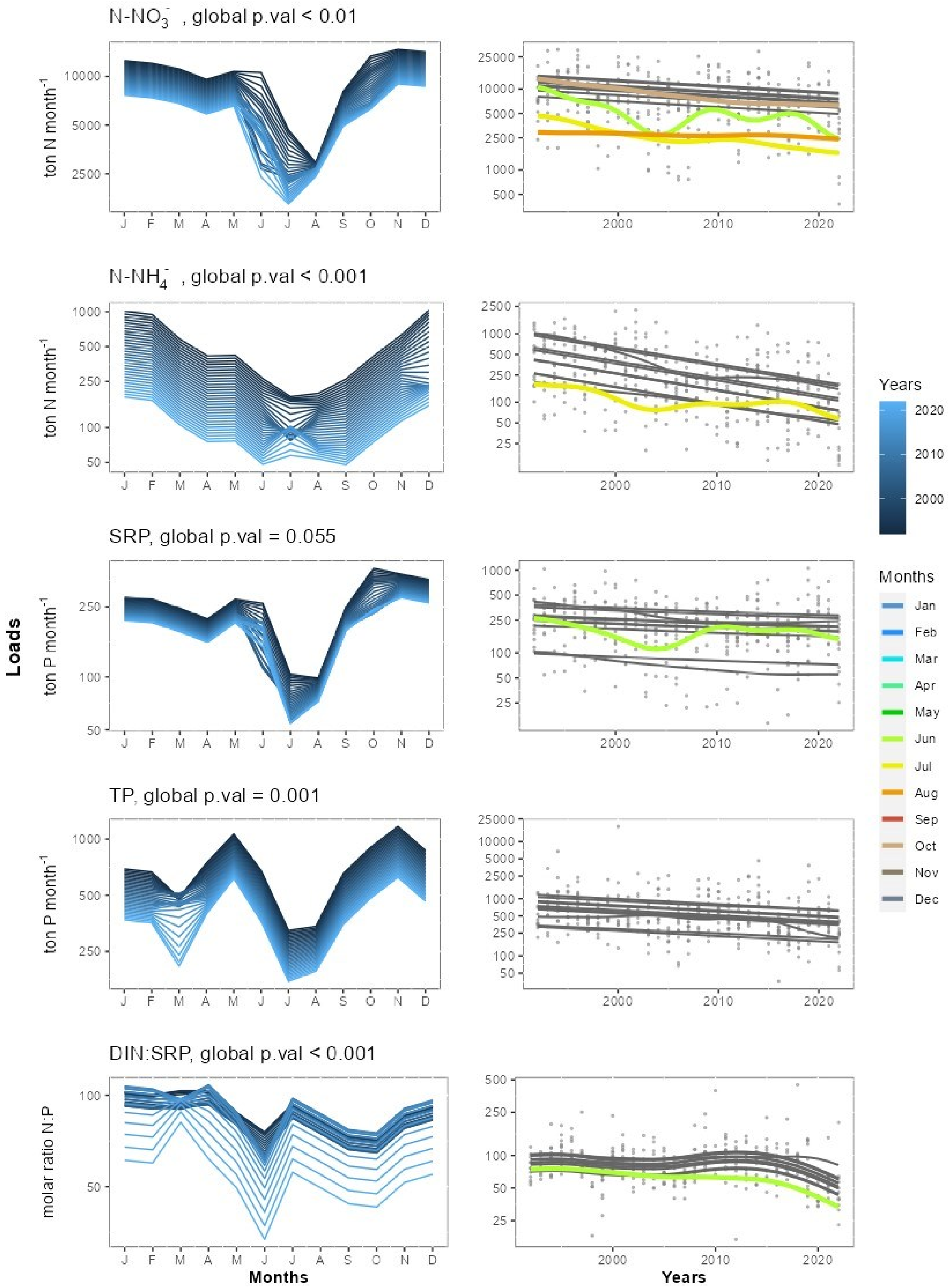

Figure 2.4 *Temporal trends of N-NO$_3^-$, N-NH$_4^+$, SRP, TP and DIN: SRP molar ratio:* ***(left)*** *long-term trend for each month. Coloured lines follow trends different from the global one.*

*Linewidth indicates the statistical significance value: thin lines not significant, wide lines significant at p < 0.05); (**right**) modelled monthly trends from 1992 to 2022.*

2.4.4 Loading responses to precipitation

Load-precipitation relationships calculated on annual data are reported in Table 2.1 . The general slope is highly significant for all nutrients, except for the DIN:SRP molar ratio. The monthly relationships between load and precipitation show a similar behaviour for $N\text{-}NO_3^-$, SRP and TP, with decreasing slopes from summer to autumn and lower or non-significant values from winter to early spring (Figure 2.5). Despite the TP trend is similar to that of SRP, its slope values are less variable among months, indicating a more stable response to rainfall. For $N\text{-}NH_4^+$ the slope is non-significant for 8 out of 12 months except for the wettest months (May, and September to November). The slope of the DIN:SRP molar ratio is non-significant for most of the year, except in July and November.

Table 2.1 *Relationship between nutrient concentration (c) and water discharge (Q) at the monthly scale (expanded plots are available in* ***Figure S. 2-1****), and nutrients load (L) vs precipitation (PPT) at the year scale (expanded plots are available in* ***Figure S. 2-2****). Significance is signed with asterisks (p < 0.05 *, p < 0.01 **, p < 0.001 ***).*

Relationships	Parameter	Intercept	Slope	St. error	R^2
log(c) – log(Q) whole data	$N\text{-}NO_3^-$	-0.55	0.18 ***	0.03	0.06
	$N\text{-}NH_4^+$	-3.88	0.15 *	0.08	0.01
	SRP	-3.42	0.08 *	0.04	0.01
	DIN: SRP	2.21	0.08 *	0.03	0.01
	TP	-3.99	0.29 **	0.04	0.13
log(L) - log (PPT) annual data	$N\text{-}NO_3^-$	3.29	1.20***	0.24	0.47
	$N\text{-}NH_4^+$	-3.66	1.73***	0.48	0.31
	SRP	1.64	0.93***	0.20	0.42
	DIN: SRP	0.76	0.30	0.21	0.06
	TP	-4.74	2.02***	0.35	0.53

2.4.5 *Concentration – water discharge metrics*

The c-Q relationships calculated with the whole dataset are reported in Table 2.1. The general slope is significant for all nutrients, decreasing from TP to SRP in the following pattern TP > N-NO3- > N-NH4+ > SRP ≈ DIN: SRP. This trend confirms that the concentration response to increasing water flux depends on nutrient form. Moreover, the CV_C/CV_Q ratio pinpoints three main groups: (1) chemostatic behaviour ($CV_C/CV_Q < 0.5$) for $N\text{-}NO_3^-$ (0.48), (2) chemodynamic behaviour ($CV_C/CV_Q > 0.5$) for SRP (0.59) and TP (0.9), (3) highly chemodynamic behaviour ($CV_C/CV_Q > 1$) for $N\text{-}NH_4^+$ (1.45).

The relationship between c and Q was also tested for each nutrient by month (Figure 1.1) to assess whether the general trend is constant along the year or is season-driven. TP monthly slope is overall positive throughout the year, although it is significant only during May, and from September to November, indicating the dominance of the mobilization regime during these months. By contrast, the $N\text{-}NO_3^-$ export pattern changes by month: log(c)-log(Q) slope is not significant during winter and spring, while from June to August it is characterized by a mobilization pattern ($b > 0$) and during October and November we observed a dilution scheme ($b < 0$). The concentration of $N\text{-}NH_4^+$ and SRP appears to be largely independent of water discharge, except in May, September and October when a mobilization pattern of ammonium occurred, and with a significant dilution pattern for SRP in colder months (November, January and February). As a clear consequence DIN:SRP molar ratio appears to be largely independent from Q; the only exception is during the colder months (Dec-Feb), where the slope becomes more significant ($p_{Dec} = 0.08$, $p_{Jan} = 0.01$, $p_{Feb} = 0.001$) and increasing from 0.16 in December, to 0.32 in February. This trend is mostly driven by the dilution pattern of SRP, because $N\text{-}NO_3^-$ follows a chemostatic scheme.

According to the general ratio (CV_C/CV_Q), $N\text{-}NH_4^+$ and TP present respectively a highly chemodynamic and a chemodynamic behaviour at monthly scale. SRP shows a general chemodynamic behaviour except during September and October, which are characterized by a chemostatic behaviour. Finally, for $N\text{-}NO_3^-$ we observed constant chemostatic behaviour.

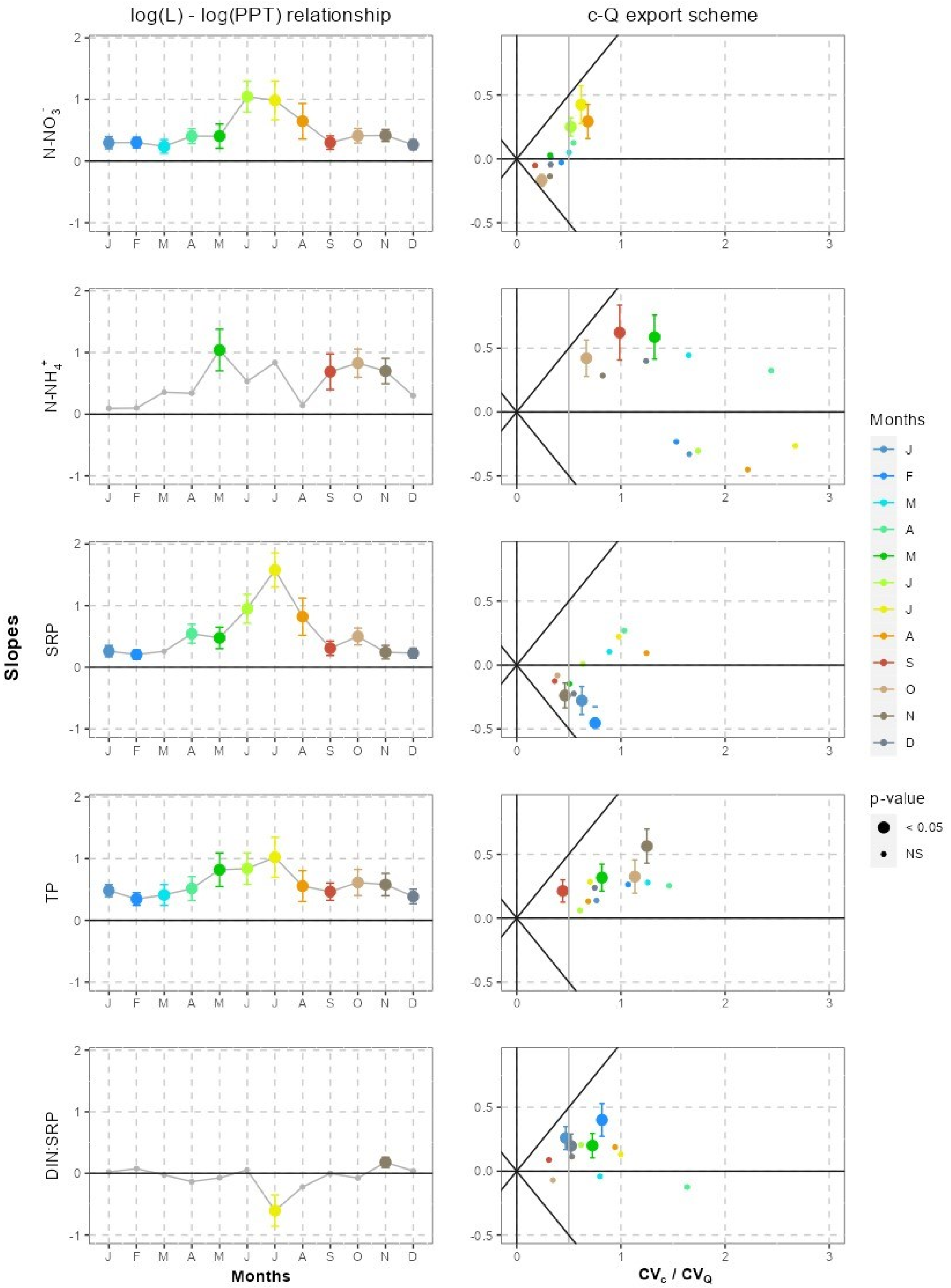

Figure 2.5 *Slope ± st. error of log-log relationship between precipitation versus nutrient loads* ***(left)****. Dot size represents the significance of log(L)-log(PPT) slope (expanded plots of monthly relationships are available on* ***Figure S. 2-4****) Export regime scheme as described by Musolff et*

*al. (2015) (**right**)). On y-axis is reported the slope ± st. error of the log(c)-log(Q) relationship, on x-axis the CV_C/CV_Q ratio. Dot size represents the significance of log(c)-log(Q) slope. Negative slope ($b < 0$) indicates a dilution pattern, while positive slope ($b > 0$) indicates a mobilization regime. When $b \approx 0$, solid grey line ($x = 0.5$) indicates the theoretical boundary which differentiates chemostatic from chemodynamic behaviour. Expanded plots of monthly relationships are available on **Figure S. 2 3**.*

2.5 Discussion

In this work we tested the interaction between the long-term and interannual trends of nutrients. Second, we estimated the basin export regime in order to identify the major drivers which control seasonal export. Finally, we characterized, at the monthly scale, the effect of precipitation on N (N-NO_3^-, N-NH_4^+) and P (SRP, TP) load export. Precipitation plays a key role in controlling nutrient export, but it varies by month and nutrient form. Nutrient concentrations co-vary with discharge along the year and they can diverge from positive to negative correlation influencing loads export. Moreover, our results showed that climate trends has been contributing to modifying monthly exported load, with a different magnitude by month.

2.5.1 Precipitation regulates loads export

The Po River basin is characterized by a biphasic precipitation pattern, which corresponds to a biphasic water discharge, as described by Montanari (2012). However, the response of water discharge to precipitation varies by season, with a maximum in summer and a minimum in winter, with the form of precipitation being responsible for this relationship. For instance, the effect is immediate in the case of rainfall and reaches the peak in summer due to water scarcity and higher agricultural demand, while from winter to early spring snowfall and snowmelt contribute to delay the increase in water flux. In fact, precipitation is stored as snow on the Alps during the winter season and then it is released from spring to early summer contributing to the Po flood during this period (Zanchettin et al., 2008).

This pattern similarly drives exported load behaviour, with some differences driven by nutrient types. The clear direction of the slope is generally interpretated as a hydrological and geological control. Solutes with a depth origin (e.g., bedrock or derived solutes) usually show a dilution scheme and solutes with surface genesis (e.g. litter and uppersoil layers) are characterised by enrichment patterns (Speir et al., 2024). We use this approach to explain the different precipitation effect on exported load by nutrient and season, and whether load increase is driven only by discharge (e.g. dilution pattern) or also by concentration growth (e.g. erosion effect).

TP and N-NH_4^+ show a coupled mobilization pattern during autumn and in May which are the wettest periods of the year. This aspect enhances the role of precipitation on the export rate, in contrast to the other months. While for TP this aspect is quite predictable, due to the erosion effect of rainfall on P particulate form, particularly in heavily impacted basins (Bieroza & Heathwaite, 2015; Dolph et al., 2019), for N-NH_4^+ it is a less predictable result. However, this appears to be consistent with Tesi et al. (2013) who found that in the Po River ammonia was positively correlated with suspended sediment (which increases during high flow events). The authors also suggest N-NH_4^+ concentration to be driven by either microbial activity (ammonification-nitrification) or ionic exchange, the rates of which are proportional to suspended sediment concentration. Finally, the observed chemodynamic behaviour associated with positive slopes during these months is likely linked to a threshold-driven transport dependant pattern. Moreover, the activation of heterogeneously distributed sources can be related to the geological spatial heterogeneity for TP, but opens further issues for N-NH_4^+ (Musolff et al., 2015).

Precipitation in form of rainfall also seems to control N-NO_3^- concentration during high flow periods from June to August. In summer, water scarcity contributes to increased stream intermittency due to higher evapotranspiration rates. Concurrently, agricultural demand favours water retention within the artificial channels, contributing to retaining N-NO_3^- in the soil or in the riverbeds. Therefore, intensive summer rainfall leads to hydrological network connectivity and can mobilise and export N-NO_3^- during high flow, especially in agricultural catchments (Bauwe et al., 2020; Cuadra and Vidon, 2011; Speir et al., 2021). In addition, the dry conditions of streams or artificial channels can favour the oxidation of ammonia to N-NO_3^-, thus increasing the N-NO_3^- pool (Merbt et al., 2016) which is washed away during first flushes.

SRP dilution pattern from December to February reduces the precipitation weights on the exported load. We associate lower SRP concentrations in wetter months with the hypothesis that point sources dominate SRP emissions (Minaudo et al., 2019) in the winter period.

2.5.2 Concentration dynamic reveals mains sources and biological controls

When the log(c)-log(Q) slope is non-clearly significant, variability of concentration against discharge (CV_C/CV_Q) indicate a degree of nutrients transformation along the continuum (Speri et al., 2024).

During summer, SRP and N-NH_4^+ show strong chemodynamic behaviour. This is generally associated with high reactivity, which may depend on biological control processes (Mussolf et al., 2015). Low flow and high light availability support stream metabolism and phytoplankton growth, which removes reactive P and N from the water column and decreases dissolved nutrient concentrations (Bernhardt et al., 2018; Tavernini et al., 2011). N-NO_3^- may also be affected by biological controls, although to a lesser extent, as confirmed by the weak chemodynamic behaviour combined with low concentrations during low flow (Moatar et al., 2017). The high variability in concentration could mask the mobilisation effect of SRP in summer, which, similarly to N-NO_3^-, seems to be more easily exported during floods (Outram et al., 2016; Zimmer et al., 2016). The assimilation of SRP could also favour an increase in TP during low flow conditions, thus masking the strong hydrological dynamics of TP dependant by particulate form erosion.

Chemostatic behaviours are generally linked to high availability and diffuse sources (e.g. agricultural sources, groundwater N legacy) (Ebeling et al., 2021; Godsey et al., 2019; Minaudo et al., 2019). This seems to be consistent with the N-NO_3^- and SRP trends in autumn and spring. The high availability of nutrients derived from chemical fertilisation or manure during wet periods may favour leaching phenomena and nutrient export via runoff (Hart et al., 2004; Robertson and Vitousek, 2009). The opposite behaviour between N-NO_3^- and SRP in winter, also confirmed by the DIN:SRP ratio, remains partly unexplained. With increasing flow rate, N-NO_3^- maintains a chemostatic behaviour while SRP decreases significantly. We hypothesise that the dry winter conditions linked to snow deposition would not affect the transport of the highly soluble N-NO_3^-. Contrarily, the conditions are likely to trigger changes in the transport of the less mobile SRP, with the SRP present on site deriving mainly from the point source as an effluent (which can be diluted in case of flow). However, this speculative explanation requires further investigation.

2.5.3 Decreasing trends are driven by changes in seasonality

The long-term analysis shows a general decreasing trend for N-NO_3^-, N-NH_4^+ and TP loads, while SRP shows an intermittent pattern. The decreasing rate varies with the nutrient and is higher for $N\text{-}NH_4^+$ than for $N\text{-}NO_3^-$ and TP, and is largely concentrated in the first decade (1992-2002), with a tendency of stabilisation in the last decade of our dataset.

Load decreasing rates are not constant in the different months (Figure 2.4). As the seasonal scheme depends on the nutrient form, the contribution to the annual load loss: (1) for TP is higher in spring and autumn, (2) for $N\text{-}NH_4^+$ decreases linearly from winter to summer and then increases, (3) for $N\text{-}NO_3^-$ is higher in autumn and lower in summer with intermediate values from winter to spring.

Our results indicate a strong relationships between nutrients, hydrological patterns and coherence of both temporal trends, suggesting that hydrology partially drives nutrient trends from 1992 to 2022. Moreover, the differences in decreasing rates among nutrients suggest that increased drought conditions have contributed to changes in export regimes and thus exported load by nutrient and month.

Our findings also show that the period with the minimum $N\text{-}NO_3^-$ load, usually occurring in Summer (Jul-Sep), varies in intensity and temporal extension (Figure 4). This decreasing phase of the load shows a progressive anticipation (from Jun-Jul to May-Jun) and the minimum of the peak, which is reached in July rather than in August, becomes more pronounced. Finally, the return to the standard higher load phase (Oct-Jun) is delayed from Sep-Oct to Oct-Nov. As such, part of the seasonal curve corresponding to summer becomes flatter and larger than in the 90s.

The SRP also shows a similar pattern, but less significant. The decreasing load of June confirms the minimum anticipation, while the weaker ($p=0.06$) significance of the October rate may suggest an autumnal shift as for $N\text{-}NO_3^-$. However, we do not have enough elements to understand which is the real behaviour of the SRP. Further studies will be necessary to verify if SRP really shows a similar pattern to $N\text{-}NO_3^-$ with a delay, and thus we are measuring a still ongoing dynamic, or if it is just a stochastic variation. The significance of the DIN: SRP ratio is strongly dependent on the trend of the last decade, confirming the steady decrease of $N\text{-}NO_3^-$ against the more fluctuating trend of SRP. Moreover the $N\text{-}NO_3^-$ shows a positive cQ relationships more frequently than SRP, which is usually dominated by dilution pattern, suggesting the decreasing trend is largely led by the hydrological factor.

The decrease in TP, depends on a decrease in the particulate form, which is also the one most dependent on erosive phenomena. The increase of extreme rainfall events in the Mediterranean, due to climate change, of the Mediterranean climate, concentrates precipitation in a few more intense events, contributing to the generation of two different problems: few intense events could export less than a long-wet period (as the decrease in flow rates seems to confirm), but rarer events may be difficult to intercept in monthly sampling, which also creates a bias. On the other hand, the increase of snowmelt (Carrer et al., 2023) might partially affect transport of particulate matter during spring and summer with unclear results. Wet-dry alternation may also have partly influenced $N\text{-}NH_4^+$. The decrease in $N\text{-}NH_4^+$, which is the largest, could be due to the sum of two factors. The main driver is the commissioning of wastewater treatment plants, which reduced the effect of the urban load, as suggested by Viaroli et al. (2018); on the other hand, similar to TP, a minor role of the hydrological factor in the wettest months cannot be excluded.

2.5.4 Wet to dry shift affects nutrients availability on receiving water bodies

The matter flow from the continental to the marine-coastal areas is regulated by precipitation, hydrological conditions and biogeochemical processes that take place in the river and its water basin. At a multi-year scale, significant relationships emerge between precipitation and flow. The long-term trend, however, is clearly affected by seasonal events, which occur especially in summer, when flow rates are low and the runoff can undergo sudden variations induced by short-term intense rainfalls.

We explored these topics through a comparison between dry and wet years, identified in Figure 2.2Figure 2.2. First of all, we grouped the months according to Montanari et al. (2023) in order to represent periods of the year that are characterized by the critical patterns of both hydrological and biogeochemical processes (Figure S. 2-4).

At a basin scale, in both wet and dry years, approximately 50% of precipitation is recorded between May and October, with minimum values in summer (Figure S. 2-4). The ratios between the quantities of water falling to the ground in wet and dry years (WET/DRY) in the different periods considered are relatively low and constant (1.3-1.5). The river flow presents a trend similar to that of precipitation, but with a significantly higher WET/DRY ratio (2.3-2.7) (Figure S. 2-4). This means that in dry years the water deficit is caused not only by the decrease in precipitation, but also by other factors, such as the increase in evaporation and evapotranspiration, and irrigation withdrawals.

In dry years from May to October, the $N\text{-}NO_3^-$ load, which represents over 90% of the reactive N, is less than 30% of the annual total and does not exceed 14% from June to September (Figure S. 2-4). A similar trend also occurs in wet years, but with a WET/DRY ratio between

2.7 and 4.1 in June-August. This means that in the period June-August, with low to absent precipitation, there is a marked decrease in the $N\text{-}NO_3^-$ load which is four times lower than the load in wet years. In other words, $N\text{-}NO_3^-$ is likely retained in arid soils or, in the absence of runoff, is conveyed and stored into groundwater due to irrigation practices (Van Meter et al., 2016).

SRP and TP (Table S.2-4) present trends similar to those of $N\text{-}NO_3^-$, which denotes how drought contributes to reducing also the river load of P. These processes might play a key role in determining the evolution of biodiversity, productivity, food webs and trophic state of the Northern Adriatic Sea, which seemed to undergo "oligotrophication", during the harsh and long drought in the 2003-2007 period (Djakovac et al., 2012; Giani et al., 2012 ; Marini et al., 2023; Neri et al., 2022).

2.6 Conclusion

The analysis of the long-term export regimes of N and P in the Po River allowed us to elucidate the role of precipitation patterns and seasonality in regulating loads, demonstrating as precipitation regime affects both water discharge and also nutrient concentrations. The analysis spanning 30 years indicates that changes in the hydrological regime have affected the timing of load export, resulting in an extension of summer conditions to autumn and a lesser extent towards spring.

The capacity of the water basin to export nutrient loads varies depending on the nutrient (N or P), the molecular form, and the month. Precipitation increases the load, but the magnitude varies depending on the month: it is more pronounced in summer and autumn than in winter and spring owing to a different relationship between concentration and discharge. In autumn rainfall drives runoff increases and then the loads, while during summer the effect is accentuated by also nutrient enrichment. Conversely, winter and spring are more influenced by precipitation as snow, which causes a decoupling between the driver (increasing precipitation) and the effect (load export), with a null or dilution effect on concentration. The observed decreases in $N\text{-}NO_3^-$ and TP over the last 30 years may therefore also be driven by seasonality, as autumn loads have become less relevant compared to the past. The results indicate that extreme weather events of higher magnitude and frequency during the late spring and summer months, together with the expected decrease in the importance of snowmelt predicted by hydrological models in the future years, may further change nutrient export with unexpected results, given the coupling between rainfall regime and N and P loads.

Since the hydrological model predicts a grater tropicalization of the Mediterranean weather this effect will be more pronounced in the coming years (Polade et al., 2017). Water discharge will be more dependent on rainfall and more characterized by extreme events suggesting an increasing importance of transport during high flows. Meanwhile, reduced snow reserves in the Alps and less persistent snow cover (Colombo et al., 2022) will change the regime and transport of Alpine rivers, although it is not yet clear how this will affect nutrient transport at the basin scale.

2.7 Supplementary materials

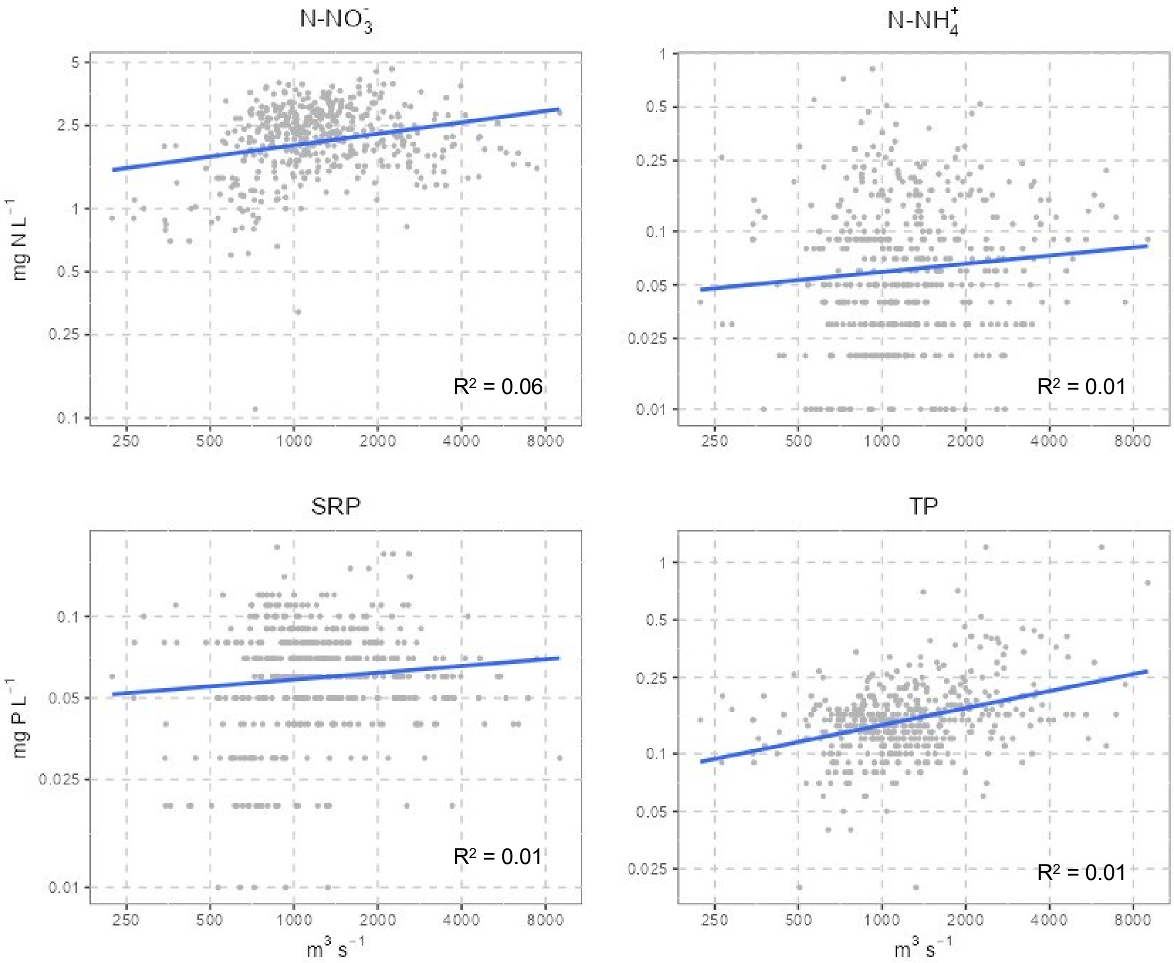

Figure S. 2-1 *log-log relationship between concentration versus water discharge with the whole dataset.* ***Table 2.1*** *provides informative data on the shown relationships.*

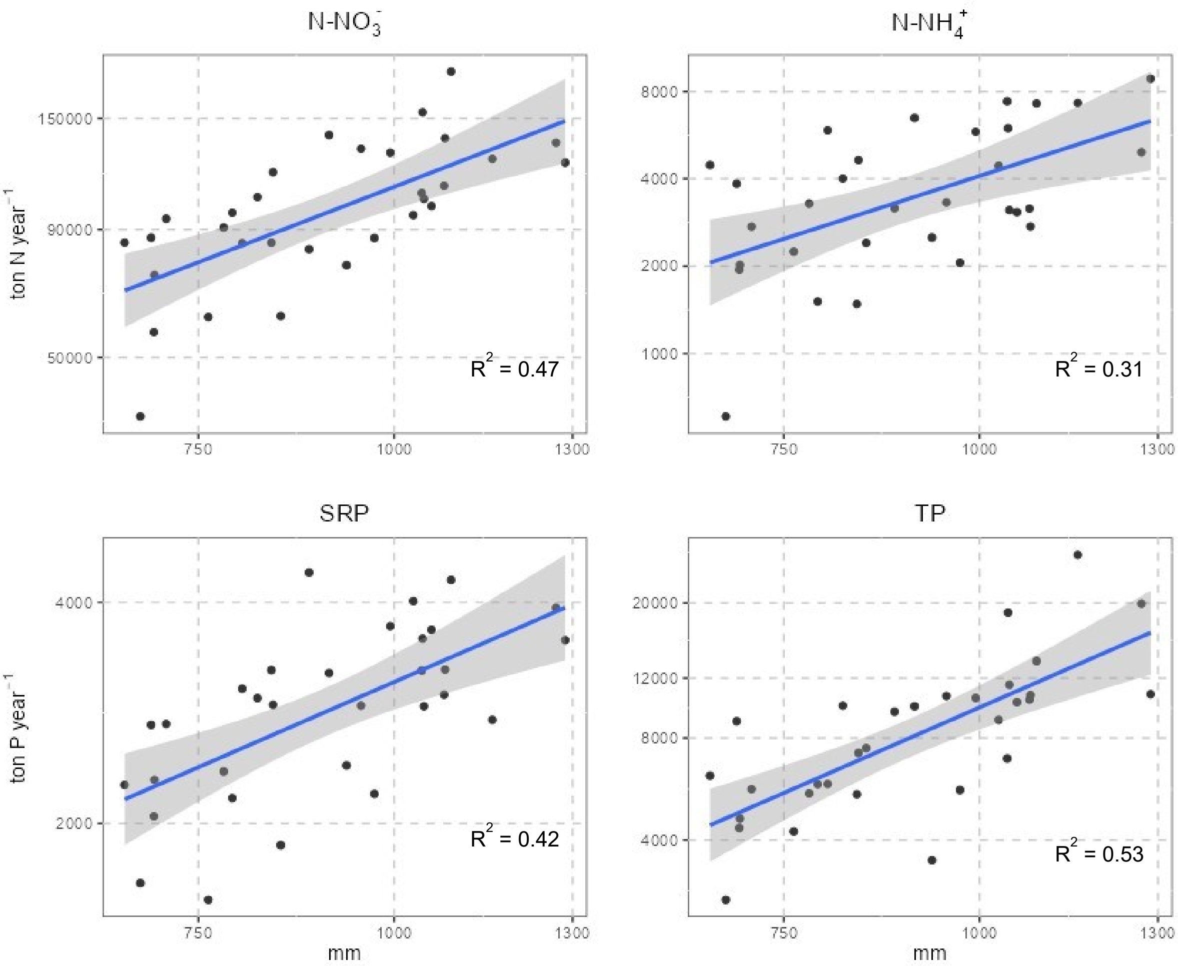

***Figure S. 2-2** log-log relationship between nutrients load versus precipitation with annual data. **Table 2.1** provides informative data on the shown relationships.*

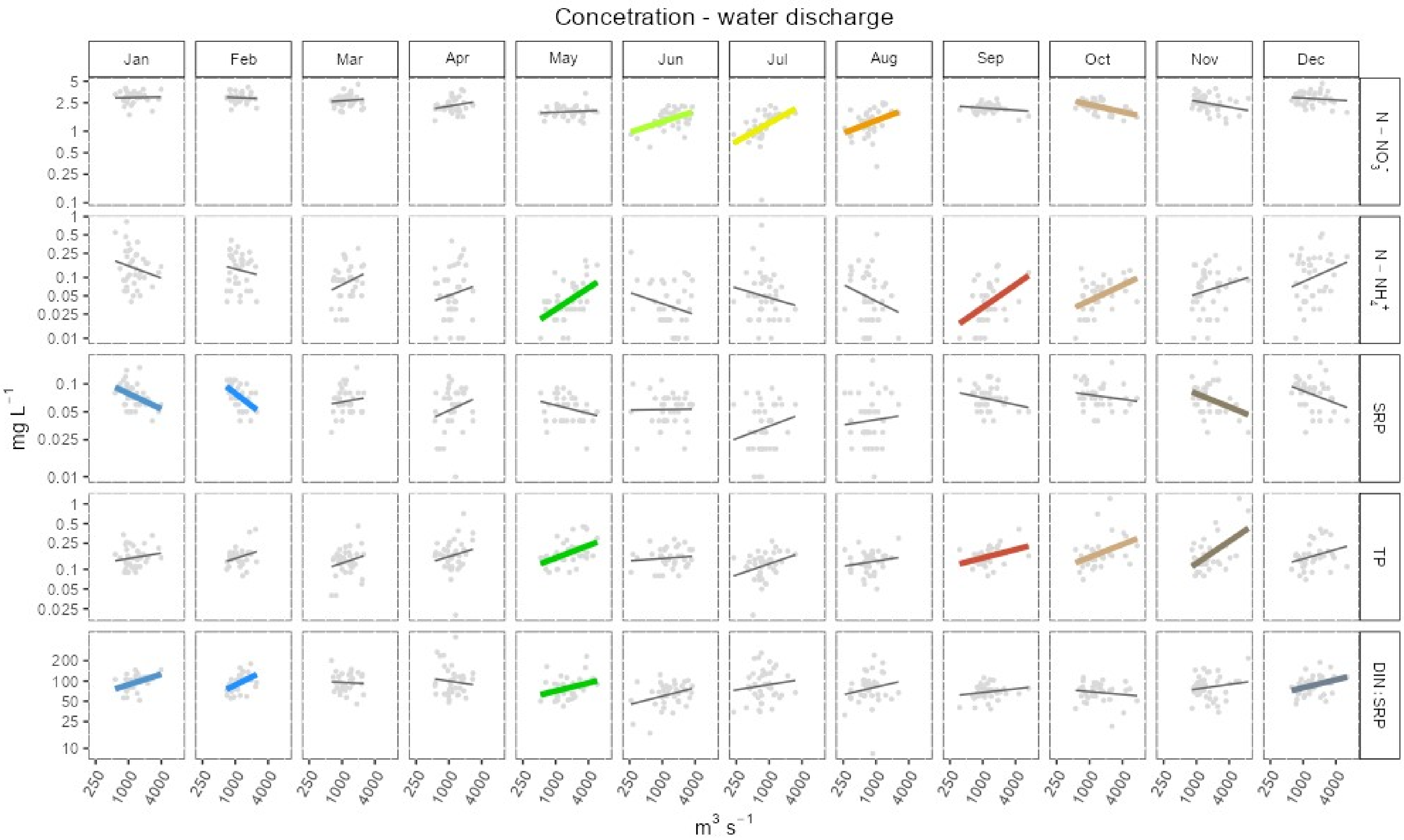

Figure S. 2-3 log-log relationship between concentration versus water discharge. The coloured line represents a significative relationship ($p < 0.05$)

Table S. 2-1 *log-log relationship between concentration versus water discharge. P-value is referred to slope.*

Nutrient	Statistic	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
$N\text{-}NO_3^-$	slope	0.01	-0.03	0.05	0.12	0.03	**0.25**	**0.42**	**0.29**	-0.05	**-0.17**	-0.14	-0.04
	p-value	0.86	0.73	0.56	0.17	0.55	0.00	0.01	0.04	0.23	0.00	0.07	0.51
	R^2	0.00	0.00	0.01	0.05	0.01	0.25	0.18	0.12	0.04	0.27	0.09	0.01
	CV_c/CV_Q	0.33	0.43	0.50	0.55	0.32	0.52	0.62	0.68	0.18	0.24	0.32	0.33
$N\text{-}NH_4^+$	slope	-0.33	-0.23	0.44	0.32	**0.59**	-0.30	-0.27	-0.45	**0.62**	**0.42**	0.28	0.40
	p-value	0.25	0.48	0.15	0.44	0.00	0.15	0.35	0.18	0.01	0.01	0.20	0.15
	R^2	0.04	0.01	0.06	0.02	0.24	0.06	0.02	0.05	0.18	0.20	0.04	0.06
	CV_c/CV_Q	1.66	1.53	1.65	2.44	1.32	1.74	2.67	2.22	0.99	0.67	0.83	1.24
SRP	slope	**-0.28**	**-0.45**	0.10	0.27	-0.15	0.01	0.22	0.09	-0.13	-0.08	**-0.24**	-0.22
	p-value	0.02	0.00	0.49	0.24	0.11	0.93	0.26	0.64	0.14	0.31	0.02	0.05
	R^2	0.16	0.28	0.01	0.04	0.07	0.00	0.03	0.01	0.06	0.03	0.14	0.10
	CV_c/CV_Q	0.63	0.75	0.89	1.03	0.51	0.64	0.98	1.25	0.36	0.39	0.46	0.55
TP	slope	0.14	**0.26**	0.28	0.25	**0.32**	0.06	**0.28**	0.13	**0.21**	**0.33**	**0.56**	0.24
	p-value	0.34	0.09	0.19	0.29	0.00	0.50	0.06	0.31	0.02	0.02	0.00	0.07
	R^2	0.03	0.08	0.05	0.03	0.20	0.01	0.09	0.03	0.14	0.15	0.32	0.09
	CV_c/CV_Q	0.77	1.07	1.26	1.46	0.82	0.61	0.70	0.69	0.44	1.13	1.25	0.75
DIN: SRP	slope	**0.26**	**0.40**	-0.04	-0.12	**0.20**	0.21	0.13	0.19	0.09	-0.07	0.11	**0.20**
	p-value	0.01	0.00	0.78	0.56	0.04	0.05	0.46	0.33	0.26	0.43	0.32	0.04
	R^2	0.20	0.23	0.00	0.01	0.11	0.10	0.01	0.03	0.03	0.02	0.03	0.11
	CV_c/CV_Q	0.47	0.82	0.80	1.64	0.73	0.62	1.00	0.94	0.31	0.35	0.53	0.52

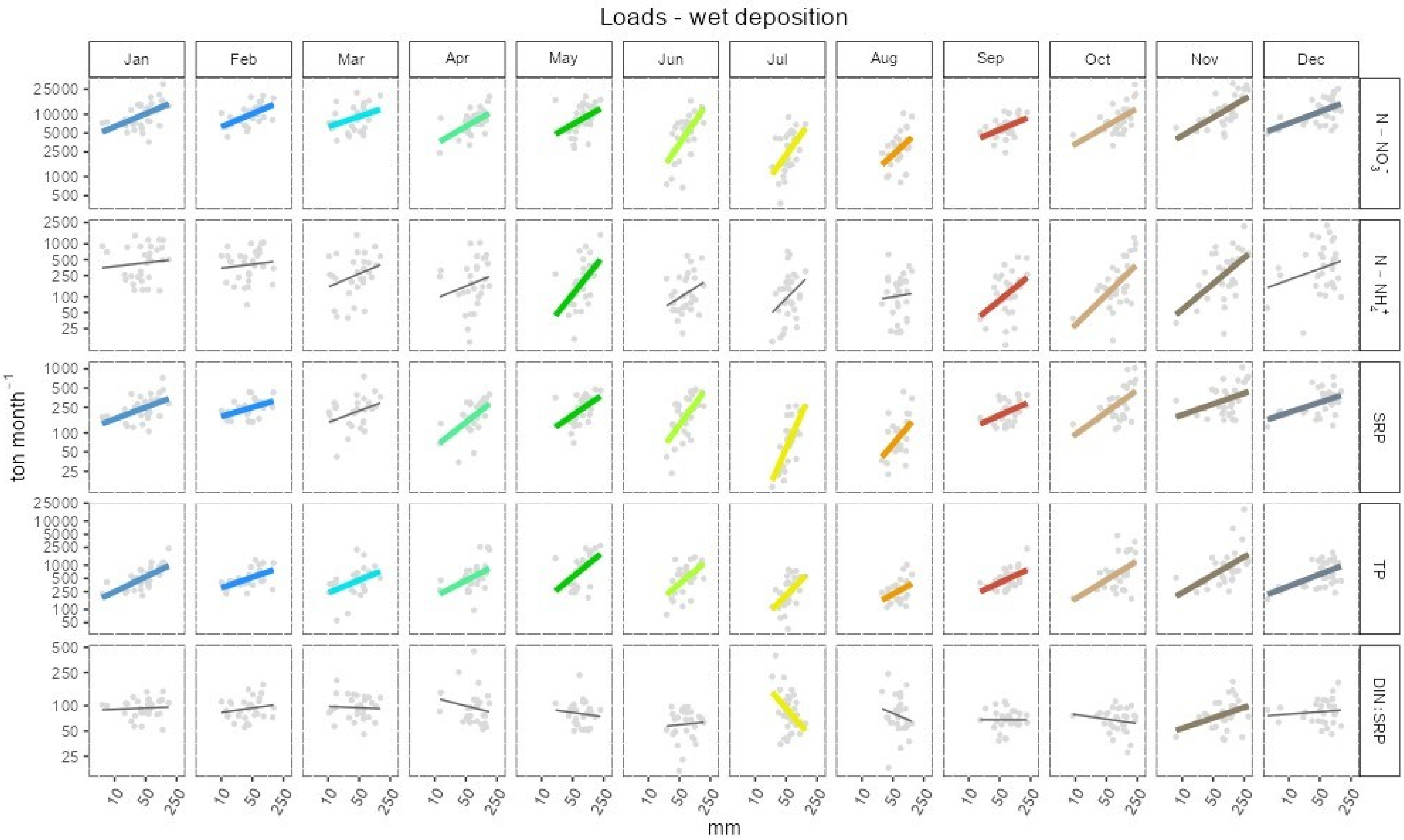

Figure S. 2-4 *log-log relationship between loads versus precipitation. The coloured line represents a significative relationship ($p < 0.05$)*

Table S. 2-2 *log-log relationship between loads versus precipitation. P-value is referred to slope.*

Nutrient	Statistic	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
$N\text{-}NO_3^-$	slope	**0.30**	**0.30**	**0.24**	**0.40**	**0.40**	**1.05**	**0.98**	**0.65**	**0.30**	**0.41**	**0.42**	**0.27**
	p-value	< 0.01	< 0.01	0.04	0.00	0.05	< 0.01	< 0.01	0.03	0.01	< 0.01	< 0.01	< 0.01
	Intercept	-3.23	-3.24	-3.09	-4.12	-4.04	-7.27	-7.43	-6.11	-3.83	-4.13	-3.83	-3.03
	R^2	0.24	0.31	0.14	0.29	0.13	0.39	0.25	0.15	0.21	0.30	0.39	0.28
$N\text{-}NH_4^+$	slope	0.10	0.10	0.36	0.34	**1.04**	0.53	0.84	0.14	**0.69**	**0.83**	**0.70**	0.30
	p-value	0.59	0.59	0.12	0.30	< 0.01	0.11	0.09	0.76	0.02	< 0.01	< 0.01	0.16
	Intercept	-5.59	-5.67	-7.08	-7.56	-10.65	-8.81	-10.04	-7.25	-9.53	-9.83	-8.86	-6.64
	R^2	0.01	0.01	0.08	0.04	0.25	0.09	0.10	0.00	0.16	0.31	0.29	0.07
SRP	slope	**0.26**	**0.20**	0.26	**0.54**	**0.48**	**0.95**	**1.58**	**0.82**	**0.31**	**0.50**	**0.24**	**0.23**
	p-value	0.01	0.01	0.06	< 0.01	0.01	< 0.01	< 0.01	0.01	0.01	< 0.01	0.04	0.01
	Intercept	-6.79	-6.57	-6.90	-8.44	-7.92	-10.12	-13.44	-10.31	-7.26	-7.90	-6.60	-6.48
	R^2	0.22	0.20	0.12	0.33	0.22	0.37	0.53	0.21	0.19	0.31	0.14	0.21
TP	slope	**0.48**	**0.35**	**0.41**	**0.52**	**0.82**	**0.84**	**1.02**	**0.55**	**0.46**	**0.61**	**0.58**	**0.39**
	p-value	< 0.01	< 0.01	0.02	0.01	0.01	< 0.01	< 0.01	0.04	< 0.01	0.01	< 0.01	< 0.01
	Intercept	-6.88	-6.36	-6.78	-7.20	-8.21	-8.64	-9.96	-8.07	-7.12	-7.55	-7.16	-6.37
	R^2	0.46	0.29	0.18	0.21	0.24	0.28	0.26	0.15	0.27	0.22	0.26	0.27
DIN: SRP	slope	0.02	0.08	-0.02	-0.14	-0.07	0.05	**-0.60**	-0.22	0.00	-0.08	**0.18**	0.04
	p-value	0.71	0.34	0.77	0.37	0.60	0.75	0.02	0.39	0.99	0.40	0.04	0.56
	Intercept	-8.43	-8.64	-8.23	-7.75	-8.18	-9.01	-5.98	-7.62	-8.65	-8.34	-9.31	-8.60
	R^2	0.01	0.03	0.00	0.03	0.01	0.00	0.17	0.03	0.00	0.03	0.13	0.01

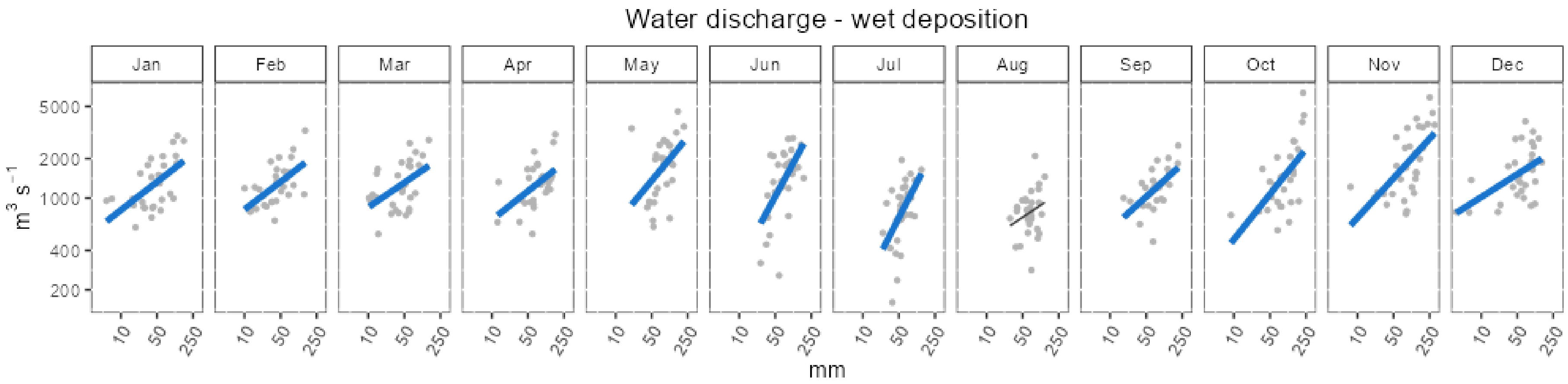

***Figure S. 2-5** log-log relationship between water discharge versus precipitation. The coloured line represents a significative relationship ($p < 0.05$)*

***Table S. 2-3** log-log relationship between water discharge versus precipitation. p-value is referred to slope.*

Nutrient	Statistic	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Q	slope	**0.31**	**0.30**	**0.27**	**0.31**	**0.48**	**0.73**	**0.77**	**0.26**	**0.36**	**0.49**	**0.43**	**0.25**
	p-value	< 0.01	< 0.01	0.01	< 0.01	0.02	< 0.01	< 0.01	0.17	< 0.01	< 0.01	< 0.01	< 0.01
	Intercept	-5.30	-5.28	-5.17	-5.48	-5.94	-7.19	-7.73	-5.75	-5.76	-6.24	-5.72	-4.94
	R^2	0.33	0.35	0.23	0.25	0.18	0.33	0.27	0.07	0.34	0.35	0.38	0.26

Table S. 2-4 *Average values of precipitation, water discharge, N-NO_3^-, SRP and TP during wet and dry years, with a focus on months along warmer period (May to October)*

Condition	UoM	Year (J-D)	MJJASO	MJJAS	JJASO	JJAS	JJA	JJ
Precipitation								
DRY	mm	705	381	315	315	248	183	109
	%	100	54	44	44	35	26	15
WET	mm	1,154	572	437	446	311	242	149
	%	100	50	38	39	27	21	13
ratio W:D		1.6	1.5	1.4	1.4	1.3	1.3	1.4
Water discharge								
DRY	Mm^3	26,497	10,946	8,669	8,580	6,303	4,121	2,875
	%	100	41	33	32	24	16	11
WET	Mm^3	63,024	28,570	22,857	20,354	14,641	11,115	7,588
	%	100	45	36	32	23	18	12
ratio W:D		2.4	2.6	2.6	2.4	2.3	2.7	2.6
N-NO_3^-								
DRY	Kt	63.6	19.4	13.6	14.7	8.9	4.3	3.0
	%	100	30	21	23	14	7	5
WET	Kt	137.5	52.2	39.8	37.7	25.3	17.5	10.4
	%	100	38	29	27	18	13	8
ratio W:D		2.1	2.7	2.9	2.6	2.8	4.1	3.4
SRP								
DRY	t	1,977	730	517	561	348	176	127
	%	100	37	26	18	17	9	6
WET	t	3,483	1,489	1,111	1,152	774	550	326
	%	100	43	32	33	22	16	9
ratio W:D		1.7	2.0	2.1	2.1	2.2	3.1	2.6
TP								
DRY	t	5,867	1,564	1,211	1,287	934	510	367
	%	100	27	21	22	16	9	6
WET	t	14,655	5,885	4,168	4,126	2,408	1,848	1,206
	%	100	40	28	28	16	13	8
ratio W:D		2.5	3.8	3.4	3.2	2.6	3.6	3.3

3 Chapter III

Net anthropogenic nitrogen/ phosphorus inputs and hydrological factors: effects on genesis, form and stoichiometry of the exported loads

3.1 Introduction

In the last 70 years, land colonisation and anthropogenic metabolism have strongly altered the processes of transport and transformation of nitrogen and phosphorus within water basins (Conley et al., 2009; Smith et al., 2009, Sutton et al. 2013). Growth in agricultural areas, intensive livestock farming, and urbanisation have changed about three-quarters of the Earth's ice-free surface of the Earth, reducing the capacity of ecosystems to absorb and treat nutrient inputs (Ellis et al., 2010; Pinay et al., 2015; Seitzinger et al., 2006). In parallel, the use of fertilisers has increased globally by more than 500%, while the production and trade of nitrogen and phosphorus in the form of animal and plant proteins has increased eight times, leading to a significant transfer of N and P across the water basin (Foley et al., 2011; Lassaletta et al., 2014). As a result, excess nutrients contaminate groundwater and increase nutrient fluxes through the river system into the sea. The resulting eutrophication is considered one of the greatest threats to many lakes, estuaries and coastal areas (Diaz and Rosenberg, 2008; Howarth, 2008; Reed and Harrison, 2016; Von Schiller et al., 2017; Wurtsbaugh et al., 2019; Le Moal et al., 2019).

Yet, the consequences of the eutrophication process are complex and depend not only on the total amount of N and P that reaches aquatic environments but also on their stoichiometric ratio and bioavailability. In fact, an imbalance in the proportion of N and P, with respect to the requirements for balanced growth of living organisms and the molecular form in which the two nutrients are found, have important effects on the growth of the community of primary producers with consequences that affect the entire trophic chain (Glibert, 2017). For example, changes in the nutrient stoichiometry can promote the growth of some algal groups at the expense of others (i.e., nitrophilous algae or nitrogen-fixing cyanobacteria under respectively nitrogen and phosphorus excess), lead to an overall impoverishment of biodiversity, and can determine the conditions for some species to produce toxins (Billen & Garnier 2007; Glibert

2017; Justice et al., 1995; Stutter et al. 2018; Penuelas & Sardans 2022). Understanding the drivers of N and P fluxes in water basins has thus been a major focus of research over the past four decades (Howarth et al., 1996; Goyette et al., 2016). However, our understanding of how the different drivers affect the transport processes of the different N and P forms, as well as their stoichiometry at the water basin level, remains challenging influencing our ability to control eutrophication (Collins et al., 2017; Maranger et al. 2018).

While inputs from human activities are very often the main source of N and P affecting freshwaters, the load exported by a river and its stoichiometry are controlled by complex processes that influence movement, mobilization, and retention across land and waterscapes (Ebeling et al. 2021; Goyette et al. 2019; Moatar et al. 2017; Shousha et al. 2023). Mass balance studies at the water basin level indicate that nitrogen and phosphorus load through hydrographic networks and their changes over time are closely linked to net inputs of human origin (Howarth et al. 1996; Goyette et al. 2016; Hong et al. 2107; Viaroli et al. 2018). However, this same approach also evidences a different relationship between anthropogenic nitrogen and phosphorus inputs and the exported fraction, which is equal to an average of 20% of the nitrogen input and 5% of the phosphorus input. However, this relationship resulted extremely variable ranging from 5-50% for nitrogen to 1-15% for phosphorus as a probable consequence of the interplay between biogeochemical characteristics of the element considered, the organization of the agricultural system and the spatial and hydrological characteristics of the water basin, such as precipitation, structure of the hydrographic network, or runoff (Hong et al., 2012; Goyette et al. 2019; Romero et al. 2021).

Large-scale analysis of the relationship between net N and P input and export evidence an influence of climate and hydrology on the fraction of the exported loads (Howarth et al., 1996; Boyer et al., 2006; Goyette et al. 2019). However, the effect of climatic factors on the amount of N and P export could vary depending on the organization of the agricultural system and the form in which N and P are exported. In particular, if the analysis of the relationship between net N and P inputs and precipitation has clarified the main dynamics of regulation between inputs and output, the weight of the different factors and how their role may vary in combination with the other characteristics of the basin are still uncertain. Moreover, if the dynamics of nitrogen are quite studied, how and which factors contribute significantly to regulate the stoichiometric N:P ratio is a less studied aspect.

The organization of the agricultural system is expected to contribute to riverine flux at varying degrees through differential effects on the form and magnitude of net N and P input. With respect to this, the concept of anthropogenic autotrophy and heterotrophy of water basins was introduced to summarize information about the organization of agricultural activities in a territory (Billen et al., 2010). The difference between autotrophy (agricultural production) and heterotrophy (human and livestock consumption) is a measure of the net export (or import) of nitrogen and phosphorus in the form of food and feed. In many net autotrophic water basins synthetic fertilizers are the single largest input while net heterotrophy is a proxy for sewage and animal manure excesses ultimately derived from the imported food and feed that support large urban populations or intensive livestock production operations. The load exported from diffuse sources (e.g., agricultural soils) is strongly linked to precipitation and runoff with a time-varying effect on the export of dissolved and particulate N and P (Dupas et al. 2015; Lasaletta et al. 2012; Romero et al. 2021). On the contrary, point sources (e.g., wastewater treatment plants) generate a more constant load over time which is apparently less subject to interaction with hydrological variables (Abbott et al., 2018; Mussolf et al., 2015). Nitrate, given its high solubility and therefore mobility is more easily exported under conditions of greater water availability through the phenomenon of soil leaching, and in the presence of permeable soils, which favours the accumulation of nitrogen in groundwater (Dupas et al., 2016; Howarth et al., 2012). On the contrary, phosphorus is more reactive and less mobile, easily adsorbed by soil particles, and is mainly exported from uncovered soils through the erosive action of rain and surface runoff (Baker et al., 2015; Dupas et al., 2018; Kalkhoff et al., 2016). Urbanisation of the territory also contributes to modifying exports with different effects for nitrogen and phosphorus: land sealing alters the intensity of floods and the hydrological regime (O'Driscoll et al., 2010), rivers rectification decreases the retention capacity and the buffering effect of perifluvial environments (Newcomer Johnson et al., 2016), while river damming affects export times and modifies the composition of the nitrogen and phosphorus pool (Maavara et al., 2020).

The in-depth study of these aspects acquires considerable importance in light of the effect of climate change in modifying the hydrological regime, which interacts with local anthropogenic dynamics generating different effects from region to region (Donelly et al., 2017). Areas such as the Alps, which are experiencing faster than expected glacier melting, and the Mediterranean in general, which is experiencing above-average temperature changes, can be considered paradigmatic cases (Gobiet et al., 2014; Segui et al., 2010). Climate change, acting on the hydrological and temperature regime, can determine important effects not only on water availability, both in a quantitative and temporal sense, but also on soil metabolism and river connectivity, altering the dynamics that regulate the export and transport of nutrients from the terrestrial to the aquatic matrix (Bernal et al., 2013; Brighenti et al., 2019; Mastrotheodoros et. 2020; Rogora et. 2018). The area of Northern Italy is characterized by both of these factors and nearby river basins, managed homogeneously from an economic and administrative point of view, are characterized at the same time by a high heterogeneity of hydrological forcings. The studies currently available in the Po Basin mainly report information on the annual and interannual variations in the nutrient load through the closure section of Pontelagoscuro (Cozzi and Giani, 2011; Romero et al., 2013; Viaroli et al., 2018). On the contrary, there is little research that analyses the contribution of individual sub-basins to load formation. In addition, the analysis of river loads presents some margins of uncertainty due to the incidence of hydrological and morphological factors that are not usually considered. Disentangling how the interplay between the organization of the agricultural system and the spatial and hydrological characteristics of the water basin, differentially influence N and P loads and their stoichiometry would therefore help to understand changes resulting from climate and land use changes.

This study aims to quantify the nitrogen and phosphorus loads generated by anthropogenic activity and exported from water basins located in Northern Italy in the period 2014-2019. Specifically, the objectives of the work are twofold: (1) to analyse how the different components that contribute to the generation of anthropogenic nitrogen and phosphorus inputs contribute to determining the nitrogen and phosphorus loads exported from catchments; (2) to analyse how differences in hydrogeological conditions influence the composition of exported nutrients and the stoichiometric ratios between N and P. In particular, we want to test the hypothesis that in heterotrophic basins the load is different than in autotrophic basins and that the degree of autotrophy or heterotrophy influences the exported load. We also expect that, given the different characteristics of the biogeochemical cycles of N and P, the effect will be different.

3.2 Materials and Methods

3.2.1 Study Area

This study was conducted in 42 basins located within the Po River Hydrographic District (Northern Italy); an area defined under the Water Framework Directive for water management (Legislative Decree 152/2006). The Po River Hydrographic District has a total surface area of

86,800 km^2 and comprises the catchment of the Po River, the major river draining to the Adriatic Sea and one of the largest rivers in southern Europe, which accounts for approximately 85% of the total surface area (74,000 km^2) of the district. Approximately 47% of the land cover is anthropized (40% agricultural and 7% urbanised) while the remaining 51% is natural (Corine Land Cover 2012). The average population density is 232 ind. km^{-2} while livestock units (1 LU = 1 adult dairy cow) account for 48 LU km^{-2} (https://www.istat.it). The spatial distribution of land use is heterogeneous with agricultural areas concentrated in lowlands where anthropogenic activity has developed a dense and capillary artificial hydrographic network, deeply interconnected and difficult to separate from the natural one (https://www.isprambiente.gov.it/it) (Figure 3.1). The territory comprises 6 different Hydro-ecoregions: Inner Alps East, Inner Alps Central, Inner Alps South, Southern Pre-Alps and Dolomites, Apennines North, Piedmont Apennines, Monferrato, Po Plain (Wasson et al., 2007). The Alpine and Apennine sides of the district have different hydrological characteristics. The Alpine side is characterised by the presence of a great number of both high-altitude small lakes and reservoirs, and five large deep subalpine lakes fed by Alpine glaciers (Lakes Maggiore, Como, Iseo, Idro and Garda) while those located on the Apennine side lack significant water storage. The south side of the water basins is therefore affected by water scarcity, and streams and rivers have an extremely variable flow regime.

The water basins included in the study (Figure 3.1) therefore encompass different climatological and physical characteristics, and regions with different socioeconomic features. They were selected based on the following criteria: (i) the catchment areas are independent; (ii) the sampling stations for concentration and discharge measurements are coupled and located close to the river mouth representing more than 80% of the catchment area; (iii) the concentration data contain at least 50 observations after the elimination of outliers; (iv) the flow data are available for more than the 80% of the entire period with daily frequency. The surface area of the basins considered varies between 106 and 8,271 km^2. Of the 42 basins analysed, 35 flow directly into the river Po and drain 88% of its catchment area, which is therefore largely represented, while the remaining 7 water basins flow directly into the Adriatic Sea. Data used in the present study to calculate input and output N and P loads were obtained from several water authorities and institutions as described in the following sections.

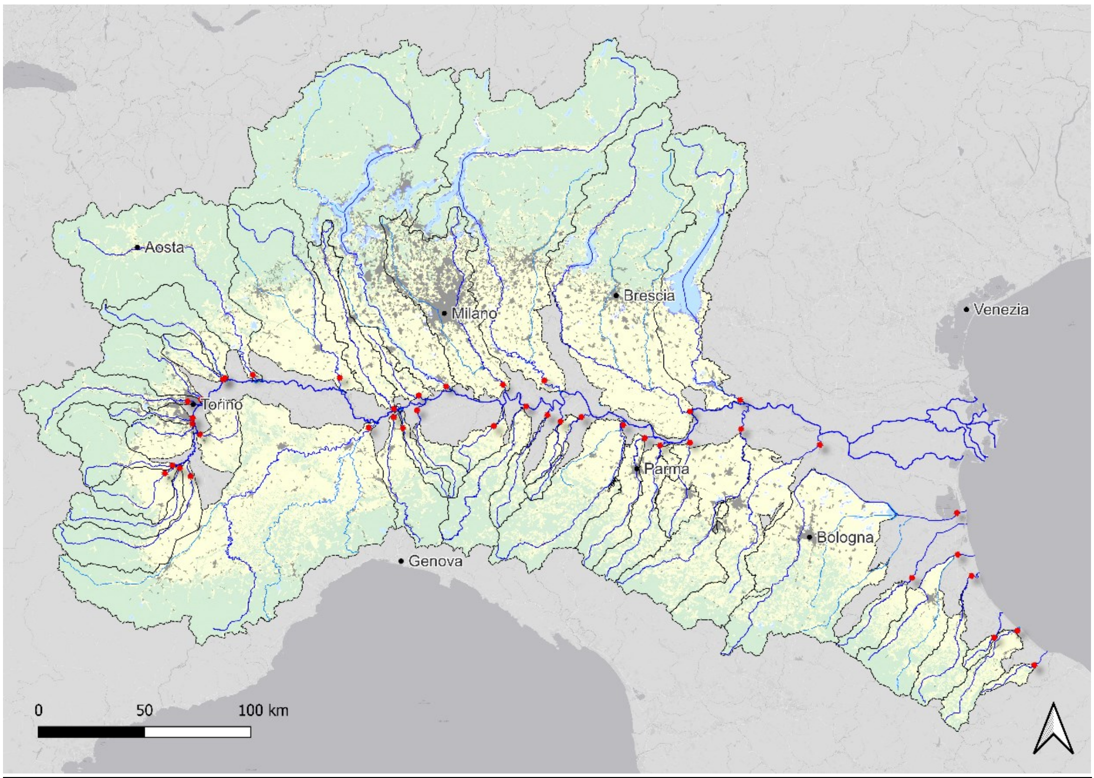

Figure 3.1 *Map of the Po River district showing the main hydrographic network, the soil use (Corine Land Cover), the studied water basins and the sampling stations for load quantification.*

3.2.2 Net anthropogenic nitrogen and phosphorus input to water basins

Net anthropogenic nitrogen (N) and phosphorus (P) inputs were calculated using the Net Anthropogenic Nitrogen Input (NANI) and Net Anthropogenic Phosphorus Input (NAPI) approaches, respectively. The methodological approach was initially introduced by Howarth et al., (1996), concerning the calculation of the N load, and then adapted and applied also for the P load (Russell et al., 2008; Hong et al., 2012; Han et al., 2013). NANI and NAPI were quantified as follows:

$$NANI = N_{DEP} + N_{FERT} + N_{FIX} + N_{FEED} + N_{FOOD}$$

$$NAPI = P_{FERT} + P_{DET} + P_{FEED} + P_{FOOD}$$

where N_{DEP} = atmospheric N deposition on total catchment 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_{FEED} and P_{FEED} = net exchange of N and P as feed; N_{FOOD} and P_{FOOD} = net exchange of N and P as food.

NANI and NAPI were first calculated at the municipal scale, which is the smallest administrative unit at which most of the national statistics are available. The data used are for 2018. To calculate the contribution of each municipality to the Nr budget, municipality-level data were then aggregated at the catchment scale by weighting each municipality based on the spatial distribution of land use areas in the water basin (Han and Allan, 2008). Fertilizer application, agricultural N fixation and net N and P food and feed imports were calculated using statistical data from different sources (Table 3.1)

Table 3.1 *Summary of statistical data sources used for the calculation of nutrient balances.*

Kind of data	Sources
Agricultural soil use	Database AGEA (Agency for Agricultural Payments) elaborated by the CREA (Council for Agricultural Research and Analysis of the Agricultural Economy)
Crop production	Data from the National Institute of Statistics (http://dati.istat.it/)
Livestock density data	National Livestock Register Data (https://www.vetinfo.it/j6_statistiche/#/)
Synthetic fertilizers	Annals of Agrarian Statistics (http://dati.istat.it/)
Population	Data from the National Institute of Statistics (http://dati.istat.it/)

Fertilizer N (N_{FERT}) and P (P_{FERT}) applications were estimated using official national data (Table 3.1) on annual synthetic fertilizer distribution at the provincial level converted into nutrient amounts by means of average N and P contents for each fertilizer type. It was assumed that, for a given year, the amount distributed in each province was equivalent to the quantity effectively applied on the agricultural land of the same province. Given the inter-annual variability associated with the use of fertilizers as a result of crop rotations, the average for the period 2017-2019 was used.

The amount of fixed N_2 (N_{FIX}) associated with alfalfa, permanent grasslands and pastures was estimated by multiplying the production of the specific N-fixing crop by the N content in harvested portions. This calculation provided the N amount fixed in the harvested aboveground tissues, which was then corrected for a coefficient (1.4) expressing the ratio of total biomass produced to harvested biomass (Carlsson and Huss-Danell 2003). For non-symbiotic N-fixation in rice paddy soils, woody crops and other arable land, was adopted a constant rate of 33, 8 and 5 kg N ha^{-2} y^{-1}, respectively (Herridge et al., 2008; Smil, 1999). Other N-fixing crops (e.g. pulses) were not considered since the occupied surfaces were negligible in the investigated catchments.

The net N and P imports with food and feed trade were calculated as the difference between production (i.e. the sum of crop and livestock production in the catchment) and consumption (i.e. sum of human and livestock intake in the catchment). The amount of N and P produced in crops and exported from agricultural land with crop harvest was calculated by multiplying the production of each crop type by the respective N and P content in the harvested portions (Rural Development Program for 2007–2013, Lombardy and Emilia-Romagna Regions). For N-fixing crops, the harvested N was assumed equal to the N amount fixed in the above-ground biomass. These terms were then divided between the amount distributed as food for humans and as feed for livestock and corrected for processing losses to include them in the NANI and NAPI computations. For each crop typology, an average value for the study period was calculated according to the FAO Food Balance Sheet of Italy (FAOSTAT http://www.fao.org/faostat/en/#data). When data were not available, published values were used (Hong et al., 2012).

Livestock consumption was calculated from livestock numbers multiplied by the specific intake parameters for each livestock category (Decree of the Italian Ministry of Agricultural and Forest Policies 4 July 2006; Crovetto and Sandrucci, 2010). Livestock production was calculated as the difference between animal intake and excretion (Hong et al., 2012).

Human consumption was estimated by multiplying the human population by N and P consumption per capita. N consumption was calculated from protein consumption, with protein = N x 6.25 (Jones, 1941). Protein consumption data for the study period were obtained from FAO statistics (FAOSTAT http://www.fao.org/faostat/en/#data) and ranged between 82 and 111 g $capita^{-1}$ d^{-1}. Human P consumption was estimated assuming N: P=5 by weight (Hong et al., 2012; Russell et al., 2008).

Annual atmospheric N deposition (N_{DEP}) was estimated using data of wet and dry deposition of oxidized N. Data were extrapolated from the EMEP-Co-operative Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe (http://www.emep.int) for years 2017-2019 and then averaged. We did not consider atmospheric P deposition.

The human use of phosphorus not associated with food consumption (P_{DET}) derives mainly from the detergents used daily by the population. This component was estimated from data on national consumption of laundry and dishwashing detergents and their average P content. The P content in detergents has changed over time due to the restrictions imposed by the implementation of national regulations. A P content in laundry detergents of 0.5% has therefore been considered (Wind, 2007; Ministerial Decree 413/1988; Legislative Decree 21/2009) and a P content in dishwashing detergents of 6% (Wind, 2007; Legislative Decree no. 21/2009). The average per capita use was estimated by dividing the national consumption by the population, multiplied by the population residing in the individual municipalities.

3.2.3 Riverine N and P loads estimation

The average annual riverine nutrient loads exported from the water basins were calculated over the period 2014-2019 for the following forms: total nitrogen (TN), nitrate ($N\text{-}NO_3^-$), ammonium ($N\text{-}NH_4^+$), dissolved organic and particulate nitrogen (OPN; calculated as TN – ($N\text{-}NO_3^-$ + $N\text{-}NH_4^+$)), total phosphorus (TP), soluble reactive phosphorus (SRP), and dissolved particulate and organic phosphorus (OPP, calculated as TP – SRP). The period was chosen in order to calculate the exported load in a time frame comparable to that for which regional statistics were available for the quantification of net anthropogenic nitrogen and phosphorous inputs and to include average years with different hydrological characteristics.

In order to accurately assess river discharge and associated nutrient loads, monitoring stations close to the river mouth were selected and, in particular, those where nutrient concentrations are regularly measured. Nutrient concentration data were derived from institutional monitoring campaigns carried out by the Regional Environmental Protection Agencies for the assessment of ecological status as required by the Water Framework Directive. Sample collection and analysis were performed in accordance with standard methods and analytical protocols adopted by regional environmental agencies (APAT—IRSA/CNR, 2003). Sampling frequency varied from once a month to once a season (from 12 to 4 per year), following ARPA monitoring protocol which is defined by the importance and dimension of the water body following Italian Law D.M. 260/10 (https://www.gazzettaufficiale.it/eli/id/2011/02/07/011G0035/sg).

For this work, we used data for the period 2014-2019 that were downloaded from the following repositories: Emilia-Romagna (https://dati.arpae.it/dataset), Piedmont (http://webgis.arpa.piemonte.it/geoportale/) and Lombardy (https://www.dati.lombardia.it).

Flow data are publicly available on a daily scale and were downloaded from the following repositories: Emilia-Romagna (https://simc.arpae.it/dext3r/), Piedmont (https://www.arpa.piemonte.it/rischinaturali/accesso-ai-dati/annali_meteoidrologici/annali-meteo-idro/banca-dati-idrologica.html) and Lombardy (https://idro.arpalombardia.it/it/map/sidro/). In the case of incomplete discharge datasets, the problem was addressed differently depending on the length of the period when data were unavailable. Flow data unavailable for periods of less than two days were recalculated by linear interpolation. For data gaps of more than two days and less than one month, data from the nearest station were used. In the case of a lack of data for periods longer than one month or when the estimate from the nearest station was not considered reliable, the entire year was eliminated.

Average annual load for the period 2014-2019 was calculated as the average of the load calculated for each single year. The annual load was calculated as the product of the discharge weighted mean concentration by the mean annual discharge (Quilbé et al., 2006) as follows:

$$L = \frac{\sum_{i=1}^{n} C_i * Q_i}{\sum_{i=1}^{n} Q_i} * \bar{Q} * k$$

where:

L = annual loading (kg y^{-1})

C_i = instantaneous concentration measured on day_i (g m^{-3})

Q_i = mean daily discharge on day_i (m^3 s^{-1})

$\bar{Q}$ = mean annual discharge (m^3 s^{-1})

k = conversion factor from g m^3 s^{-1} to kg y^{-1}.

3.2.4 Additional hydrological and geospatial characteristics of the catchments

Precipitation data were extracted from E-OBS raster files provided by the Copernicus Climate Change Service (https://surfobs.climate.copernicus.eu/) with a grid resolution of 0.1° (Cornse et al., 2018). The mean annual cumulative precipitation (r) was calculated as the sum of the mean daily precipitation per basin in a year.

The average elevation of each catchment was calculated from DEM files of the Geographic Information System of the Commission (CISCO) (https://ec.europa.eu/eurostat/web/gisco) processed by the Copernicus service with a resolution of 2.5° using the software QGIS and sf R package (Pebesma & Bivand, 2023).

Areal runoff (r.off) was estimated from discharge data as the ratio of the annual discharge, calculated as the sum of the daily flow at the basin closure station, to the catchment area. The coefficient of variation of the flow (CV_Q), used as an indicator of the flow regime, was calculated as the ratio between the standard deviation and the mean annual discharge.

3.2.5 Statistical analysis of the data

Factors influencing the loads of the different forms of nitrogen and phosphorous exported from catchments were assessed by applying a multiple linear regression model. Loads were analysed in relation to the independent variables shown in Table 3.2. In this way, the role of continuous and factorial variables and their possible interaction was specifically assessed. Before conducting the statistical analysis, all the variables included in the model were normalised for the area, logarithmic transformed to decrease skewness, and finally scaled because variables with different units or dimensionless were present. Since the feed and food components of NANI and NAPI can take both positive and negative values, two additional categorical variables have been created to distinguish between autotrophic and heterotrophic catchments. Thus, the continuous variable indicates the magnitude of the flux, while the second categorical variable indicates its direction and takes a positive value if the load is imported into the catchment, or a negative one if it is exported. The variance inflation index (VIF) was applied to exclude the presence of collinearity between the variables (Graham 2003) by considering variables with a VIF < 5 as not-correlated (Mason et al., 2003).

Table 3.2 *Variables included in the statistical model. Type columns differentiate continuous (Cont.) from factor variables.*

Code	Denomination	Type	Units
	NANI		
Fer	Fertilization N	Cont.	Kg N km^{-2} y^{-1}
Dep	Deposition N	Cont.	Kg N km^{-2} y^{-1}
Fix	Fixation N	Cont.	Kg N km^{-2} y^{-1}
Feed	Net N feed balance	Cont.	Kg N km^{-2} y^{-1}

Food	Net N food balance	Cont.	Kg N km^{-2} y^{-1}
Feed (+ *or* -)	N feed balance direction	Factor	+ *or* -
Food (+ *or* -)	N food balance direction	Factor	+ *or* -
	NAPI		
Fert	Fertilization P	Cont.	Kg P km^{-2} y^{-1}
Det	Detergent P	Cont.	Kg P km^{-2} y^{-1}
Feed	Net N feed trade	Cont.	Kg P km^{-2} y^{-1}
Food	Net N food trade	Cont.	Kg P km^{-2} y^{-1}
Feed (+ *or* -)	N feed trade direction	Factor	+ *or* -
	Geo-hydrological		
r_off	Runoff	Cont.	mm y^{-1}
CVq	Variation coefficient of water discharge	Cont.	ratio
r	Precipitation	Cont.	mm y^{-1}
Z_mean	Mean altitude	Cont.	m

For each nutrient, we started from the general model, including all the variables shown in Table 3.2, the interaction between the continuous and factorial variables of feed and food, and the interaction between fertilisation and hydrological variables. A simplified intermediate model was selected (with lower AIC) by applying the stepward selection technique, corrected for both directions (backward and forward), through the *stepAIC* function of the *MASS* package (Venables et al., 2002). From this intermediate model, the non-significant variables were excluded by exclusively applying the backward selection technique as described in the approach by Zuur et al. (2009). In order to assess the performance of the different models, the AIC (Akaike information criterion) and BIC (Bayesian information criterion) values were estimated, and then the simplest possible model was selected considering the consistency between the two indices. Model validation was performed by analysing the assumptions via the graphical output of the residuals as described in Zuur et al. (2009). Since OPN and OPP were not directly measured, we found some statistical issues, probably driven by errors associated with different sampling and analytical methods. The TON model had problems with heterogeneity of variance, so it was recalculated using a GLS (generalised least square) by weighting the variance for the variable r.off as described by Zuur et al. (2009). While OPP presents 6 basins where SRP weights more than 85% of TP affecting the global results. In the OPP linear model, we considered these basins as outliers, and we tested the hypothesis without them as suggested by Zuur et al. (2010). Data processing was carried out with R (R Core Team, 2019) and models informations were analysed using parameters (Lüdecke et al., 2020), *effectsize* (Ben-Shachar et al., 2020) and *performance* (Lüdecke et al., 2021) packages.

3.3 Results

3.3.1 Net anthropogenic nitrogen and phosphorus input to catchments

The average net anthropogenic nitrogen and phosphorus inputs were 7,074 kg N km^{-2} y^{-1} and 1,168 kg P km^{-2} y^{-1} respectively, but with high spatial variability between the catchments (Figure 3.2). Considering individual catchments, NANI ranged from 2,039 to 19,258 kg N km^{-2} y^{-1} (CV = 0.5), while NAPI ranged from 114 to 3,257 kg P km^{-2} y^{-1} (CV = 0.7).

Overall, 54% of NANI was supported by direct inputs on agricultural land (fertiliser inputs and nitrogen fixation) while 35% was imported as feed for farmed animals. The components constituting anthropogenic N and P loads were also heterogeneously distributed among the different sub-basins (Figure 3.2). Atmospheric deposition ranged between 306 and 1,359 kg N km^{-2} y^{-1} but was the least important component in most of the catchments (average 10%). Nitrogen input due to the application of chemical fertilisers, the most important component of NANI (37%), averaged 2,425 kg N km^{-2} y^{-1} and ranged between 234 and 11,397 kg N km^{-2} y^{-1} with higher loads in the northern catchment. Nitrogen fixation (1,430 kg N km^{-2} y^{-1}, 20% of NANI) was between 281 and 8,398 kg N km^{-2} y^{-1} with a distinct spatial variation and higher inputs in the southern basins.

On average, the investigated area was found to be net heterotrophic, with a nitrogen trade in proteins for livestock forage and human food that averaged 2,531 kg N km^{-2} y^{-1}. The majority of the nitrogen input is due to the trade in animal feed products (average 2,486 kg N km^{-2} y^{-1}). In 78% of the catchments, the net N import is between 45 to 9,730 kg N km^{-2} y^{-1} (average 2,886 kg N km^{-2} y^{-1}), while in the remaining catchments, the net export ranges from 538 to 1,677 kg N km^{-2} y^{-1} (average 1,374 kg N km^{-2} y^{-1}). In contrast, the average trade of nitrogen in food is only slightly positive at 44 kg N km^{-2} y^{-1}. Furthermore, the majority of catchments (27 out of 42) were found to be net autotrophic, with an average net nitrogen export in food of 1,061 kg N km^{-2} y^{-1}.

Net anthropogenic phosphorous input was dominated by the net trade of feed and food (average 833 kg P km^{-2} y^{-1}) while import of chemical fertilisers accounted for about 27% of the net phosphorous load (420 kg P km^{-2} y^{-1}) and only 2% was due to detergents. All catchments were found to be net heterotrophic in terms of trade-in products intended for animal feeding, with a phosphorus input ranging from 29 to 3,019 kg P km^{-2} y^{-1} (average 962 kg P km^{-2} y^{-1}).

Conversely, trade in products for human consumption was on average negative (average value of $^{-1}$29 kg P km^{-2} y^{-1}), resulting in a net phosphorus export in 83% of the basins (-8 to 1,054 kg P km^{-2} y^{-1}).

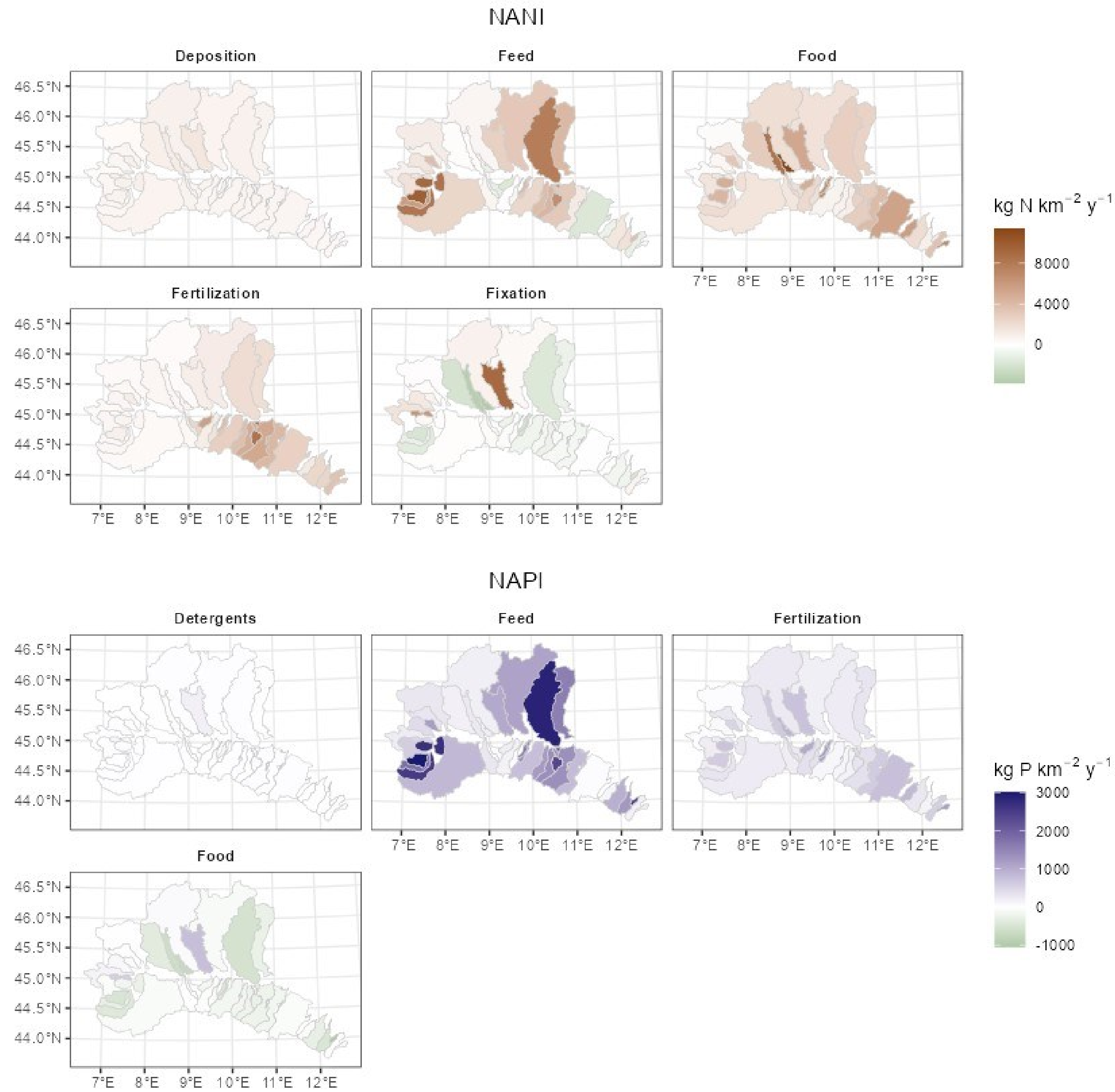

Figure 3.2 *Net anthropogenic nitrogen (NANI, kg N km^{-2} y^{-1}), and phosphorus (NAPI, kg N km^{-2} y^{-1}) inputs and their components in the catchments of the Po River district.*

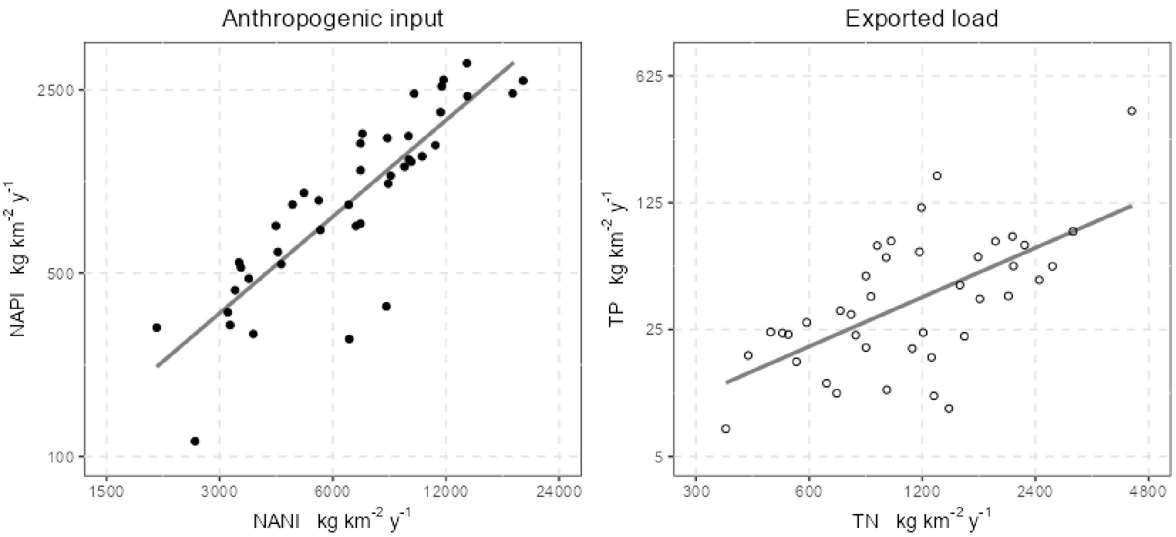

Figure 3.3 *On the left side relationship between anthropogenic input (NANI vs NAPI): b-slope=0.6, $p<0.001$, $R^2=0.74$. On the right-side relationship between exported loads (TN vs TP): b-slope=0.5, $p<0.001$, $R^2=0.5$**.** Plots show a log-log scale.*

3.3.2 Exported loads of nitrogen and phosphorus from catchments

The catchments exported an average of 1,571 kg N km^{-2} y^{-1} TN (ranging from 193 to 4350 kg N km^{-2} y^{-1}) and 61 kg P km^{-2} y^{-1} TP (ranging from 2 to 402 kg P km^{-2} y^{-1}). The TN load consisted on average of 73% of the inorganic fraction (68% N-NO_3^-, 5% N-NH_4^+) and 27% of the sum of organic and particulate fraction, while the TP load consisted on average of 56% of the reactive fraction (SRP) and the remaining 44% is the sum of the organic and particulate fraction (PP) (Figure 3.4).

The TN load exported was a fraction comprised between 3 and 66% of NANI while the TP load was a fraction comprised between 0.2 and 32% of NAPI. Overall, therefore, the catchments preferentially retain phosphorus over nitrogen, with repercussions on the stoichiometric ratios between the two elements. Nitrogen and phosphorus were found to be strongly correlated both as net anthropogenic inputs and as exported loads. The district-wide average molar ratio of NANI: NAPI was 13:1 (median=16, min=9, max=52), while the TN: TP and DIN: SRP ratios were found to be 58:1 (median=67, min=17, max=340) and 76:1 (median=112, min=27, max=654) respectively. Only a slightly significant relationship ($p < 0.01$, $R^2= 0.15$) was found between net anthropogenic nitrogen input and exported total nitrogen load, while the relationship between net anthropogenic phosphorus input and total phosphorus load was not significant ($p = 0.18$, $R^2=0.02$) (Figure 3.3).

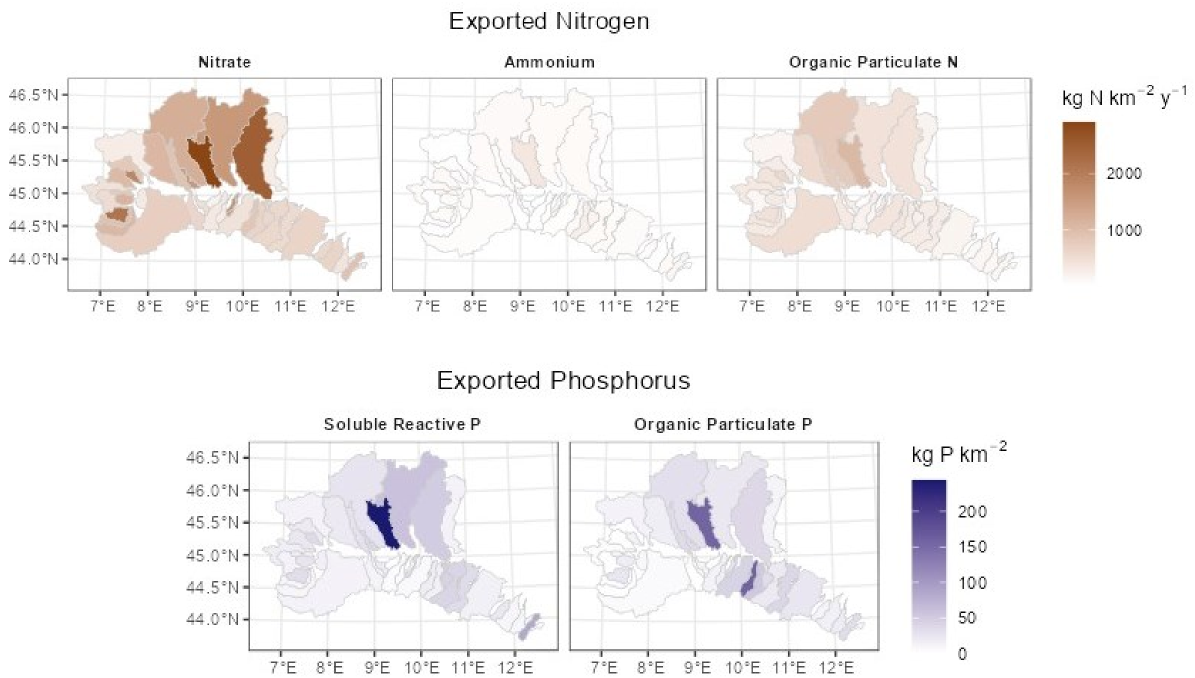

Figure 3.4 *N and P exported load, showed by single forms.*

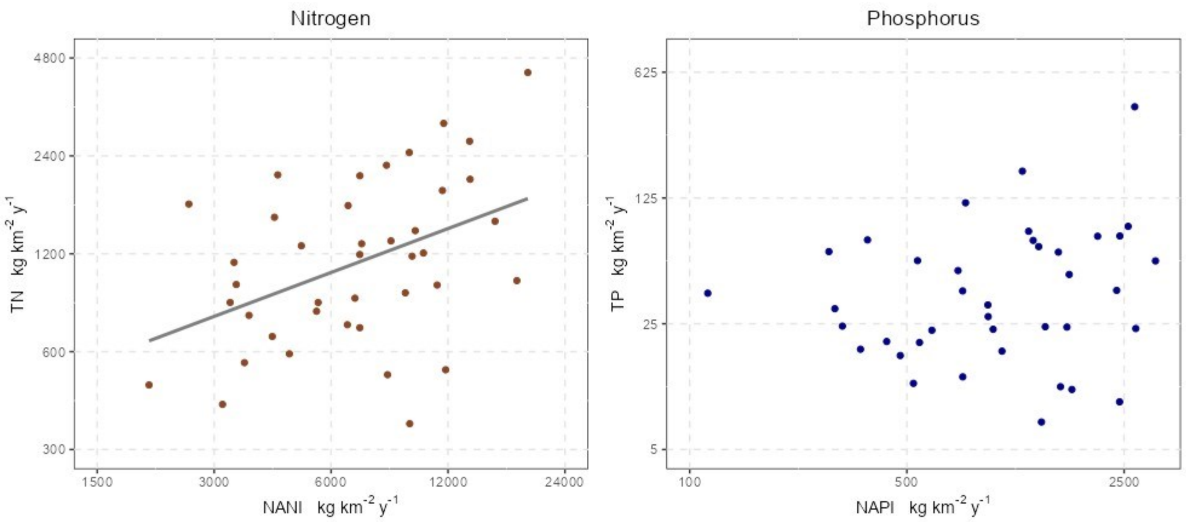

Figure 3.5 *Relationship between anthropogenic nutrients input and exported nutrients load (NANI vs TN and NAPI vs TP)**.** Plots show a log-log scale.*

3.3.3 *Physical features of the catchments*

The 42 catchments were characterised by a high degree of hydrogeological diversity (Figure 3.6). The average altitude was between 97 and 1,841 m.a.s.l., while the average slope varied between 0.8° and 27°. Regarding hydrological aspects, the mean precipitation was 908 mm (min=676, max=1.297), the runoff was 479 mm (min=60, max=1.214) and the CV_Q was 1.8 (min=0.4, max=5.2).

Precipitation and runoff were spatially independent (R^2=0.2, p=0.2), confirming the hydrological heterogeneity of the district and the presence of different factors that may influence the hydrological balance, while runoff and CV_Q were inversely correlated (R^2 = -0.59, $p < 0.01$). The median of the mean altitude was 497 m.a.s.l. (min=97, max=1,851), the mean slope was 14.4 (min=0.9, max=27.2) and the mean temperature was 12.6 (min=4.8, max=14.3). These three variables were highly correlated (R^2z-m = 0.9, $p < 0.01$; R^2z-T = -0.95, $p < 0.01$), so it was decided to keep only the mean elevation in the model.

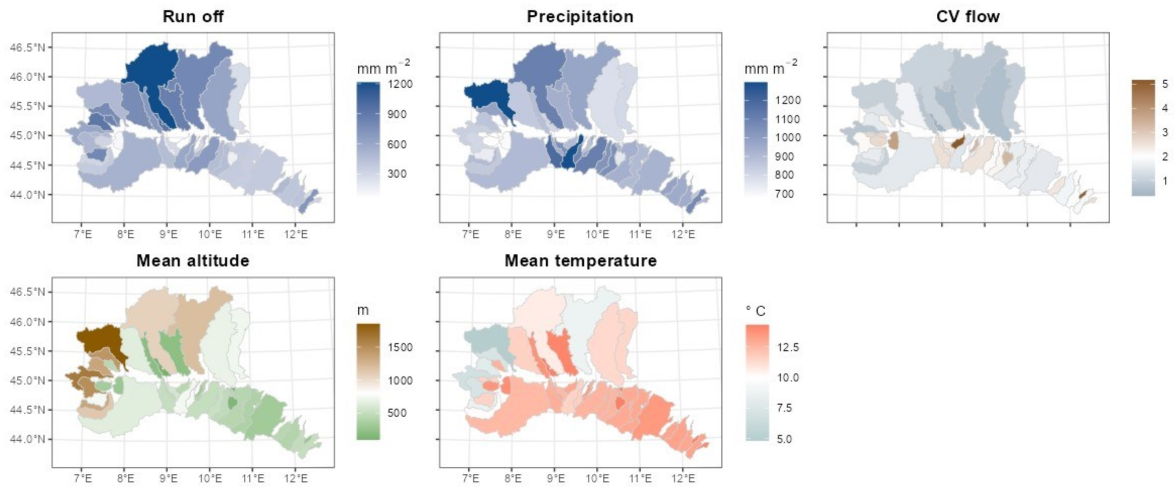

Figure 3.6 *Hydrological and physical features of the studied catchments*

3.3.4 *Factors influencing nitrogen and phosphorus loads exported from catchments.*

The multiple regression models evidenced that different factors influenced the exported N and P loads depending on the nutrient its molecular form (Figure 3.7 and Table 3.3). The model identified for each nutrient has a high level of prediction, with R^2 of 0.57 for N-NH_4^+, 0.64 for SRP, 0.70 for PP, 0.74 for TP, 0.73 for ON, 0.82 for N-NO_3^-, and 0.84 for TN.

The condition of autotrophy and heterotrophy in the farming and civil sectors significantly affected the exported load of all nitrogen forms. The feed factor always resulted significant, while the food factor was significant only for inorganic forms, with a greater effect on N-NH_4^+ than on N-NO_3^- (Figure 3.7). Net heterotrophic catchments, which import nitrogen in the form of both feed and food, had a significantly higher intercept for N-NO_3^- and N-NH_4^+ compared to net autotrophic catchments.

The variability of the nitrate load exported from the catchments was primarily influenced by N fertilisation and N trade in animal feed products while trade in food products and nitrogen fixation were found to be insignificant. Specifically, the N-NO_3^- load was positively correlated with the N input associated with fertilisers and the net import of animal feed, while it was negatively correlated with net exports. Of the physical variables, only runoff had a positive influence on N-NO_3^- load. Flow variability and total precipitation, on the other hand, did not appear to affect the load.

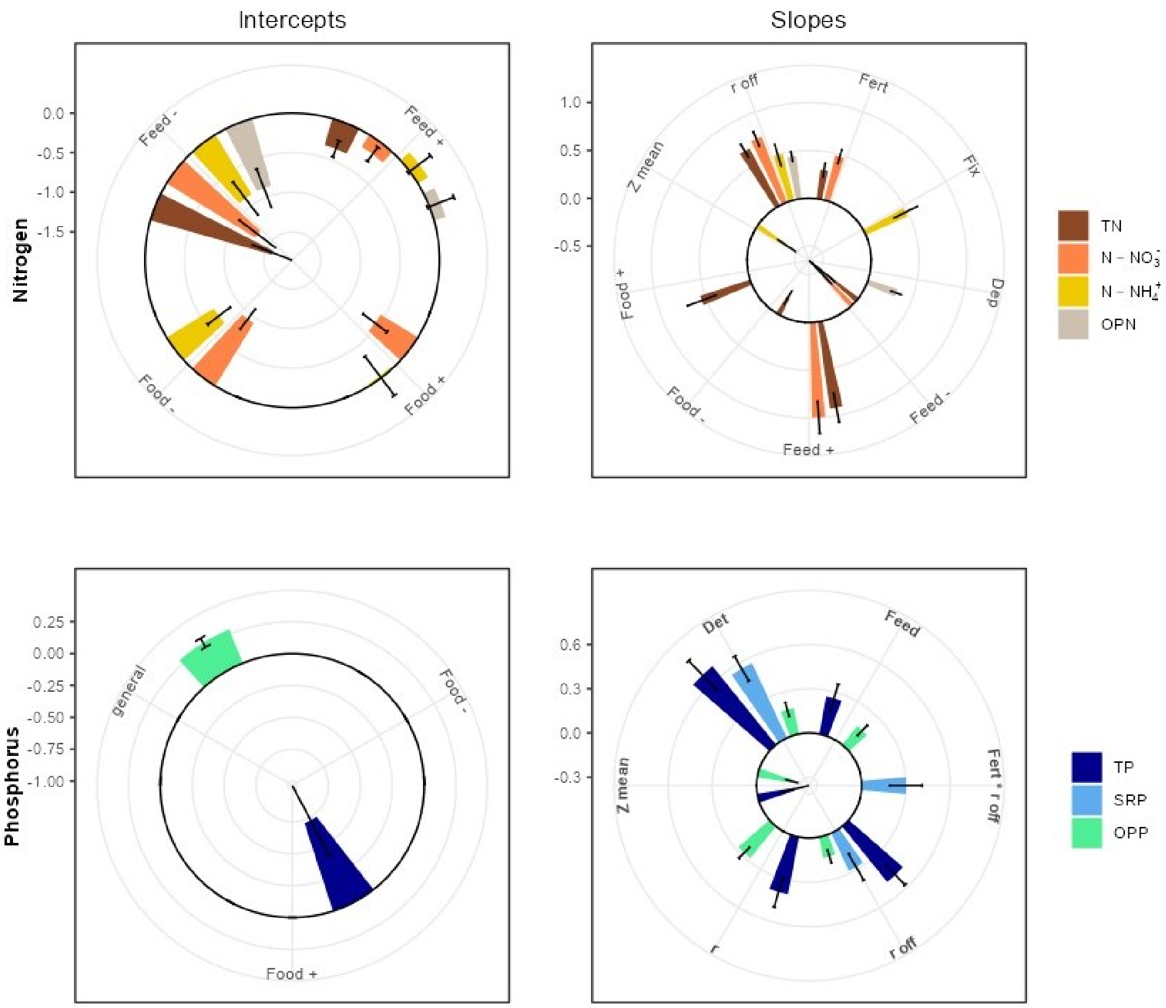

Figure 3.7 *Effect of tested variables on N and P forms loading export. Only significant variables ($p < 0.05$) are reported and are presented with a 95% confidence interval. Significant intercepts represent the role of factor variables and modulate the response of slopes.*

Regarding the loading of reactive phosphorus and organic and particulate phosphorus, only the net food heterotrophy was significant. The autotrophic condition does not seem to play a role in modifying the intercept. The only form that exhibits a positive intercept independent of the autotrophy or heterotrophy condition is the particulate form. The variability of the phosphorus load exported from catchments was primarily influenced by the import of phosphorus contained in detergents, which was associated with an increase in the load of both soluble reactive phosphorus and organic and particulate phosphorus. Fertilizer input only correlated with the SRP export, with the effect depending on the level of runoff. Trade in food products did not affect the variability of the load of both phosphorus forms while the net import of products intended for animal feed had a positive effect on the load of OPP. Run-off and precipitation increased OPP export, while mean altitude had a negative effect.

3.4 Discussion

In most of the studied catchments, anthropogenic management has altered the processes of accumulation and transport of nitrogen and phosphorus. Through the gradient of anthropogenic pressures and physical characteristics, the exported load of the different forms of N and P is determined by agriculture and livestock farming and hydrological conditions, but in different ways depending on the form considered.

3.4.1 Agriculture as a major driver of N and P input to catchments

The catchments analysed in this study are characterized by a great combination of anthropogenic pressures and hydrogeological characteristics. Anthropogenic pressures are among the highest in the Mediterranean basin (Romero et al., 2021) and are generated by the consistent development of the agricultural and farming sectors that have occurred in this area since the 1950s of the last century and by the presence of urbanised areas such as the cities of Milan, Turin, Bologna, and their suburbs (Viaroli et al., 2018). The anthropogenic inputs of N and P in the catchments were correspondingly high. In 2010, the average load calculated for the territory corresponding to the Po River basin was 8,751 kg N km^{-2} y^{-1} while that of phosphorus was 1,177 kg P km^{-2} y^{-1} (Viaroli et al., 2018). The mean values quantified in this study, considering only the catchments included in the Po River basins, were 7,135 kg N km^{-2} y^{-1}, and 1,203 kg P km^{-2} y^{-1} indicating that the loads have undergone little change. Overall, a comparison of the NANI and NAPI quantified in this study with those of other catchments shows that mean values are at the upper end of the range (Table 3.4). The average value is about three times higher than that of European countries, North America, China, and India, while in the most anthropized catchments, the average nitrogen (~ 26000 kg km^{-2} y^{-1}) and phosphorus (~4000 kg km^{-2} y^{-1}) are up to 3 times greater than in the most affected areas worldwide (Table 3.4). The input of synthetic fertilisers and the net import of feed are the most important factors contributing to NANI and NAPI and the territory is on average heterotrophic. Consequently, N and P's demand to maintain livestock farming and human needs exceeds the agricultural sector's production capacity. The correlation between NANI and NAPI and the number of animals farmed highlights in particular how the livestock sector influences N and P fluxes (Figure 3.8).

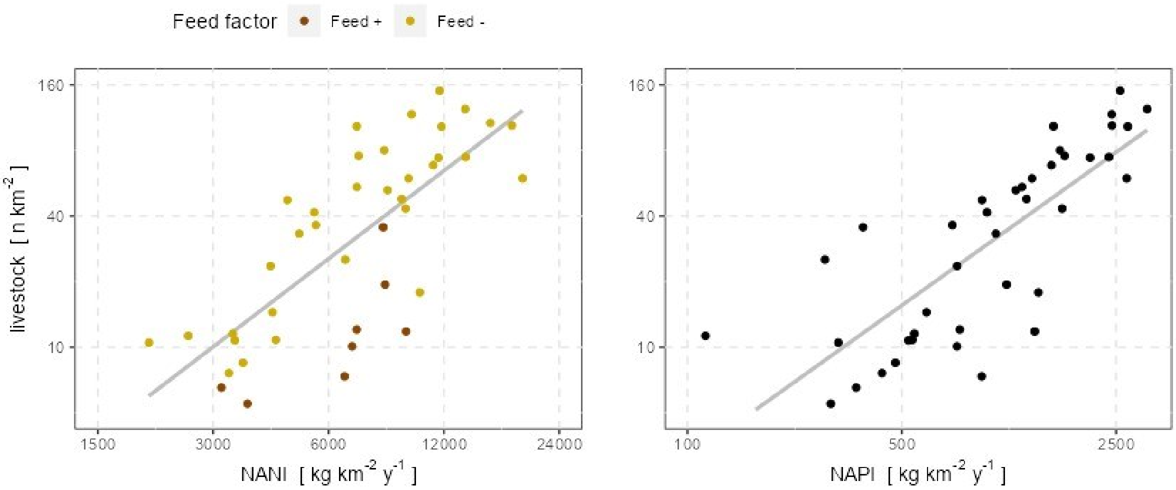

Figure 3.8 *Correlation between anthropogenic input and livestock density. The left panel shows the correlation with NANI (R^2= 0.8, p<0.001), right panel the correlation with NAPI (R^2= 0.8, p<0.001). Plots show a log-log scale.*

Table 3.3 *Linear model of nitrogen and phosphorus inputs vs exported load. Are reported only results from the best model after the model selection process. All data were log-transformed and scaled.*

NITROGEN

Forms	Intercepts				Slopes									Model	
	Feed -	Feed +	Food -	Food +	Fert	Feed -	Feed +	Food -	Food +	Fix	Dep	r off	Z mean	adj R^2	Method
TN	-1.59	-0.35	-	-	0.31 ***	-0.46 ***	0.92 ***	-0.2 **	0.55 ***	-	-	0.68 ***	-	0.85	LM
$N\text{-}NO_3^-$	-1.31	-0.18	-0.93	-0.55	0.48 ***	-0.48 ***	1 ***	-	-	-	-	0.74 ***	-	0.82	LM
$N\text{-}NH_4^+$	-0.88	0.15	-0.7	-0.03	-	-	-	-	-	0.48 ***	-	0.5 ***	-0.37 ***	0.57	LM
OPN	-0.88	0.15	-	-	-	-	-	-	-	-	0.32 ***	0.45 ***	-	0.75	GLS

PHOSPHORUS

Forms	Intercepts			Slopes						Model	
	Food -	Food +	General	Det	Feed	Fert * r off	r	r off	Z mean	adj R^2	Method
TP	0.12	-0.6	-	0.68 ***	0.26 **	-	0.39 ***	0.49 ***	-0.26 **	0.74	LM
SRP	-	-	-	0.56 ***	-	0.3 ***	-	0.28 ***	-	0.64	LM
OPP	-	-	0.29 ***	0.18 ***	0.16 ***	-	0.28 ***	0.14 ***	-0.24 ***	0.70	LM

Surprisingly, no spatial correlation has been identified between runoff and precipitation, while there is an inverse correlation between runoff and CV_Q (Figure 3.9C). The influence of precipitation on discharge is influenced by various factors, such as the average slope, temperature, and the structure of the hydrographic network, both natural and artificial. A detailed analysis of the factors that regulate hydrological characteristics in the district is beyond the scope of this study, but the observed decoupling between precipitation and runoff can be interpreted as an index of the high heterogeneity between the investigated basins. This heterogeneity is likely due to the presence of large lakes, glaciers, large dams, agricultural activities such as rice paddies with high water demand, different soil permeability, different average temperatures, and different orography (Bruno et al., 2021; Castaldini et al., 2019). Based upon these considerations, the negative correlation between runoff and CV_Q, consents to consider runoff as an index of temporal hydrological continuity within the basin, which orders the basins according to a moisture gradient and persistence of the hydrological continuum (aquifer-river continuity, fluvial intermittency, etc.). Conversely, the precipitation variable, which is not correlated with runoff, contributes little to explaining the difference between dry and wet basins and can be interpreted as a proxy of soil erosion.

Table 3.4 *Linear N and P budgets comparison with the literature. NANI and NAPI are normalized for the catchment area and expressed in kg N km^{-2} y^{-1} and kg P km^{-2} y^{-1}.*

	NANI	**NAPI**	**N Exported**	**P exported**
Po river	8751±634	1177±101	1478±375*	34±9*
European Average, 2000 (1)	3700	-	811	-
Lake Michigan Catchment, 1987-1997 (2)	3115	-	-	-
Mississippi Catchment,1987-1997 (2)	2156	-	-	-
Ebro Catchment, 2000 (3)	5118	-	394	-
St. Lawrence catchment, 1960-2010 (10)	609-7885	44-1838	125-1890	9-97
Danish straits, Baltic Sea catchments, 2010 (5)	8779	1251	-	-
Bothnian Bay, Baltic Sea catchments, 2010 (5)	332	31	-	-
Lake Erie catchment (US), 1964-1007 (6)		28-1438	-	6-80
Mainland China 2009 (7, 8)	5013	465	-	-

India Average (9)	4016	-	-	-
Minnesota (US), (11)	-	2-1131	-	3-121
St. Lawrence Basin (Canada), (4)	609-7885	44-1838	125-1890	9-97
This study	2039-19258	114-3257	193-4331	2-402

*Expressed as DIN and SRP

References: (1) Billen et al., 2011, (2) Hong et al., 2013; (3) Lassaletta et al., 2012; (4) Goyette et al., 2019; (5) Hong et al. 2017; (6) Han et al., 2011; (7) Han et al., (2014) ; (8) Han et al., (2013) ; (9) Swaney et al., (2015) ; (11) Boardman et al., 2019.

The high pressures affect the exported loads that are higher than those observed in other catchments; however, there is no constant relationship between imported and exported loads. In fact, the variability of the exported loads of different forms of nitrogen and phosphorus was higher than the variability of NANI and NAPI. In addition, only a slight relationship was observed between NANI and TN, while the effect of NAPI on the TP export was not evident. Previous studies have shown that river nutrient loads depend on several factors, including the amount of N and P generated in the catchment and the basin's capacity to retain and metabolise these loads (Goyette et al., 2019; Romero et al., 2021). The type of pressure determines not only the form of the N and P input but also the length of the nutrient's path to the aquatic compartment. These two aspects determine the possibility (initial chemical form) and the time (path length) of interaction with hydrological variables. Factors that influence nutrients transport and/or dissipation can be attributed to the distribution and intensity of precipitation, runoff, the structure of the hydrographic network, and the maintenance of natural components of river systems that can break down and reduce eutrophication loads (e.g. wetlands, secondary network vegetation). The study partially confirms previous observations regarding the relationship between N and P loads and runoff. This relationship is expected since the load is calculated from the runoff. However, this work shows that the contribution of hydrological variables differs depending on the form considered.

3.4.2 Contribution of different anthropogenic pressures and hydrogeological features to exported N loads.

The exported load of total nitrogen and nitrate was mainly influenced by two components of NANI: net imports of organic nitrogen through the feed trade and, to a lesser extent, fertiliser inputs. The amount of N exported from the net heterotrophic catchments was 1.8 times greater than from the autotrophic catchments. However, the regression analysis indicates that the loads of river N increase in relation to both the amount of nitrogen imported to and (to a lesser extent) exported from the catchments with feed trade. These results support previous observations that both high autotrophic and heterotrophic catchments export elevated amounts of nitrogen through the hydrographic network (Swaney et al., 2015). The underlying mechanisms are likely different. The positive effect of net exports in net autotrophic catchments can be considered an indirect effect of the input of fertilisers necessary to sustain agronomic production (Billen et al., 2010). On the other hand, the impact of feed imports can be interpreted by considering that most of this input is transformed by the farming system into manure, which represents an additional N input to the soil and contributes to the generation of an excess that can be leached (Soana et al., 2011). Previous studies have shown that livestock manure is a significant source of diffuse nitrogen pollution, especially in areas with high livestock density, where traditional dairy cattle farming has been replaced by industrial pig farming (Martinelli et al., 2018; Soana et al., 2011; Pinardi et al., 2018). The higher intercept in net heterotrophic catchments compared to autotrophic ones suggests that in these sites the condition of heterotrophy determines a higher N export, even in the absence of anthropogenic pressures. The higher intercept likely reflects a progressive accumulation of nitrogen in the catchment as a result of years of application of livestock manure on the soil. We have no direct evidence of nitrogen accumulation in soils due to the retention and transformations in the root zone of organic nitrogen from crop residues and manure, but several studies support this hypothesis both in relation to nitrogen retention in groundwater and in the unsaturated zone followed by its release period into surface waters (Bartoli et al., 2012; van Meter, 2016; Martinelli et al., 2018). This interpretation is also consistent with the higher export of OPN in heterotrophic catchments, which comprise between 75-90% of the manure nitrogen pool (Anon, 2010). Thus, our results support the view that the degree of heterotrophy of a catchment is an indicator of the disconnection between the livestock and agricultural sectors, and consequently, between the production of nitrogen from animal waste and the capacity of the catchment to dispose of it (Billen et al., 2014). Overall, these results suggest that catchments that tend towards equilibrium (i.e. low net feed trade) are likely to be more efficient systems either in the use of anthropogenic nitrogen or in its removal from the natural compartment.

Runoff has a greater impact on nitrate exports than on ammonium and organic nitrogen. At a general level, the relationship between N and runoff is due to the high solubility of $N\text{-}NO_3^-$. This results in a linear increase in nitrogen export as water availability increases, driven by soil leaching (Crasweel et al., 2021). Conversely, the less mobile ammonium or particulate forms must first be eroded by the action of precipitation in order to be exported (Dolph et al., 2019; Ehrhardt et al., 2021). On the Alpine side of the Po River, where runoff and therefore water availability is greater, the leaching of nitrates from cultivated land to groundwater is also accelerated by extensive irrigation with submersion of heavily fertilized and manure-amended soils (Perego et al., 2012; Provolo et al., 2005).

Finally, the role of nitrogen-fixing crops must be considered in the agricultural soil balance. A study reconstructing crop yield in 124 countries found that countries using a higher proportion of nitrogen inputs from symbiotic fixation rather than synthetic fertilizers are more efficient in their use of nitrogen (Lassaletta et al., 2014). The results obtained in this work are partially coherent as nitrogen fixation was found to be significant only in relation to the export of ammonium, which constitutes a small fraction of the total N export. This relationship can be explained by the particular concurrence of several aspects of which nitrogen fixation is an index. The area of nitrogen-fixing crops is correlated with livestock density in feed-importing basins (Figure 3.9A). Moreover, since nitrogen-fixing crops do not require nitrogen fertilisation, catchments with higher livestock numbers and large surface areas of nitrogen-fixing crops have a smaller surface area for spreading livestock manure. In this case, nitrogen fixation could be interpreted both as a proxy for livestock density and as the imbalance between the production of nitrogen from manure and the agricultural soil's ability to process it. Finally, it should be noted that most of the nitrogen fixation is concentrated in the driest catchments where the effect of drought is greater. A recent study has shown that the fixation capacity decreases in drought conditions and part of the fixed N can be excreted in the soil, and then reabsorbed by plants in better conditions (Doellette et al., 2023). However, drought can also reduce plant uptake and nitrification or denitrification in soils (Hartmann et al., 2013).

Regarding N-NH_4^+ export, the interaction between agricultural components and catchment characteristics is confirmed by the variable of mean altitude. Since Z_mean is collinear with temperature (Figure 3.6) and positively correlated with agricultural land (and negatively with natural areas Figure 3.9B), flatter basins tend to be more agricultural and have higher temperatures. Tesi et al., (2013) observed that in the Po River, ammonia was positively correlated with suspended particulate matter during high-flow events. Open agricultural soils are more susceptible to erosion than forested soils (Ramos et al., 20119) and if sudden and intense precipitation occurs, the soil may experience rapid erosion, resulting in the loss of the accumulated ammonium. In addition, drought conditions increase N-NH_4^+ availability since microbial processes continue, while plant N uptake decreases with drought (Homyak et al., 2007).

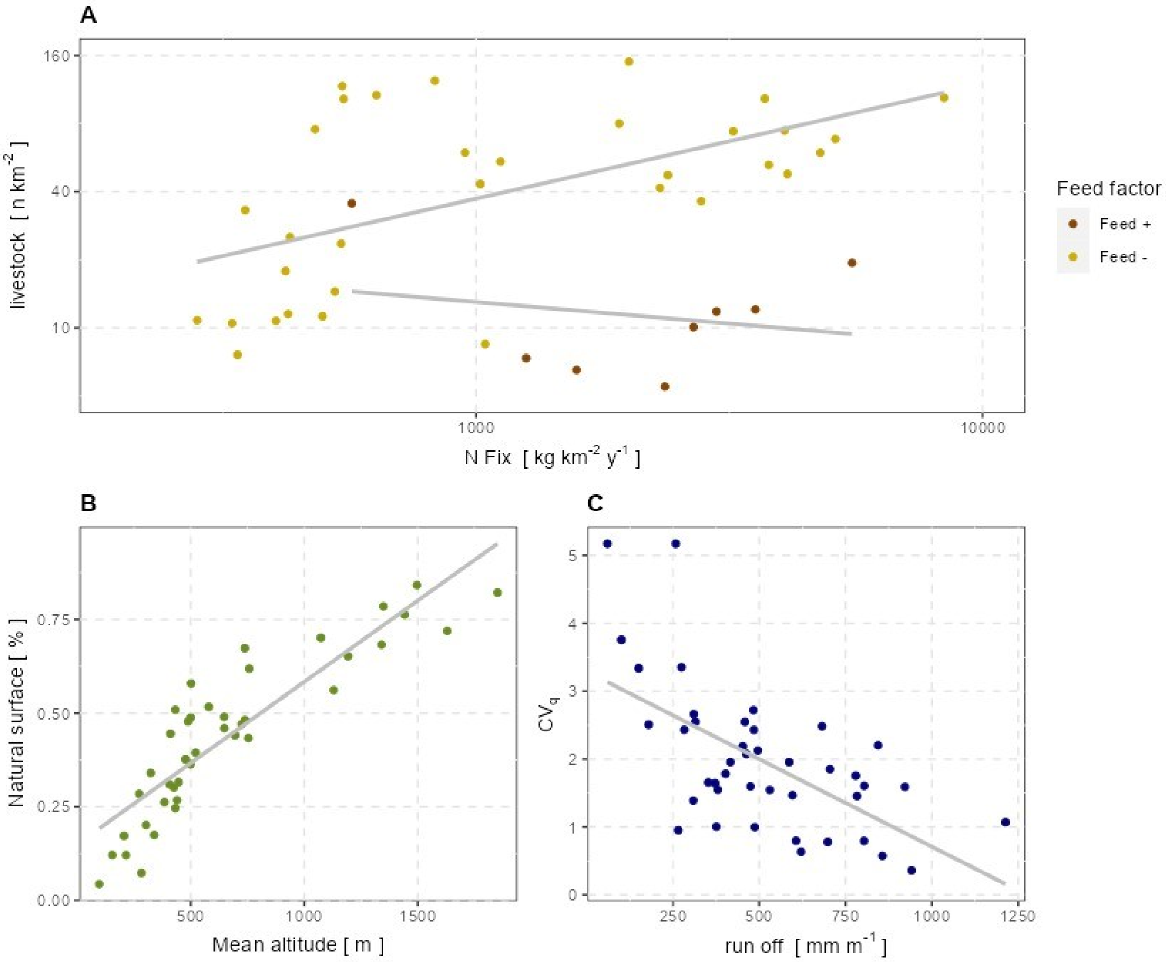

Figure 3.9 *(A) Correlation between N fixation and livestock density. Data are divided following feed factors: Feed + as importers (R^2=0.6, $p < 0.01$), Feed – as exporters (R^2=0, p=0.9).* ***(B)*** *Correlation between mean altitude (Z_mean) and natural surface (%), R^2=0.6, $p<0.001$.* ***(C)*** *Correlation between run-off and CV_Q, R^2=-0.8, $p<0.001$. Plots show a log-log scale.*

The effect of net N input related to trade in food products was less evident and not clearly interpretable. A partial explanation is that the primary fate of this input is the wastewater treatment plants. The presence of sewage collectors and treatment plants contributes to lengthening the path of nutrients through additional compartments, making it more difficult to interpret this variable. Nitrogen deposition is another input that includes some of the secondary effects related to human presence which, however, compared to other countries (i.e. USA, China), contributes only 10% of the total budget. This component of the NANI was found to be positively associated only with the OPN load. This is consistent with the results of several studies in which atmospheric deposition may play a role in contributing to the formation of organic compounds through the non-negligible content of organic nitrogen in aerosols and atmospheric particulate matter (Calderon et al., 2007; Cornell et al., 2011; Matsumoto et al., 2019). Furthermore, deposition promotes the formation and export of organic nitrogen in forest ecosystems, as a consequence of the fertilization of natural soils (Brookshire et al., 2007; Cheng et al., 2019).

3.4.3 Contribution of different hydrogeological features and anthropogenic pressures to exported P loads

In contrast to N, the P organic and particulate pool constitutes around 45% of the TP load and the hydro-geological characteristics of the catchments play a more significant role in regulating the export. Specifically, the export of total phosphorus and OPP increases with total precipitation and runoff, while the export of the soluble inorganic form increases with runoff as a function of fertilization levels. These results can be explained by the lower mobility and solubility of phosphorus compared to nitrogen. The high solubility of nitrate results in nitrogen export driven by soil leaching (Crasweel et al., 2021). Phosphorus, on the other hand, is poorly mobile and tends to accumulate easily in the soil-bound to metals such as calcium, iron or aluminium (Panagos et al., 2022). Therefore, the transfer of phosphorus to the aquatic environment occurs primarily due to the erosion of particulate forms (Kleinmann et al., 2011; Menezes-Blackburn et al., 2018, Goyette et al., 2018, Dolph et al., 2019; Ehrhardt et al., 2021). The positive intercept of the total phosphorus not linked to any anthropogenic pressure can be interpreted as an indication of export that depends on the erosion of rocks containing phosphorus, regulated by hydrological factors.

The regression analysis showed that the increase in feed imports is associated with a higher export of organic and particulate form. This increase in exports may be due to the fact that manure is one of the few sources of organic phosphorus (Reddy et al., 2000; Turner et al., 2003). Previous studies have shown that during flood times, more than 70% of phosphorus can be exported in the form of particulate phosphorus due to the erosive effect of rainfall. This form of export is dominant in areas where livestock manure is used as fertilizer (Dolph et al., 2019). The negative correlation between P export and catchment slope may reflect the impact of tile drainage on flat agricultural catchments, which may facilitate downstream transport of P from the same year's application or due the historical sources.

Phosphorus input through fertilization and the use of detergents were found to be the strongest factors associated with the loss of inorganic phosphorus from catchments. Of particular interest is the interaction between runoff and fertilizer application, which most likely underlies the complex interplay between fertilizer application and soils' ability to accumulate phosphorus before it is leached (Goyette et al., 2018). Historical analyses have revealed that the continuous application of fertilizers, which is often more than crop demand, has led to a progressive increase in both the amount of TP and the potentially bioavailable P (Rubæk et al., 2013; Panagos et al., 2022). Consequently, the observed relationship could depend on a multiplicity of factors such as the loss of recently applied P after the soil buffering capacity becomes saturated (Dolph et al., 2019), senescent crop residues and vegetation within the canals, typical of wetter catchments (Elliott, 2013). However, further studies are needed to define whether this exported P is of natural origin or depends on an accumulated release period of P.

The component linked to the use of detergents reflects the direct effect of the population and by extension takes into account not only the direct effect of detergents but is also indicative of the production of urban wastewater. Human presence is the only variable to explain the increase in SRP in watercourses, introduced by point sources such as wastewater discharge and helps to explain some of the PP that could result from both unretained suspended solids and the presence of algal biomass due to the P fertilization effect in rivers (Dolph et al., 2019).

3.4.4 Nutrient stoichiometry in exported loads

The transport of nitrogen and phosphorus through the catchments modifies the stoichiometric ratio between the two nutrients. The fraction of NANI and NAPI exported by the different catchments as total nitrogen and total phosphorus was found to be on average 18% (N) and 6% (P). These findings are in line with prior research indicating a greater relative export of nitrogen in comparison to phosphorus (25% for N and 5-10% for P, Hong et al., 2012; Swaney et al., 2012; Goyette et al., 2016). Due to differences in fractional export, the N:P ratio increases by approximately 9 to 7 times depending on whether only the inorganic forms or the total are considered. The exported load's N:P ratio remains of the same order of magnitude as that measured at the Po basin's closure station in Pontelagoscuro, despite high variability (Viaroli et al., 2018). The regression analysis does not suggest any relationship between catchment features and N:P ratio making it difficult to understand the factors regulating the observed change.

The change in the N:P ratio could depend on the different biogeochemical cycles that characterize the two elements. Phosphorus and nitrogen have different accumulation and release period dynamics, the former being accumulated mainly in soils while the latter in water (REF). N:P ratio in crop soil compartments subject to intensive agricultural practices has been estimated as low as 6 (Romero et al., 2021). This difference adds to the spatial variability of N and P inputs a temporal dynamic linked to the delay between immobilization and the release period. As such, higher leaching rates of N compounds lead to much higher N:P ratios in rivers and groundwaters than in croplands. On the other hand, the capacity for net accumulation and elimination of N depends fundamentally on the rate of renewal of groundwater and the connection with the subsurface aquifer. The difference between the input-to-output ratio could be explained by the combination of these dynamics, which determine a different short-term buffering effect for N and P. Where P is substantially accumulated to a greater extent than N and/or a partial release period effect of N could be observed in conditions of high groundwater renewal (Dolph et al., 2019; Ehrhardt et al., 2021).

3.5 Conclusion

Control of eutrophication requires a clear understanding of factors regulating N and P transport along the hydrological continuum. In this study, the comparison between the different components of NANI and NAPI and the loads of different N and P forms helped to highlight less evident dynamics and possible control factors.

The Po River District has a high human presence, which contributes to some of the highest levels of NANI and NAPI globally. However, the spatial relationship between anthropogenic input and exports was found to be insignificant for P and only slightly significant for N, while the single components contributing to NANI and NAPI have varying degrees of influence on the formation and magnitude of the river load magnitude and composition. The N and P river loads are also clearly affected by different hydrological factors, which play a different role depending on the nutrient being considered. The condition of autotrophy and net heterotrophy influences the fate of N and river loads. The results confirm the idea that the degree of heterotrophy of a catchment is an indicator of the disconnection between the livestock and agricultural sectors. catchments that tend towards equilibrium are likely to be more efficient systems in the use of anthropogenic nitrogen.

In the case of nitrogen, water availability is central, as it ensures hydrological continuity within the basin and promotes N exports. For P, in addition to run-off, rainfall also plays a significant role in contributing to the erosion phenomenon, particularly in uncovered soils such as agricultural ones. This suggests a pulsating dynamic in the regulation of the export of this nutrient.

Overall, the analysis indicates that in the absence of human pressures, the exported load of N would be extremely low, tending to zero, while the load of OPP would still be positive. This suggests a possible natural origin of OPP or the presence of a legacy of P in the soil, which is exported only during river flood phases. These findings indicate that erosion-promoting factors can also affect the distribution of elements, primarily by mobilising pre-existing sources of phosphorus in particulate form. On the other hand, it also highlighted how the interaction between individual sources with hydrological variables can contribute to generating a similar export load starting from different initial conditions. This aspect could help to explain the strong alteration of the N:P ratio between input and output load.

4 Chapter IV

Hydraulic management and flood effect regulate sink and source behaviour altering nutrient stoichiometry of irrigation-dammed reservoirs

4.1 Introduction

Nitrogen (N), phosphorus (P), and silicon (Si) are essential elements that support primary production in both terrestrial and aquatic environments (Verburg et al., 2013; Serediak et al., 2014). In recent decades, human activities have significantly altered the biogeochemical cycles of N, P, and Si. This has resulted in increased loads being transported through the hydrographic network and substantial changes in the structure and functioning of receiving water bodies (Harrison et al., 2009; Carey & Fulweiler, 2016). However, the hydrographic network has a dual role. It transports nutrients downstream, which can lead to eutrophication in aquatic systems. Additionally, it modifies the nutrient amount and the initial composition of the nutrient cocktail through metabolism and transport action (Hilton et al., 2006, Dodds & Smith 2016, Wurtsbaugh et al., 2019).

The total and relative amount of nutrients transported through the hydrographic network are determined by various control factors related to the characteristics of a river basin. These factors include the type of anthropogenic pressures, hydrological and geological characteristics, and biological transformations (Ebeling et al., 2021; Goyette et al., 2019; Moatar et al., 2017; Shousha et al., 2023). The hydrographic network is a complex system comprising interconnected aquatic subsystems that act as reactors and filters (Meybeck & Vörösmarty, 2005). Whitin this system, natural deep and shallow lakes and reservoirs are biogeochemical reactors that modify longitudinal connectivity and affect dissolved nutrient transport (Harrison et al., 2009; Verburg et al., 2013; Frings et al., 2014; Nizzoli et al., 2018; Maavara et al., 2020; Scibona et al., 2022).

The regulation of river systems, including the construction of dams, river regulation, canal creation for navigation and transport, and the building of irrigation and water-diversion schemes, has always been crucial for the exploitation of water resources for civil and industrial purposes (Vörösmarty et al., 2015). For example, reservoirs, estimated to number around 2.8 million (with reservoir areas >103 m^2), provide approximately 30-40% of irrigation and 16% of energy production through stored water (Maavara et al., 2020a). Over 60% of rivers longer than 1000 km have interruptions or barriers along their course but these numbers are expected to change due to the continuous request of water and hydroelectric power generation (Grill et al., 2015 Grill et al., 2019). In Europe, an estimated density of 0.74 weirs per km^2 has been identified, of which approximately 10% are classified as dams, while the rest are categorized as weirs, ramps, or other structures (Beletti et al., 2020). Therefore, rivers are under sustained pressure from fragmentation and loss of connectivity, resulting in changes to their flow regime and affecting many fundamental processes and functions. Dams, in particular, interrupt the river continuum and can significantly alter hydrological dynamics and nutrient transport (Maavara et al., 2020a). The construction of dams for water storage increases the residence time along the river continuum and the reservoirs become biogeochemical reactors that alter the overall amount of the load and its composition. Recent modelling synthesis have shown that reservoirs retain an average of approximately 21% of transited load of Si, 43% of P, and 35% of N worldwide (Maavara et al., 2020a; Van Cappellen & Mavaara, 2016). The retention capacity of P is higher compared to Si and N, and the Si: N and Si P ratios have generally increased which can partially balance the N and P excess caused by anthropogenic inputs (Maavara et al., 2020b).

However, although reservoirs act as filters along the river continuum, the retention capacity of the different nutrients is highly variable, and some aspects such as residence time, morphometry, age of the reservoir and water stratification can contribute to modifying the normal transport dynamics. Maavara et al., (2014) found that Si retention increases as residence time decreases. This is due to particulate Si formation and subsequent sedimentation, which is sustained by higher diatom growth rates compared to other algal species. Conversely, the very high retention capacity of P is likely related to its sedimentary cycle, where the particulate component tends to be easily deposited by gravity and the soluble component can react with carbonates and become trapped in the sediment (Maavara et al., 2015). Nitrogen has a more complex dynamic partially due to its exchange with the atmosphere. Part of the accumulated nitrogen in the sediment can be lost by denitrification (~ 30%), and if nitrogen is limiting in the water column, nitrogen-fixing communities can develop, contributing to an input of about 19%. This estimation, performed by Akbarzadeh et al. (2019) takes into account the N:P ratio to determine the potential effect of N-fix. In the long term, the progressive accumulation of sediment, which varies depending on the size and age of the reservoir, saturates the capacity to retain nutrients and reservoirs can switch from sink to source (Maavara et al., 2020). In the short term, seasonal dynamics may activate processes that cause a release period of part of the pool accumulated in the sediments. This can occur, for example, due to the onset of anoxia phenomena that influence the release period of phosphorus and nitrogen (Fovet et al., 2019). It has also been observed that the position of the outflow not only affects the temperature of the release period water but also on nutrients concentration and stoichiometry due to waters currents or reservoir stratification (Lindenschmidt et al., 2019; Carr et al., 2020).

Finally, the alteration in hydrological characteristics should be considered when analysing nutrient transport. The recharge period and release period phases in irrigation reservoirs can differently alter the seasonal dynamics of N, P and Si transport by modifying the natural hydrological regime and the c-Q relationship upstream and downstream of the reservoir (Ammar et al., 2015; Shaughnessy et al., 2019; Yan et al., 2021). Floods, especially for reservoirs built in intermittent streams which are characterized by a hydrological extreme, concentrate the input of nutrients into the reservoir in short moments which can be followed by algal explosions related to a momentary excess of nutrients (Brito et al., 2017). At the same time, the erosive effect is reduced, and the effect of the interruption compared to the moments of normal flow rate is different for N and P (Tang et al., 2023; Zhao et al., 2020). The release period of water in summer or during periods of low flow modifies the availability of nutrients downstream of the dam and consequently the stream metabolism, which has been observed to respond quickly to the variations in flow and nutrients determined by the management of the dam (Patil et al., 2022; Rohlfs et al., 2018), with consequences for both the benthic and hyporheic zones (Aristi et al., 2014; Kasahara et al., 2022).

Thus, within a dynamic of nutrient elimination or release period, secondary variables can contribute to modulating the stoichiometric ratio differently with very different effects depending on the environment considered. Furthermore, in semi-arid environments, the impact of flow regulation on nutrient cycling appears to be more significant than that of land use, unlike in humid environments (Calderon et al., 2023). This is particularly important in the Mediterranean area, where climate change is causing a serious imbalance in the management of water resources. On the one hand, higher temperatures can lead to an increase in water demand especially for irrigation purposes, on the other hand, altered precipitation regimes increase flood risks and decrease water availability when the demand is higher (Gorguner, M., & Kavvas, 2020; Nunes et al., 2017; Rocha et al., 2020). The importance of understanding these aspects could be even greater, as highlighted by Dopico et al. (2022), as communities may respond to hydrological stress by requesting the construction of new reservoirs. Therefore, understanding the regulation dynamics of biogeochemical processes in these environments is becoming increasingly important due to the direct and indirect effects of climate change.

However, while the effectiveness of dams as filter elements that retain nutrients within catchments has been extensively evaluated through global models and estimates, there are still several points that require further clarification. Global models can be used to illustrate the overall impact of dams, but assessing the local effects of each dam is challenging due to the unique characteristics of each one. This is because the sink or source behaviour is defined by multiple variables, whose weight varies depending on climatic conditions, flow regime management, and the nutrient being considered. Numerous studies have been conducted on large plants, while the function of small reservoirs primarily used for irrigation is relatively less researched (Maavara et al., 2020b). Furthermore, these reservoirs, due to their particular seasonal dynamics characterized by autumn/spring recharge and summer release phases, can have a pulsating behaviour that, in the face of overall annual retention, a biogeochemical asynchrony between inflowing and outflowing stream sectors that can seasonally alter the transport of nutrients.

This study investigates the impact of reservoir management on nutrient transport and stoichiometry in Mediterranean basins with intermittent fluvial regime. We focused on how the spatial and temporal discontinuity, due to the dams, alters hydrology and seasonality on nutrients cycling. More specifically, the objectives of the work are: (1) to define how reservoir presence change downstream nutrient availability; (2) to identify the effect of the natural hydrological regime and the hydrological regime induced by the reservoir (stream flow *and* reservoir level); (3) to estimate the in-out load balance. We hypothesize that the shift from natural pulsating behaviour, driven by the torrential character of the river, to the artificial, due to reservoir level, may decrease retention capacity and contribute to a delay in nutrient transport.

4.2 Materials and Methods

4.2.1 Study Area

The experimental units of this study were Arda and Tidone streams located in the Northern Apennines in the province of Piacenza (Northern Italy) (Figure 4.1).

Both streams are characterized by the presence of two large dams that allow the storage of water for agricultural purposes (Arda) and for drinking water storage and agricultural purposes

(Tidone). The Arda Stream dam, which was completed in 1934, creates a reservoir with a maximum volume of approximately 11.3 million m^3 and a maximum depth of around 45 m. The catchment area is 106 km^2, with 58% being forested or semi-natural areas and the remainder being agricultural land. Urban areas occupy less than 1% of the area. The dam on Tidone Stream was completed in 1928, forming a reservoir with a maximum volume of approximately 7.6 million m^3 and a maximum depth of around 38 m. The catchment area covers 96 km^2, with 50% consisting of forested and semi-natural areas, and 49% being utilized for agricultural purposes. In contrast, less than 0.5% of the total area is used for urban purposes. In both catchments the population density is very low, amounting to 17 and 29 inhabitants km^{-2} for the Arda and Tidone respectively, and substantially distributed in sparsely distributed houses.

Table 4.1 *Use of soil (%) of the catchment area of the last sampling station, based on CLC 2018.*

Corine Land Cover 2018	Arda	Tidone
Urbanized areas	0.6%	0.3%
Arable land	1.3%	7.7%
Permanent pasture	-	2.1%
Varied agricultural areas	39.5%	39.6%
Woods	55.7%	41.0%
Bushes	1.0%	9.0%
Sparse vegetation	1.3%	-
Lake	0.6%	0.3%

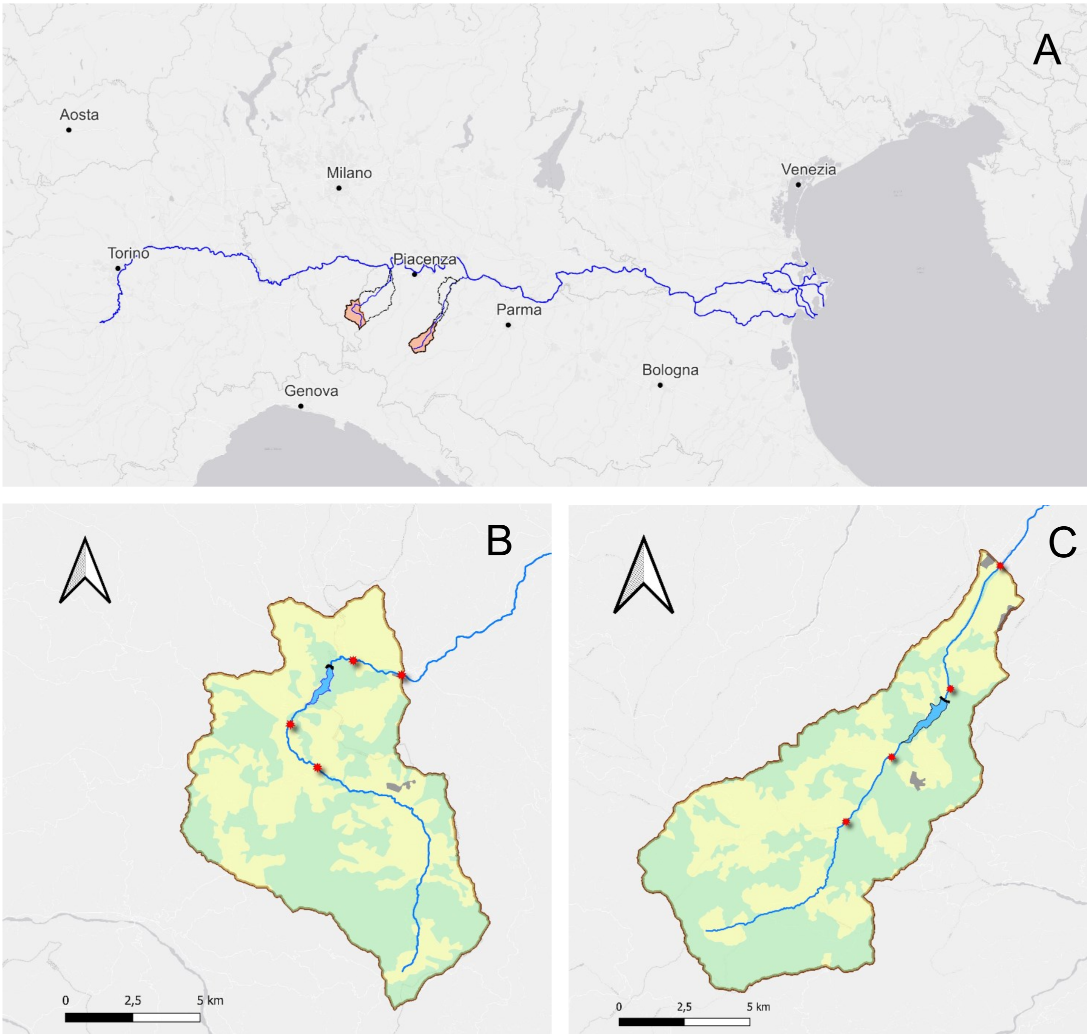

Figure 4.1 *Upper Panel: position of the Tidone and Arda catchments (**A**). Lower panel: land use map of the Tidone (**B**) and Arda (**C**) catchments (yellow = agricultural land, green = semi-natural areas), the thick blue line in the catchments show the river's mainstem, the red dots represent the sampling stations.*

4.2.2 Water sampling and analysis

The survey took place over 12 months (October 2021 − September 2022). Two stations were selected before and after the reservoir in each catchment, positioned on the main course of the streams, to account for longitudinal variability. Stations were distanced between each other by about ~ 3 km (Figure 4.1). At each station, a minimum of one sample per month was taken, resulting in 13 dates for each of the four sites. Samples were collected during both base flow and high flow events to account for the impact of flow variability on water chemistry. For each sampling date and station, three replicate water samples were collected along a cross-sectional transect.

Following collection, an aliquot for each replicate was immediately filtered (Whatman GF/F) and stored in polyethene vials and in glass vials, the former ready for dissolved silica (DRSi), ammonium ($N-NH_4^+$) and nitrate ($N-NO_3^-$), and the latter for soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP) and total dissolved nitrogen (TDN) analyses. A second aliquot for each replicate was filtered on pre-weighed filters (Whatman GF/F), with the material collected on filters being subsequently analysed for the measurement of total particulate phosphorus (PP) and particulate nitrogen (PN). Total phosphorus (TP) was estimated as TDP + PP, while total nitrogen (TN) was estimated as TDN + PN. A third aliquot of water for each replicate was filtered (Whatman GF/F) for Chl-a determination.

$N-NH_4^+$ (Koroleff 1970), $N-NO_3^-$ (APHA 1998), SRP (Valderrama 1981) and DRSi (Golterman et al., 1978) in stream water were determined using standard spectrophotometric methods (Shimadzu, UV-1900i). An analytical blank undergoing the same procedure as the samples, including filtration, was always analysed to correct for sample contamination. Filters with particulate matter were oven-dried at 70 °C for 24 h. Subsequently, they were analysed to quantify particulate N and P following an alkaline digestion (Maher et al., 2002). Filtered samples were processed with the same digestion to determine total dissolved N and P. Chl-a was extracted with acetone 90% v/v overnight at 4 °C and quantified spectrophotometrically (UV-1900i) after filtration (Whatman GF/C fibreglass filters) of the extract (Jeffrey & Humphrey, 1975).

4.2.3 Hydrological schema description

Hydrological data as daily cumulated precipitation, inflow and outflow discharge and lake volume were obtained by the Piacenza Consortium for Water Management (https://www.cbpiacenza.it/).

We identified two main hydrological schemes, one upstream and the second downstream.

In the upstream section, two classes (low flows and high flows) were identified as shown in Figure 4.2. The cut-off between high and low flow classes was identified by applying the flow duration curve (Searcy, 1959) as discharges that occur below (high) or above (low) 20% of the time (https://streamflow.engr.oregonstate.edu/analysis/flow/index.htm). Based on this method the cut of was identified at 0.24 m^3 s^{-1} for Tidone and 0.45 m^3 s^{-1} for Arda. For the outflow section, the two classes were identified concerning the volume of the reservoir using 50% of the volume as the cut-off value. With a volume less than 50% of the maximum capacity, the reservoir was considered empty, while with upper volume was considered as full. (Figure 4.3)

4.2.4 Statistical Analysis

All statistical analyses were carried out with R software (https://www.R-project.org/). Concentration values measured at the two stations within the same stream reach were averaged (i.e. the two stations upstream and downstream), while the two streams (Arda and Tidone) were considered as replicates. A linear mixed model (LMM) was applied to analyse the differences between upstream and downstream stations considering the different hydrological conditions: low or high flow for the upstream reach, and full or empty reservoir for the downstream reach. We used the *lme* function of the *nlme* R package (Pinheiro et al., 2023) to execute the model. To resolve heterogeneity issues, we applied *varIdent* variance structure on hydrological conditions. To isolate the hydrological role from the seasonal effect, the month was considered as a random variable. The significance of random effect was tested by comparing *lme* and *gls* models with and without random effect with *anova* function as suggested by Zuur et al. (2009). When necessary to obtain normality of residuals data were transformed using log or square root. Method validations were carried out both graphically and with the *performance* R package (Lüdecke et al., 2021). Orthogonal contrasts were executed using the *emmeans* R package (Lenth et al., 2018) in order to test differences: up vs downstream, high vs low flow, and full vs empty reservoir.

4.2.5 Nutrient loads estimation

The nutrient loads were estimated according to the retention capacity which is strictly dependent on the management of the reservoir. Therefore, the study year (October 2021 - September 2022) was divided into two periods: the first is the recharge period and the second is the release one. We used water release period start dates, corresponding to 2022-06-08 for Arda Basin, and 2022-06-03 for Tidone Basin, as dividing points (Table 4.4, Figure 4.7). Fill and empty conditions indicate the reservoir water level, whereas recharge and release periods indicate the process which modifies the water level condition.

The nutrient loadings into and out of the reservoirs were calculated as the product of the discharge weighted mean concentration by the mean discharge (Quilbé et al., 2006) as follows:

$$L = \frac{\sum_{i=1}^{n} C_i * Q_i}{\sum_{i=1}^{n} Q_i} * \bar{Q} * k$$

where:

L = period loading (kg m^{-1})

C_i = mean daily discharge on day_i day_i (g m^{-3})

Q_i = mean daily discharge on day_i (m^3 s^{-1})

$\bar{Q}$ = mean period discharge (m^3 s^{-1})

k = conversion factor from g m^3 s^{-1} to kg $period^{-1}$.

The daily mean load was separately calculated for each period (recharge and release) and then was multiplied by the length (number of days) to obtain the total load per period. The annual load was finally calculated by summing up the two intervals. Following this scheme, we estimated the nutrient load for each stream sector: separately upstream and downstream, both Arda and Tidone. Finally, reservoir net nutrient retention was estimated as the difference between the nutrient total loading flowing into the reservoirs and the nutrient total load exported through the reservoir's outlet, calculated as:

$$R = \frac{L_{in} - L_{out}}{L_{out}} * 100$$

where:

R = retention capacity (%)

L_{in} = inflow annual load (kg)

L_{out} = outflow annual load (kg)

4.3 Results

4.3.1 Hydrological characteristics

The two reservoirs were very similar, both in terms of the natural hydrological dynamics in the upstream reaches that were characterized by a torrential regime and in the timing of the water release period downstream. The average discharge at the inlet and outlet for the Arda was 0.40 m^3 s^{-1} (coefficient of variation (CV) = 2.5) and 0.32 m^3 s^{-1} (CV = 1.0), while for the Tidone it was 0.16 m^3 s^{-1} (CV = 1.7) and 0.17 m^3 s^{-1} (CV = 1.2).

Between October and May, a minimum discharge (between 0 and 0.25 $m^3 s^{-1}$ for Arda and between 0 and 0.11 $m^3 s^{-1}$ for Tidone) was established for both reservoirs to allow their recharge period, so that the accumulated volume was released period between June and September. During the period under consideration in this study, northern Italy experienced rainfall significantly below the seasonal average (Montanari et al., 2023). As a result, the Arda and Tidone reservoirs were only filled up to 60% (Arda) and 40% (Tidone) of their maximum allowable capacity. This had a significant impact on the flow rate of the Tidone, which decreased to 0.05 $m^3 s^{-1}$ during the release phase in August and September and continued to decrease progressively to 0.01 $m^3 s^{-1}$ $m^3 s^{-1}$. The reservoirs' water balance was positive for the Arda, retaining approximately 20% (2.7 million m^3), and negative for the Tidone, with a release period of around 6% (0.3 million m^3). The estimated average retention time (HRT) for the basins was about 7 months, with approximately 7.1 months for Arda and 7.5 months for Tidone.

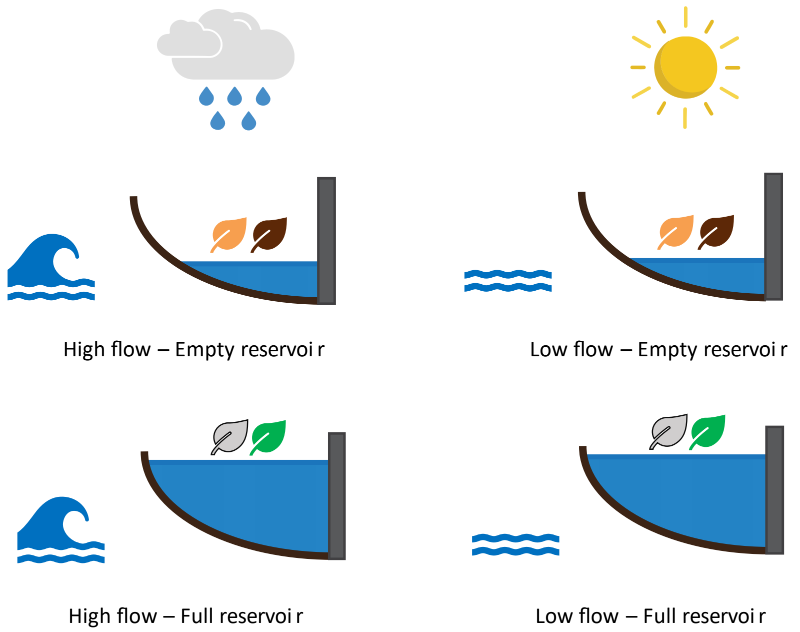

Figure 4.2 *Possible combinations of the different hydrological conditions: upstream (low or high flow) and downstream (full or empty reservoir). Coloured leaves indicate seasons in which hydrological conditions are more probable: summer (orange), autumn (brown), winter (grey) and spring (green).*

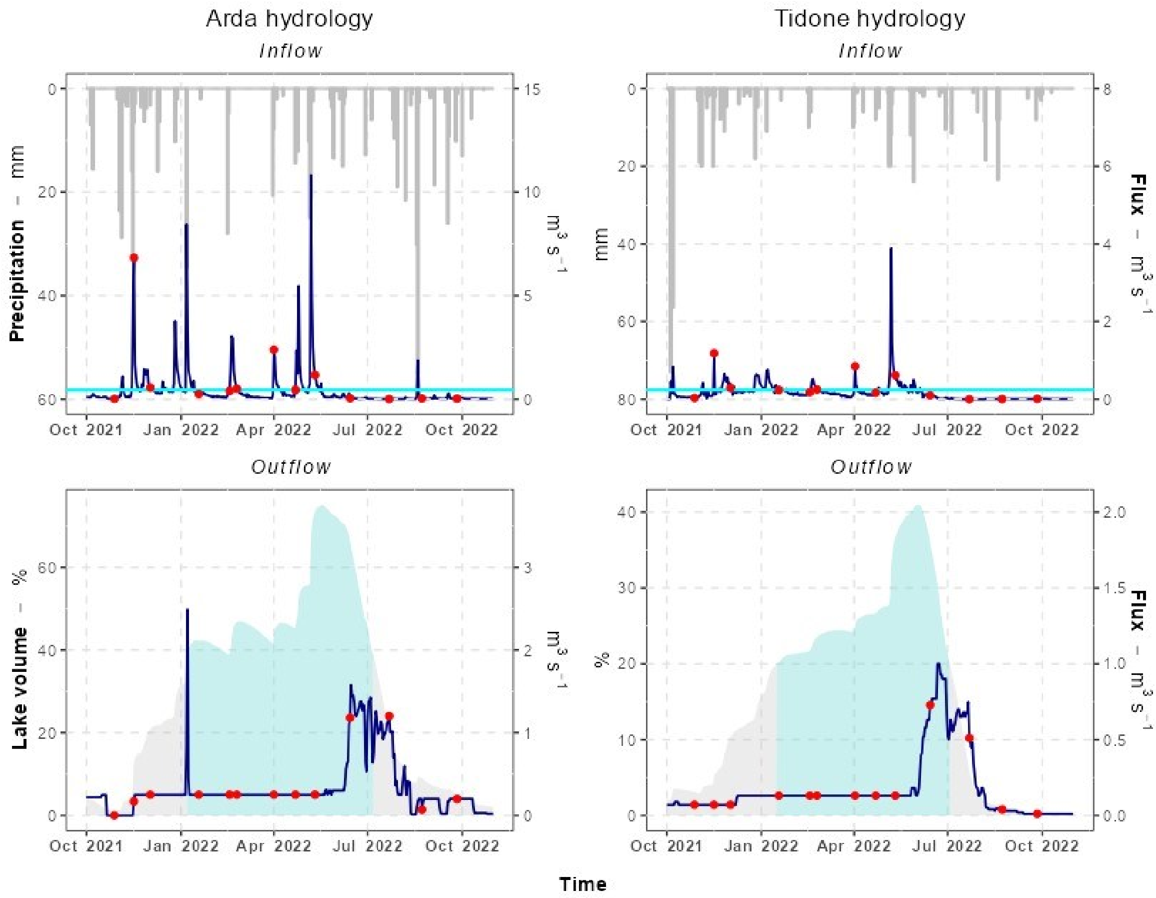

Figure 4.3 *Daily hydrological patterns of Arda and Tidone streams. On the top side is shown the dynamic of the inflow section, with precipitation (grey bar), water discharge (blue line) and water discharge threshold (cyan line) (Arda threshold = 0.45 m^3 s^{-1}; Tidone threshold = 0.24 m^3 s^{-1}). On the bottom side is showed dynamic of the outflow section, with the coloured area representing the reservoir recharge period percentage (grey area indicates empty period, cyan area filled period), and water discharge (blue line). Red points indicate sampling days according to the hydrological scheme.*

4.3.2 Role of hydrology and seasonality in controlling N, P and Si concentrations

The effect of reservoirs on upstream-downstream nutrients concentrations was evaluated taking into account the different hydrological conditions as a fixed effect and seasonality as a random effect (Table 4.2, Figure 4.4). The downstream concentration of nitrogen (PN and DIN), phosphorus (PP only), and Chl-a was significantly higher ($p < 0.01$) compared to the upstream. In contrast, the reactive forms of P and Si remained constant along the longitudinal gradient. Upon analysing individual differences, it is evident that the hydrological conditions upstream of the dam had a significant impact ($p < 0.01$) only on particulate compounds (PN and PP), with higher concentrations observed during flood phases.

Conversely, downstream hydrological conditions significantly modified ($p < 0.01$) all forms of phosphorus and PN, with higher values observed when the reservoir was empty. The statistical model with the random term showed significant differences and higher likelihoods for TN, DIN, TP, and DRSi, suggesting a potential influence of seasonality on the availability of these nutrients.

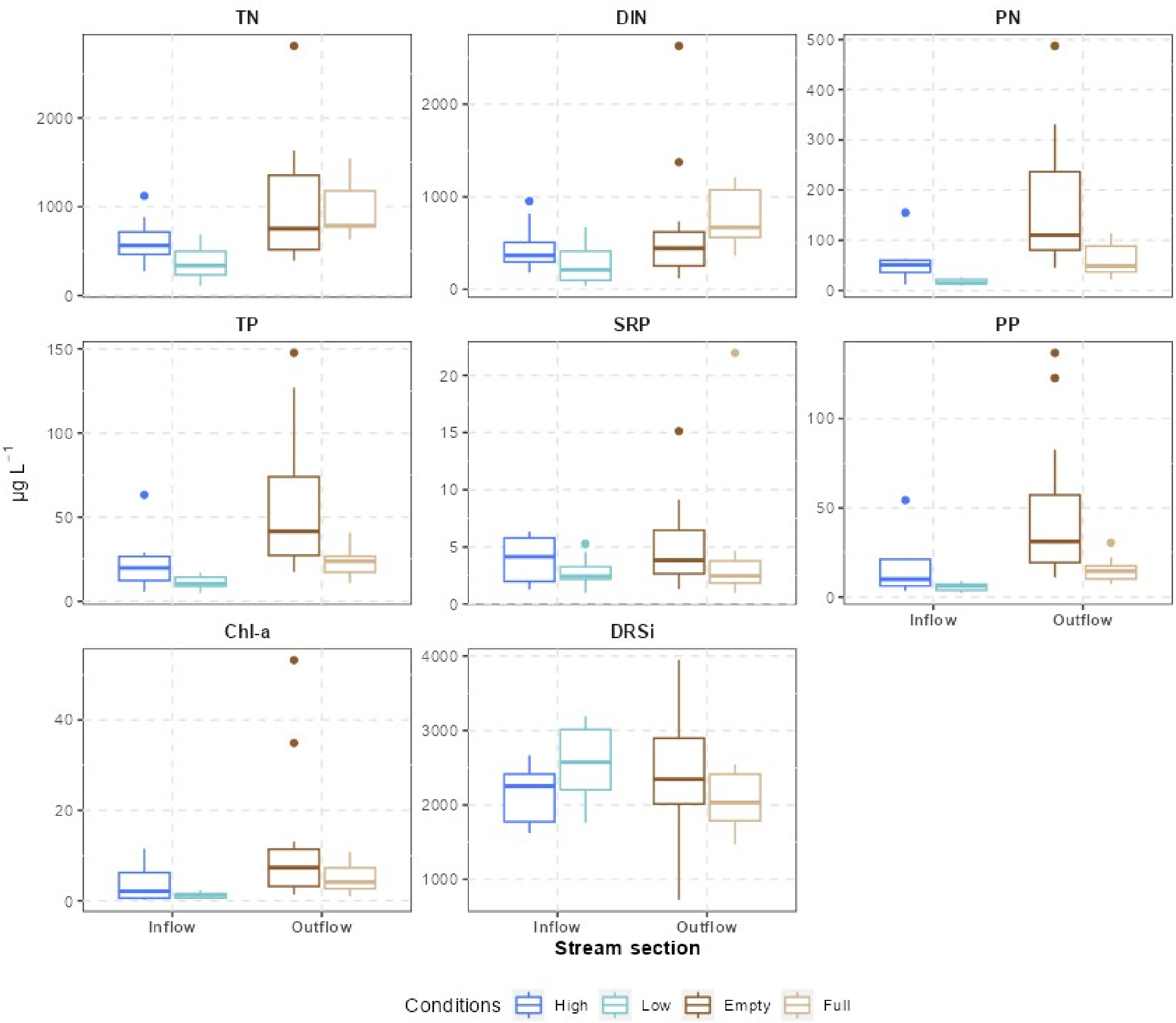

Figure 4.4 *Boxplot of TN, PN and DIN (total, particulate and dissolved inorganic Nitrogen), TP, PP and SRP (total, particulate and soluble reactive Phosphorus), Chl-a (Chlorophyll-a) and DRSi (dissolved reactive Silica). Inflow stations are divided into* ***low*** *and* ***high*** *flow interval, while outflow stations are divided into* ***empty*** *and* ***full*** *reservoirs. Plots are printed with different y scales. Significant contrasts are marked in* ***Table 4.1****.*

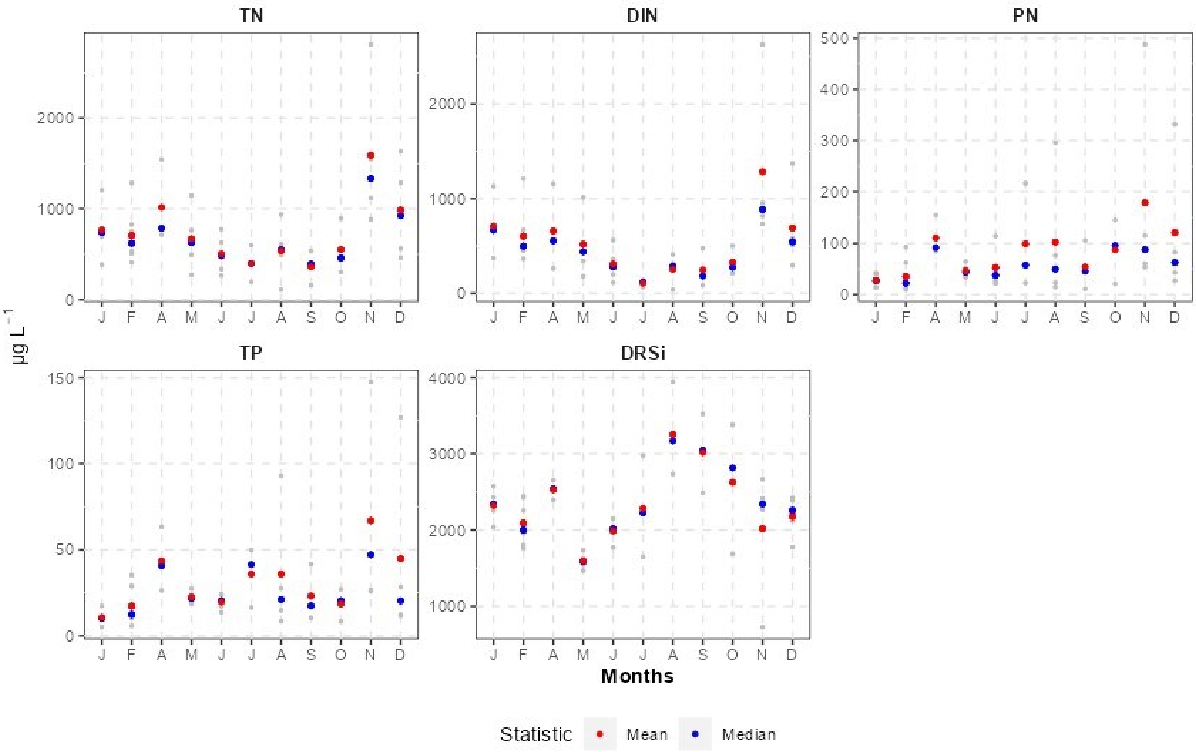

***Figure 4.5** Concentrations of nutrients which present a significative random effect (TN, DIN, PN, TP and DRSi – **Table 4.2**), showed by month. Red dots represent the mean, while blue dots the median.*

***Table 4.2** Statistical tests' p-values. The first three rows show the p-value of the differences between inflow-outflow, wet-dry flow, and empty-full reservoir. The last row reports the p-value of the differences between the AIC model, which should be interpreted as the effect of time.*

Test	Effect	TN	DIN	PN	TP	SRP	PP	DRSi	Chl-a
contrast	inflow - outflow	< 0.001	< 0.001	< 0.001	< 0.01	-	< 0.001	-	< 0.001
contrast	wet - dry	-	-	< 0.001	< 0.05	-	< 0.01	-	-
contrast	empty - full	-	-	< 0.01	< 0.01	-	< 0.01	-	-
AIC	Random effect	< 0.05	< 0.01	< 0.01	< 0.05	-	0.544	< 0.01	-

4.3.3 Role of hydrology and seasonality in controlling N, P and Si stoichiometry

To assess the effect of reservoirs on nutrients stoichiometry the upstream and downstream molar ratios between the different forms of N and P, and between the reactive forms of N, P and Si were compared (Figure 4.6). Globally, an excess of nitrogen and silica compared to phosphorus was measured for both dissolved reactive forms (DIN: SRP: DRSi = 395:1:1014) and total forms (TN: TP = 80:1). The high difference between DIN: SRP and TN: TP is to be analysed by considering the different contribution of the particulate forms to the total pool (PN: PP = 9:1) (Figure 4.5). The dam has a significant effect on the ratio of dissolved forms, but not on particulate forms. The DIN: SRP ratio doubled (from 250 to 520), and the DRSi: DIN ratio dropped by 2/3 (from 8 to 3). Only downstream of the dam does the hydrological condition affect the stoichiometric ratio, resulting in an imbalance in favour of nitrogen (both TN and DIN) compared to phosphorus (Figure 4.6). The seasonal random variable was significant ($p < 0.01$) only for TN: TP and DIN: SRP, which show a similar trend, and for the DRSi: DIN ratio (Figure 4.6).

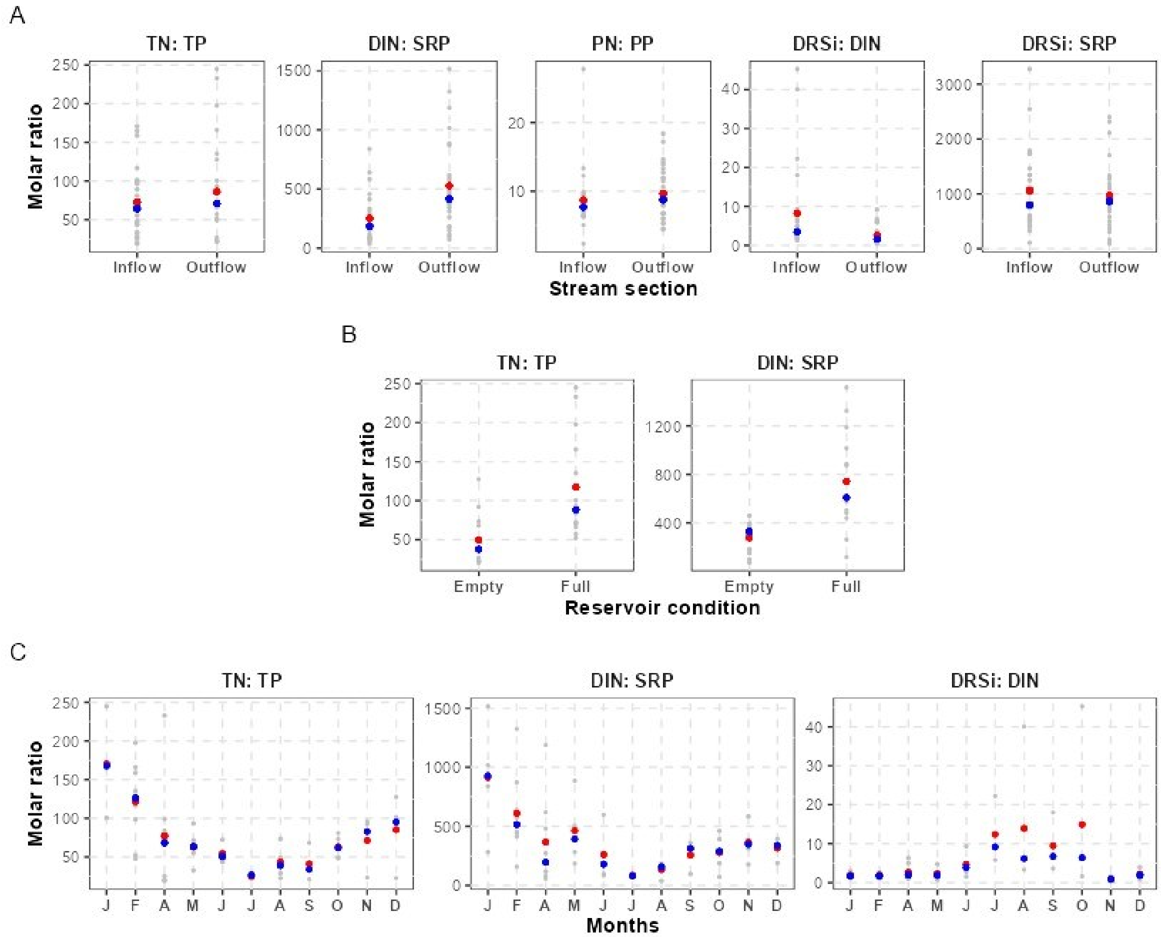

Figure 4.6 *(A) Molar ratios between TN: TP, DIN: SRP, PN: PP, DRSi: DIN, DRSi: SRP comparing inflow and outflow stations;* ***(B)*** *molar ratio of TN: TP, DIN: SRP comparing reservoir condition;* ***(C)*** *molar ratio of TN: TP, DIN: SRP, DRSi: DIN comparing significant temporal trends. Red dots represent the mean, while blue dots the median.*

Table 4.3 *Statistical tests' p-values. The first three rows show the p-value of the differences between inflow-outflow, wet-dry flow, and empty-full reservoir. The last row reports the p-value of the differences between the AIC model, which should be interpreted as the effect of time.*

Test	**Effect**	**TN: TP**	**PN: PP**	**DIN: SRP**	**DRSi: DIN**	**DRSi: SRP**
contrast	inflow - outflow	-	-	<0.001	<0.001	-
contrast	wet - dry	-	-	-	-	-
contrast	empty - full	< 0.05	-	<0.05	-	-
AIC	Random effect	< 0.05	-	<0.001	<0.001	-

4.3.4 Nutrients loads and retention in relation to reservoirs hydrological management

The loads are presented separately for the two basins as the lakes have a different hydrological balances (Table 4.4, Figure 4.7). Overall, the Arda basin received a mean annual TN loading of 10.8 ± 3.1 tons N y^{-1} and exported 7.7 ± 1.4 tons N y^{-1}, equivalent to a 27±23% net TN retention. Total P load was 0.4 ± 0.1 tons P y^{-1} in the inlet and 0.5 ± 0.1 tons P y^{-1} in the outlet, equivalent to a 13±59% net release period of TP. Mean annual DRSi loading was 31.6 ± 1.5 tons Si y^{-1} at the inlet and 21.9 ± 1.1 tons Si y^{-1} at the outlet equivalent to a 30±8% net DRSi retention. (Table 4.5)

The Tidone basin received a mean annual TN loading of 4.8 ± 1.7 tons N y^{-1} and exported 5.0 ± 0.9 tons N y^{-1}, equivalent to a 2±41% net TN retention. Total P load was 0.3 ± 0.2 tons P y^{-1} in the inlet and 0.2 ± 0 tons P y^{-1} in the outlet, equivalent to a 67±74% net TP retention. Mean annual DRSi loading was 11.7 ± 0.8 tons Si y^{-1} at the inlet and 10.8 ± 0.6 tons Si y^{-1} at the outlet equivalent to a 9±12 % net DRSi retention. While the net retention may be positive or negative, the high variability involved suggests caution in managing these results.(Table 4.5)

Table 4.4 *Annual hydraulic retention. Positive values indicate a net retention while negative values indicate a net release period.*

Site	**Volume M m^3**		**Retention**	**Number of days**	
	in	out		Release period	Recharge period
A	13.5	11.0	18%	145	251
T	5.5	5.8	-6%	150	246

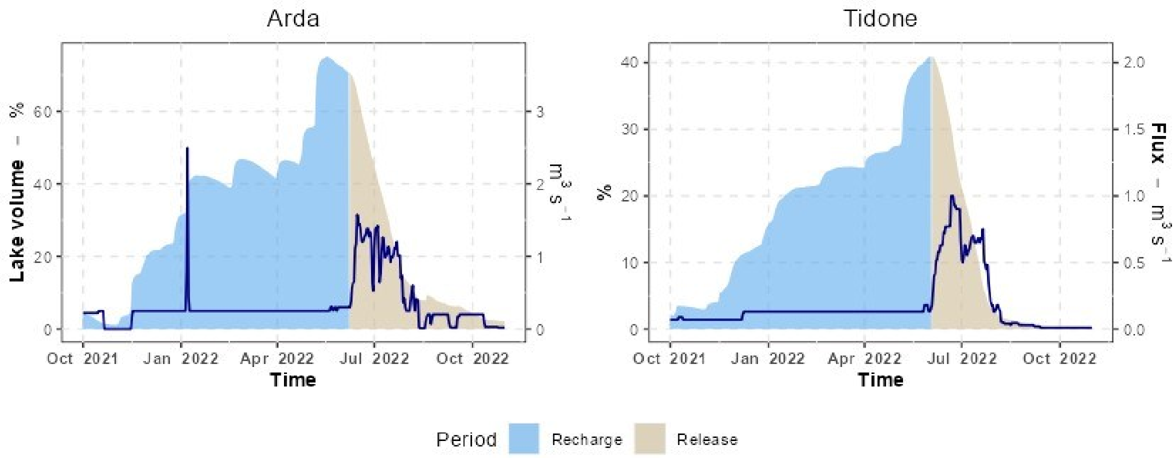

Figure 4.7 *In the plot is presented the water flux at the outflow with the respective lake volume. This scheme follows reservoir management dividing the year between recharge and release periods.*

Table 4.5 *Annual retention percentage calculated. Positive values indicate a net retention while negative values indicate a net release period.*

Site	TN	DIN	PN	TP	SRP	PP	DRSi
Arda	27 ± 23	46 ± 37	-59 ± 67	-12 ± 53	32 ± 21	-13 ± 59	30 ± 8
Tidone	2 ± 41	-19 ± 38	74 ± 73	59 ± 69	48 ± 55	67 ± 74	9 ± 12

The contribution of each period to the load varies depending on the station's location and the nutrient in question. The recharge period, which spans three seasons (autumn, winter, and spring), accounts for approximately 98% of the TN and TP incoming load and about 95% of the DRSi load in both lakes. In contrast, the load that was release period shows a more balanced ratio between the recharge period and release period phases. The recharge phase contributes approximately 60% (TN) and 45% (DRSi) loads for both streams, while the contribution to TP load was 52% in Arda and 36% in Tidone streams.

In terms of pool composition, DIN accounts for the majority of TN (61% of the load), while PP is the dominant form of TP (69% of the load) (Figure 4.9). These proportions (DIN > PN for nitrogen and PP > SRP for phosphorus) are consistent across all stations and periods but may not always align with the global average.

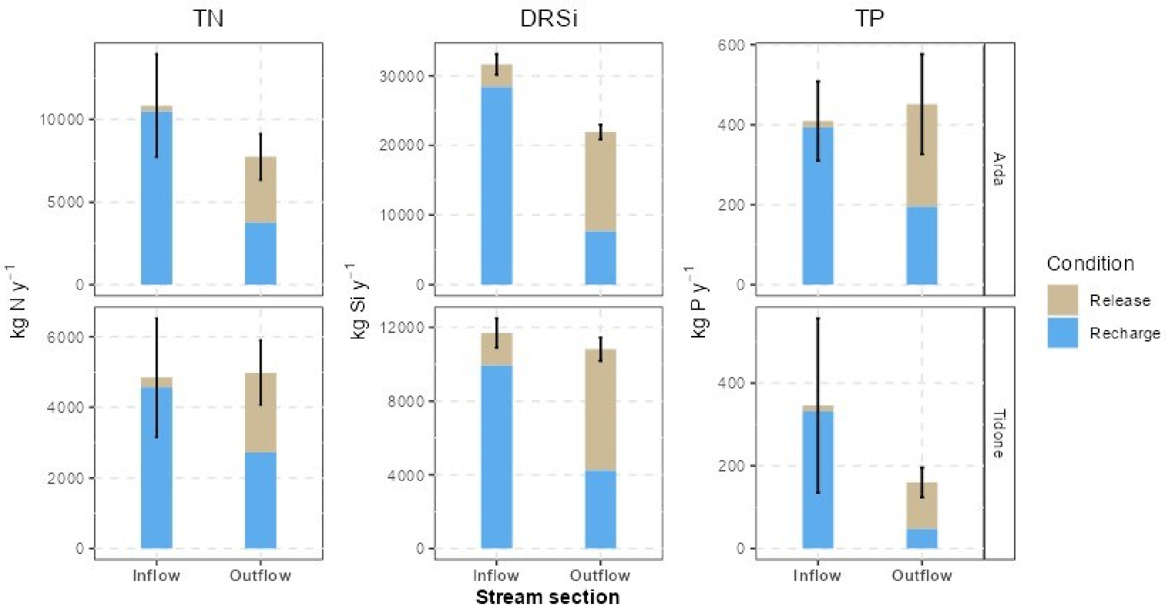

***Figure 4.8** Barplot of TN, DRSi and TP load ± se (kg y^{-1}) at the inflow and outflow stations. Colour represents the two periods: the **release period** and the **recharge period** (see figure 4.7).*

When comparing the composition of the pool between the recharge period and release periods for the stations upstream of the dam, the particulate component for N drops from 17% to 7% and for P drops from 83% to 47%. However, for the downstream stations of the dam, the particulate component for N rises from 10% to 18%. The two dams exhibit opposite behaviour when comparing the two moments for phosphorus, with the PP decreasing for Arda (from 90% to 76%) and increasing for Tidone (from 57% to 71%).

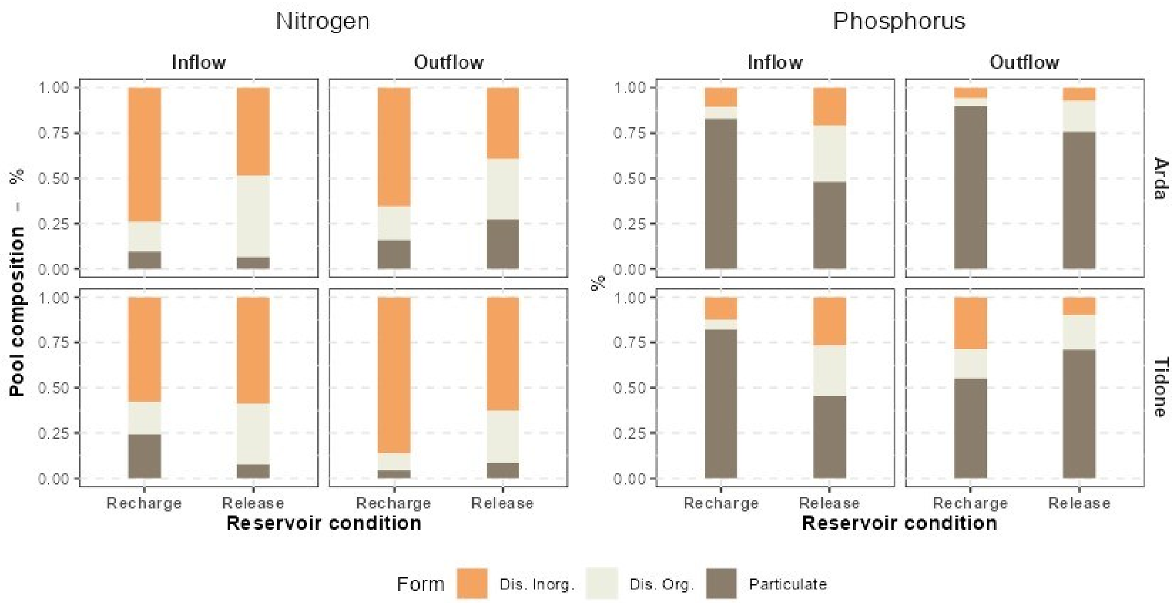

Figure 4.9 *Percentage composition of N and P load pool at inflow (low and high flow) and outflow stations during recharge period and release period periods (see figure 4.7). Colour represents the main nutrients forms: Dissolved Inorganic forms (DIN or SRP), Dissolved Organic forms (DON or DOP), and particulate forms (PN or PP).*

4.4 Discussion

This work evaluated the effect of two dams on nutrient transport along an upstream-downstream gradient, with a focus on the dynamics of the recharge and release period of the reservoirs. The results demonstrate that the two reservoirs act as biogeochemical reactors by transforming a fraction of the external N and P loads. Such behaviour is clearly evidenced by the concentration changes between upstream and downstream stations as well as changes in nutrient ratios. This is not unexpected, as many other studies have shown that biogeochemical processes occurring in reservoirs or lakes regulate nutrient transport in the hydrographic network (Kijowska-Strugała et al., 2016; Shaughnessy et al., 2019; Webster et al., 2021; Yan et al., 2021). Contrary to what was observed in other studies, the presence of the two reservoirs does not result in a clear reduction of nutrients loads. Such a result is not straightforward, but it is probably a consequence of concentration changes coupled with the management of the reservoir's hydrology that influences the timing of discharge in the two streams. Overall, the combination of reservoir metabolism and discharge determines a change in the relative proportion of nutrient loads as shown by the increase in nutrient ratios with DIN: SRP and DIN: DRSi molar ratios being greater in the outflowing water relative to inflowing. These results are in line with the few studies that had previously addressed the large-scale effect of reservoirs on nutrient stoichiometry (Bartoszek et al., 2016; Grantz et al., 2014), but the underlying mechanisms are likely different (see below).

4.4.1 Role of Different Control Factors on nutrients concentrations and stoichiometry

The two reservoirs act as biogeochemical reactors by changing nutrient concentrations, but the contribution of the hydrological and seasonal control varies depending on the nutrient and its form. Following the significance of the tested relationships, it is possible to order the nutrients according to a gradient from purely hydrological to purely seasonal control. According to this gradient PP > PN ~ TP > TN ~ DIN > DRSi, where at the extremes there are the PP, for which all strictly hydrological variables are significant, and the DRSi for which only the seasonal component is significant. No SRP is reported in this list because no factors were found to be significant.

Particulate forms of N and P are primarily influenced by hydrological and physical factors. Upstream of the reservoir, the role of hydrology is mainly linked to the erosive action of precipitation. Downstream, the results are less intuitive, as one would expect a buffering effect of the two artificial basins. The presence of the impoundment by increasing residence time should increase sedimentation rates and reduce the concentration of particulate matter suspended in the water column. This buffering effect was observed when the reservoirs were full, while an increase in particulate material concentration was measured when the reservoirs were empty. Such contrasting behaviour can be explained considering that the dams have a mouth at their base to allow the outflow of water. During the recharge phase, water currents inside the reservoir likely facilitate the resuspension and export of solid material that has accumulated over the years at the bottom of the lakes. The duration of the dam's recharge phase (approximately 75% of the time) is much longer than flood phases in upstream reach (which occur 20% of the time). Therefore, downstream particulate nitrogen and phosphorus concentration is higher for a longer period than in the upstream section. In contrast, flood phenomena (upstream) or filling level (downstream) do not affect DIN and DRSi, but their levels are strongly influenced by seasonal dynamics likely related to biological control at higher temperatures. The concentration of DIN downstream of the reservoirs increased in contrast to DRSi and SRP concentrations, indicating a potential role of lake processes in the inorganic nitrogen release period.

The observed changes in stoichiometric ratios coherently mirror the different behaviour of concentrations. Overall, an increase in the DIN: SRP and DIN: DRSi ratio was observed. If this result seems consistent with what was synthesized by Maavara et al. (2020a) and found by other authors (Yan et al., 2021), the underlying mechanisms are different. In fact, the ratio increase does not occur because of a greater SRP or DRSi retention capacity, but it is due to a greater release period of DIN, especially in the full reservoir condition.

Soluble P is commonly considered a limiting factor (Kekc & Lepori, 2012) and reservoirs with a higher rate of P retention compared to N are often located downstream or within an agricultural context and are affected by bloom phenomena that can favour P burial thanks to sedimentation of organic P (Grantz et al., 2014; Pearce et al., 2017). On the contrary, the reservoirs considered in this study are located upstream of intensive crops and large population centres and there are no relevant anthropogenic sources of P. In addition, most of the P enters only during flood phases in particulate, poorly bioavailable, form and is quickly deposited on the bottom by gravity.

In this research biogeochemical nitrogen transformations within the lakes were not measured, but the higher DIN concentrations at the outlet suggest a predominance of processes that produce inorganic forms (net mineralization of organic nitrogen) over those that consume them (assimilation by primary producers and/or denitrification). In particular, the recharge-release dynamics expose the sediments of the lake in contact with the atmosphere, which influences the amount and forms of dissolved inorganic nitrogen accumulated in sediments. Exposure to sunlight and intense desiccation alter the physico-chemical properties of the accumulated organic matter, exposure to air induces cell lysis during drying and modifies sediment properties by increasing oxygen penetration (De Groot & Van Wijck, 1993). In turn, increased oxygen availability in the sediment influences rates of microbial nitrification, which oxidizes ammonium to nitrate which is highly soluble (Burgin & Hamilton, 2007; Merbt et al., 2016). Therefore, sediment inundation during the recharge period could promote diffusion to the water column of the high NO_3^- concentrations accumulated in drying sediments. Conversely, sediment exposure reduces the period in which the sediment can potentially be in anoxic conditions when the denitrification process is favoured and thus nitrate elimination.

The dynamics of the main forms affect TP and TN. DIN, which dominates the N pool, characterizes the seasonal behaviour of TN, while PP, which is the majority in TP, defines the hydrological control of phosphorus. TP and PN are the only two forms controlled by both hydrological and seasonal factors. This could be related to the role of organic components, which have not been specifically analysed in this work. The constant TN: TP ratio between the upstream and downstream stations masks the opposing dynamics of the different forms of the analysed nutrients. The increase in DIN: SRP (upstream-downstream) should correspond to the increase in TN: TP that is not recorded because of the increase in the export of particulate matter, which has an unbalanced ratio towards PP (PN: PP = 8:1).

4.4.2 Nutrient Retention capacity in relation to the management of the reservoirs

Reservoirs are considered to be 'in-stream' reactors that enhance nutrient retention due to sedimentation and gaseous elimination. One of the main descriptors used to estimate the effect of a reservoir on nutrient transport is its retention capacity (Maavara et al., 2020). The results of this work show that a general tendency towards net nutrient retention is difficult to define. Considering the three main forms analysed (TN, TP and DRSi), the net retention capacity or release period is extremely uncertain for both reservoirs, with the exception of DRSi for the Arda reservoir. These results are in contrast to previous studies, which have shown that reservoirs retain on average about 21% of the Si, 43% of the P and 35% of the N loads transported worldwide (Maavara et al., 2020a; Van Cappellen & Mavaara, 2016). However, the high variability is consistent with other studies (Powers et al., 2015; Yan et al., 2021) where the uncertainty associated with retention capacity was estimated to be even greater than 50% and both positive and negative rates were measured on the same reservoir but in different years.

Such variability suggests that caution should be considered when extrapolating these results. Accurate evaluations for budgets of nutrients in reservoirs rely in fact on high-frequency data of hydrology and water quality. In this work, daily hydrological parameters were recorded while water quality data were collected with a monthly frequency. The short period of observation (one hydrological year) and the monthly sampling frequencies could have introduced uncertainty in the calculated net retention rates. For example, the year when the study was conducted was characterized by an exceptionally high drought (Montanari et al., 2023) with only a few periods of intense precipitation. Under such conditions, upstream of the reservoirs, short phases of high flood alternate with long base flow periods, while more constant and predictable discharges characterized the downstream reach. Hydrological differences upstream and downstream of the dams related to the recharge period-release period cycle could explain the variability associated with nutrient loads. The high variability of the TN and TP retention is in fact linked to the propagation of the variability related to the particulate forms, which is always higher than 50% in both basins. Compared to the dissolved components, particulate forms are present only under high flows and their concentration can vary very quickly (especially upstream of the reservoirs), resulting in a high level of uncertainty. In this case, the associated error is therefore related to the variability of the concentration, which is in turn influenced by the interaction with the hydrological dynamics.

Additionally, prolonged water scarcity has retarded the reservoir’s recharge period and likely altered the normal biogeochemical functioning by reducing the actual water retention time. Nutrient retention depends on hydrological characteristics and depends on average residence time (Maavara et al., 2020). The two reservoirs, during the studied period, had a relatively low residence time of about 7 months and a recharge period release period cycle that was completed in one year. The relatively short residence time could partially explain the low nutrient retention capacity. One way to overcome this uncertainty would be to increase the period of observation including years with different hydrological conditions and dam management.

The estimated load retention capacity seems to partially contradict the upstream-downstream trends of nutrients concentrations. This is particularly true for nitrogen, which has an average higher concentration downstream compared to upstream, but negligible retention. This could be explained by the combination of seasonal dynamics (higher N concentration during winter months), reservoir management (higher water retention in winter) and lake nitrogen transformations. During the winter period, the upstream/downstream N concentration increase does not translate into a load increase because of the upstream/downstream discharge decrease at the outlet driven by water retention within the reservoir. During summer, the dam is emptied but the N concentration, although greater downstream than upstream is equal to or lower than in the winter period. In addition, the higher winter concentration explains the higher TN downstream load during the recharge period phase compared to the downstream load during the release period phase.

Despite the absence of a clear effect of the two reservoirs on nutrient transport at the year time scale (i.e. the time scale that includes a complete recharge period-release period cycle), what emerges is a change in the timing of transport. The reservoirs are primarily filled during periods of stream flooding in autumn and spring and emptied during the summer months (mainly between June and July) for irrigation purposes. This management strategy results in minimal downstream flows during periods when flows should be naturally higher (from October to May) and the presence of water downstream during the summer period when minimum flows or intermittent river conditions are more common (from June to October). As such, dam functioning creates a temporal discontinuity in the natural hydrological dynamics and nutrient transport. This concept was developed by Hayes et al. (2018) for Mediterranean basins primarily used for irrigation purposes. However, the recharge period and release period phases only partially overlap with the natural seasonal dynamics that affect different hydrology and nutrients concentration resulting in a high variability of loads. Primary producers follow a seasonal cycle linked to temperature and light availability that gradually increases and decreases, passing from a maximum during the summer season to a minimum during the winter season. In contrast, the strictly hydrological dynamics are characterized by a pulsating behaviour. This has an impact on the reservoir fill level, where a single flood event can contribute to more than 10% of the reservoir volume, resulting in a sudden increase in the water volume and nutrient input. Similarly, the release period is concentrated in a few weeks, so the reservoir quickly turns from being full to empty, and a large part of the accumulated nutrients are exported.

4.5 Conclusion

The results of this study demonstrate that the two reservoirs act as biogeochemical reactors by transforming the external nutrient loads. Such behaviour is clearly evidenced by the concentration changes between upstream and downstream stations and by changes in nutrient ratios with DIN: SRP and DIN: DRSi molar ratios being greater in the outflowing water relative to inflowing. However, nutrients transformation does not result in a clear reduction of loads on an annual scale and in some cases, a slight net release period was detected. Nevertheless, the cyclicity of the recharge period and release period alters the natural hydrological and biogeochemical dynamics of rivers with implications for the seasonal distribution of nutrient loads. By analysing concentration changes and associated loads in relation to the recharge period and release period periods, it is evident that in-lake biogeochemical processes, coupled with altered river hydrology due to reservoir management, result in a temporal discontinuity in nutrient loads.

Reservoirs managed for irrigation purposes in a Mediterranean climate could potentially not only alter the relative amount of nutrients along the hydrographic network but also create a temporal discontinuity in the natural hydrological connectivity. As a result, nutrient transport would be out of phase with the natural seasonal dynamics. This effect could become more pronounced if climate change leads to longer droughts and more variable rainfall, as less water is released during replenishment periods. Additionally, nutrient disequilibrium and excess delivery of particulate forms could impact the structure and functioning of downstream reach. For much of the year the reservoirs release period particulate nutrients which tend to settle more rapidly under the low flow conditions observed downstream and accumulate on riverbeds. Such a nutrient pool could represent an additional source of P and N during summer months when metabolic processes are higher. Further research is, therefore, needed in order to provide new insights with which to understand the role of reservoirs in regulating nutrient transport – these studies would serve as a guide for the maintenance of water quality and the ecological conservation of water systems.

5 Conclusions

This study aimed to enhance understanding of how nitrogen and phosphorus dynamics respond to changes in hydrology and human activity. The research analysed at different spatial and temporal scales the relationships between hydrology, human pressures, catchment characteristics and seasonality with N and P loads in the Po River Hydrographic District and its catchments. More specifically three main questions were addressed:

- How precipitation patterns have influenced the evolution of nitrogen and phosphorus river loads from 1992 to 2022, in a highly anthropized catchment (the Po River basin);
- What different anthropogenic nutrients input and hydro-geological characteristics influence N and P river loads and their ratios;
- How hydrology and water accumulation in artificial reservoirs affect the transport of nitrogen, phosphorus, and silicon, as well as their stoichiometry.

The main hypotheses were that modifications to the hydrological continuum, whether in terms of altered precipitation patterns or changes to river flow, can significantly impact the transport of excess nitrogen and phosphorus resulting from human activity. Additionally, due to differences in the biogeochemistry of nitrogen and phosphorus, these hydrological factors can also alter the stoichiometry of these two elements. The results of this thesis highlight that climate regimes interact with human activities in complex ways that alter both the absolute magnitude of nutrient loads to rivers as well as their composition across space and time. Anthropic activities affect these two factors, either on a large scale (climate change) or on a local (hydrographic network modification), altering natural transport and recycling and shifting load export between seasons (dry and wet periods), between years (legacy) and across space.

The analysis of the long-term N and P export regime of the Po River in relation to hydrological variability allowed us to explain the role that precipitation pattern and seasonality play in regulating loads. The results show that the rainfall regime affects not only water resources but also nutrient concentrations. The catchment's ability to export nutrient loads varies depending on the nutrient (N or P), the molecular form, and the season. We found that:

- Precipitation increases the load, but the effect varies depending on the season. It is more pronounced in summer and autumn than in winter and spring owing to a different relationship between concentration and discharge. In autumn rainfall drives runoff increases and then the loads, while during summer the effect is accentuated by also nutrient enrichment. Conversely, winter and spring are more influenced by precipitation as snow, which causes a decoupling between the driver (increasing precipitation) and the effect (load export), with a null or dilution effect on concentration.
- The analysis spanning 30 years indicates that changes in the hydrological regime have affected the timing of load export, resulting in an extension of summer conditions towards autumn and a lesser extent towards spring. The observed decreases in $N\text{-}NO_3^-$ and TP over the last 30 years may therefore also be driven by seasonality, as autumn loads have become less relevant compared to the past.
- The rise in extreme weather events during the late spring and summer months, coupled with the anticipated decline in the significance of snowmelt as predicted by hydrological models in the future years, may further change nutrient export, given the coupling between rainfall regime and N and P loads.

The contribution of hydrology to nitrogen and phosphorus loads about anthropogenic nutrient sources was then examined. The diversity of landscapes within the Po River District enabled the comparison of catchments with varying levels of anthropogenic influence, pressure distribution show as the high pressures affect the exported loads that are higher than those observed in other catchments. While, geographical and hydrological characteristics, suggest that the seasonal trend identified in the previous chapter could depend also on the spatial variability of the Po River sub-basins. Results demonstrated that:

- The levels of NANI and NAPI are not sufficient to explain the high spatial diversity of river N and P loads: the relationship between anthropogenic input and exports was found to be insignificant for P and only slightly significant for N. In contrast, the individual components contributing to NANI and NAPI and the hydrological characteristics have different effects on the loads of the different forms of N and P exported by the catchments. N is more easily exported from wetter basins, while P is more dependent on the erosive action of rainfall.

- The relationship between single N sources and exported loads showed that the organization of the agrifood system strongly influences river loads. TN was mainly exported as $N\text{-}NO_3^-$ and the regression analysis indicates that feed trade and fertilizer application are the major drivers of $N\text{-}NO_3^-$ loads. More specifically $N\text{-}NO_3^-$ export increased in relation to both the amount of nitrogen imported to and exported from the catchments and those that tend towards equilibrium (i.e. low net fluxes associated with feed trade) are more efficient systems in the use of anthropogenic nitrogen.
- The imbalance (high concentration of intensive livestock farming) creates a decoupling between animal farming and crop production that may contribute to saturating the capacity of basins to remove nitrogen. In wet basins this corresponds to an increase in exports, in drier basins N export regime became similar to the P one, strongly dependent by high flow periods.
- Due to differences in fractional export, the N:P ratio increases by approximately 9 to 7 times depending on whether only the inorganic forms or the total are considered. Because of the effects that N:P ratios have on ecosystem functioning a better understanding of what features alter the relative N and P fluxes throughout the catchments is required.

The high seasonality of rainfall and the lack of natural water storage features (especially on the Apennines side) have led to the construction of artificial reservoirs to regulate the variability of water availability. These reservoirs are essential to sustain the intensive agricultural sector that characterizes the Po River plain but also affect the characteristics of the hydrographic network, the hydrological continuity and the seasonality of water resources. Analysis of Arda and Tidone reservoirs shows that the two reservoirs act as biogeochemical reactors by transforming the external nutrient loads. The analysis showed that:

- Nutrients transformation does not result in a clear reduction of loads on an annual scale and in some cases, a slight net release period was detected. These reservoirs through hydrological alteration, dampen the effect of floods during the recharge phase of the reservoirs, while the accumulated guarantee the water during the summer season when streams used to be dry.

- The alternance of the recharge-release phases alters the biogeochemical dynamics of rivers resulting in a temporal discontinuity of nutrient transport. As a result, nutrient transport is out of phase with the natural seasonal dynamics and this nutrient load represents an additional source of P and N during the summer months when metabolic processes are more intense.
- Because of the expansion of Mediterranean climate conditions in southern Europe, this effect could become more pronounced if climate change leads to longer droughts and more variable rainfall, as less water is released during replenishment periods.

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